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"Behavioral Response of *Drosophila melanogaster* to *Piper longum*: A Study on Pupation Site Preference"

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Abstract

This study investigates the effects of *Piper longum* on the pupation behavior of *Drosophila melanogaster* larvae. The research focuses on the larval stage, where insects exhibit pupation site preference (PSP), a critical factor influencing their survival and successful emergence as adults. In addition, the influence of *Piper longum* , commonly used in Ayurvedic medicine, on PSP is examined. Piperine, a key bioactive alkaloid in *Piper longum*, is noted for its anticancer, antioxidative, antibacterial, antiapoptotic, and antidiabetic properties. Meanwhile, *Piper longum* affects PSP, demonstrating varied preferences for pupation sites among Drosophila treated with different concentration of *Piper longum*. This research underscores the importance of understanding the genetic and environmental interactions affecting PSP, with potential implications for enhancing insect health and survival through targeted herbal treatments.

Key words: *Drosophila melanogaster*, Pupation site Preference, *Piper longum*

Introduction:

Many insects stop feeding toward the end of the third larval instar, become less negatively phototactic, and start to roam before pupating and changing into adult flies (Bainbridge etal.,1981;Sawin-McCormack etal., 1995).Often, roving larvae depart from their feeding source to explore their surroundings. The larva starts pupariation, a developmental stage that happens right before pupation when the larva becomes immobile, everts its spiracles, and cuticle tanning begins, after around 6 to 8 hours of wandering at 25˚C. Pupation is a critical and intricate process for holometabolous insects, making them vulnerable to predators and environmental challenges due to their limited movement. To protect themselves, insects seek safe pupation sites to guard against predators and extreme conditions. This process varies among species and habitats, making the selection of pupation sites crucial for the insect lifecycle.

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In *Drosophila*, pupation site preference (PSP) determines successful adult emergence and survival. The *Drosophila* life cycle includes four stages: egg, larva, pupa, and adult. During the larval stage, the insect explores various surfaces to pupate. PSP is a critical step in preadult development, as the location chosen by larvae can significantly impact their future survival as pupae (Sameoto and Miller, 1968).

Research on *Drosophila* species has revealed that both biotic (sex, density, locomotory path, developmental period, and digging behavior) and abiotic (temperature, humidity, moisture, light, and pH) factors significantly influence pupation site selection. Genetic factors also play a role in PSP, with relevant genes found on autosomes showing little or no dominance. Interspecific differences in PSP are regulated by loci on chromosomes X and II, while intraspecific variation is highly polygenic, with major effects from loci on chromosome III (Reference)

Despite extensive research, there is limited awareness of the differences in PSP among locally adapted *Drosophila* species. PSP in *Drosophila* is affected by environmental interactions and genetic makeup, but the behavior of regionally adapted species remains largely unknown. Using *Drosophila* as a model, this study aims to identify parameters influencing PSP survival rates.

Previous studies have shown that PSP is correlated with salivary gland secretions (glue proteins). PSP is a trait exhibited by late third instar larvae and has been analyzed using pupation height measurements and site choice percentages. Various *Drosophila* subgroups, including *melanogaster*, *virilis*, *repleta*, and *immigrans*, show different preferences for pupation sites. Most species prefer wheat cream agar media, while some select glass or cotton (Shrik et al., 1988; Shivanna et al., 1996; Vandal et al., 2003). Genetic investigations have indicated that the preference for pupation sites is influenced by single gene changes (Desouza et al., 1970).

Taylor (1976) suggested that habitat selection and behavior play significant roles in defining population genetic structure. Studies on pupation site preference in *Drosophila melanogaster*, *D. simulans*, *D. mauritiana*, and their hybrids showed that *D. melanogaster* larvae prefer glass, while *D. simulans* larvae prefer media. Progenies of direct and reciprocal crosses of D. melanogaster and *D. simulans* prefer wheat cream agar media for pupation.

