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Piper longum Extract Enhances Starvation Resistance in *Drosophila melanogaster*

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Abstract

Starvation resistance is a critical trait for animals, particularly those in environments with fluctuating and unpredictable food availability. This study explores the impact of *Piper longum*, commonly known as Indian long pepper, on the starvation resistance of *Drosophila melanogaster*. Starvation resistance involves various behavioral, physiological, and metabolic adaptations and is influenced by an animal's nutritional history and dietary composition. Piperine, a bioactive alkaloid in *Piper longum*, is known for its antitumor, antioxidative, antibacterial, antiapoptotic, and antidiabetic properties, which contribute to enhanced longevity and overall health.

This study investigates how different concentrations of *Piper longum* affect the starvation resistance of *Drosophila melanogaster*. The results show that *Piper longum* significantly improves the flies' ability to withstand extended periods of food deprivation. Both qualitative and quantitative dietary impacts were considered, highlighting the importance of food composition in starvation resistance. This research provides new insights into the potential use of *Piper longum* as a dietary supplement to enhance starvation resistance, contributing to broader applications in ecological and evolutionary studies.

Keywords: Diet, starvation resistance, *Drosophila melanogaster*, mated

Introduction

The ability of an animal to withstand a lack of food is referred to as starvation. The ability to endure extended periods of food deprivation is known as starvation resistance (SR). Starvation is the most common environmental stress experienced by animals living in environments where food availability fluctuates unpredictably. Starvation resistance is a phenotypic trait of significant organismal, ecological, and evolutionary importance. It involves various behavioral, physiological, and metabolic adaptations (Lee and Jang, 2014) [18].

Starvation can occur either suddenly or gradually. Prolonged starvation, such as that caused by seasonal changes and a lack of food sources, can result in animal death (McCue, 2010) [20]. Foraging ability is closely linked to reproductive fitness in many animal species that face food scarcity.

An animal's nutritional history and status determine its resistance to starvation. The physiological capacity to endure starvation depends on the nutritional value of its diet. As a heterotroph, *Drosophila melanogaster* requires food to develop, reproduce, and store energy. The rate and frequency of food consumption, along with the efficiency of digestion and nutrient assimilation, influence the nutrition an animal receives. While lipid stores are primary factors in determining a *Drosophila's* starvation resistance, the relative amounts of proteins, carbohydrates, and lipids also play crucial roles.

A living organism's life history traits, including susceptibility to disease, fecundity, reproduction, longevity, and stress tolerance, are significantly influenced by the quantity and quality of its food. Dietary impacts can generally be divided into two categories: quantitative (availability of food) or qualitative (food composition). Since animals derive their energy and other nutrients from food, the quantitative impact is evident. The rate and frequency of food consumption, digestion, and nutrient absorption determine the nutrition an animal derives from its diet. In certain animals, this feeding response is regulated by feedback loops influenced by nutrient quantity and quality (Simpson *et al*., 2004) [26].

The fruit fly, *Drosophila melanogaster*, has served as a main model organism for research into the immediate and long-term reasons of starvation resistance (SR). In *Drosophila* SR, the majority of studies have examined genetic or evolutionary responses to varying degrees of extreme food scarcity (Hoffmann & Harshman, 1999; Matzkin *et al*., 2009) [10, 19]. However, the environment significantly influences the phenotypic expression of SR and its fitness implications (Pijpe, Brakefield & Zwaan, 2007) [22]. Since the physiological ability of animals to survive starvation depends on their nutritional status and dietary history, the nutrient

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composition of food is the most significant factor affecting SR among various environmental variables (Jensen *et al*., 2010) [11].

Any environmental factor that has the potential to negatively alter an organism or biological system is referred to as stress. As stated by Karan *et al*. (1998) [13], stress resistance characteristics in Drosophila often vary along latitudinal clines, indicating that these traits may be modified by selection directly or indirectly. Individuals of many species have to suffer from starvation or inadequate nutrition. Positive selection for starvation stress resistance is expected in environments where food is likely to be less accessible or unpredictable in the near future. When insects are fed a diet that is nutritionally unbalanced, as is commonly the case when they are restricted to foods that are low in protein relative to carbohydrates (P:C), compensatory feeding for the limiting component results in an excess intake of other nutrients (Raubenheimer and Simpson 1999) [23]. As a result, fitness may decline and lipid storage may increase (Simpson *et al*. 2004; Warbrick-Smith and associates. 2006) [26, 28].

