International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 06-08-2023, Accepted: 21-08-2023, Published: 07-09-2023
Volume 8, Issue 3, 2023, Page No. 50-55

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

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Abstract
The amount and quality of nutrients consumed by organisms have a strong impact on stress resistance, life history traits and reproduction. The balance between energy acquisition and expenditure is crucial to the survival and reproductive success of animals. The ability of organisms to adjust their development, physiology or behaviour in response to environmental conditions. The availability of the food and nutrition is important factor for the flies or organism which cope with the various types of environmental stress. 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 Heat resistance. The results reveals that the Jeeni millet fed flies had the greater resistance to the heat than the Wheat cream agar media which had the least resistance to heat as well as the mixed diet had more or less equal resistance of heat as the Jeeni millet. Further in the present study, males are more sensitive to the heat resistance than those of the females which shows greater tolerance to the heat, in all the three diet. Further among mated and virgin flies, mated males and females were more resistant to the heat than those of unmated males and females in all the three diets. Hence these studies suggests that Jeeni millet enhances the heat resistance results in *D. melanogaster.*

Keywords: Nutrition, *Drosophila melanogaster*, heat resistance, mated, virgins

Introduction

Life-history features like illness susceptibility, fertility, reproduction, lifespan, and stress resistance are significantly influenced by the quantity and quality of nutrients consumed by organisms. Studies that examine the effects of diet frequently evaluate how people react physiologically and morphologically when exposed to various nutrient quality and quantity. In order to adapt to environmental changes, organisms have evolved a variety of techniques, such as phenotypic plasticity and adaptability (Lynch and Gabriel, 1987; Stearns, 1989; Meyers and Bull, 2002) [14, 25, 15]. Extreme environmental factors and stress may have a negative impact on a organism’s physiology and life-history features.

The genetic variations in stress tolerance leads to adaptive change relies on the frequency of environmental challenges faced by the organism and the corresponding physiological costs (Hoffmann *et al*., 1991) [9]. Environmental stress is defined as the lack of normal nutrients caused by inadequate or unsuitable food resources, and it has been proposed that stress related to few resources has an effect on populations of most species (White, 1993) [27]. Selection most likely influences stress resistance features either directly or indirectly because *Drosophila* stress resistance traits frequently differ across latitudinal clines (Sisodia and Singh,2010) [21]. Numerous elements influence an organism's ability to withstand stress. A response to climatic fluctuations may involve physiological stiffening, coma, or the creation of chemicals that enable an organism to withstand temperature extremes. (Sørensen *et al*., 2003, 2005; Lalouette, 2007) [23, 24, 11]. Also an organism may compensate for nutritional stress and reduced body size by extending its growth period or altering its energy allocation to growth, hence postponing the reproductive period (Reichling, 2000; Lobe, 2006) [19, 13].

Because the climate changes significantly with geographic factors, geographic gradients are of particular relevance in the study of climatic adaptation. Although a number of environmental factors may have an effect on an individual's physiology, temperature is believed to be one of the greatest and is therefore of significant selection significance (Clarke, 2003; Hoffmann *et al*., 2003a) [3, 9]. It is possible that temperature has a significant influence on the distribution and development of species, but it is less certain which aspects of the thermal environment operate as the primary drivers of thermal selection. Although the exact method of adaptation is unknown, it is commonly acknowledged that temperature selection is extremely important and that *Drosophila* are likely to be subjected to stressful situations.

According to Hoffman *et al*. (2003), Sinclair *et al*. (2007), and Kristensen *et al*. (2008)a [9, 20, 10]. *D. melanogaster* is frequently employed as a model organism in studies of physiological and evolutionary responses to various types of stress. However, little is known about how nutrition impacts life history features and performance under heat stress (Watts *et al*., 2006) [28] and the role of diet in experimental designs is frequently understated (Prasad *et al*., 2003) [17]. As a result, there is a rising need to explore the role that diet may have in the variance of features that are crucial for fitness.

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; Edge *et al.* 2005) [16, 5]. 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 heat resistance and other environmental stress of the organism. Therefore the study is under taken to address the effect of jeeni millet traditional mix on the heat resistance in the *D. melanogaster*

Materials method

Material and methods

The jeeni millet traditional mix was purchased from the Appollo pharmacy shop, Jayalakshmi puram, Mysuru, Karnataka, India. This jeeni millet traditional mix used to prepare the experimental media.

