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A COMPARATIVE STUDY OF EFFECT OF *STEVIA REBAUDIANA* PURE AND CRUDE LEAF POWDER ON THE STARVATION RESISTANCE OF *DROSOPHILA MELANOGASTER*

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Abstract:

The cells in our body require energy for performing an array of tasks. Food and sleep nourish us with the energy we need. One of the main foods that provide us with energy is sugar or sweeteners. The plant *Stevia rebaudiana Bretoni* is the source of stevia, a naturally occurring sweetener. A physiological characteristic that is frequently measured in insects such as *Drosophila malanogaster* is starvation resistance. Starvation resistance refers to the ability of an organism to survive and function for an extended period of time without food or with limited food intake. Several studies have shown that different Drosophila species exhibit varying levels of starvation resistance, indicating genetic variation underlying this trait. One way to gauge an insect population's resistance to famine is to track how long it takes for them to perish. They were placed in 1% non nutritive agar media. The duration of hours the flies were able to survive without food was recorded by observing the vials at 2 hours interval till all the flies had perished. The data is analyzed and discussed.

Key words: *D.melanogaster*, diet, starvation, stevia, stevia dried leaf, mated.

Introduction

The cells in our body require energy for performing an array of tasks. Food and sleep nourish us with the energy we need. One of the main foods that provide us with energy is sugar or sweeteners. If you regularly eat and drink foods and beverages with a lot of added sugars, the empty calories can build up resulting in weight gain. It may also raise your risk of serious health problems including diabetes and heart diseases. Sweeteners have long been seen in many different dishes and refreshments. A lot of individuals are looking into non nutritive sugar alternatives as a means of reducing food cravings as a result of public's growing concern over diabetes, obesity and health during the past few decades. Some utilize substitutes for sugar, commonly referred to as artificial sweeteners. They taste precisely like sugar, but they contain fewer calories. Some have no calories at all. A lot of sugar substitutes taste sweeter than sugar. Very little is needed to sweeten food and drinks. One such substitute for sugar is stevia.

The plant *Stevia rebaudiana Bretoni* is the source of stevia, a naturally occurring sweetener. For many years, this plant has been utilized to produce pure and dried leaf extracts which are used as flavoring agent. Stevia has a more complex and enduring flavor than other sweeteners. A lot of powdered sugar has a bitter or licorice-like taste when they absorb too much. 95% of the freshness in stevia plants comes from their steviol glycosides.It has been discovered that stevia extract, or rebaudioside-A, is 300 times sweeter than regular sweetener. It is less bitter compared to stevia dried leaves. Although rebaudioside-A and stevioside are the most prevalent and thoroughly studied steviol glycosides, over thirty other steviol glycosides have been reported in the scientific literature to date. Some distinctive effects in stevia powder have long ledge life and high inversion sufferance (Mahendra, Supreet, 2019).

Eating and drinking sugar sweetened foods and beverages can have a big impact on the glycemic index of each meal and diet overall (Willett et al. 2002). Furthermore, eating too many high calories, high glycemic foods can raise blood levels of insulin and glucose after meals and may cause hormonal and metabolic changes that increase appetite and encourage the storage of fat (O'Keefe and Bell, 2007). Stevia may play a role in regulating food intake because it has been shown to improve insulin sensitivity in rat models (Chang et al. 2005) and to have positive effects on the blood glucose and insulin levels in human investigations (Curi et al. 1986, Gregersen et al. 2003). No favorable side effects were noted in safety investigations (Barriocanal et al. 2008). The Joint Food and Agriculture Organization or World Health Organization Expert Committee on Food Additives has authorized stevia for use as a sweetener (Joint Food and Agriculture Organization or World Health Expert Committee on Food Additives, 2005).

A physiological characteristic that is frequently measured in insects such as *Drosophilamalanogaster* is starvation resistance. Starvation resistance refers to the ability of an organism to survive and function for an

extended period of time without food or with limited food intake. Studies have shown that stevia leaf extract may possess starvation resistance properties that can help improve metabolic health and promote weight loss. Several studies have shown that different Drosophila species exhibit varying levels of starvation resistance, indicating genetic variation underlying this trait. Generally, Drosophila species that inhabit environments with fluctuating food availability tend to have higher levels of starvation resistance compared to those living in more stable environments. This suggests that natural selection has played a role in shaping the evolution of starvation resistance in Drosophila.

