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The Chia Seed Affects on the Rate of Development and Percentage of Hatchability in *Drosophila melanogaster*

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Abstract

Drosophila melanogaster is a widely used model organism for studying embryonic development, where traits such as developmental rate and hatchability serve as important indicators of pre-adult fitness. Nutritional factors, particularly diet composition, can significantly impact these developmental outcomes. In this study, flies were reared on wheat cream agar (control) and chia seed treated (5g, 10g, or 15g of chia seeds) to investigate the effects of chia seed supplementation on rate of development and percentage of hatchability from larva to pupa and pupa to adult in *D.melanogaster*

The group receiving 15g of chia seeds demonstrated the shortest developmental time from larva to pupa and from pupa to adult, followed by the 10g group. In contrast, the 5g chia seed supplementation group exhibited delayed development relative to the control. These results suggest that higher levels of chia seed supplementation may provide nutritional or physiological benefits that promote faster maturation, whereas lower concentrations may exert inhibitory effects.

Hatchability patterns mirrored the developmental trends. Larva-to-pupa survival rates increased with chia seed supplementation, reaching a maximum in the 15g group. Pupal-to-adult emergence was significantly highest in the 15g and 5g treatments, whereas the 10g group showed reduced emergence compared to the highest and lowest supplementation levels. Overall, these findings indicate that chia seed supplementation contains a great source of omega 3 fatty acids, fiber, protein and antioxidants. Particularly at higher concentration chia seed supplementation improves both rate of development and percentage of hatchability in *D. melanogaster*, likely through enhanced nutritional support affecting energy metabolism and developmental pathways.

Keywords: *Drosophila melanogaster,* chia seed supplementation, development rate.

Introduction

Environmental variation plays a crucial role in shaping fecundity and physical condition, largely through its effects on nutrition, which are influenced by the availability and diversity of food sources. Many species in natural habitats face challenges in meeting the nutritional demands required for both somatic maintenance and reproductive development (Raubenheimer and Simpson, 1999) [34] Life-history traits such as fertility, disease resistance, lifespan, and stress tolerance are directly impacted by the quality and quantity of nutrients consumed (Hoffmann and Parsons, 1991; Rion and Kawecki, 2007; Lee *et al.,* 2008) [19, 31, 23]. Since food is the primary source of both energy and essential nutrients, diet significantly influences an organism's entire life history (Sterner and Schulz, 1998; Taylor *et al*., 2005) [37, 38]. Experimental manipulation of diet has proven valuable in understanding energy allocation strategies in animals (Chown and Nicolson, 2004; Cruz-Neto and Bozinovic, 2004) [9, 10]. Survival and reproductive success in animals depend on a balance between energy intake and expenditure, involving the processes of food consumption, digestion, and energy allocation toward maintenance, growth, and reproduction (Pough, 1989; Sibly, 1991; Karasov, 1986) [28, 20]. Optimal development requires a specific balance of nutrients, and imbalances in macronutrients—such as fats, carbohydrates, or proteins—can adversely affect physiological processes including growth and reproductive output (Bauerfeind and Fischer, 2005; Wang and Clark, 1995) [6, 39]. In *Drosophila melanogaster*, for instance, protein deficiency has been shown to reduce fertility and inhibit growth, with protein often being a limiting factor for fruit-feeding insects (Mattson, 1980; Adams and Gerst, 1991; Hendrichs *et al*., 1991; Markow *et al*., 1999, 2001) [26, 1, 24].

Increased egg-to-adult viability has been observed in diets enriched with sucrose and protein, with such nutritional enhancements also improving female survival rates (Djawdan *et al*., [37] development, as it significantly affects both viability and long-term adult performance.

Previous experimental studies on *Drosophila* species have highlighted the role of natural food sources in promoting developmental fitness. For example, organic fruits such as chikku and watermelon were found to significantly enhance pre-adult fitness in certain *Drosophila* species (Geeth and Krishna, 2015) [15]. While avocado and yogurt did not show a

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marked improvement in pre-adult development, they did contribute positively to larval-to-pupal transitions, pupal viability, and developmental rate in D. *melanogaster* (Cleona Alexander and Krishna, 2018) [2]. Additionally, research by Alwyn D'souza and Krishna (2015) [13] reported that an alternative natural energy drink supported early developmental processes in *D. melanogaster*, reinforcing the idea that specific dietary compositions can have profound effects on both pre-adult development and overall fitness.

