



The effect of the jeeni millets traditional mix on the longevity in *Drosophila melanogaster*

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Abstract

Nutrition has long been recognized as an important factor for influencing both the health span and lifespan in a variety of animals, including flies. In the present study the flies of *Drosophila melanogaster* flies are cultured in the wheat cream agar media, Jeeni millet media and mixed media to understand the effect of Jeeni millet traditional mix on the longevity. The results revealed that flies fed with Jeeni millet were significantly lived shorter compared to the wheat cream agar which lived longer and mixed diet fed flies which showed average life span. Further in the present study. Among the male and females, females were showed the longer life span than the males in all the three diets. Hence this study suggests that the consumption of the Jeeni millet showed the detrimental effect on the Longevity in the *D. melanogaster*. Thus, the longevity sequences of flies in our experiments as follows wheat cream agar>Mixed >Jeeni millet. Suggest that Consumption Jeeni millet reduces the life span in *D. melanogaster*.

Keywords: Nutrition, *drosophila melanogaster*, longevity, Jeeni millet

Introduction

Diet is a significant extrinsic factor that can influence an organism's growth, development, and survival (Sisodia and Singh, 2012) [20]. It was demonstrated that dietary limits without starvation affect lifespan and reproductive output in a variety of taxa, including mammals and nematode worms research underlined that food had a major impact on life span and reproductive output. Piper *et al.*, (2011) [17]. The diet that an organism consumes and its impact on life history traits can be divided into two categories: quantity, which depends on food availability, and quality, which depends on the nutritional makeup of food (Sisodia and Singh, 2012) [20]. The lifespan of many animal species, including flies, has been demonstrated to increase with dietary modifications.

Numerous factors, including extrinsic factors like predation, malnutrition, and other environmental factors, as well as intrinsic elements like aging rates, have an impact on the lifetime of wild animals. Genes that affect various fitness traits and nutrition signaling pathways also have an impact on longevity, which is a quantitative trait. Lifespan is a quantitative trait that is impacted by a number of variables, including sex, age, genetic make-up (epigenetics), and environment (Paaby and Schmidt, 2009) [16]. Extended lifespan in *Drosophila* is frequently linked to a variety of characteristics, including lipid content, development time, body size, biochemical defenses, and resistance to environmental stressors (starvation, desiccation, and cold), which can correlate positively or negatively (Vermeulen and Loeschcke, 2007; Wit *et al.*, 2013; Deepashree *et al.*, 2017) [24, 25, 7].

According to Finch (1990) [8]. and Charlesworth (1994) [5], aging is the steady functional decrease of an organism that results in death. Although a number of hypotheses have been put up to explain the evolutionary pressures involving fitness and reproduction that affects a species' longevity (Hughes and Reynolds, 2005) [9].

In many species, there are disparities between the average lifespan of the sexes, and females often outlive males (Lints

et al., 1983; Austad and Fischer, 2016) [13, 4]. The reported variations in lifespan between the sexes are frequently inferred from wild populations and attributed to risk-taking behavior, feeding habits, and sexual competition, which may have little to do with sex differences in intrinsic aging rates (Austad and Fischer, 2016) [4]. The sexual selection theory (Trivers, 1972) [22]. postulates that lifelong investment and reproductive effort trade-off, resulting in sex differences in ROS generation and antioxidant defenses.

Millets are nutri-cereals, which are known to be exceptionally nutrient-dense and high in protein, carbohydrates, essential fatty acids, dietary fiber, B vitamins, and minerals including calcium, iron, zinc, potassium, and magnesium. Millets include significant nutrients such as resistant starch, oligosaccharides, lipids, antioxidants such phenolic acids, avenanthramides, flavonoids, lignans, and phytosterols, which are thought to be responsible for a number of health advantages (Miller, 2001) [15]. In addition to minerals and vitamins, it contains phenolic components such phenolic acids, flavonoids, and tannins as well as insoluble fiber and peptides, carbs, and protein-rich foods.

The jeeni millet health mix has the following nutritional value per 100g: 69.4g of carbohydrates, 13.57g of protein, 399Kcal of calories, 7.49g of fat, 110mg of calcium, 4.5g of iron, and 0.6g of natural sugars.

Now a days the people are enormously consuming the Jeeni Millet traditional mix by all age people due it's nutritional and health benefits. The several studies shows that the consumption of the millet would reduce the diabetes, control the blood pressure, also helps in the wound healing and also shows the positive effects on controlling the cardiovascular diseases etc in different model organism, but there is no evidence documented about how the millets effect on the longevity of the organism. Therefore, the study is under taken to address the effect of jeeni millet traditional mix on the longevity in the *Drosophila melanogaster*.