One way to gauge an insect population's resistance to famine is to track how long it takes for them to perish. Animals have developed a variety of responses to sudden drops in nutritional availability. According to Koehn RK and Bayne RL (1989) stress is defined as exposure to any environmental element that lowers an organism's fitness. Because stress resistance traits in *Drosophila* often vary across latitudinal clines (Karan et al., 1998), it is likely that selection affects resistance traits either directly or indirectly (Karan et al.1998).For starvation, there is good evidence that an increase in the lipid content of adults underlies increased resistance to starvation. Some data suggest that this trait accounts for almost all the variation in starvation resistance (Ary and Lawrence, 1999). A fundamental technique for assessing the impact of both hereditary and non genetic aging-related factors is lifespan measuring (Finch, Hummer et al. 2001).The variety of responses include changes in metabolic rate, altered sleep and locomotor activity, and the induction of foraging behavior (Schmidt 2014, Stahl et al. 2017, Sternson et al. 2013, Yurgel et al. 2014). Strong resilience exists to starvation impacted by evolutionary and ecological history. According to Brown EB, Slocumb ME et al. (2019) *D.melanogaster* offers a potent model for utilizing experimental evolution to study features linked to starvation resistance. Lack of food is a major source of stress, and the majority of animals do, in fact, occasionally go starvation. For *Drosophila* and many other species, diet is a significant environmental component that greatly affects lifespan. Furthermore, sustaining normal physiology depends on the appropriate management of carbohydrate homeostasis, which includes one of the primary energy storage molecules—lipids and their metabolism. These molecules have a multitude of functions, such as storing energy as glycogen and serving as substrates for glycolysis, which is a necessary energy source for biosynthetic reactions in developing animals.

Rebaudioside-A and stevioside make up the majority of stevia. Stevioside can lessen the fat buildup in mice that is brought on by hunger. It can lower plasma glucose levels by increasing insulin sensitivity and stimulating engineering secretion (Park M, Sharma A, Back H 2022). One of the main ways in which stevia leaf extract may enhance starvation resistance is through its impact on blood sugar levels. Stevia leaf extract contains compounds called steviol glycosides, which do not have any effect on blood sugar levels. This means that consuming stevia leaf extract does not cause spikes in blood sugar or insulin levels, which can help regulate

hunger and appetite. Hence, the current study looked at the effects of stevia on starvation resistance in *Drosophila* melanogaster.

Materials and methodology

Establishment of stock

The experimental stock of *Drosophila melanogaster* was obtained from *Drosophila* stock center, Manasagangotri, University of Mysore. *D.melanogaster* is one of the most widely used and one of the most understood of all model organism. The flies obtained were redistributed and raised in different culture bottles containing wheat cream agar media (100g of jiggery, 100g of wheat powder, 10g of agar agar was boiled in 1000ml of double distilled water. 7.5ml of propionic acid was added at last). Twenty flies (10 males and 10 females) were introduced into culture bottles and maintained at a temperature of 22^{o}C ± 1^{o}C with a relative humidity of 70% in 12 hours dark: 12 hours light cycle. The virgin flies were isolated in pupa stage and cultured in test media. The test media containing 1%, 2% and 3% natural sweetener, stevia powder (pure extract) based media and the flies grown in normal wheat agar media were used as control. In the same way, the test media containing 1%, 2% and 3% dried stevia leaves powder based media and the flies grown in normal wheat agar media were used. The results of both the products ie stevia extract and dried stevia leaves were compared and were discussed. Five day old flies were isolated from the culture and raised in control, stevia extract media and dried stevia leaves media. It was maintained in the conditions mentioned above. They were allowed for five days in the corresponding media, control and test and then used for studying different parameters.

Starvation resistance in mated flies

After pupation, the adult flies were allowed to grow in respective media for 5 days. Then these 5 day old mated flies were taken for starvation resistance test. They were placed in 1% non nutritive agar media. The duration of hours the flies were able to survive without food was recorded by observing the vials at 2 hours interval till all the flies had perished. A total of 5 replicates of 10 flies each were run separately for each group. Males and females were considered separately for this experiment. The data is subjected to statistics.

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 carried out for the data obtained for starvation resistance. A graph of concentration v/s survival time in hours was plotted for both stevia pure extract and stevia dried leaf extract. The graph of the two was compared.

Results

Fig.1, fig.3, and fig.5 represent the effect of pure stevia on the starvation resistance of mated males of *D.melanogaster* raised in control diet and treated media. According to the data obtained the starvation resistance was found high in stevia treated media compared to control. In between the concentration groups the SR is found to be high in 2% stevia treated media which is significant with $p < 0.05$, df = 3 and F =20.676. Fig.2, fig.4 and fig.6 represent the SR of mated females of *D.melanogaster*. It was found that the SR is high in stevia treated media compared to control. In between the concentration groups the SR is more in 1% stevia treated media which is significant with p , 0.05, df = 3 and F = 62.037. Fig. 7 and fig. 8 represents the comparison of SR of both mated males and mated females. It was found that the SR is significantly high in females compared to males. The value of F= 65.574 between media, F= 41016 between sex and F=17.765 between media and sex.