Piper longum Linn., commonly known as Indian long pepper, has been used in Ayurvedic medicine to treat various respiratory conditions (Kumawat *et al*., 2012) [15]. Historically, different parts of the plant have been used to treat a wide range of illnesses (Sultana *et al*., 2019) [27]. Piperine, a bioactive alkaloid isolated from the *Piper* species, has demonstrated numerous benefits, including antitumor, antioxidative, antibacterial, antiapoptotic, and antidiabetic effects (Buranrat & Junking, 2022; Choi *et al*., 2013; Derosa *et al,* 2016; and Park, 2019; Jwa *et al*., 2012; Yang *et al*., 2015; Zarai *et al*., 2013) [2, 4, 6, 21, 12, 29, 30]. These positive effects indirectly enhance longevity and overall health. Lee *et al*. investigated piperine's effects on lifespan, physiological functions such as feeding, fecundity, and resistance to environmental stresses. However, the impact of long pepper on starvation resistance remains undocumented. This study aims to address this gap by examining the impact of long pepper on starvation resistance.

Materials and Methodology

Preparation of *Piper longum* Media: The fruit extract powder of *Piper longum* was obtained from the Government Ayurveda Medical College and Hospital, Mysuru, Karnataka, India. This powder was used to prepare the experimental media.

Establishment of Stock: The experimental stock of the Oregon K strain of *Drosophila melanogaster* was acquired from the *Drosophila* Stock Center, Department of Studies in Zoology, University of Mysore, Mysuru. This stock was cultured in bottles containing wheat cream agar media, which was prepared by boiling 100 g of jaggery, 100 g of wheat cream rava, and 10 g of agar in 1000 ml of distilled water, with 7.5 ml of propionic acid added. The flies were maintained under laboratory conditions, with 70% humidity, a 12-hour dark/light cycle, and a temperature of 22°C.

Experimental Media Preparation

The flies were cultured on various diet media for the experimental study:

Wheat Cream Agar Media: Prepared by boiling 10 g of agar in 1000 ml of distilled water, then adding100 g of jaggery, and 100 g of wheat cream rava powder, 7.5 ml of propionic acid.

10 mg Long Pepper Media: Prepared by boiling 10 mg of Long pepper powder, 100 g of jaggery, 100 g of wheat cream rava, and 10 g of agar in 1000 ml of distilled water, with 7.5 ml of propionic acid added

20 mg Long Pepper Media: Prepared similarly by boiling 20 mg of Long pepper powder, 100 g of jaggery, 100 g of wheat cream rava, and 10 g of agar in 1000 ml of distilled water, with 7.5 ml of propionic acid added.

Experimental Procedure

The flies emerging from the wheat cream agar media and other experimental media were maintained under the same laboratory conditions as mentioned above. The flies grown in normal wheat agar media were used as the control group, while those grown in 10 mg and 20 mg Long pepper media were used as the test groups. These flies were then used to study the starvation resistance in *Drosophila melanogaster.*

The flies were cultured in wheat cream agar media and long pepper media (10 mg and 20 mg). To study starvation resistance, five-day-old mated male and female flies obtained from the cultured media (control and test media) were used. The flies were anesthetized using ether, and males and females were separated. The separated male n female were kept in 10 vials. These vials were kept at 22°C under constant light conditions. The duration of hours the flies survived without food was recorded by observing the vials at 2-hour intervals until all the flies had perished. The data were then subjected to statistical analysis.