Materials method

The jeeni millet traditional health mix was purchased from the Apollo pharmacy shop, Jayalakshmi puram, Mysuru, Karnataka, India. used for prepare the experimental media.

Establishment of stock

Experimental Oregon K strain of *D. melanogaster* used in the study was collected from *Drosophila* stock center. 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 $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

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 rava powder, 10g of agar boiled in 1000ml distilled water and 7.5 ml of propionic acid added to it.; Jeeni millet traditional mix (Referred as Jeeni millet) media: Jeeni millet media was prepared from 100g of jaggery, 100g of Jeeni millet traditional mix powder, 10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it; Mixed (Wheat cream+ Jeeni millet (1:1) media): Mixed media is prepared from 100g of jaggery, 50g of wheat cream powder and 50g Jeeni millet mix powder,10g of agar boiled in 1000ml of distilled water and 7.5 ml of propionic acid added to it.] 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 longevity experiments in *D. melanogaster*.

Experimental procedure

Longevity

From the wheat cream agar, Jeeni millet, mixed media bottle, virgin females and unmated flies were isolated within 3 hours of eclosion from their respective media. These flies were aged for 5days. Virgin female and unmated males are individually aspirated into mating chamber. If mating occurs, these mated pairs were transferred to vial containing their respective media, once in seven days until their death. And note down the number of flies died every day. A total of twenty pairs were made separately for each of the wheat cream agar and Jeeni millet and Mixed media.

Results

Analysis of Survival curve

Survival curve was calculated for longevity of males and females. Two functions that are dependent on time are of particular interest: The survival function and Hazard function. The survival function $S(t)$ is defined as the probability of dying at time t having survived until that time. The graph of $S(t)$ against t is called the survival curve. The Kaplan- Meir method was used to estimate this curve from observed survival times without assuming an underlying probability distribution. Two survival curves were compared using a statistical hypothesis test called the log-rank test, which is used to test null hypothesis that there is no difference between survival curves, i.e., the probability of an event occurring at any point of time is for each media 20 trials were made for each of the Wheat cream agar, Jeeni millet, Mixed diet. The Figure 1A and Figure 2 revealed that the females flies lived longer than the male flies in all the three diets. Further the Jeeni millet diet flies showed that the significantly shorter life span compared to wheat cream agar and mixed diet.

Table 1 (A): Mean and median for survival time for Control (wheat cream agar), Jeeni millet (JM) and Mixed diet Treated male flies

| Groups | Mean ^a | | | | Median | | | |
|---------|-------------------|------------|-------------------------|-------------|----------|------------|-------------------------|-------------|
| | Estimate | Std. Error | 95% Confidence Interval | | Estimate | Std. Error | 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound | | | Lower Bound | Upper Bound |
| Control | 48.800 | 2.975 | 42.969 | 54.631 | 56.000 | 5.963 | 44.313 | 67.687 |
| JM | 40.400 | 1.763 | 36.945 | 43.855 | 40.000 | 3.354 | 33.426 | 46.574 |
| Mixed | 46.600 | 2.799 | 41.114 | 52.086 | 50.000 | 2.236 | 45.617 | 54.383 |
| Overall | 45.267 | 1.529 | 42.269 | 48.264 | 48.000 | 1.919 | 44.238 | 51.762 |

a. Estimation is limited to the largest survival time if it is censored.

Table 1 (B): Test of equality of survival distribution for Control (wheat cream agar), Jeeni millet (JM) and Mixed diet treated male flies.

| | Chi-Square | df | Sig. |
|--------------------------------|------------|----|------|
| Log Rank (Mantel-Cox) | 16.670 | 2 | .000 |
| Breslow (Generalized Wilcoxon) | 8.220 | 2 | .016 |
| Tarone-Ware | 11.925 | 2 | .003 |

Test of equality of survival distributions for the different levels of Groups.

Table 2 (A): Mean and median for survival time for Control (wheat cream agar), Jeeni millet (JM) and Mixed diet treated female flies.

| Groups | Mean ^a | | | | Median | | | |
|---------|-------------------|------------|-------------------------|-------------|----------|------------|-------------------------|-------------|
| | Estimate | Std. Error | 95% Confidence Interval | | Estimate | Std. Error | 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound | | | Lower Bound | Upper Bound |
| Control | 53.800 | 1.852 | 50.170 | 57.430 | 50.000 | 2.795 | 44.522 | 55.478 |
| JM | 43.000 | 1.720 | 39.629 | 46.371 | 46.000 | 2.191 | 41.706 | 50.294 |
| Mixed | 48.400 | 2.908 | 42.701 | 54.099 | 54.000 | 5.477 | 43.265 | 64.735 |
| Overall | 48.400 | 1.387 | 45.682 | 51.118 | 50.000 | .622 | 48.781 | 51.219 |

Estimation is limited to the largest survival time if it is censored.

