



## **Effect of Spirulina on Rate of development and percentage of hatchability in *Drosophila melanogaster***

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### **Abstract**

Nutrition consumed by an organism provides energy for growth, development, reproduction and survival of an organism. Pre-adult development is one of the important stages in the survival of an organism. In the present study flies of *D. melanogaster* reared in different concentration of spirulina (2.5g, 5g, 10g) and control media. Flies raised in different concentration of spirulina showed significant influence pre adult development. According to the results obtained in our experiment, the rate of development from larva to pupa was found to be faster in flies raised in control media. Whereas, the rate of development from larva to pupa is slower in flies raised in spirulina treated media. However, the rate of development from pupa to adult was faster in 10g spirulina treated media. In contrast, the rate of development from pupa to adult was found to slower in flies raised in control media. The percentage of hatchability was greater in spirulina treated flies compared to control flies both in case of larva to pupa and pupa to adult hatchability. Further, the rate of hatchability was greater in flies raised in 10g spirulina treated media and the flies raised in control media have showed a least rate of hatchability. Thus, these studies suggest that spirulina increases pre adult fitness in *D. melanogaster*.

**Keywords:** Nutrition, Spirulia, Pre-adult fitness, *Drosophila*

### **Introduction**

In general diet can be categorized as either quantitative (i.e., food availability) or qualitative (i.e., food consumption). Out of these two, the quantitative effects are evident since animals obtain their energy and other nutritional needs from food; subsequently, under a variety of natural conditions, there is a positive relation

between food availability and fitness of an organism. On the other hand, qualitative effects are frequently divided into two categories, namely Nutritional deficiencies and nutrient deficiencies. (Pough, 1989; Sibly, 1991). Both internal and extrinsic factors that are known to influence all biochemical, physiological, and developmental changes that occur in an organism have an impact on the overall growth, development, and

reproduction of an organism. (Sterner and Schulz, 1998; Taylor *et al.*, 2005)

The level of balance depends on how food intake, digestion, and the distribution of newly acquired energy among various tasks like maintenance, growth, and reproduction interact. (Karasov, 1986). Therefore, research into how organisms govern their use of energy and its impact on pre-adult development in creatures has been significantly supported by experimental changes to animal diet.

In earlier studies on species of *Drosophila*, (Geeth and Krishna., 2015) proved that the organic fruits chikku and watermelon had significant influence on pre adult fitness. The avocado and yogurt had showed a positive effect on larva to pupa, pupal to adult viability and rate of development in *D.melanogaster* but their study did not had pre-adult development benefits on *D.melanogaster* (Cleona Alexander and krishna.,2018). Further Alwyn's D Sowza and Krishna (2015) who while studying in *D.melanogaster* have also found that the consumption of alternative natural energy drink was beneficial in preadult development. However, the effect of spirulina on preadult fitness have not found. Therefore, the present study has been undertaken.

The blue-green alga spirulina (*Arthrospira platensis*) is a small filamentous organism with a spiral form. Proteins (55-70%), anti-inflammatory compounds, and antioxidants such carotenoids, -carotene, phycocyanin, and phycocyanobilin are all found in this cyanobacterium. Spirulina is consumed by humans and other animals due to its wide variety of nutritional qualities and pharmacological effects.(Kumar *et al.*, 2017). "Superfood" status is given to spirulina. Spirulina is gaining popularity as a food supplement because of the touted health advantages; it can either be used as a powder or taken as capsules.(Grosshagauer *et al.*, 2020).However, there is no work conducted on the effect spirulina on the reproductive fitness in species of *Drosophila*. Therefore present study has been undertaken in *D.melanogaster*.

Due to its numerous health benefits, spirulina is now widely used by individuals as a nutritional supplement. Numerous studies demonstrate that consumption of spirulina has a good effect on reducing triglycerides and bad cholesterol. Additionally, it aids in the management of illnesses connected to the metabolic syndrome. In fact, a number of studies have showed that taking spirulina supplements can help with the treatment of a variety of cancers (Konickova *et al.*, 2014).