Establishment of stock

Experimental Oregon K strain of *Drosophila 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 cream rava,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 22^0 C ± 1^0 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 cream rava powder, 10g of agar boiled in 1000ml distilled water and 7.5 ml of propionic acid added to it.; Jeeni millet traditional mix (Refered 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) media: Mixed media is prepared from 100g of jaggery, 50g of wheat cream rava 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 under the same laboratory conditions as mentioned above were used to study the heat resistance experiment in *D. melanogaster*.

Experimental procedure

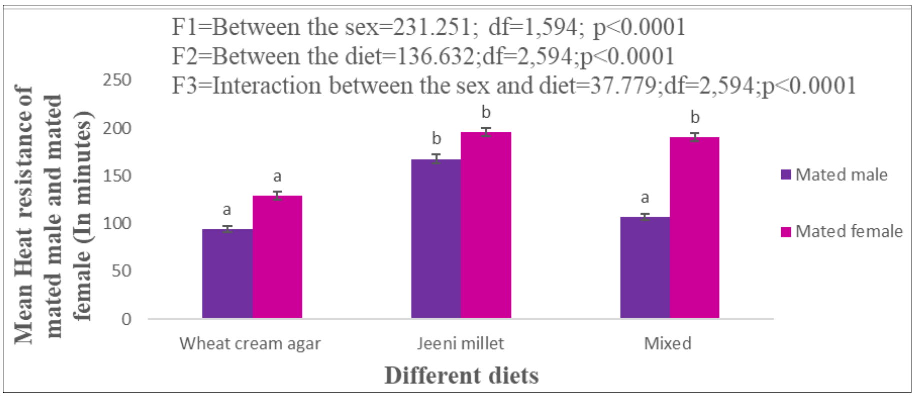
Heat resistance: To study the heat resistance, five days old virgins flies (male and female) and the mated flies (male and female) obtained from the wheat cream agar, Jeeni millet and mixed diet. Twenty male and Twenty female flies were obtained from the each media or diet were transferred to the empty glass vials separately and kept in the incubator at the 37^0 C (sub lethal temperature). These vials were placed in incubator and observed at every 5 minutes interval, vials were taken out and the number of dead flies in each vial was noted. A totally five replicates were carried out for each Wheat cream agar, Jeeni millet and Mixed diet.

Results

Effect of the jeeni millet traditional mix on the heat resistance in the mated male and female of *D. melanogaster.*

The mean and standard error value of the heat resistance of mated male and female flies raised with Jeeni millet, Mixed and Wheat cream agar media are provided in the figure 1. According to data it was noticed that Heat resistance was greater in the jeeni millet media compared to the wheat cream agar and mixed diet. Further the result was also found that the mated female had the greater heat resistance than mated males in different diet.

The above data were subjected to the Two way ANOVA followed by the Tukey's post hoc test showed the significant heat resistant variation between the diets, sex and interaction between the diets and sexes. However, the significant variation was observed between diets. As well as the mated male of the Jeeni millet had the greater significant variation compared to the wheat cream agar media and Mixed diet. However nonsignificant variation was observed between the mixed and wheat cream agar males. Further the mated females fed with the Jeeni millet and mixed diet showed the significant variation compared to the wheat cream agar media as well as the nonsignificant variation was observed between the females of Jeeni millet and mixed diet.



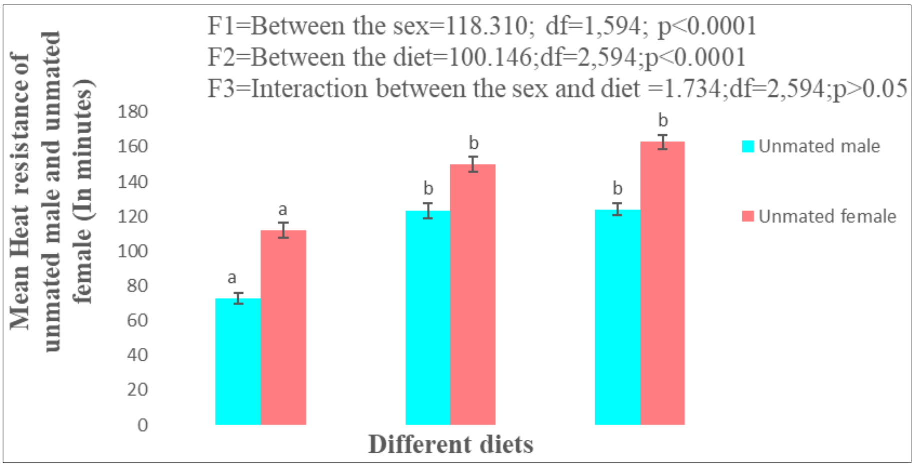
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Fig 1: Effect of the Jeeni millet traditional mix on the Heat resistance in the mated male and female of *D. melanogaster*.