Fig. 9, fig.11 and fig 13 represent the effect of starvation resistance of dried stevia leaf on SR of mated males of *D.melanogaster*. It was found that the SR is high in dried stevia leaf treated media than the control. In between the concentration groups the SR is high in 2% dried stevia leaf which is significant with $p < 0.05$, df = 3 and F = 39.524. Fig.10, fig. 12 and fig.14 represent the effect of dried stevia leaf on the SR of mated females of *D.melanogaster*. It was found that the SR is high in dried stevia leaf than in control. In between the concentration groups the SR is significantly high in 2% dried stevia leaf with $p< 0.05$, df = 3 and F = 85.530. Fig.15 and fig.17 represents the effect of dried stevia leaf on the SR of both males and females. It was found that SR is significantly high in females than in males. The value of F=115.044 between media, F=19.901 between sex and F=2.407 between media and sex.

Fig. 17 represents the comparison of SR of both mated males and mated females raised in control, pure stevia and dried stevia leaf. It was found that the SR of both mated males and mated females is high in pure stevia treated media than in dried stevia leaf.

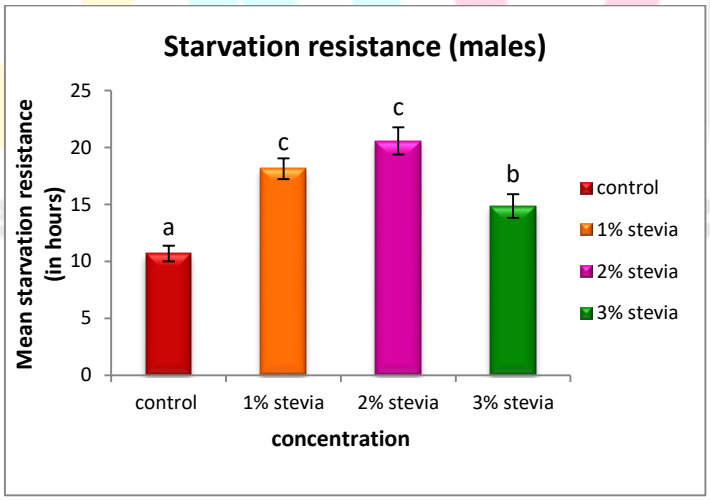

Fig.1 : Effect of stevia (pure) on starvation resistance in mated males of *D.melanogaster*.

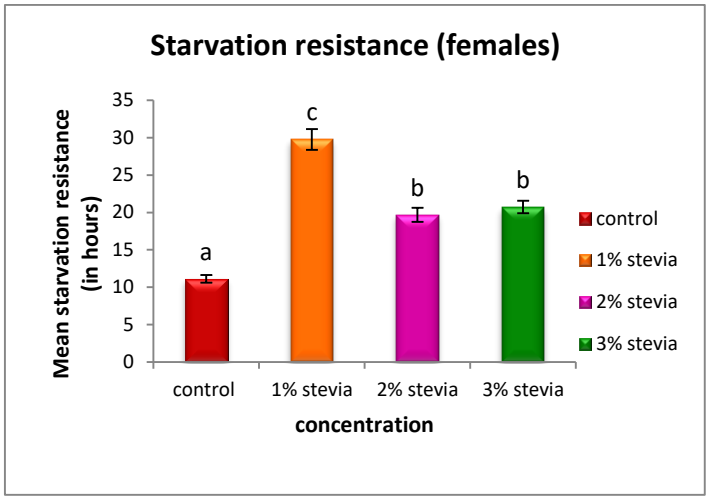

Fig.2 : Effect of stevia (pure) on starvation resistance in mated females of *D.melanogaster*.

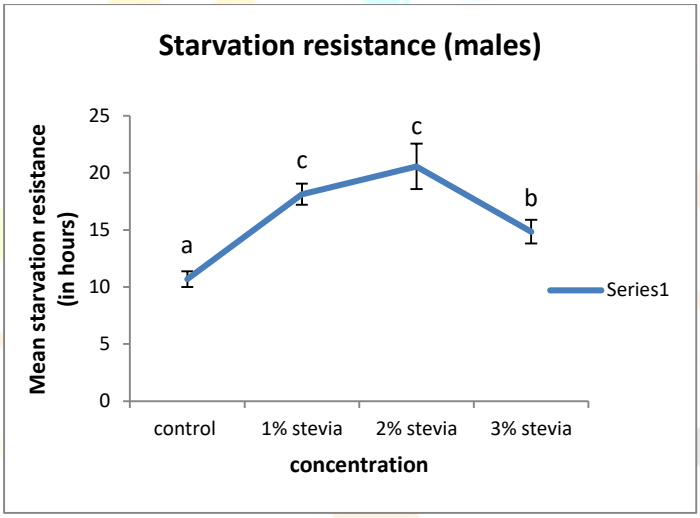

Fig.3 : Effect of stevia (pure) on starvation resistance in mated males of *D.melanogaster*.

