Impact Factor (SJIF): 6.126 E-ISSN: 2583-1615, P-ISSN: 3049-3498

International Journal of Research in Academic World

Received: 19/April/2025 **IJRAW:** 2025; 4(5):144-147 **Accepted:** 23/May/2025

The Effect of Chia Seed on Pupation Site Preference in *Drosophila melanogaster*

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Abstract

Pupation site preference (PSP), which includes habitat selection, is an essential part of *Drosophila melanogaster* preadult development. Larvae's choice of location can have a big impact on how long they survive as pupa. Using three different concentrations of Chia seeds (5g, 10g, and 15g) and wheat-cream agar media (control), the larval pupation site preference (PSP) in *D.melanogaster* was examined in a lab setting. Our findings demonstrated that PSP varies considerably depending on the concentration. Larvae preferred to pupate on the culture bottle's medium, which were significantly higher in all three concentrations, regardless of concentration. A smaller proportion of larvae chose their pupation site across the wall, and the results varied depending on the medium concentration. The findings indicated that the greatest number of pupations occurred in larvae fed 5 g of chia seed concentration, whereas the least amount of PSP was observed in larvae fed 10 g, 15 g, and wheat cream agar media. Therefore, our research indicates that the nutritious component in the 5 g concentration of chia seeds aids in the larva's pupation on the culture bottle's media. Consequently, our findings demonstrated that the quality and quantity of the nutrients present in different concentrations of Chia seeds can affect Pupation site preference in *D.melanogaster*.

Keywords: Pupation site preference (PSP), Diet, *D.melanogaster*, Pupa, Larva.

Introduction

The four phases of a *Drosophila's* life cycle are egg, larva, pupa, and adult. The insect travels to different surfaces to pupate during the larval stage (Moreno *et al*, 2007). Because the place that larvae choose can have a major impact on their future survival as pupae, pupation site preference (PSP), a crucial stage in *Drosophila* preadult development that involves habitat selection, is important (Sameoto and Miller, 1968) [26].

Research on *Drosophila* species has shown that the selection of pupation sites is significantly influenced by both biotic (sex, density, length of locomotory path, developmental phase, and digging activity) and abiotic (temperature, humidity, moisture, light and dark, and pH) factors (Zhang *et al*, 2019). Additionally, studies have demonstrated that genetic factors have a role in PSP, and the genes that regulate PSP are located on autosomes with little to no dominance (Bauer and Sokolowski, 1985) [5]. According to a genetic study on pupation, a single gene alteration is mostly responsible for the difference between larvae that prefer to pupate on media and those that prefer the bottom of the media bottle (de Souza *et al*., 1970) [14].

Another important factor influencing pupation site preference is the release of glue protein, a salivary gland protein. The amount of adhesive proteins released determines the pupation site (Shivanna *et al*., 1996) [30]. According to them, larvae that release half as much glue protein tend to pupate on glass surfaces, those that release more glue protein tend to pupate on cotton, and those that release very little glue protein tend to pupate on media surfaces.

Numerous Drosophila subgroups, such as *D. melanogaster, D. virilis, D. repleta, and D. immigrant,* have been studied for it. According to the study, *D. replete* favours glass, but *D. immigrants* prefer to pupate on wheat cream agar media. Recent discoveries have focused on the *D.melanogaste*r group. *D. melanogaster* used glass for pupation, but *D. simulans and D. mauritiana* preferred wheat cream agar media. For pupation, the majority of the species under analysis preferred maximum wheat cream agar media, whereas just a small number chose glass. Cotton was employed for pupation by *D. rajasekari and D. gigberosa* (Shirk *et al*., 1988; Shivanna *et al*., 1996; vandal *et al*., 2003) [29, 30, 37].

Several experiments on pupation site preference have been conducted using different products such as Whey protein by (Asniati Jabbar *et al.*; 2024) [2], Ensure nutritional supplement by (Shilapashree *et al*.; 2024), Avocado and Probiotics (Cleoan and Krishna, 2018). From the above experiments they found that the quality and quantity of their products on different concentration affected the pupation site preference in *Drosophila melanogaster*. There are several studies on how various Drosophila species' PSP is affected by temperature,

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humidity, light, glue protein, pH, and intra and interspecific competition (Seema and Girish, 2019; Divya Singh *et al.*, 2022; Manning and Markow, 1981; Bezerra Da Silva *et al.*, 2019; Hodge and Simon, 2001; Vandal *et al.,* 2008; Shivanna *et al.,* 1996) [28, 15, 23, 7, 19, 30].