More recent studies investigated commercial supplements. Whey protein, when incorporated into larval media, supported robust growth conditions that favored consistent development, though specific acceleration in development or hatch rate was moderate (Manaswini D. Kashyap *et al* 2024) [11] Mass gainer at optimal concentrations was associated with improved developmental conditions, allowing for faster progression from egg to adult and suggesting enhanced rate of development and stable hatchability (Aysha Barira H. M. *et al* 2024) [5] Spirulina, rich in nutrients, promoted healthier larval stages and better pupation, indirectly reflecting improvements in developmental rate and successful hatching (Shreejani H. K. *et al*.2023) [33]. Notably, Ensure®, while slightly delaying the rate of development, led to a significantly higher percentage of hatchability when mixed with wheat cream agar, suggesting a trade-off where survival during embryonic stages is prioritized (Suma s. *et al* 2024) [37].

However, the impact of chia seeds on preadult fitness and percentage of hatchability has not yet been explored, which prompted the present study.

Chia seeds, harvested from *Salvia hispanica L*., a member of the Lamiaceae family, have evolved from being a traditional dietary staple among Indigenous populations in Central and South America to becoming a globally recognized health food. In terms of composition, chia seeds provide a notable range of macronutrients and bioactive compounds. They are composed of approximately 30–33% fat, 15–25% protein, 18–30% dietary fiber, and 26–41% carbohydrates, with ash content accounting for about 4–5%, indicating a substantial mineral presence (Muñoz *et al.,* 2013) [27]. Additionally, chia seeds are naturally high in polyphenolic antioxidants such as quercetin, kaempferol, myricetin, and caffeic acid. These compounds not only stabilize the polyunsaturated fatty acids in the seed oil but also offer protection against oxidative stress, which is linked to various metabolic disorders (Reyes-Caudillo *et al.,* 2008) [30].

Materials and Methods

Chia seeds used in this study were procured from Loyal World, a retail grocery outlet located in Mysore, Karnataka. The seeds, marketed under the True Elements brand, were used to prepare the experimental medium. Prior to application in the treatment process, the seeds were finely ground into a powder and stored under appropriate conditions for further use.

Establishment of Experimental Stock

The experimental Oregon K strain of *Drosophila melanogaster* was collected from *Drosophila* Stock center University of Mysore, Mysuru were cultured using a wheat cream agar-based medium, prepared by boiling 100 g of jaggery, 100 g of rava powder, and 10 g of agar in 1000 ml of distilled water, followed by the addition of 7.5 ml of propionic acid.

All flies were kept under controlled laboratory conditions with a temperature of 22°C ± 1°C, 70% relative humidity, and a 12:12 hour light-dark cycle.

The above cultured flies were used to establish the experimental flies with different diet media

a) **Wheat Cream Agar Medium (Control):** This media was prepared 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.
b) **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.
c) **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.
d) **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.

Flies that developed on these control and treated media were maintained under consistent laboratory conditions as mentioned above and were used to assess parameters such as rate of development and % of hatchability.

Experimental Procedure

To assess the effect of Chia seed supplementation on developmental rates in flies, approximately 20 adult flies (10 males and 10 females) that had been reared in standard media were selected. These flies were transferred into separate culture bottles containing either control media or media supplemented with 5g, 10g, and 15g of chia seeds. The flies were left in the bottles for 3 hours to allow egg-laying. After this period, the adults were removed, and the bottles were left undisturbed for 24 hours to allow eggs to hatch into first instar larvae.

Rate of Development Analysis

Thirty first instar larvae were collected from each treatment group by carefully scooping from the media. These larvae were then placed into fresh vials containing their respective media: control, 5g, 10g, and 15g chia seed-supplemented media. The larvae were monitored regularly to record the exact time they entered the pupal stage. Observations continued until the adult flies emerged from the pupae, and the time taken for these transformation were recorded in hours.

Hatchability Percentage Assessment

Another set of thirty first instar larvae from each treatment group (control and chia seeds treated groups) was introduced into vials with their corresponding media. These larvae were observed until pupation, and the total number of pupae formed was recorded in percentage. Following this, the number of pupae that successfully hatched into adult flies was also recorded in percentage. Experiment was conducted separately and continuously for all the control and treated group studied.