Four to six hours later, larval/pupal apolysis gives way to pupation(Bainbridge etal.,1981;Bodenstein 1950;Ashburner etal., 2005).The animal undergoes four days of metamorphosis and is still vulnerable to many of the same threats it encountered as a larva, but it is now immobile inside its pupal case. The genetic and cellular processes that work together to shape this innate variation in behaviour remain a mystery, although earlier research has shown impacts related to the three primary chromosomes. Possible cellular processes might involve changes in sensitivity, reaction, adaptability, or immunity to environmental factors important for choosing a place to lay eggs, like temperature, light, and moisture(Bauer etal., 1988)

A correlation between pupation distance and the rate of larval mental development has been reported, and experiments with selected lines have observed a variety of larval behaviours, such as locomotion, Digging speed and geotaxis, vary depending on responses correlated with selection for pupal distance. These observations indicate that pupal spacing may not be a single behaviour but a manifestation of the integration of several simpler behavioural responses. Studies of pupal spacing and associated "mingling" behaviour have used a variety of experiments, however, all of these methods focus on the end point of wandering behaviour: end positions end of the pupa(Caseres etal , 1987; Wong etal.,1997). The pupation site preference (PSP) is an important developmental event in *Drosophila* because the location that the larvae select can have a major impact on their survival as pupae(Someoto and Miller, 1968). PSP is frequently assessed based on two factors. Pupation Height is a measure of how far larvae must move upward from the surface of food to pupate, and Pupation Site Preference is a measure of the percentage of larvae that pupate on different surfaces, such as cotton, glass, or media. When immobile, pupae employ antipredator strategies to evade predators. Therefore, choosing a pupation site is crucial to an insect's life cycle. The pupation site preference (PSP) of *D. melanogaster* controls the adult organisms' ability to

successfully emerge. In the current study, the efficacy of the *Piper longum* on pupation behavior o*f Drsophila melanogaster.*

Piper longum linn,. Often referred to as Indian long pepper, this plant has been used to treat a number of respiratory disorders in Ayurvedic medicine. (Kumawat et al. (2012). Many plant components have been utilized historically to treat a variety of ailments, including the seed, the root, the leaf, and the entire plant.(Sultana et al., 2019).

Piperine is a bioactive alkaloid molecule that has been isolated as a dietary phytochemical from the *Piper* species. Studies have shown that piperine has a wide range of beneficial effects, including anticancer, antioxidative, antibacterial, antiapoptotic, and antidiabetic ones.(Buranrat and Junking 2022; Choi et al. 2013; Derosa & Co. Park, 2019; Jwa et al., 2016. 2012; Yang and associates. 2015; Zarai et al. (2013). The efficacy of the seed powder on the pupation site preferences of the *D. melanogaster lavae*

Materials and methods

The fruit extract powder of *Piper longum* was acquired from the Government Ayurveda Medical College and Hospital, Mysuru, Karnataka, India. Used for the preparation of experimental media.

Experimental stock

Experimental Oregon K strain of *Drosophila melanogaster* used in the study was collected from *Drosophila* stock centre. 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 powder,10g of Agar was boiled in 1000ml 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 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 Soji powder, 10g of agar boiled in 1000ml distilled water and 7.5 ml of propionic acid added to It.; *Piper longum* media was prepared from 100g of jaggery,100g of wheat powder,10g of Agar was boiled in 1000ml Distilled water, 7.5 ml of propionic acid and 10mg of *Piper longum* powder was added for the 10mg/l concentration of *Piper longum* media. Another 20mg/l concentration of *Piper longum* media was prepared from 100g of jaggery,100g of wheat powder,10g of Agar was boiled in 1000ml Distilled water, 7.5 ml of propionic acid and 20mg of *Piper longum* powder was added

The flies emerged from the wheat cream agar media and other experimental treated media were maintained under the same laboratory conditions as mentioned above and were used to study the Pupation site preference Experiments in *Drosophila melanogaster*.

Pupation site preference

Adult male and female flies were collected and 10 male and 10 female flies were placed in culture bottles containing wheat-cream agar media, different concentration of 10mg/l of Lp(*Piper longum*) and 20mg/l of Lp. The flies were allowed to mate for one day and the females were allowed to lay eggs. These parental flies were removed from the culture bottle. The eggs were allowed to hatch, form larvae and pupae. The number of pupae produced were counted and recorded. The culture bottle was divided into 3 regions i.e. on media, wall of bottle and neck of the bottle. The number of pupae in each region were counted and recorded. The data was subjected to statistics and the graph of pupation site preference was plotted.

Statistical analysis

The data obtained were analyzed using IBM SPSS version 29.0. Mean, standard error, one way ANOVA, and Tukey's Post – Hoc test and two way ANOVA were carried out for the data obtained for pupation site preference.

A graph of site preference v/s number of pupae was plotted for both stevia pure extract and stevia dried leaf extract. The graph of the two was compared.