## **Materials and Methods**

### **Establishment of stock**

The Oregon K strain of *D. melanogaster*, procured from the Drosophila stock centre, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysuru, was used to establish the experimental stock. Wheat cream agar media (100g of jaggary, 100g of wheat powder, 10g of Agar was boiled in 1000ml of distilled water and 7.5 ml of propionic acid was added to avoid the fungal growth.) was used to culture the flies and were maintained in laboratory conditions such as humidity of 70% and 12:12 Photoperiod (dark and light cycles) and temperature of 22°C ± 1°C. These flies were utilized to conduct our experiment.

### **Establishment of experimental stocks**

Flies cultured in wheat cream agar media were considered as control flies. Spirulina treated flies were obtained by using different concentration of spirulina i.e., 2.5g,5g and 10g was mixed thoroughly with 100ml of wheat cream agar media. Both control and spirulina flies were maintained in afore mentioned laboratory conditions.

### **Effect of Spirulina on rate of development in *Drosophila melanogaster***

To analyse, the effect of spirulina on the rate of development around 20 flies in which 10 male and 10 female flies raised in control media and different concentration of spirulina treated media were collected. The collected flies were

introduced into vials and allowed them for 24 hours. Later, the flies were removed from the vial and allowed the vial as such for 24 hours to obtain 1<sup>st</sup> instar larvae from the eggs laid by the flies. Ten first instar larvae were collected by scooping out the media from the vial. The collected first instar larvae were fed with their respective nutrient media i.e., control and 2.5g, 5g and 10g spirulina treated media. The treated larvae in all the vials were kept under control to monitor the developmental stages of larvae and percentage of hatchability. The timings were recorded at every stage of each larva to get the data to analyse the rate of development.

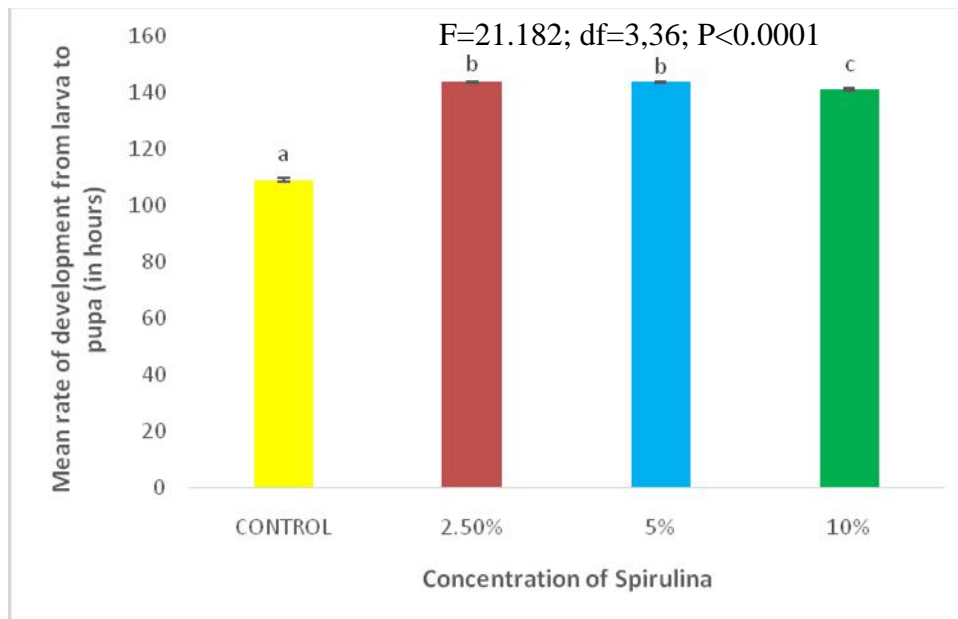
## Results and Discussion

### Effect of Spirulina on rate of development in *D.melanogaster*

Diet is an important environmental factor affecting growth, development, reproduction and survival of an organism. The quality and quantity of nutrients availability varies in different diets. Therefore the present study has undertaken to investigate the positive and negative impacts of spirulina on the pre-adult development of *D.melanogaster*.