Result Analysis

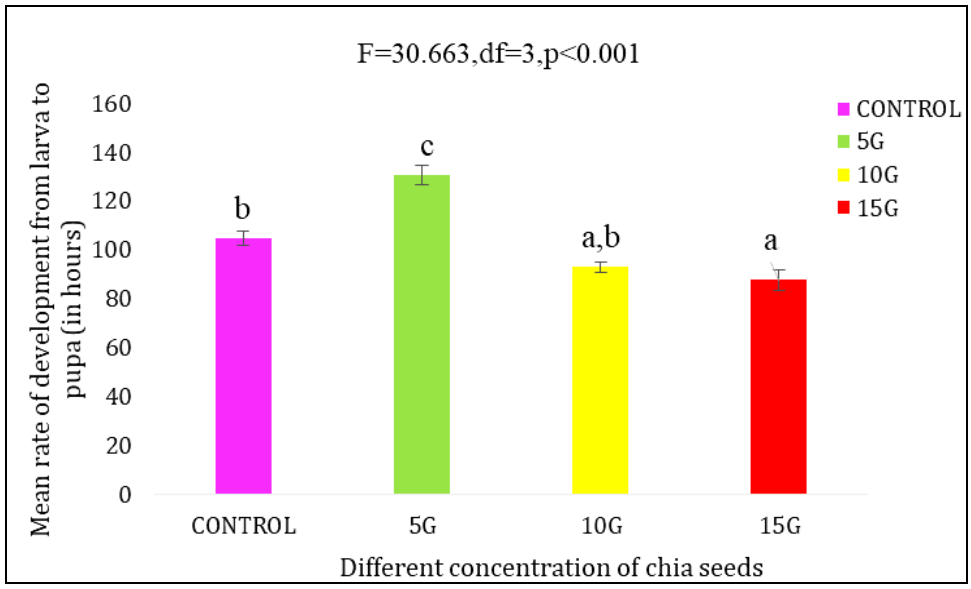

Fig 1: Effect of the chia seed supplemented diets on the rate of development larva to pupa in *D. melanogaster*

Distinct letters on the bar graph represent statistically significant differences among the various concentrations, as determined by Tukey's post hoc test at the 0.05 significance level.

Figure 1 shows the mean and standard error values of larva-to-pupa development rate in *D. melanogaster* reared on diets with different chia seed concentrations (Control, 5g, 10g, and 15g). According to the data, the development was slowest in flies fed with 5g chia seed-supplemented diet, followed by the control group, while faster development was observed in the 10g and 15g chia seed diets—with 15g showing the shortest development time.

The experimental data were analyzed using one-way ANOVA, followed by Tukey's post hoc test, which revealed statistically significant differences in the rate of development among the different diet groups. A significant difference was observed between the 5g group and all other groups, while the control group also showed a significant difference when compared with the 15g group by Tukey's post hoc test. However, the difference between the control and 10g group was not statistically significant, as indicated by the Tukey's post hoc test. Similarly, there was no significant difference between the 10g and 15g groups.

Thus, according to Tukey's post hoc analysis, larvae fed with the 5g chia diet took significantly longer to develop compared to those fed with the 15g chia diet, which had the fastest development rate.