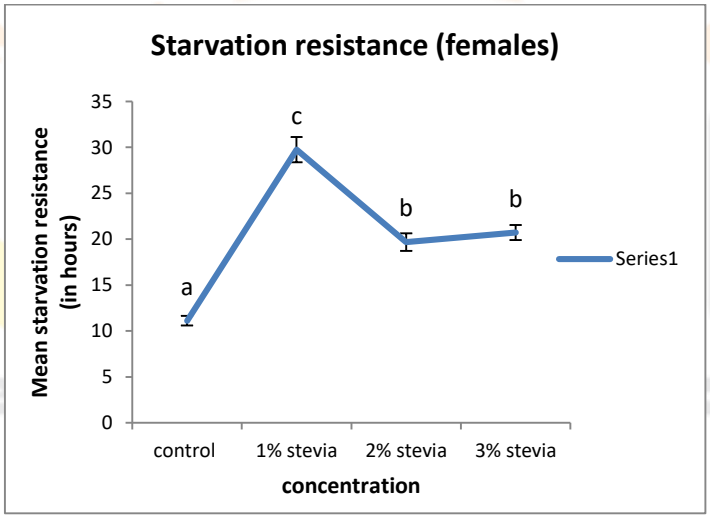

Fig.4 : Effect of stevia (pure) on starvation resistance in mated females of *D.melanogaster*.

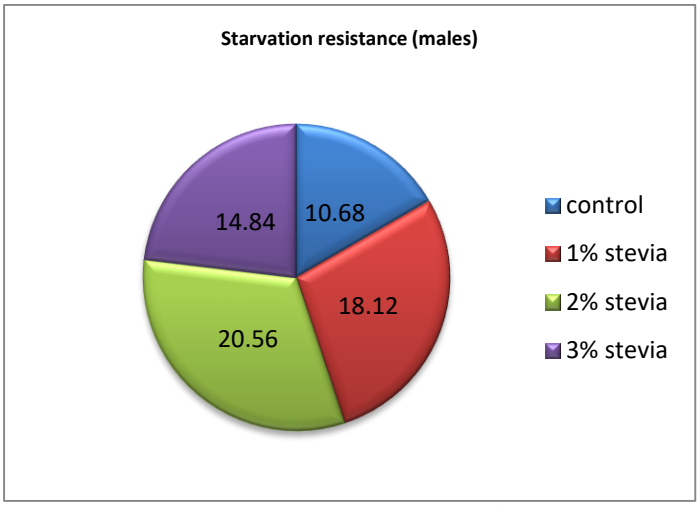

Fig.5 : Effect of stevia (pure) on starvation resistance in mated males of *D.melanogaster*.

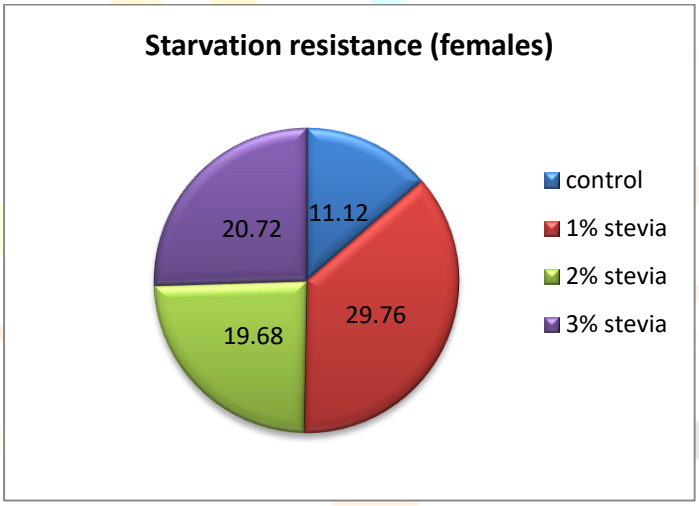

Fig.6 : Effect of stevia (pure) on starvation resistance in mated females of *D.melanogaster*.

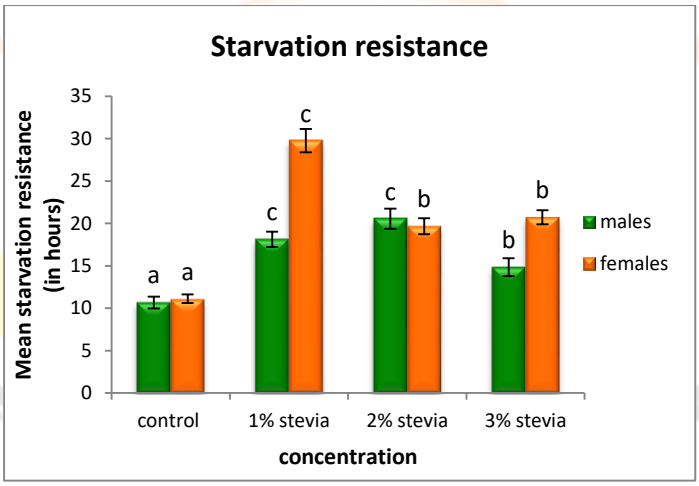

Fig.7 : Effect of stevia (pure) on starvation resistance in mated males and mated females of *D.melanogaster*.