Table 2 (B): Test of equality of survival distribution for Control (wheat cream agar), Jeeni millet (JM) and Mixed diet Treated female flies.

| | Chi-Square | Df | Sig. |
|--------------------------------|------------|----|------|
| Log Rank (Mantel-Cox) | 17.267 | 2 | .000 |
| Breslow (Generalized Wilcoxon) | 13.363 | 2 | .001 |
| Tarone-Ware | 15.266 | 2 | .000 |

Test of equality of survival distributions for the different levels of Groups.

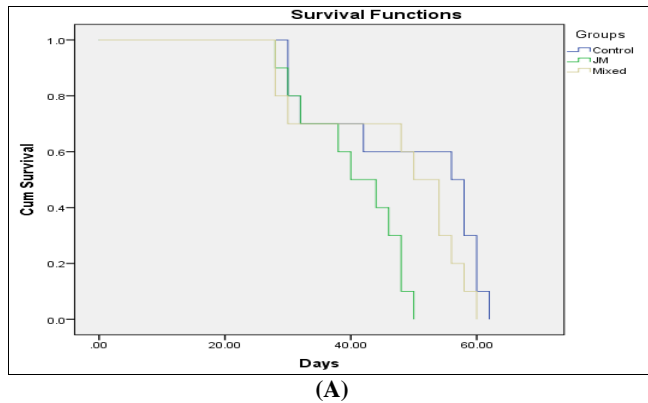


Fig 1: The Effect of the jeeni millet traditional mix on the longevity of the male (mated) and female (Mated) in *D. melanogaster*.

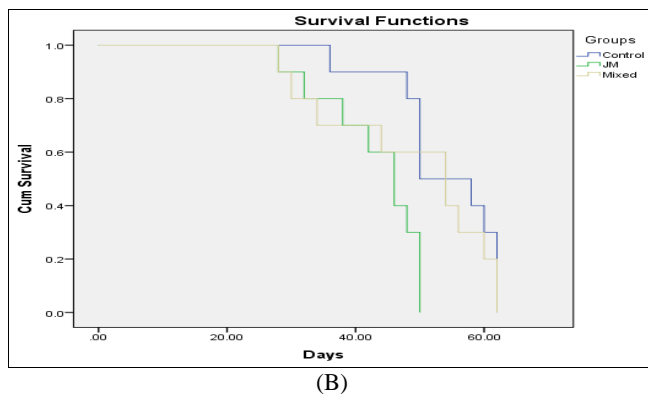


Fig 1: Sex difference in the survivability of as determined by the Kaplan–Meier survival analysis of *D. melanogaster* [A] Male [B] Female. The graphs represent the survivorship of male and female flies housed separately in vials (n=60; 20 vials with 10 flies each per group per sex). The log-rank test revealed a statistically significant difference in the survivorship between males and females (Chi square value for male=16.670, P<0.0001. Female=17.267 and P<0.0001).

Discussion

Aging is a complex biological process characterized by a gradual decline of biological functions. Elucidation of the molecular mechanisms, pathways and physiological processes implicated in longevity are of primary importance for medical and biological research. Lifespan is a quantitative trait influenced by various factors including sex, age, genetic background (epigenetics) and environment (Paaby and Schmidt, 2009) [16] and also the diet. The nutritional diet is one of the important external factors which influenced on the reproduction, stress, growth and development and survivability of the organism. Further the quality and quantity of the nutrients present in the diet is the at most important for the survivability or life span of the organism.