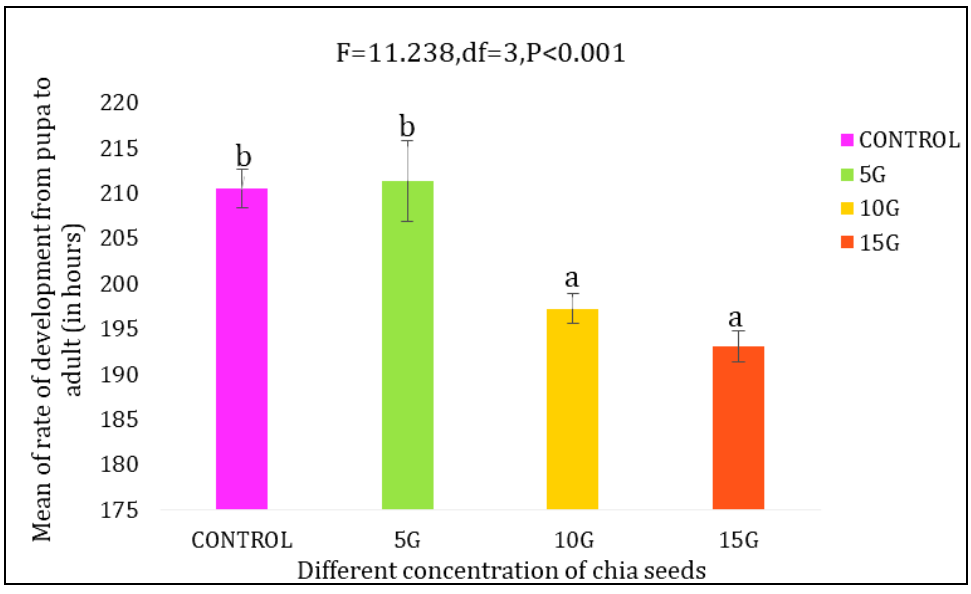

Fig 2: Effect of chia seed-supplemented diets on the rate of development from pupa to adult in *D. melanogaster.*

Distinct letters on the bar graph represent statistically significant differences among the various concentrations, as determined by Tukey's post hoc test at the 0.05 significance level.

Figure 2 showed the mean and standard error value of pupa to adult rate of development in *D. melanogaster* flies which were cultured in the different diet (Control, 5g, 10g, and 15g). The flies fed with 5g of chia seeds showed the slowest development, followed by the control group. In contrast, those given 10g and 15g chia seed diets developed more quickly, with the 15g group showing the fastest rate of development. The results were analyzed using one-way ANOVA followed by Tukey's post hoc test. A significant difference was observed between the 5g group and the 10g and 15g groups. The control group also showed a significant variation compared to the 15g group. However, there was no significant difference between the 10g and 15g groups, as they shared the same significance letter.

Overall, the findings suggest that higher concentrations of chia seeds, particularly 15g, improved the rate of development from pupa to adult in *D. melanogaster.*

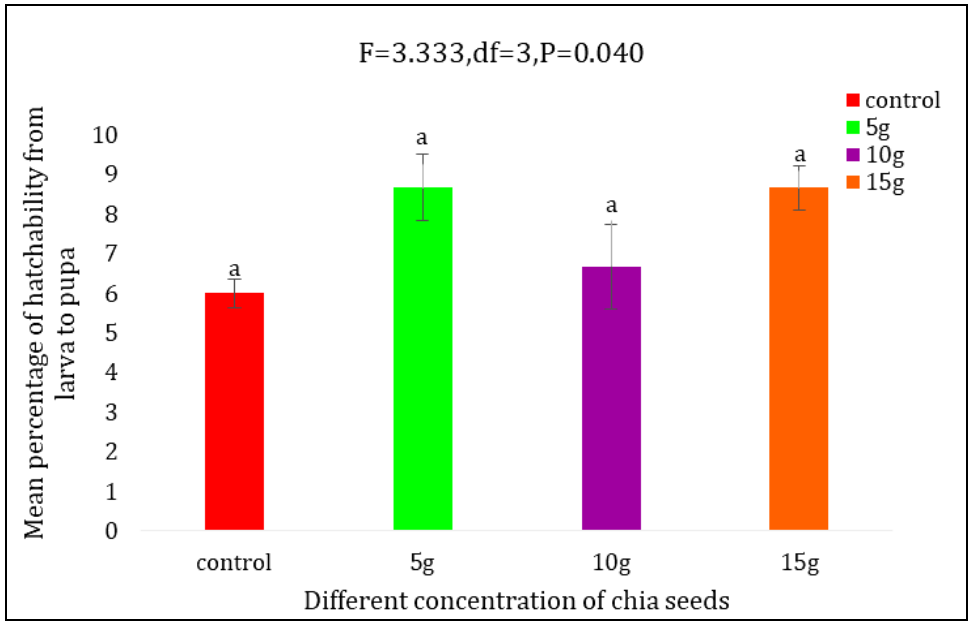

Fig 3: Effect of chia seed-supplemented diets on the % of hatchability of larva to pupa in *D. melanogaster*