From the above literature we found that no other experiments were conducted on Chia seed regarding pupation site preference. Chia seeds offer numerous health advantages, including great source of omega 3 fatty acids, Fiber, protein and antioxidants. They can support heart health, digestive health and help with weight management. (Cristiane Freitas Rodrigues *et al.*, 2018) [12]. Therefore, the current study was conducted to investigate how the Chia seed affects *D. melanogaster* pupation site preference.

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. It has been demonstrated that these natural antioxidants may offer protection against the damaging effects of reactive oxygen and nitrogen species, which are significant in conditions associated with obesity. (Cristiane Freitas Rodrigues *et al.*, 2018) [12].

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 treatment system, they are ground into a powder and stored.

Establishment of Stock

The *Drosophila* stock centre, Department of Studies in Zoology, University of Mysore, Mysore, provided the experimental Oregon K strain flies of *Drosophila melanogaster* used in the study. These flies were cultured in wheat cream agar media (100g of jaggery, 100g of wheat cream rava, and 10g of agar in 1000ml of distilled water with 7.5ml of propionic acid). The above flies used for experiment were kept in a lab setting with a 70% humidity level, 12 hours of darkness and 12 hours of light cycles, and a temperature of 22° C + 1° C.

Establishment of Experimental Stock

The flies obtained from wheat cream agar medium [control] was prepared by adding 100g of jaggery, 100g of wheat cream rava powder, 10g of agar cooked in 1000ml of distilled water, and 7.5ml of propionic acid. The treated media containing Chia seed of different concentration such as 5g,10g and 15g were prepared by following method. [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. The10g 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 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 these media were maintained using 70% humidity level, 12 hours of darkness and 12 hours of light cycles, and a temperature of 22° C + 1° C. These flies were used in the below experiment.

Pupation Site Preference Experiment

Twenty flies (10 male and 10 female) were put separately in the control and 5g, 10g, and 15g Chia seed medium culture bottles and left for three hours. The flies were later taken out of the culture bottles, and the bottles were left for a few days until the pupa appeared in various chia seed concentrations. The number of larvae pupated at three different sites (on the media, on the glass wall, on the cotton of culture vials) were counted and tabulated.

Result and Discussion

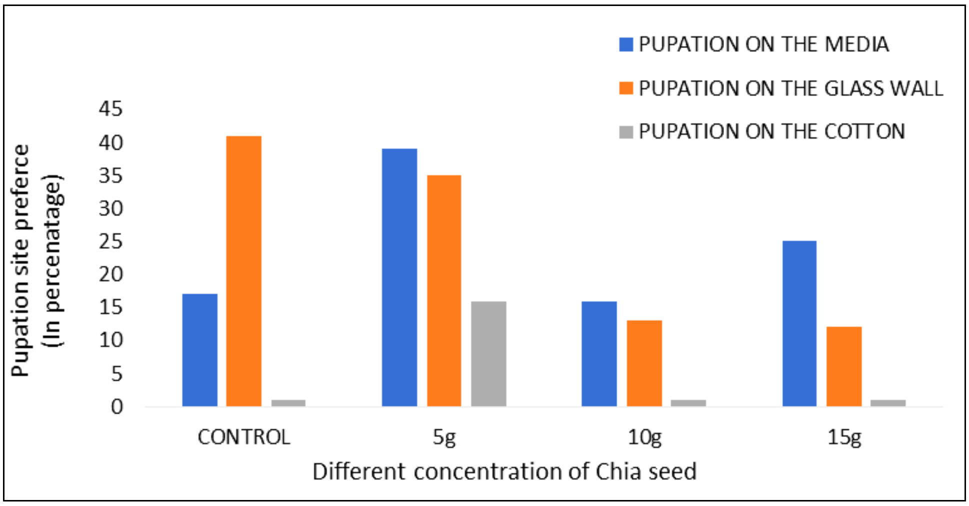

Fig 1: Effect of Chia seed on pupation site preference in *Drosophila melanogaster.*

The tendency of an insect larva to select a particular area for pupation—the stage of life where it changes into an adult—is known as pupation site preference or pupation site behaviour. Because the location that the larva chooses might have a significant impact on its subsequent survival as a pupa, larval pupation site preference (PSP) is a crucial step in *Drosophila* pre-adult behaviour development (Sameoto and Miller, 1968) [26]. By counting the number of larvae pupating on various surfaces, including cotton, glass, and food, PSP has been investigated in various *Drosophila* species (Barker 1971; Shik *et al.* 1988; Shivanna *et al.* 1996 [30]; Shivanna and Ramesh 1997; Vandal *et al.* 2003 [37]). Numerous elements, including the insect's species, the biotic, abiotic and genetic factors might affect this choice. Nutrition is also one of the major abiotic factors affecting pupation site preference in species in *Drosophila.* (Asniati Jabbar *et al*.; 2024) [2]

Our research showed that larvae chose distinct pupation sites across various media (Figure 1). Less pupae were generated on the cotton and more on the bottle/culture bottle media in both control and Chia seed treated flies.