Result

Fig 1, fig 2, and fig. 3 represent the effect of Long pepper on the starvation resistance of mated male and female flies of *D. melanogaster* raised in control diet and treated media. Fig 1 represent the effect of starvation resistance of Long pepper on SR of mated males of *D. melanogaster.* Fig 2 represent the effect of long pepper on the SR of mated females of *D. melanogaster.* According to the data obtained, Long pepper administrated flies have showed more resistance to starvation compared to control media (wheat cream agar media) in both male and female flies. In between the concentration groups, the starvation resistance is found to be high in 10mg long pepper media. In the data analysis of starvation resistance, In male flies it is significant with $P<0.05$, df=2 and F=79.257, And in female flies it is significant with $P<0.05$, df=2 and F=117.724. In 10mg of Long pepper administrated flies, mean value of male flies is 29.60 and of female flies is 21.44, therefore is says that male flies are having more resistance to starvation compared to female flies. In 20mg of Long pepper administrated flies, mean value of male flies is 23.80 and the mean value of female flies is 18.08, hence here also the male flies showed more resistance to the starvation than female flies.

Fig 3 Represent the comparison graph on starvation resistance in male and female *Drosophila* fed with control and treated media. According to the data obtained the Starvation Resistance was found high in male *Drosophila* fed with treated media compare to control. In between concentration groups Starvation resistance is high on 10mg *Piper longum* compared to 20mg long pepper which is insignificant with $P < 0.05$ df = 2 and F=13.237.

Statistical Analysis

The data obtained were analyzed using IBM SPSS version 29.0. Mean, standard error, one-way ANOVA, two-way ANOVA, and Tukey's Post-Hoc test were performed on the data obtained for starvation resistance. A graph of concentration media and survival time in hours of male and female flies was plotted.

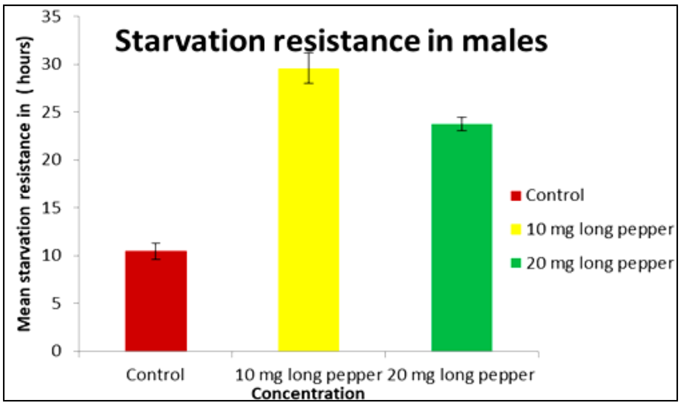

Fig 1: Effect of long pepper on starvation resistance in mated males of *D. melanogaster.*

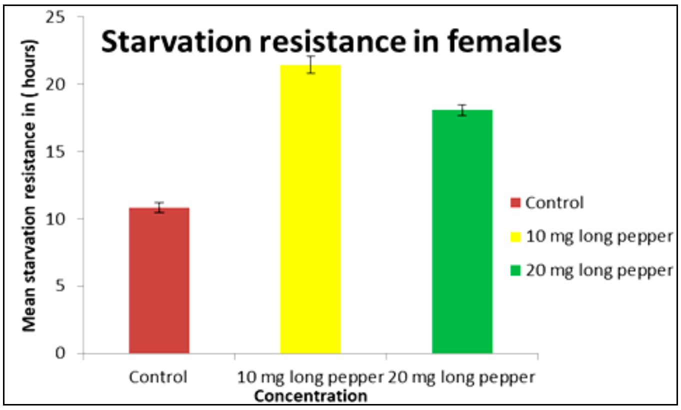

Fig 2: Effect of long pepper on starvation resistance in mated females of *D. melanogaster.*

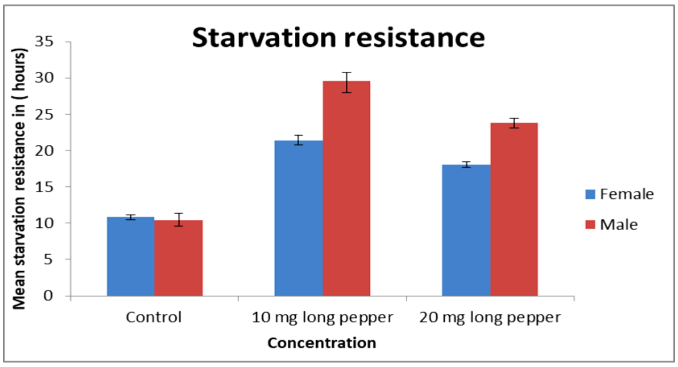

Fig 3: Effect of long pepper on starvation resistance in mated males and mated females of *D. melanogaster.*