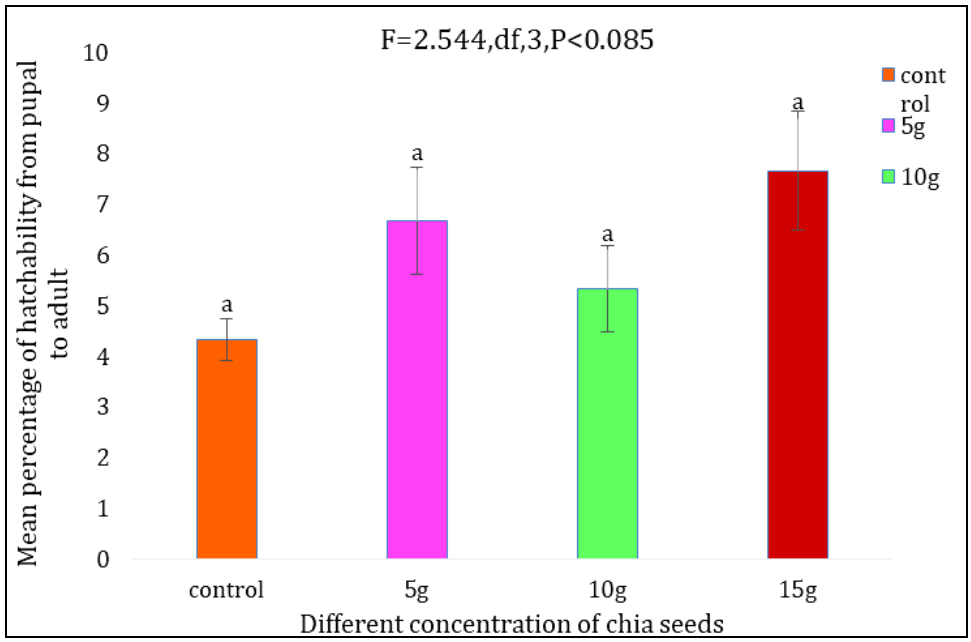

Fig 4: Effect of chia seed-supplemented diets on the % of hatchability of pupa to adult in *D. melanogaster*

Same letters on the bar graph represent statistically non-significant differences among the various concentrations, as determined by Tukey's post hoc test at the 0.05 significance level.

Figure 3 and 4 illustrates the effect of chia seed-supplemented diets on the percentage of hatchability from larva to pupa and pupa to adult in *D.melanogaster.* The flies were raised on four different diets: a standard control diet, and diets supplemented with 5g, 10g, and 15g of chia seeds. Among these, the control group and the 10g chia seed group showed the lowest hatchability rates, indicating a potential negative impact at lower and moderate chia seed concentrations. In contrast, the 5g and 15g groups exhibited higher hatchability percentages, suggesting an improvement in larval survival with increased chia seed concentration.

Statistical analysis using one-way ANOVA followed by

Tukey's post hoc test revealed statistically non-significant difference between any of the groups

Discussion

The Effect of Chia seed on the Rate of Development in *D. melanogaster*

The nutritional environment is a critical determinant of various life history traits in developing organisms, influencing survival, developmental timing, body morphology, lifespan, and reproductive capacity (Simpson and Raubenheimer, 2012) [35]. Among these factors, the quality and quantity of dietary nutrients play a pivotal role, providing the necessary energy and building blocks for physiological development and function. In the present study, we investigated the effects of varying concentrations of chia seed extract on the preadult fitness parameters specifically, developmental rate and hatchability percentage in *D. melanogaster.*

Chia seeds are widely acknowledged for their comprehensive nutrient profile, which includes high-quality proteins, omega-3 fatty acids, fiber, antioxidants, vitamins, and minerals. Given these bioactive compounds' crucial involvement in metabolic pathways, hormonal regulation, and energy utilization, chia seed extract served as a promising dietary supplement to explore its potential effects on developmental and survival outcomes.

Experimental groups were fed diets supplemented with 5g/10g/15g of chia seed powder per 1000 ml of food, alongside a control (wheat agar) group. As shown in Fig. 1, larvae in the control and 5g groups exhibited delayed development, indicating that absent or low chia supplementation does not significantly enhance early growth. In contrast, 10g and 15g chia treatments accelerated larva-to-pupa transition, suggesting a threshold effect where sufficient chia-derived nutrients improve energy metabolism. Similarly, Fig. 2 shows faster pupa-to-adult development in the 10g and 15g groups, supporting the role of optimal supplementation in enhancing metabolic efficiency and developmental progression.

In our study, the enhanced developmental rates observed with higher concentrations of chia seed extract may be attributed to its comprehensive nutrient profile, which includes high-quality proteins, omega-3 fatty acids, fiber, antioxidants, vitamins, and minerals. These components are crucial for metabolic pathways, hormonal regulation, and energy utilization, which are essential for growth and development.