Effect of the Jeeni millet traditional mix on the Heat resistance on the unmated male and female of *D. melanogaster*.

The mean and standard error value of the heat resistance of unmated male and female flies raised with Jeeni millet, Mixed and Wheat cream agar media are provided in the figure 2. According to data it was noticed that heat resistance was greater in the mixed media compared to the wheat cream agar and Jeeni millet diet. The result was found that the unmated female had the greater heat resistance than mated males in different diet.

The above data were subjected to the Two way ANOVA followed by the Tukey's post hoc test showed the significant heat resistant variation between the diets, sexes and also interaction between the sexes and diets. The significant variation observed between the males and females of jeeni millet and mixed with the wheat cream agar diet as well as the Nonsignificant variation was observed between the Jeeni millet and Mixed diet.



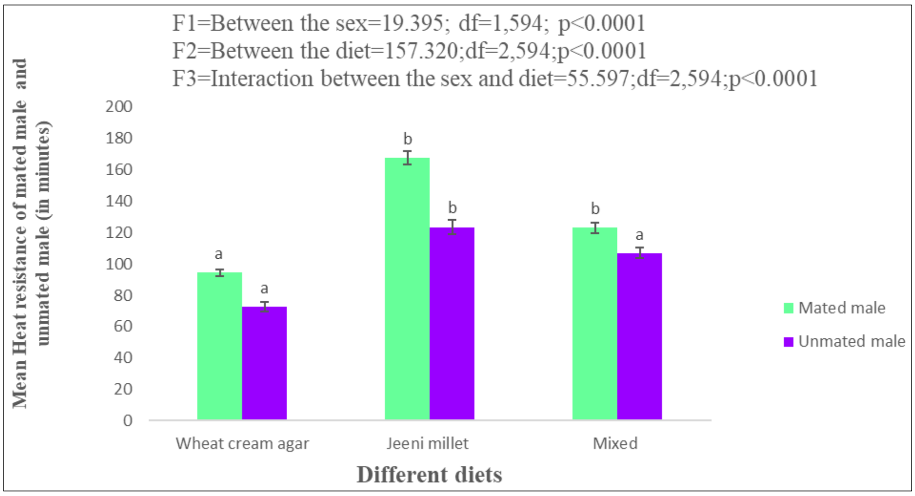
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Fig 2: Effect of the jeeni millet traditional mix on the heat resistance on the unmated male and female of *D. melanogaster*.

Effect of the Jeeni millet traditional mix on the Heat resistance of the mated male and unmated male in *D. melanogaster*.

The mean and standard error value of the Heat resistance of mated male and un mated male flies raised with Jeeni millet, Mixed and Wheat cream agar media are provided in the Figure 3. According to data it was noticed that heat resistance was greater in the jeeni millet compared to the wheat cream agar and mixed diet. The result was found that the mated male had the greater Heat resistance than unmated males in different diet.

The above data were subjected to the Two way ANOVA followed by the Tukey's post hoc test showed the significant heat resistant variation between the sexes, diets and also interaction between the diets and sexes. Significant variation observed between the mated males of the wheat cream cream agar and the mixed and jeeni millet diet, whereas Jeeni millet diet had the nonsignificant variation with mixed. The unmated males of the Jeeni millet had the significant variation compared with the wheat cream agar and mixed diet as well as the Nonsignificant variation of unmated males was observed between the wheat cream and the mixed diet.