Results and analysis

Fig.1 represents the effect of normal wheat agar media on the pupation site preference of *D.melanogaster*. From the graph it is observed that the the pupation site preference is more on the neck of the bottle than on media and wall region which is significant with df = 2, F=58.334 and P<0.005.Fig.2 represents the effect of 10mg/l Lp on the pupation site preference of *D.melanogaster*. The pupation site preference is more on the neck of the bottle which is significant with df= 2, F = 14.293 and P< 0.005. Fig.3 represents the effect of 20mg/l of Lp on the pupation preference of *D.melanogaster*. The pupation site preference is more on the neck of the bottle which is significant with df= 2, F = 75.851 and P< 0.005. Fig.4 represents the comparative effect of *Piper longum* on the pupation site preference of *D.melanogaster*. The pupation site preference is more on the neck of the bottle in all three concentration which is significant with p< 0.005. The value of F=1.541 between media, F=82.853 between site preference and F=0.422 between media and site.

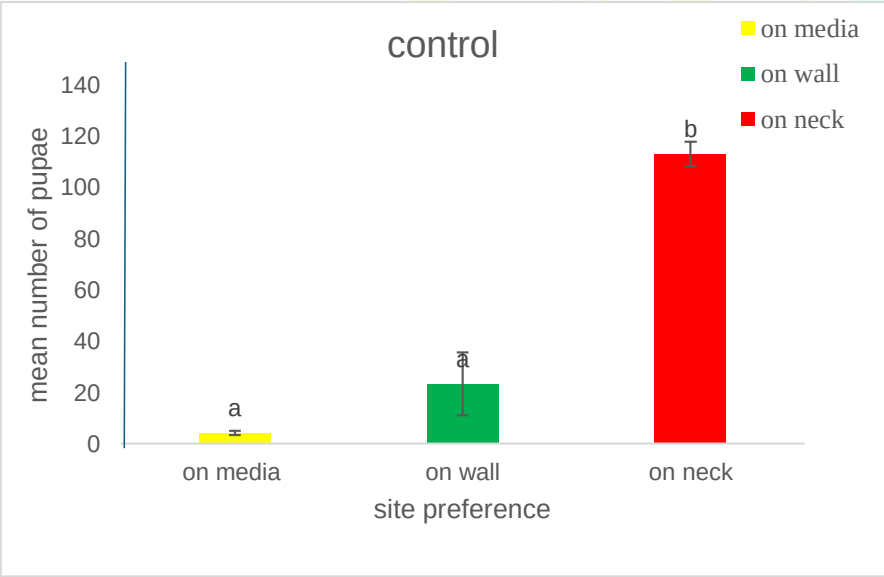

Fig.1: Effect of normal media on the pupation site preference of *D.melanogaster.*

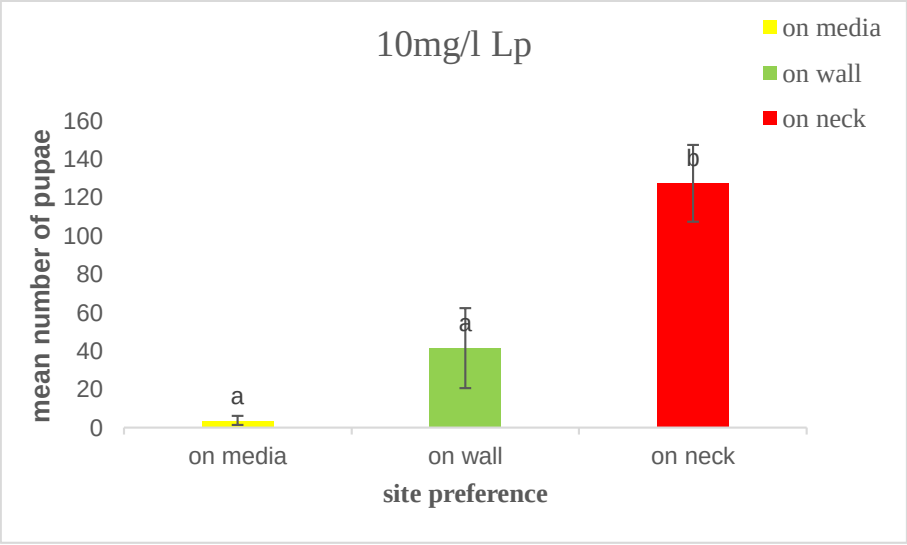

Fig.2: Effect of 10mg/l of Lp on the pupation site preference of *D.melanogaster*.