Mean±standard error of the rate of development from larva to pupa in flies raised in control and spirulina treated media were provided in **Figure1**. According to the data obtained showed that flies raised in 2.5g and 5g spirulina treated media had significantly faster rate of development compared to the flies fed with control media and 10g spirulina treated media. Whereas the rate of development was found to be lower in flies raised in control media.

**Figure 1: The effect of different concentration of spirulina diet on the rate of development from larva to pupa of *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration)].**

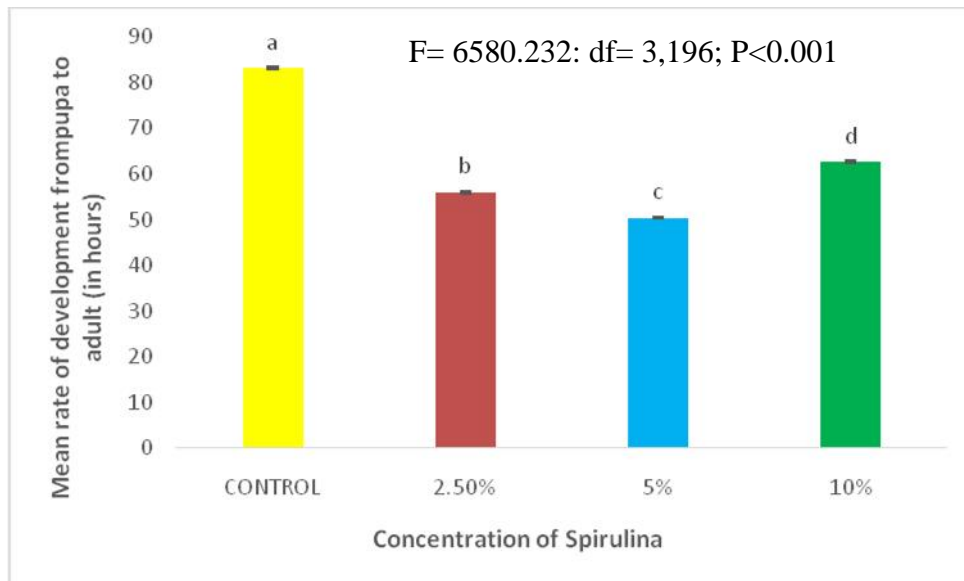


The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 levels

One way ANOVA followed by the Tukey's Post hoc test carried out on above data revealed the significant variation in the rate of development from larva to pupa between the flies of different diet (**Figure 1**). Tukey's post hoc test showed that the flies fed with control and 10g spirulina treated media had significant faster rate of development from larva to pupa than those of 2.5g and 5g spirulina treated media. Further nonsignificant variation in rate of development from larva to pupa was found between the flies raised in 2.5g and 5g spirulina treated media.

The **Figure 2** shows the mean value of rate of development from pupa to adult in flies fed with control and different concentrations of spirulina treated media. According to the data obtained showed that flies raised in control media had significantly faster rate of development from pupa to adult compared to the flies fed with spirulina treated media(2.5g, 5g and 10g). Whereas the rate of development was found to be lower in flies raised in 5g spirulina treated media.

**Figure 2: The effect of different concentration of spirulina diet on the rate of development from pupa to adult of *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration)].**



The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 levels

**Effects of spirulina on the percentage of hatchability in *D. melanogaster*.**

One way ANOVA followed by the Tukey's Post hoc test carried out on above data revealed the significant variation in the rate of development from pupa to adult between the flies of different diet (Figure 2). Tukey's post hoc test showed that the flies fed with control and different concentration of spirulina treated media (2.5g,5g and 10g) had significant rate of development from pupa to adult. The observed variation in rate

of development in control and spirulina treated flies was due to the nutrients available in wheat cream agar media and spirulina media. According to the data obtained indicate that larvae which fed on spirulina emerge faster. It is noteworthy that larvae fed on spirulina treated media are nearly twice the mass of those compared to the larvae fed on wheat cream agar media. and they do not require more time to achieve this size. Larvae would therefore spend less time converting the substrates into metabolized nutrient stores than

when raised on different concentrations of spirulina treated media, which is likely because spirulina provides greater nutrient availability, possibly attributable to differences in media preparation.