The jeeni millet traditional mix contains the various nutrients (per 100 g) such as 69.4g of carbohydrates, 13.57g of protein, 399Kcal of calories, 7.49g of fat, 110mg of calcium, 4.5g of iron, and 0.6g of natural sugars. Therefore, present study has been undertaken in *D. melanogaster* to know the effect of Jeeni millet traditional mix on the longevity in the *D. melanogaster*. In the present study mean values (Figure 1 A -B and Table 1A and 2A) of by the Chi square test (Table 1B and 2B) revealed significant variation in the life span of flies in the all three different diets i.e., the flies fed with the Jeeni millet were lived significantly shorter than those of the flies fed with the wheat cream agar media which lived longer and average life span was observed in the flies fed with mixed diet. This suggests the amount quantity, quality of nutrients present in the wheat cream agar, Jeeni millet, mixed diet is responsible for the variation in the longevity lifespan in the *D. melanogaster*. The jeeni millets contains the high proteins and high carbohydrates contains along with some amount minerals which may influence on the physiology of the flies may results reducing the longevity than mixed and wheat cream agar media. Nutrient intake has a profound influence on lifespan across a wide range of species, with both very low and very high levels of dietary protein (Piper *et al.*, 2011) [17]. Our study also supported by Brook *et al.*, (2021) who while studying in the *Drosophila* also found the high protein and carbohydrates ratios was reduced the life span in flies and. Theodore *et al.*, (1999), suggest that nutrition should be neutral for selecting life span in *Drosophila* and also proved that the lifespan is decreased under conditions of high nutrition as nutrition increases, lifespan decreases. In most insects, dietary restriction, and in particular a reduced protein intake, increases lifespan (Simpson *et al.*, 2012). Alwyn Dsouza and Krishna, (2015) [1], also found that flies grown on natural energy drink had consumed greater quantity of food and lived significantly longer than those flies grown on synthetic energy drink which showed the least longevity and normal media. As well as several studies also suggested that the high concentration of antioxidants in the diet and supplemented with the other diet also reduces the life span in the flies (Le Bourg, 2001; Magwere *et al.*, 2004) [12, 14].

These above all studies suggests that the nutritional variation in the dietary components is responsible for the increases or decreases in the life span in the *D. melanogaster*. The Jeeni millets are mixture of the different millets and it is nutritional rich food contains the high protein and high carbohydrates and millets usually contains antioxidants may designed as ‘high nutrition’ which may results the decreased the life span in the flies fed with Jeeni millet.

Several studies suggested that longevity is positively correlated with stress resistance in *Drosophila* (Rose 1984; Arking 1987; Rose *et al.*, 1992) [18, 3, 19]. However, our present study revealed that negatively correlation between the longevity and stress resistance which is supported by the study of Kiran and Krishna, (2023) [10, 11]. who while studying in the *D. melanogaster* found that the starvation and heat resistance were found to be greater in the Jeeni millet and Jeeni millet supplemented media treated flies than wheat cream agar treated flies.

In laboratory species like *C. elegans*, *D. melanogaster*, and *Mus musculus*, differences in lifespan between males and females are frequently noted (Tower and Arbeitman, 2009; Austad and Fischer, 2016) [21, 4]. Many species have longevity disparities between the sexes, and females frequently outlive male (Lints *et al.*, 1983; Austad and Fischer, 2016) [13, 4]. The reported variations in lifespan between the sexes are frequently inferred from wild populations and attributed to risk-taking behavior, feeding habits, and sexual competition, all of which may have nothing to do with sex-specific changes in intrinsic aging rates (Austad and Fischer 2016) [4]. Understanding sex-specific aging can be helped by examining longevity variations within a species. The lifespan disparities between male and female flies have been observed, and these findings may be explained by the fundamentally different energy requirements of the two sexes (Hunt *et al.*, 2011; Magwere *et al.*, 2004; Velasco and Medina, 2014) [14, 23]. In the present, The Survival cures and log rank test analysis between the male and females evidently revealed the longevity between the sexes that is females are significantly lived longer than the males in all the three different diets. Several studies suggests that the females have the larger gut than the male helps to consumes the more amount of nutritional food in turn extends the life span in females. Lipid metabolism also helps to enhances the Life span.

According to Vermeulen *et al.*, (2005) [24], oxidative stress resistance to a stressor like paraquat may shorten a *Drosophila*'s lifetime. According to recent research by Archer *et al.*, (2013) [2], in the *Gryllobates sigillatus* cricket, there are sex differences in oxidative stress and lifespan. In *Drosophila* treated with paraquat, sex differences in oxidative stress-mediated mortality were also documented (Chaudhuri *et al.*, 2007) [6]. While induction of catalase activity was significantly higher in older females than in men of the same age, longer-lived females displayed stronger SOD activity that persisted after ethanol exposure. In the present study, also found that females are lived longer than the male flies in all the three diets, however in our study we do not measures the oxidative stress in the flies.

There are several studies which shown that the variation in the environmental condition such as temperature, light, age and stress also influenced on the survival of the flies and these factors also influence on the life span of the flies in the sex specific manner. How ever in our study we maintained the flies in the laboratory condition, the same aged flies which fed with the different diets used to study the longevity the hence observed variation in the experimental results is due to the variation in the quantity, quality of the nutrients in the diet.

Hence from our study in *D. melanogaster* we can conclude that the nutrition is one of the key factors which influenced on the longevity of the organism. The flies raised on the jeeni millet media showed the least longevity than the wheat cream agar and mixed diet. Thus, longevity of sequences of our experiments as follows that wheat cream agar>Mixed >Jeeni millet. Suggests that the consumption Jeeni millet reduces the life span in the in the *D. melanogaster*.

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