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Effect of caffeine nutritional supplement on the pupation site preference in *Drosophila melanogaster*

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Abstract
Preference for a pupation site is a crucial phase in *Drosophila's* pre-adult behavioral development since the location a larva chooses may significantly affect its chances of surviving as a pupa. In this study, PSP was investigated using *D. melanogaster* larvae cultivated in wheat-cream agar and combined (wheat-cream agar + caffeine powder) media. Pupae in wheat cream agar media tended to pupate more frequently in the following order: on the media's surface, on its wall, and next to the cotton plug. However, the larvae cultivated in the test (caffeine) media prefer to pupate more frequently on the media bottle wall, then close to the cotton plug, and less frequently on the media surface. In contrast, the PSP varies depending on the concentration of caffeine media (2.5g, 5g, and 10g). In 2.5g, the larvae prefer to pupate primarily on the media bottle wall, followed by the media surface and less on the cotton plug; in 5g, the larvae prefer to pupate primarily on the media bottle wall, followed by the cotton plug and less on the media surface; and in 10g, the larvae prefer to pupate primarily on the media bottle wall, followed by the cotton plug and very little on the media surface.
This implies that the varying media concentrations were the primary cause of the PSP variance. *D.melanogaster* larvae prefer to move away from the media surface and pupate more on the middle wall and close to the cotton (neck) when there is a higher concentration of media present. This study examines how caffeine intake affects *D. melanogaster* larvae's choice of pupation place. The pupation sites of larvae raised on media with different caffeine concentrations (2.5g, 5g, and 10g) were noted and examined in connection to the distance from the food source.

Keywords: *Drosophila melanogaster*, pupation site preference, nutrija caffeine

Introduction

A key ecological and behavioral characteristic of holometabolous insects like *Drosophila melanogaster* is preference for the pupation site. Because the location of pupation affects exposure to microbial pathogens, temperature fluctuations, desiccation, and predation, it can have a substantial impact on the survival of larvae (Sokolowski *et al*., 1986). Larval behavior includes selecting a preferred pupation site, feeding, and skipping. A vital aspect of the *Drosophila* life cycle is the larvae's choice of location, which is a critical developmental event in *Drosophila* and can significantly affect the larvae's survival as pups (Someoto and Miller, 1968). PSP is frequently evaluated using two criteria. Pupation height quantifies the distance the larvae must travel upward to pupate away from the food surface, and pupation site preference evaluates the proportion of larvae that pupate on different surfaces like cotton, walls, and the surface of the media.Numerous *Drosophila* species have been the subject of studies on PSP and pupation height (Cleoan and Krishna, 2018; Seema and Girish, 2019; Shivanna *et al*., 1996; Vandal *et al*., 2008; Singh and Pandey, 1991; Casares and Carracedo, 1986,1987; Bauer and Sokolowski, 1989; Folgleman and Markow, 1982) [21, 25]. When third instar larvae migrate to pupation sites, a number of extrinsic factors are known to cause different responses. These include light (Manning and Markow, 1981; Rizki and Davis, 1953) [15], moisture (Sameoto and Miller, 1968) [19], gravity (Markow, 1979) [16], larval density (Singh and Pandey, 1953), larval development time (Markow, 1979) [16], and the presence of other species (Rizaki and Davis, 1953).
It has been demonstrated that a wide range of abiotic factors affect pupal behavior variations within and between species (Markow, 1979; Sokolowski and Hansell, 1983) [16]. Even biotic parameters, such as density, sex, developmental time, digging habit, and locomotory path length, are significant in PSP (Beltrami *et al*., 2010; Sokal *et al*., 1960; Munoz *et al*., 2005) [4, 23].
In a number of *Drosophila* species, pupation behavior has also been found to be controlled by genetic variables (Bauer and Sokolowski, 1985, 1988; Garcia-Florelz *et al*., 1989) [3, 10]. For example, pupation height is a polygenic characteristic that successfully responds to bidirectional selection, according to Joshi and Mueller (1993) [13]. In a recent study, it was discovered that *D. annassae's* preference for pupation height (in shell vials) was under polygenic control, with the majority of the variations being additive in character (Pandy and Singh 1993) [22].
Researchers found that whereas interspecific variance is extremely polygenic, with loci on chromosome lll, loci on chromosomes X and II influence differences in interspecific pupation behavior of *Drosophila sechellila* and *Drosophila simulans* (Erezyilmaz and Stem, 2013) [8].
PSP and glue proteins, which are produced by the larvae's salivary glands, are related. Research on glue proteins shows that the larvae tend to move away from the media's surface the more glue proteins the salivary glands express (Shivanna *et al*., 1996) [21].
Stressors from the environment, such as chemicals in food, might affect this behavior. Caffeine, a plant-derived alkaloid that is frequently present in coffee, tea, and chocolate, is one such substance. It has been demonstrated that caffeine influences insect behavior and physiology in a variety of ways and functions as a natural pesticide in plants. Through its effects on adenosine receptors and other neurochemical pathways, caffeine administration in *Drosophila* can modify