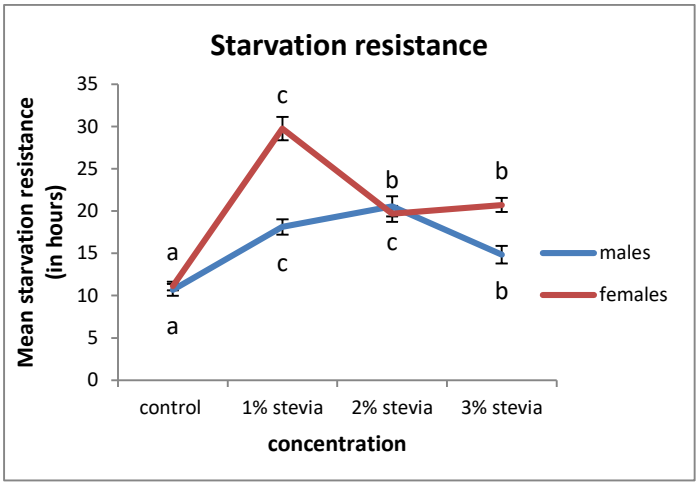

Fig.8: Effect of stevia (pure) on starvation resistance in mated males and mated females of *D.melanogaster*.

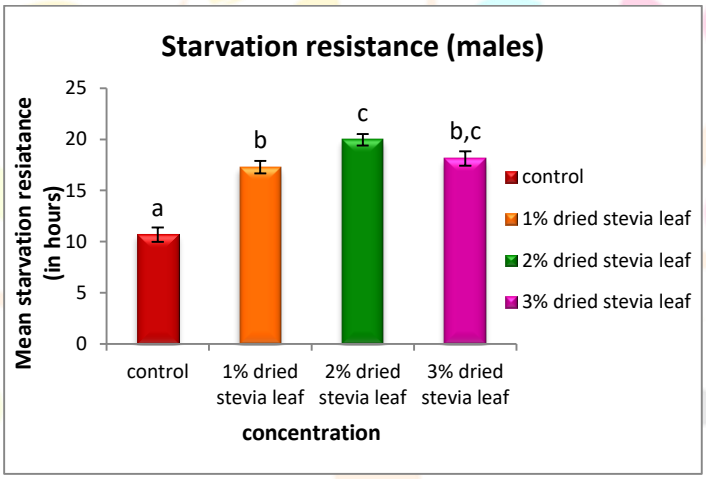

Fig.9: Effect of dried stevia leaf on starvation resistance in mated males of *D.melanogaster*.

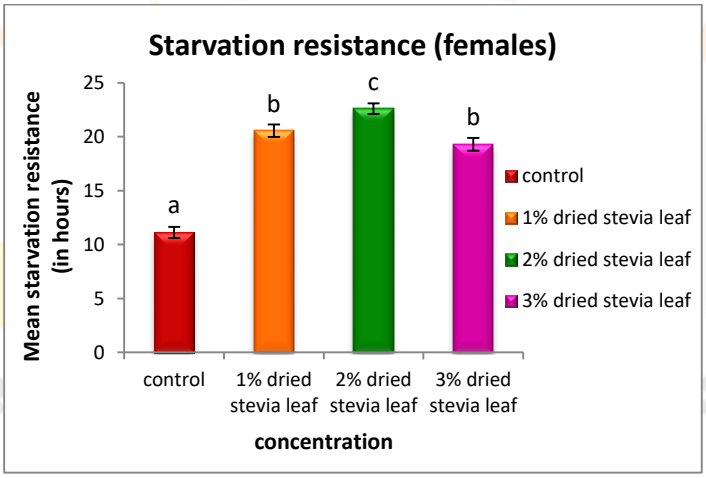

Fig.10: Effect of dried stevia leaf on starvation resistance in mated females of *D.melanogaster*.

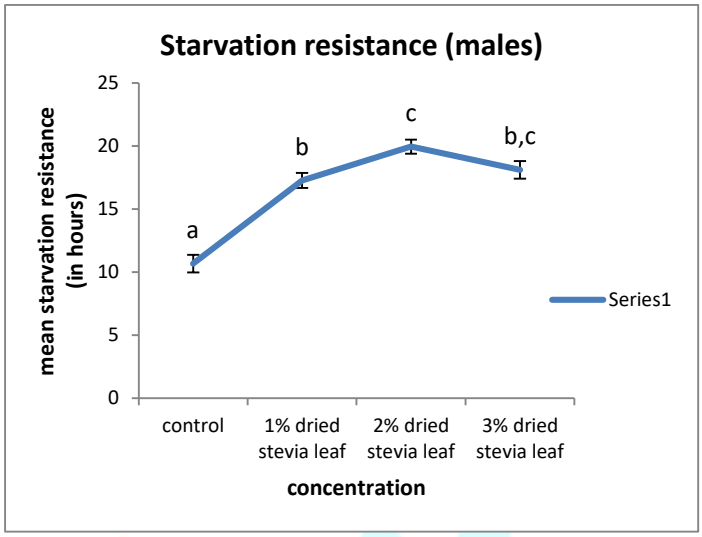

Fig.11: Effect of dried stevia leaf on starvation resistance in mated males of *D.melanogaster*.