According to our current study, larvae raised in control media had a tendency to select pupation sites where they had a better chance of surviving; that is, the majority of the larvae prefer to use the bottle's media, which is fed wheat cream agar media, as their ideal pupation sites. And the fact that so few larvae chose the cotton of the bottle and the glass wall indicates that *D. melanogaster* had a higher chance of surviving at the culture media of the bottle as opposed to the cotton and wall site.

Significant amounts of dietary fibre (18–30%), ashes (4–5%), proteins (15–25%), lipids (30–33%), and carbohydrates (26–41%) are also present in chia seeds. They also include a lot of antioxidants, including caffeic acid, myricetin, quercetin, and kaempferol, which help stabilize the oil even though it contains a lot of polyunsaturated fatty acids (PUFAs). These natural antioxidants have been shown to provide defence against the harmful effects of reactive oxygen and nitrogen species, which are important in obesity-related disorders. Rodrigues, Cristiane Freitas *et al*. (2018). [12]

Compared to larvae reared on a diet low in yeast, those raised on a diet high in yeast exhibit higher fertility but worse longevity and famine resistance (Chippindale *et al.,* 1993) [11]. This suggests that the variation in the timing of growth is influenced by both proteins and carbohydrates (Schwarz *et al*., 2013) [27].

Research on PSP in *D. jambulina* revealed that the larvae tend to pupate on food at higher temperatures (30°C) and on cotton plugs at lower temperatures (21°C). According to the studies by Seema and Girish (2019) [28] found that higher temperatures acted as an inducer for the production of large amounts of glue protein, which aids the pupa in attaching food for pupation, while lower temperatures resulted in less glue protein being produced, allowing larvae to migrate along the edges of the container for pupation on cotton. However, throughout our study, constant laboratory temperatures were maintained. Consequently, the variance in PSP was not caused by changes in temperature.

Larvae prefer to pupate close to the lowest pH resource rather than the highest, according to the numerous research looking at the connection between pH and pupation distance.

This implies that larvae would travel farther to become more fit and that acidic resources affect development time (Hodge *et al*., 1996 [19]; Vandal *et al*., 2008). Since we kept the pH constant during our study, variations in pupation site preference could not be caused by this factor.

Humidity also had an effect on the PSP. In wet condition, larvae pupate on the wall and adjacent to or on the cotton because the surrounding dampness may make it difficult to create a pupal chamber, according to Divya Singh *et al.* (2022) [15]. Larvae pupate on media with a high water content when it's dry outside. From our experiment, the larvae more tend to pupate in media rather than cotton or glass wall.

Additionally, the salivary gland protein known as glue protein production had a considerable impact on the choice of pupation site. *Drosophila* larvae produce a glue just before pupariation, which helps the pupa stay affixed to a substrate during the metamorphosis process. These proteins, referred to as salivary gland secretion proteins (Sgs), have rapidly changed both within and between species. The glue dries a few minutes after expectoration and spreads between the substrate and the body (Borne *et al*, 2021). The quantity of adhesive proteins secreted in *D. melanogaster* determines the pupation site (Shivanna *et al*, 1996) [30]. Since we did not measure the amount of glue proteins secreted by the larvae in our experiment, we were unable to conclude that this variation in pupation location choice was caused by this quantity.

Based on this study, we can say that different protein and rich in omega 3 fatty acid ratios had an impact on the pupation site in *Drosophila*. Since the pupal stage is immobile and cannot tolerate changes in the environment, food availability, and scarcity, the larva's preferred pupation location is crucial to the pupae's survival.

In our Experiment light, humidity, pH, and larval density was maintained constantly and even the same species were used. However, variation in PSP across different media was influenced by less competition, viscosity, Chia seed concentration. Hence observed variation in our experimental results was due to the quality and quantity of the nutrients present in different concentrations of Chia seed.

Conclusion

Our research showed that PSP differs between media; in control, a higher percentage of pupae were detected on the wall and media, however in different chia seed concentrations (5g, 10g, and 15g), they preferred media for pupation. Pupa numbers on the medium were higher in the 15 g concentration of Chia seed than in the 5g and 10g concentrations. In summary, PSP was an adaptable trait that the Drosophila employed to defend itself against diseases, desiccation, and predators, and primarily to improve the survival of the developing adult flies. Additionally, we found that variations in the quantity and quality of nutrients provided at various concentrations were the cause of the variance in PSP in various media.

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, our beloved seniors Mr. Kiran and Miss Anusree for their guidance.

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