Previous works have demonstrated that nutrient-poor diets can result in smaller sized adults (Vijendravarma et al., 2010). The lower larval mass and smaller adult size of *Drosophila* may represent poorer nutrition. It is also noteworthy that as adults spirulina treated flies consume food at a significantly greater rate. This added food intake may directly contribute to their improved health or it may simply be a product of their significantly greater size. Taken together, our findings indicate that the protein rich spirulina treated media contains a nutrient balance that optimizes reproduction rates but it found to be negatively influencing lifespan. Further it significantly enhances the pre adult fitness in *D.melanogaster*.

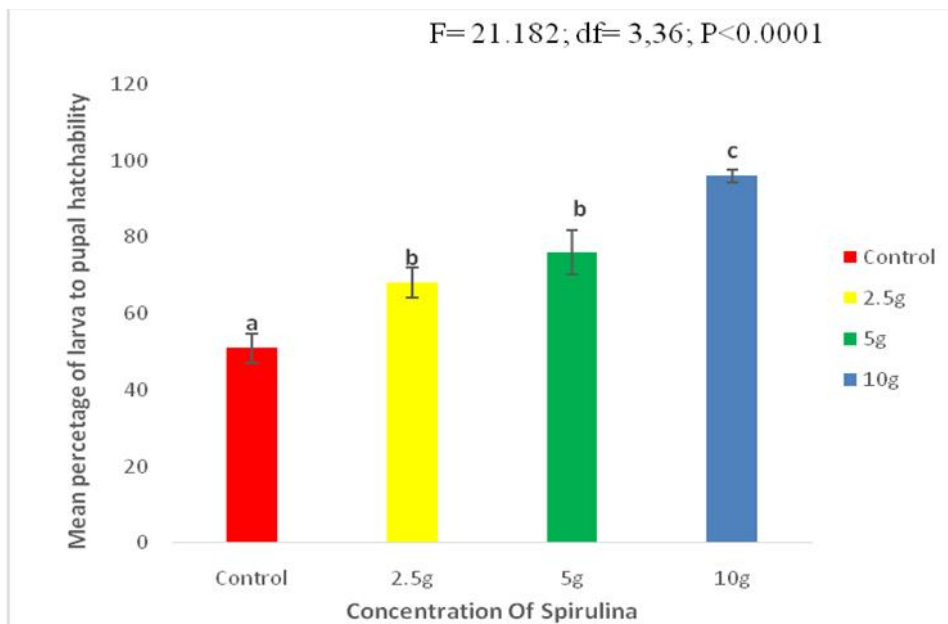
Recent studies have also carried out to analyze the pre-adult fitness in *D.melanogaster* using various diet. Chabra et al., (2013) have showed the effect of organic fruits and vegetables on preadult fitness. They found that rate of preadult development was significantly greater in flies fed

with organic fruits. Similarly, (Geetha and Krishna., 2015) who while studying in *D.melanogaster* have also found that the influence of organic fruits i.e, chikku and watermelon on pre-adult development. Further, Alwyn's D Sowza and Krishna., (2015) also found the effect of alternative natural drink was beneficial in preadult development compared to the synthetic and natural juice. From all these studies it was noticed that the quantity and quality of nutrients present in the diet had an effect on pre-adult development in *D.melanogaster*.

**The effect of spirulina on percentage of pre-adult hatchability:**

Mean±standard error of the larva to pupal hatchability in flies raised in control and spirulina treated media were provided in **Figure 3**. According to the data obtained showed that flies raised in 10g spirulina treated media had significantly greater rate of hatchability from larva to pupa compared to the flies fed with control media, 2.5g and 5g spirulina treated media. Whereas the rate of hatchability from larva to pupa was found to be low in flies raised in control media.