locomotor activity, shorten lifespan, hinder reproduction, and change stress responses (Nath *et al*., 2013; Mustard, 2014)

There are numerous studies on how various *Drosophila* species' PSP is affected by temperature, humidity, light, intra and interspecific competition, PH, and glue protein (Seema and Girish, 2019; Divya Singh *et al*., 2022; Manning and Markow, 1981; Bezerra *et al*., 2019; Hodge and Simon, 2001; Vandel *et al*., 2008; Shivanna *et al*., 1996) [5, 7, 11, 15, 21]. However, no other animal model has been used to examine the impact of varying nutrija caffeine powder concentrations on pupation location preference behavior. Therefore, the goal of the current study is to determine how nutrija caffeine powder affects *Drosophila melanogaster* pupation location preference.

Materials and Methodologies

"The caffeine powder (Nutrija caffeine)" a prelockoutsupplement (orange flavour) was purchased from www.amazon.com,and used as a dietary component in the experimental setup.

Establishment of Stock: The Oregon K starin of *D. melanogaster* flies was acquired from the *Drosophila* stock center DOS in zoology, University of Mysore, Manasagangotri, Mysuru. The resultant flies were raised in bottles with 1000 milliliters of boiling distilled water, 100 grams of jaggery, 100 grams of rava powder, and 10 grams of agar to prevent fungal growth. A lab environment with a 12:12 light and dark cycle, a temperature of 22+10 C, and a relative humidity of roughly 70% RH was employed to house these flies. utilizing different diet media.

Establishment of experimental stock

Control media :100 grams of jaggery, 100g of rava powder and 10g of agar were combined with 1000ml of boiling distilled water and 7.5ml of propionic acid to create wheat cream agar media.

2.5g of caffeine media: 2.5g of caffeine powder ,100 grams of jaggery, 100g of rava powder and 10g of agar were combined with 1000ml of boiling distilled water and 7.5ml of propionic acid to create wheat cream agar media.

5g of caffeine media: 5g of caffeine powder,100 grams of jaggery, 100g of rava powder and 10g of agar were combined with 1000ml of boiling distilled water and 7.5ml of propionic acid to create wheat cream agar media.

10g of caffeine media: 10g of caffeine powder,100 grams of jaggery, 100g of rava powder and 10g of agar were combined with 1000ml of boiling distilled water and 7.5ml of propionic acid to create wheat cream agar media.