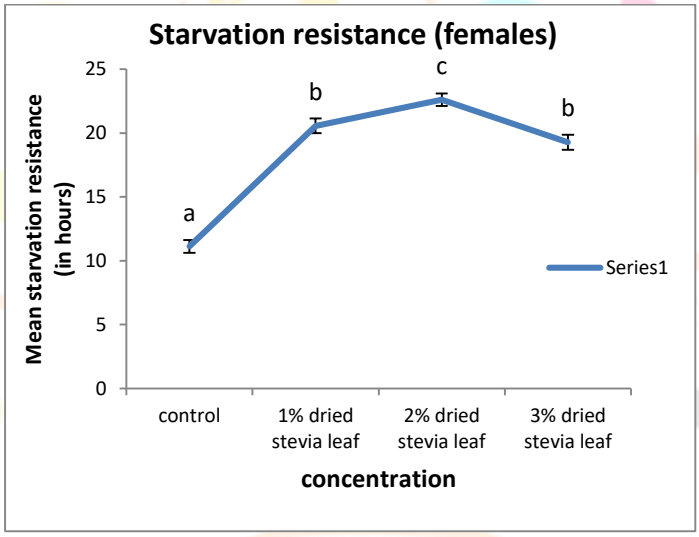

Fig.12: Effect of dried stevia leaf on starvation resistance in mated females of *D.melanogaster*.

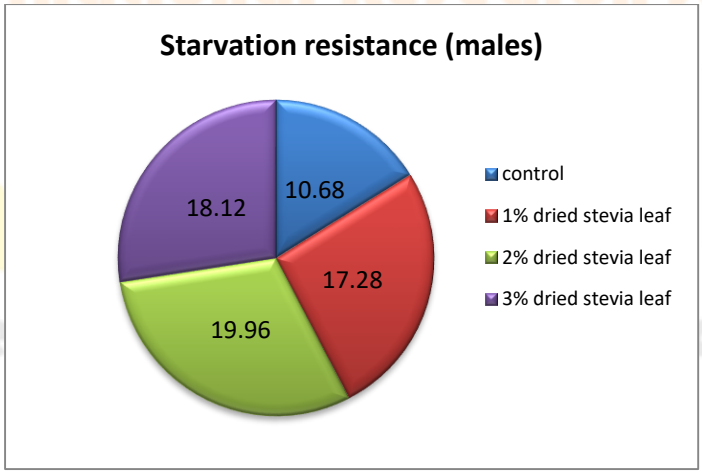

Fig.13: Effect of dried stevia leaf on starvation resistance in mated males of *D.melanogaster*.

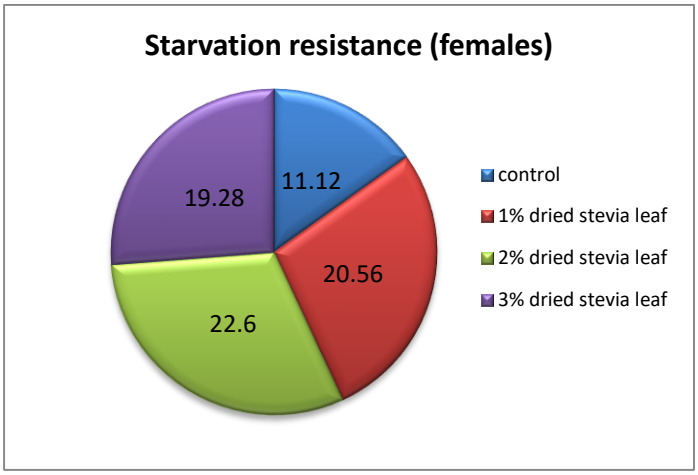

Fig.14: Effect of dried stevia leaf on starvation resistance in mated females of *D.melanogaster*.