Collectively, these studies underscore the significance of both the quantity and quality of dietary nutrients in supporting the growth and survival of juvenile *D. melanogaster*. Our findings contribute to this body of knowledge by demonstrating that chia seed extract, at optimal concentrations, can serve as an effective dietary supplement to enhance pre-adult fitness parameters in *D. melanogaster*

Several studies have reported positive effects of dietary interventions on *Drosophila melanogaster* development. Rodrigues *et al.* (2015) [32] found that a balanced protein-to-carbohydrate ratio reduced larval development time. Geetha and Krishna (2014) [15] showed that organic fruits like chikku and watermelon accelerated pre-adult development, while D'Souza and Krishna (2015) [13] noted similar benefits from natural energy drinks. Chabra *et al*. (2013) [8] observed faster development with organic over non-organic fruits, and Alexander and Krishna (2018) [2] found that yogurt enhanced development, likely due to probiotics improving gut health.

Conversely, some interventions had negative impacts. Layalle *et al.* (2008) [22] reported developmental delays under poor nutrition, and Kaya *et al.* (2016) [17] found that a high-casein diet extended the larval stage. Guler *et al*. (2014) [16] linked protein restriction to prolonged larva-to-pupa transition. Similarly, the current study showed that low doses of chia seed extract (5g) did not significantly improve early growth. Reduced yeast content, as noted by Rodrigues *et al*. (2015) [32], also delayed pupal eclosion, underscoring the importance of adequate protein.

Our study demonstrated a significant positive impact of chia seed supplementation on the pre-adult development of *D. melanogaster*. Both the 5g and 15g chia extract groups exhibited higher hatchability rates compared to the control, with the 15g group showing the highest larva-to-pupa and pupa-to-adult viability. This suggests that the bioavailable nutrients in chia seeds—particularly high-quality proteins and essential fatty acids—play a critical role in improving organismal viability during the vulnerable stages of development.

These findings underscore the importance of nutrient quality and composition in developmental success. Since the experiments were conducted under uniform environmental conditions (controlled temperature and light), the observed variation in developmental outcomes is attributed solely to the differences in diet. This reinforces the conclusion that the amount, quality, and type of dietary nutrients are key determinants of egg-to-adult viability in *D. melanogaster.*

The Effect of Chia Seeds on Pre Adult Viability in D. *melanogaster*

Several studies have shown positive effects of specific diets on *Drosophila* viability. Sisodia and Singh (2015) [36] reported increased egg-to-adult viability on carbohydrate-rich diets, while D'souza and Krishna (2015) [13] observed enhanced pre-adult survival with natural energy drinks compared to synthetic ones. Likewise, feeding on organic fruits like sapodilla and watermelon improved hatchability, emphasizing the value of nutrient-rich, natural food sources.

In contrast, some dietary components had negative effects. Alexander and Krishna (2018) [2] found that avocado and yogurt reduced egg-to-adult viability, while Rodrigues *et al.* (2015) [32] noted higher survival only up to pupariation on high-protein, low-carbohydrate diets, indicating that imbalanced macronutrient content can limit full development.

Overall, these findings highlight the complex role of diet in developmental success. Our results partially align with these studies, showing that chia seed supplementation can support pre-adult survival, reinforcing the benefits of natural dietary inputs with a balanced nutritional profile.

Importantly, the present study was conducted under tightly controlled laboratory conditions with uniform temperature and light cycles (22°C ±1°C; 12:12 light:dark photoperiod), thereby isolating the effect of dietary variables. Therefore, the observed developmental and hatchability differences can be confidently attributed to the nutritional variations introduced by different concentrations of chia seed extract.

In conclusion, the findings suggest that chia seed extract acts as a potent natural supplement capable of enhancing preadult fitness in *D.melanogaster* when provided at appropriate concentrations. While low supplementation levels (5g) offer limited benefits, intermediate (10g) and high (15g) concentrations significantly enhance developmental speed and hatchability. Thus, chia seed extract, rich in essential macro- and micronutrients, may serve as a valuable dietary enhancer for promoting survival and rapid development in model insect species. These insights not only contribute to understanding

dietary impacts on insect physiology but also offer broader implications for nutritional management in biological research and applied entomology.

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