**Figure 3: The effect of different concentration of spirulina diet on larva to pupal hatchability of *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration)].**

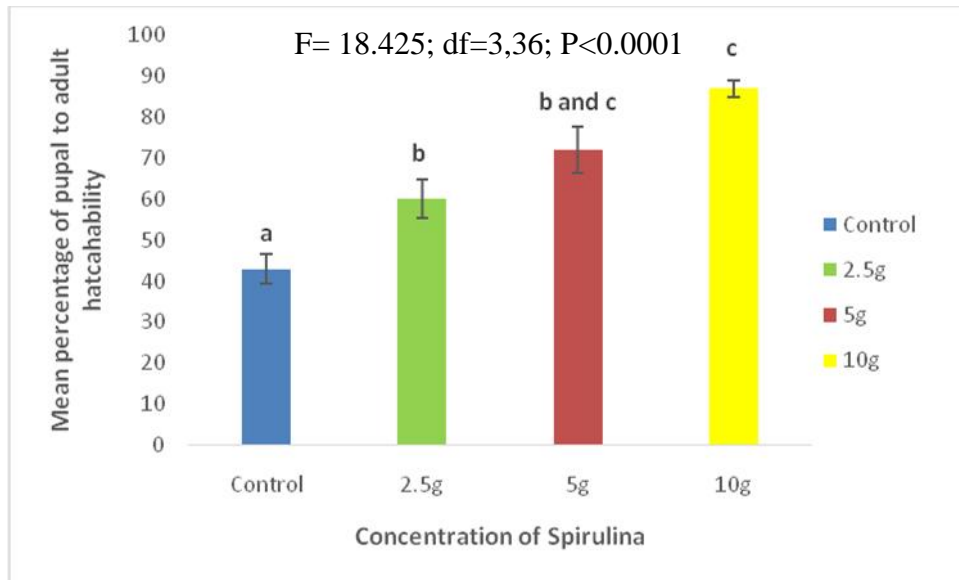


The different letters on the bar graph indicate the significant variation between the different diet by tukey's post hoc test at 0.05 levels

One way ANOVA followed by the Tukey's Post hoc test carried out on above data revealed the significant variation in the rate of hatchability from larva to pupa between the flies of different diet (Figure 3). Tukey's post hoc test showed that the flies fed with control and 10g spirulina treated media had significant rate of hatchability from larva to pupa than those of 2.5g and 5g spirulina treated media. Further non significant variation in rate of hatchability from larva to pupa was found between the flies raised in 2.5g and 5g spirulina treated media.

The **Figure 4** shows the mean value of rate of hatchability from pupa to adult in flies fed with control and different concentrations of spirulina treated media. According to the data obtained showed that flies raised in 10g spirulina treated media had significantly greater rate of hatchability from pupa to adult compared to the flies fed with control and spirulina treated media(2.5g, 5g). Whereas the rate of development was found to be low in flies raised in control media.

**Figure 4: The effect of different concentration of spirulina diet on percentage of hatchability from pupa to adult hatchability in *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration)]**



**The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 levels**

One way ANOVA followed by the Tukey's Post hoc test carried out on above data revealed the significant variation in the rate of hatchability from pupa to adult between the flies of different diet (Figure 4). Tukey's post hoc test showed that the flies fed with control and different concentration of spirulina treated media (2.5g,5g and 10g) had significant rate of hatchability from pupa to adult. Further non significant variation in rate of hatchability from pupa to adult was found

between the flies raised in 2.5g and 5g spirulina treated media.

Physical variables like light, temperature, moisture, and so on, as well as chemical factors such as pH, affect viability. The pre adult viability consisting of different stages which involves Egg to Larval viability larvae to pupae viability and pupae to adult viability It was found that from figure 2, figure 3, shows there was a significant



variation in the pre adult development in flies fed on Spirulina diet had great, larvae to pupae and pupae to adult viability compared to the flies fed on control media.

This suggests that the spirulina provides greater quantity of nutrients and energy required for the pre adult development of larva to pupa and pupa to adult stage, whereas the control media do not equally support energy and required nutrients to increase the pre adult fitness. The rate of development of *Drosophila* tends to decrease with increasing temperature. (Al-Saffar *et al.*, 1995; Gilbert and De Jong, 2001; Hartwell *et al.*, 2011). As rate of development increases, the viability will be faster. This is because both control and spirulina treated flies were cultures under the same lab conditions the only difference was the nutrients present in the diet. Thus we conclude that spirulina treated diet increases the rate of development and percentage of hatchability in *D.melanogaster*.

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