Results and Discussion

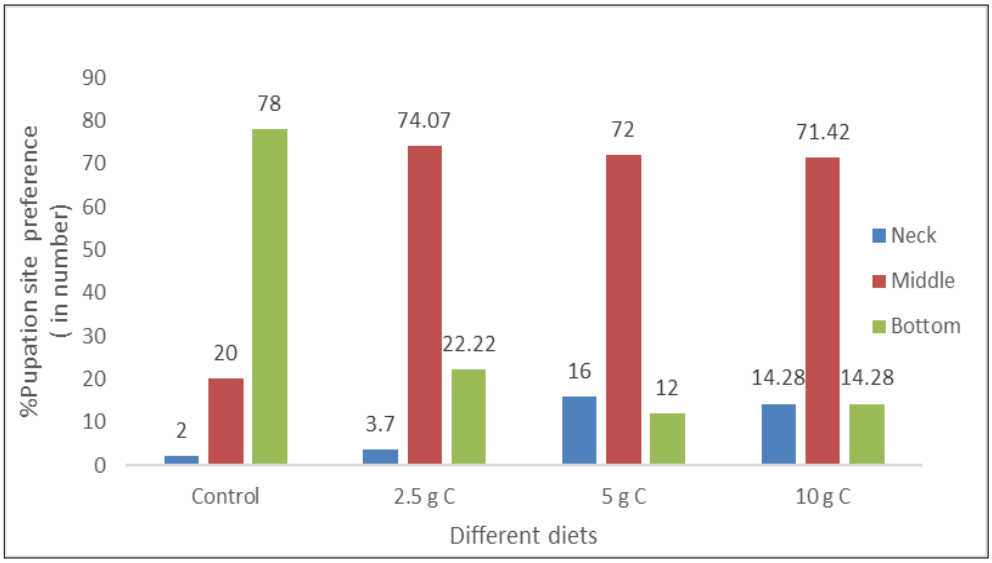

Fig 1: Effect of nutrija caffeine powder on the pupation site preference of the *Drosophila melanogaster*

Pupal behavior is important in *Drosophila* development because the location the larvae choose may affect their chances of surviving as pupae (Someoto and Miller, 1968). PSP is primarily influenced by a variety of internal factors, such as the amount of glue protein secreted in the salivary gland, locomotory path length, sex, digging behavior, developmental time, and genetic factors, as well as numerous external factors, such as light, temperature, humidity, PH, larval density, and interspecies.

Figure 1 demonstrated that the larvae choose distinct pupation sites in various mediums. In control media, a higher proportion of pupae were discovered on the mrdia's surface, followed by the media bottle's wall, and a lower proportion were discovered close to or on the cooton plug. In contrast, a higher proportion of pupae were discovered on the media bottle's wall in the test media (caffeine media), followed by those on the cotton plug and the media's surface.

Based on our experiment's above results, we can infer that media concentration also affects PSP. For example, when media contains more caffeine, the larva prefers to move away from the media surface and pupate more on the middle wall and close to the cotton (neck).

As a result, the elevated caffeine content suggests that it is hazardous, demonstrating that the concentration is also a significant factor influencing *D. melanogaster's* PSP.Because we constantly maintained similar amounts of light and dark phases, light had no effect on the variance in pupation site preference in our experiment. In contrast to our findings, some research indicates that if PSP was affected by light, the photopositive *Drosophila melanogaster* prefers dark areas to pupate. Research on PSP

in *Drosophila melanogaster* revealed that the larvae tended to pupate on food at higher temperatures (300 C) and on cotton plugs at lower temperatures (210 C).

Seema and Girish (2019) 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 fluctuation in PSP was not caused by changes in temperature.

According to a large body of research on the connection between PH and pupation, larvae prefer to pupate close to the lowest PH resource rather than the highest. This implies that larvae would travel farther to become more fit and that acidic resources have an effect on development time (Hodge *et al*., 1996; vandal *et al*., 2008) [25]. Since we kept the PH constant during our research, variations in a PSP could not be caused by this.

Singh and Pandey (1993) [17, 22] found a correlation between high PSP and increased larval density, which in turn causes intraspecific competition and feeding needs. As one of the anti-annibalistic strategies, crowding and food deprivation make *D. melanogaster* more likely to engage in larval cannibalism and migrate to high pupation sites (Bezerra Da Silva *et al*., 2019) [5]. However, in our experiment, the larval density was lower, so a higher percentage of pupa were found on the middle media bottle wall.

Humidity also had an effect on the PSP. Larvae pupate on the wall and on the cotton plug in wet conditions since the surrounding wetness may pose problems for the pupal chamber, according to Divya Singh *et al*. (2022) [7]. Our results confirm the above claim that because the coffee medium was viscous, a higher percentage of pupae could be seen close by or on the wall, after which the cotton of the media bottle was used to shield them from the moisture.

Our experiment's above results indicate that the amount of caffeine in the media has an impact on PSP as well. The larva prefers to move away from the media surface and pupate more on the middle wall and close to the cotton (neck) when the concentration of caffeine in the media is higher. As a result, the elevated caffeine content suggests that it is hazardous, demonstrating that the concentration is also a significant factor influencing *D. melanogaster's* PSP.

Conclusion

According to our research, PSP changes depending on the kind of media. In control media, a higher percentage of pupae were located on the media's surface, but in test media (caffiene), a higher percentage of pupae were detected on the media bottle's wall. It can be inferred that PSP was a versatile trait that the *Drosophila* employed to defend itself against desiccation, illness, and predators, and primarily to boost the survival of the developing adult flies. Additionally, we found that variations in PSP among media were caused by variations in the media's caffeine concentration.

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