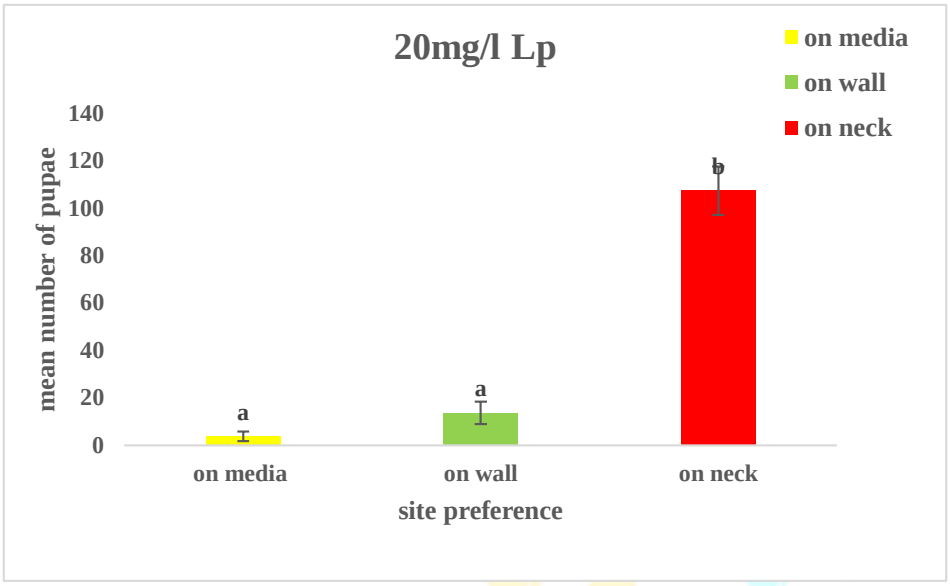

Fig.3: Effect of 20mg/l of Lp on the pupation site preference of *D.melanogaster*.

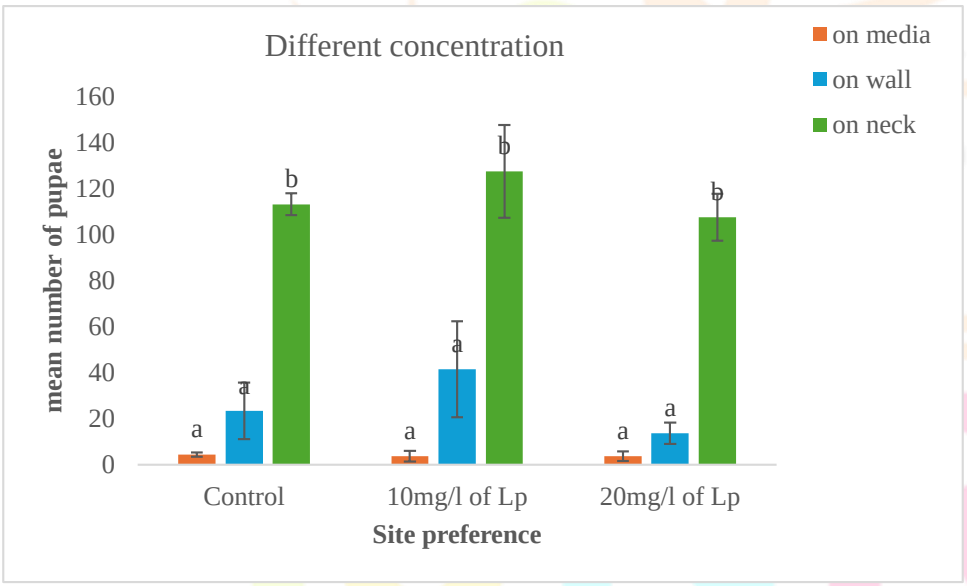

Fig.4: Comparison of effect of *Piper longum* on the pupation site preference of *D.melanogaster*

Results compared:

Pupation Site Preference on Normal Wheat Agar Media

Fig. 1 illustrates the pupation site preference of *Drosophila melanogaster* on normal wheat agar media. The data indicates a significant preference for pupation on the neck of the bottle compared to the media and wall regions. Statistical analysis shows a significant difference ($df = 2$, $F = 58.334$, $P < 0.005$), with the majority of larvae selecting the neck of the bottle as their pupation site.

Effect of 10 mg/L *Piper longum* (Lp) on Pupation Site Preference

Fig. 2 demonstrates the effect of a 10 mg/L concentration of Piper longum on the pupation site preference of *D. melanogaster*. Similar to the control group, larvae exhibited a significant preference for the neck of the bottle over the media and wall regions. This preference was statistically significant ($df = 2$, $F = 14.293$, $P < 0.005$).