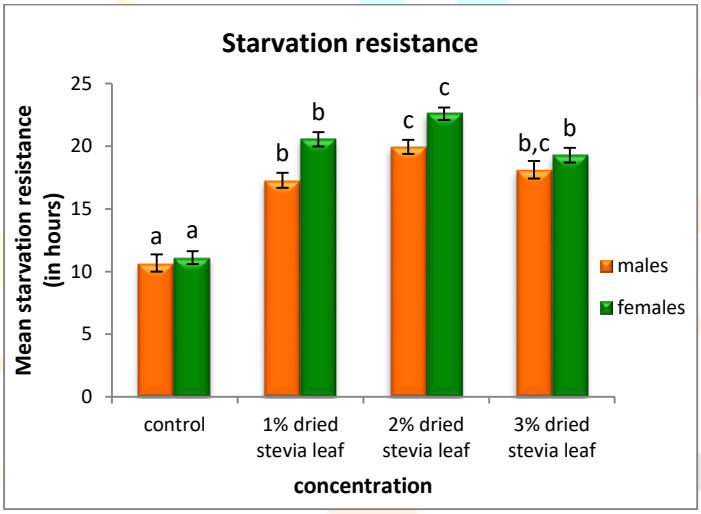

Fig.15 : Effect of dried stevia leaf on starvation resistance in mated males and mated females of *D.melanogaster*.

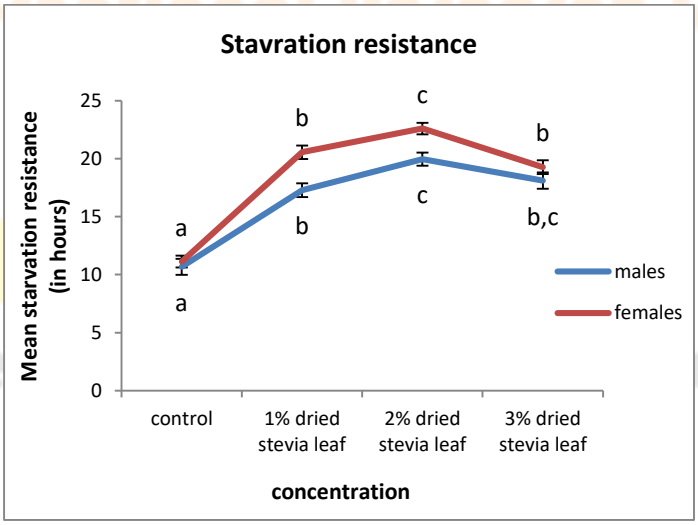

Fig.16 : Effect of dried stevia leaf on starvation resistance in mated males and mated females of *D.melanogaster*.

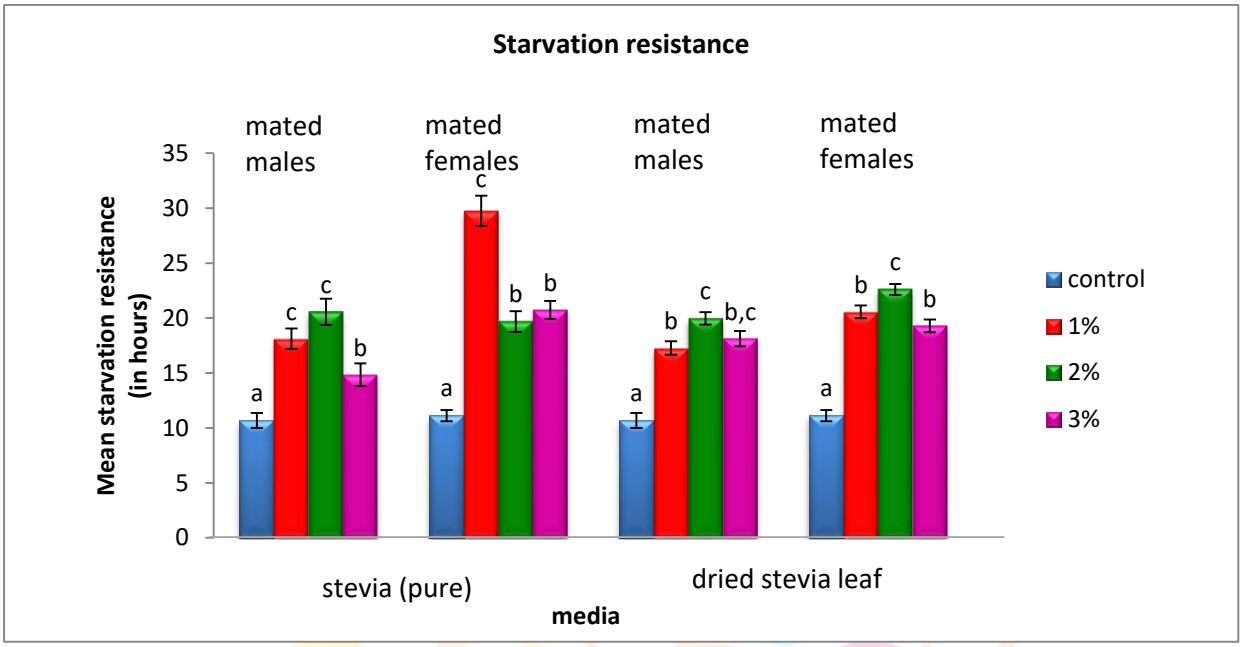

Fig. 17: Effect of pure stevia and dried stevia leaf on the starvation resistance of mated males and mated females of *D.melanogaster*.