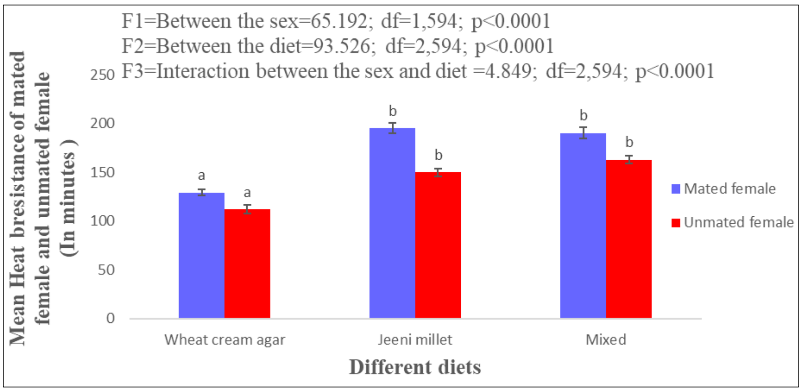
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level

Fig 3: Effect of the Jeeni millet traditional mix on the Heat resistance of the mated male and unmated male in *D. melanogaster*.

Effect of the Jeeni millet traditional mix on the Heat resistance of the mated female and unmated female in *D. melanogaster.*

The mean and standard error value of the heat resistance of mated male and un mated male flies raised with Jeeni millet, Mixed and Wheat cream agar media are provided in the figure 4. According to data it was noticed that Heat resistance was greater in the Jeeni millet and mixed diet compared to the wheat cream agar. The result was found that the mated females had the greater heat resistance than unmated (Virgins) females in different diet.

The above data were subjected to the Two way ANOVA followed by the Tukey's post hoc test showed the significant heat resistant variation between the sexes, diets and also interaction between the diets and sexes. The Jeeni millet and mixed diet had significant variation of the mated and unmated females with the wheat cream agar diet. The nonsignificant variation was observed between the mated and unmated females of mixed and Jeeni millet diet.



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

Fig 4: Effect of the Jeeni millet traditional mix on the Heat resistance of the mated female and unmated female in *D. melanogaster.*

Discussion

Variation in stress related traits in insects in other organisms has been widely studied because it underlies the ability of insects to adopt and counter the effects of changing climatic conditions. Nutrition and sex and genetic variations are also known to influence a species ability to tolerate thermal stress.

Temperature rises more gradually in nature than in the majority of lab studies, and extremities often do not vary significantly from day to day. Therefore, the majority of organisms that have previously encountered high temperatures will have triggered the heat shock response and the genetic diversity in resistance to brief exposure to a high temperature that is important for adjusting to a warmer environment. Survival in the presence of high temperatures depends on the capacity to withstand the physiological stress, with selection acting on genetic variation for the amount of a heat shock protein produced, for its amino-acid sequence, or for the thermal stability of structural or enzymatic proteins. the year. (Koushik and Krishna, 2014) [4].

The availability of the food and nutrition is important factor for the flies or organism which cope with the various types of environmental stress. In the present study, the results (Figure 1, Figure 2, Figure 3, Figure 4) revealed that significant variation in the Thermal stress or heat resistance in the three different diet. It shown that the flies fed with Jeeni millet had the greater resistance to heat or thermal stress than those of the flies fed with wheat cream agar media which had the least resistance to the heat as well as the mixed diet fed flies had more or less equal resistance to heat as the jeeni millet flies. This suggest the nutrient availability, quality in the food was influenced on the variation in the heat resistance of the flies. The jeeni millet is rich with the protein and carbohydrates along with the few minerals which may provide the energy to withstands the heat resistance in the *Drosophila melanogaster* and as well as jeeni millet supplemented diet also enhances the heat resistance whereas the wheat cream agar media may still need the extra energized food to cope up with thermal stress. and we also explain the study result that flies developed on Jeeni millet and Jeeni millet supplemented diet flies cope up with heat shock faster than flies developed on Wheat cream agar diet. The physiological explanation for an increased heat knockdown tolerance among flies developed on protein enriched medium is unknown. Our study also supported by the Sisodia and Singh, (2012) [22], who also found that the flies raised on the protein enriched diet had higher heat resistance than the flies fed with carbohydrates. It is unknown what physiological factors contributed to flies raised on protein-enriched medium having a higher heat knockdown resistance. One option might be associated with the production of heat shock proteins, which are known to be crucial for dealing with various types of stress (Srensen *et al*. 2005; Sinclair *et al*. 2007) [24, 20]. Heat shock protein synthesis is also a component of the physiological processes underpinning fast heat hardening. In addition, according to Anderson *et al*. (2010) [1], Hsp 70 is more highly expressed in fly larvae raised on protein-enriched media as opposed to larvae raised on protein-deficient medium. This may be related to our study results because the experimental diet is rich in the protein and carbohydrates content. How ever we does not quantify the Hsp's proteins in the flies of our experiment.