Effect of 20 mg/L *Piper longum* (Lp) on Pupation Site Preference

Fig. 3 shows the pupation site preference of *D. melanogaster* in the presence of 20 mg/L *Piper longum*. Again, the larvae significantly preferred the neck of the bottle, with the statistical analysis confirming this preference ($df = 2$, $F = 75.851$, $P < 0.005$).

Comparative Effect of *Piper longum* on Pupation Site Preference

Fig. 4 compares the pupation site preferences across different concentrations of *Piper longum*. The data reveals a consistent preference for the neck of the bottle across all tested concentrations (10 mg/L, 20 mg/L, and a higher concentration not specified in the provided data). The analysis showed insignificant differences between site preferences ($F = 82.853$, $P>0.005$), between the media types ($F = 1.541$) or the interaction between media and site preference ($F = 0.422$).

Discussion

The site chosen for pupation Can have a significant impact on survival in different environmental circumstances. For instance, animals that pupate near the moist food source in drier climates tend to survive longer than those that relocate farther away. On the other hand, animals pupating near food in moist conditions are less likely to survive. This could be because of an increased risk of microbial attack or because other larvae churn up and liquefy the substrate, which can lead to drowning and suffocating(Rodriguez etal., 1992; Markow 1979).Thus, in order to lower their danger, larvae must accurately analyze their surroundings and choose pupation positions that are suited for the setting. Previous research has shown the natural variability in the *D. melanogaster* pupation position, which is often assessed in relation to the food source. These studies have also examined the effects of other environmental conditions, including temperature, humidity, light, and crowding.5–14 Notably, in a particular environmental situation, the tendency to pupate close to or far from the food is also substantially influenced by genetic factors(Sokolowski etal., 1983;Sisodia etal., 2005) Multiple research findings have also shown that there's a gender difference, with male pupae typically being found farther away from the food source compared to females(Bauer etal., 1985;Bauer 1984; casares etal., 1987).Numerous studies have examined the impact of abiotic and biotic factors on larval pupation height in various *Drosophila* species and strains (Manning and Markow, 1981; Ringo and Wood, 1983; Sokolowski and Bauer, 1989; Singh and Pandey, 1993a & b). Genetic research revealed that the second and third chromosomes act additively on pupation distance, with the third pair having a greater influence (Bauer and Sokolowski, 1985, 1988; Casares and Carracedo, 1986b). Our results consistently show that *Drosophila melanogaster* larvae prefer to pupate on the neck of the bottle, regardless of the presence or concentration of *Piper longum*. This suggests that the physical location within the environment is a more significant factor in pupation site selection than the chemical composition of the media. In the control group with normal wheat agar media, the larvae's preference for the neck of the bottle may be due to its elevated position, which could offer a more stable and suitable environment for pupation compared to the media or wall regions. The consistent preference observed in the treated groups (10 mg/L and 20 mg/L *Piper longum*) further supports the hypothesis that location is a critical determinant in pupation site selection. The statistical significance of the results ($P < 0.005$) across all experiments underscores the robustness of these findings. The variation in F-values between different site preferences indicates a strong, consistent trend that is not significantly affected by the type of media used. Further, when larvae pupate at a higher position, this increases the chance of successful hatching and thus may reflect better fitness in normal *D. melanogaster* populations (Buck et al., 2000) . But pupal height choice is influenced by the combined response to selection (Joshi and Mueller, 1996; Chippindale et al., 1997; Prasad et al., 2001), independent of several factors. Other such as food texture, temperature (Vandal and Shivanna, 2007), light (Paranjpe et al., 2004) and larval density (Sokal et al., 1960; Joshi and Mueller, 1993).Therefore, evaluating

larval decisions regarding developmental time and larval height in relation to diet availability would be useful for studying its long-term effects, especially is for longevity

Conclusions:

This study demonstrates that *Drosophila melanogaster* larvae exhibit a strong preference for pupating on the neck of the bottle over other available sites, irrespective of the media's chemical composition. The addition of *Piper longum*, at concentrations of 10 mg/L and 20 mg/L, does not alter this preference. These findings suggest that the physical environment plays a more crucial role in pupation site selection than previously anticipated.

Future research could explore the underlying mechanisms driving this preference, potentially examining factors such as humidity, light exposure, and temperature gradients at different bottle locations. Additionally, investigating other environmental and chemical variables could provide a more comprehensive understanding of the determinants of pupation site selection in *D. melanogaster*

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