Discussion

Starvation resistance is often measured in physiolgical studies with *Drosophila* and other insects. It is directly relevant to general issues of the evolution of stress resistance (Hoffmann & Parsons 1991; Huey et al 2004). In the present study two products of stevia powder and stevia leaf extracts treated Drosophila flies were observed to know the efficacy of these two products to make flies to toler of starvation. Several studies have shown that insects face many nutritional challenges during different seasons of the year resistance to starvation may enhance their survivability in the absence of the food. Mated males raised on stevia-treated media displayed higher starvation resistance compared to the control group. Among the concentration groups, the 2% stevia-treated media showed the highest starvation resistance, which was statistically significant ($p < 0.05$, $df = 3$, $F = 20.676$), whereas mated females raised on stevia-treated media exhibited higher starvation resistance compared to the control group. The highest starvation resistance was found in the 1% stevia-treated media, significantly different from other groups ($p < 0.05$, $df = 3$, $F = 62.037$).Starvation resistance was significantly higher in females compared to males in stevia-treated media ($p < 0.05$, $df = 3$, $F = 41.016$).Mated males raised on dried stevia leaf-treated media showed higher starvation resistance compared to the control group. The highest resistance was observed in the 2% dried stevia leaf-treated media, which was statistically significant ($p < 0.05$, $df = 3$, $F = 39.524$).Mated females raised on dried stevia leaf-treated media exhibited higher starvation resistance compared to the control group. The highest starvation resistance was found in the 2% dried stevia leaf-treated media, significantly different from other groups ($p < 0.05$, $df = 3$, $F = 85.530$). Males exhibited optimal starvation resistance at 2% concentration of pure stevia, while females showed the highest resistance at 1% concentration.

This suggests a gender-specific response to pure stevia, with males requiring a higher concentration to achieve optimal starvation resistance. The lower optimal concentration for females could be due to different metabolic or physiological mechanisms influencing nutrient absorption and stress resistance. Both males and females showed the highest starvation resistance at 2% concentration of dried stevia leaf. However, the magnitude of resistance was higher in females compared to males.

Dried stevia leaf appears to enhance starvation resistance effectively in both genders, but females benefit more. This indicates that the bioactive compounds in dried stevia leaf might be more effective or better utilized by females. Females exhibited significantly higher starvation resistance compared to males in stevia-treated media. The gender-specific differences in starvation resistance suggest that females may have more efficient metabolic pathways or physiological adaptations that enhance their ability to withstand starvation. This is consistent with findings in other studies showing that females often have better stress resistance capabilities.

There is a good evidences that an increase in the lipid content of adults underlies increased resistance to starvation Chippindale et al 1996 opines that this traits accounts for most of the variation in starvation and experiments with many selected lines of Drosophila have shown lipid and starvation levels is positively corelated (Jan et al 2021). The present work also supports this, resistance is enhanced in the stevia treated groups probably accumulation of good fat in the cuticle responsible for enhanced starvation resistance. Preclinical research on Rats and mice have also associated with increased body weight after treatment (Harshman & Schimid 1998; Djawadn et al 1998).Further, pharmacokinetics of steviosides and Rebaudioside A is absorbed and rapidly eliminated in the urine in the form of Stevioglucoronide. Hence, stevia is less toxic and reduces the potential side effects of the compound

Starvation causes the accumulation of lipid droplets in the liver, a somewhat counterintuitive phenomenon that is nevertheless conserved from flies to humans. Rion and Kawecki (2007) have proposed that SR 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 energetic reserves required for tolerating starvation. All these physiological mechanisms are intimately linked to nutrition and are likely to be coupled with traits or processes that are associated with nutrient acquisition and allocation. Out bred populations of fruit flies display highly variable starvation resistance, as well as traits that are associated with starvation resistance, including developmental timing, sleep and feeding behaviors (Folguera et al. 2008). The primary component influencing starvation resistance is the nutritional makeup of the food (Evagelia et al. 2023).The antioxidant abilities of the stevia powder may help flies to sustain more starvation compared pure powder.

Conclusion

The study demonstrates that stevia (both pure and dried) significantly affects the starvation resistance of *Drosophila melanogaster*, with notable gender-specific responses. Pure stevia enhances starvation resistance more effectively than dried stevia leaf, with different optimal concentrations for males and females. Females

generally show higher resistance to starvation compared to males, highlighting the importance of considering both the form and concentration of dietary supplements and their potential gender-specific effects. Further research is needed to understand the mechanisms behind these observations and explore the practical applications of stevia in enhancing stress resistance and other health-related outcomes.

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