The pattern of sexual dimorphism in stress resistance has been shown to differ considerably depending on strain, mating status, age and assay condition (Goenaga *et al.*, 2012) [7]. The several study, also shown that the normally the females are have the greater stress tolerance than the males. In our study the among the male and females, The Figure1 and Figure 2 revealed that the females were had the greater resistance to heat than those of the male flies in all the three diet. The several studies also shown the females had the greater stress resistance than the males. Our results suggests that, may due to the females are, ingested more food and thus accumulated greater quantity of lipids compared with males.as well as the females have the greater fat and the protein content which is efficiently used for the energy metabolism than the males (Carvalho *et al*. 2006; Lee *et al.* 2013) [2, 12]. This may results the females are greater resistant to heat than the males

As well as we also noticed the heat resistance. also varies in the mated and unmated conditions, and sexual differences also influences the thermal stress of the flies. In the present study, According to the results (Figure 3), among the mated male and unmated male, The mated males shown the greater heat resistance than the virgins male flies, as the earlier studies mating may not be a harmful process but could be beneficial to males with reference to resistance to heat. And mating rate and activity may be is advantageous for the females in *D. melanogaster*. So, we can explain that like females, the post mating may be results the large food intake by increasing the gut size in males this may results the greater heat resistance than the virgins flies. However, in our study we doesn’t measure amount of food intake thus results the utilization of more energy to enhances the thermal resistance in the *D. melanogaster.* As well as another possible explanation is that the mated male flies may be continuously expose to the pheromones released by females during the copulation may alters the male physiology that may be results the greater resistance to the heat than the virgin males. In contrast our study, several studies shown that mated males are shown that sexual activity in mated male transfer the sperms and accessory gland proteins (Acp’s) during mating and looses it’s energy results the sensitive to the heat than the virgins flies.

Among the mated and unmated females, according to the Figure 4, the mated females are had the greater resistant to the heat resistance than the unmated (Virgins) females. Our study result also confirms the works of Goenaga *et al.*, (2012) [7], who while works on *D. melanogaster* have also demonstrated that mated females exhibit an increased tolerance to stress in comparison to virgins. As well as, the food intake has been shown to increase in female Drosophila after mating (Ravi Ram and Wolfner 2007, Carvalho *et al*., 2006) [18, 2], and these findings may offer a physiological justification for the differences in stress resistance between mated and virgin females. Further, may be due to the mating process gives the nuptial gifts to the females, i.e. Males transfer accessory gland proteins to females during the mating process, and females transfer seminal gland proteins during copulation. The seminal fluid, which is transported to the female along with sperm during copulation, is made up of a complex protein mixture that is produced and secreted by accessory glands (Wolfner, 2002) [28]. Accessory gland proteins (Acps) cause behavioral and physiological changes in mated females (Gillott, 2003) [6]. This may results the greater heat resistance in mated females compared to virgins So, from our results we can explains that the mating condition is advantageous to influencing the physiological behaviour, which helps to cope up and withstand the heat resistance in the both sexes of the *D. melanogaster*

Even though, numerous intrinsic and extrinsic factors, including diet, social interactions, environmental temperature and age, genetic variation also can affect stress resistance in an organism, But in our study we provide same temperature and same aged flies fed with different diet flies were taken to study the heat resistance in the *D. melanogaster.* Hence the variation in the amount, quantity, quality of the nutrients present in the diet was responsible for the variation in the heat resistance in the *D. melanogaster*.

Hence, by our experimental study we can conclude that, the flies developed with Jeeni millet and jeeni millets supplemented (1:1) diet had the greater resistance to the heat than the wheat cream agar media, suggesting that the Jeeni millet increases the heat resistant in *D. melanogaster*.

Acknowledgement

The authors would like to thank the Chairman Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru, and aslo extended the gratitude to *Drosophila* stock centre, National facility, University of Mysore for providing the facilities to carry out the major project work.

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