Discussion

Figures 1, 2, and 3 illustrate the effect of Long pepper on the starvation resistance of mated male and female *Drosophila melanogaster* raised on control and treated media. Figure 1 shows the effect of Long pepper on the starvation resistance of mated male flies. According to the data, flies administered Long pepper exhibited greater resistance to starvation compared to those raised on the control media (wheat cream agar). Among the concentration groups, flies fed 10 mg Long pepper media displayed the highest starvation resistance. The analysis for male flies showed statistical significance with $P < 0.05$, df = 2, and F = 79.257. Figure 2 illustrates the effect of Long pepper on the starvation resistance of mated female flies. Similar to the males, the females administered Long pepper also demonstrated increased starvation resistance compared to the control group. The highest resistance was observed in the 10 mg Long pepper group. The data analysis for female flies indicated statistical significance with $P < 0.05$, df = 2, and F = 117.724. The mean starvation resistance for male flies in the 10 mg Long pepper group was 29.60 hours, while for female flies it was 21.44 hours, indicating that male flies exhibited greater resistance to starvation compared to female flies. In the 20 mg Long pepper group, the mean starvation resistance for male flies was 23.80 hours, and for female flies, it was 18.08 hours. Again, male flies showed higher resistance to starvation than female flies. Figure 3 compares the starvation resistance of both mated males and females raised on control and treated media. The results indicate that both mated males and females exhibited higher starvation resistance in the groups treated with Long pepper compared to the control group. There are ample amount of work on the starvation resistance among animals focused on the lipid storage may have positive effect on the starvation, that is prolonged starvation resistance is due to accumulation of lipid content in the cuticle of insects. There is good evidence that an increase in lipid content in adults underlies increased resistance to starvation. Chippindale *et al.* (1996) [3] opined that this trait accounts for most of the variation in starvation resistance, and experiments with many selected lines of *Drosophila* have shown that lipid levels and starvation resistance are positively correlated. The present work supports this, showing enhanced resistance in the *Piper longum* treated groups, likely due to the accumulation of beneficial fats in the cuticle. Starvation causes the accumulation of lipid droplets in the liver, a somewhat counterintuitive phenomenon that is conserved from flies to humans. Rion and Kawecki (2007) [24] proposed that starvation resistance can be enhanced by

i). Increasing energy storage,
ii). Slowing the rate at which energy is expended, and
iii). Lowering the minimum level of body energy reserves required to tolerate starvation.

All these physiological mechanisms are intimately linked to nutrition and are likely associated with traits or processes related to nutrient acquisition and allocation. Outbred populations of fruit flies display highly variable starvation resistance, along with traits associated with starvation resistance, including developmental timing, sleep, and feeding behaviors (Folguera *et al.*, 2008) [7]. The primary factor influencing starvation resistance is the nutritional makeup of the food (Lampiri *et al.*, 2023) [16]. The present work supports that the starvation is reduced in fly groups treated with *Piper longum* in higher concentration. The starvation reduction is expressed in a dose dependent manner. The compound *Piper longum* has potential to reduce lipid accumulation in tissues may be a good candidate to reduce obesity much work has to be done on this aspects.

Conclusion

Higher Resistance with Long Pepper: Flies administered Long pepper showed significantly higher resistance to starvation compared to the control group.

Optimal Concentration: The 10 mg Long pepper group demonstrated the highest starvation resistance in both males and females.

Gender Differences: Male flies exhibited greater starvation resistance than female flies in both concentration groups.

These findings suggest that Long pepper can significantly enhance the starvation resistance of *Drosophila melanogaster*, with males benefiting more than females. The results also indicate that the 10 mg concentration of Long pepper is more effective than the 20 mg concentration, highlighting the importance of optimizing the dosage for maximum benefits.

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