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The effect of the Jeeni millet traditional mix on the starvation 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. So diet is one of the important external environment factor which influence on the 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 starvation resistance. The results revealed that the flies fed with the mixed media had the greater starvation resistance than flies fed with the jeeni millet which had the average resistance to starvation and whereas flies raised on the wheat cream agar media which showed the least resistance to starvation. This suggest that mixed diet provided the more energy and food to withstand the starvation for loner period. As well as the study also reveals that the females were more starvation resistance than the males in all the three diet. Further among mated and virgin flies, virgin males and females were more resistant to the starvation than those of mated males and females in all the three diets. This study suggests that the supplementation of jeeni millet along with the wheat cream agar in the diet in equal proportions would provides the sufficient energy and food storage to with stand the starvation for longer periods. Thus the starvation resistance sequences in the present study as follows: Mixed>Jeeni millet >Wheat cream agar media.

Keywords: Diet**,** Starvation resistance, *Drosophila melanogaster*, Mated, Virgins.

Introduction

The ability of animals to withstand prolonged periods of food deprivation is called ‘starvation resistance’, which is a phenotypic trait of great organismal, ecological and evolutionary significance given that starvation is the most ubiquitous environmental stress faced by animals inhabiting environments where food availability fluctuates and is unpredictable (McCue, 2010). Starvation, desiccation and exposure to extreme heat or cold are most stressful to insects among environmental challenges. A population exposed to new and stressful environment, may

acclimatize itself either by developing phenotypic compensation through increased competency in acclimation, or by evolving macromolecules that are either more tolerant to functional disturbance or better able to retain functional efficiency in the unfavourable environment. In this respect, unsuitability or insufficient food resources are frequently encountered in nature and starvation is recognized as a major stress in natural populations of *Drosophila* species.

A variety of factors may known to effect organisms stress tolerance, through physiological as well as behavioural changes. Which inturn affect life history traits such as fecundity, fertility, longevity and stress resistance, it was also shown that climatic changes met by an organism may also cause physiological changes such as, hardening process, coma, production of metabolites, making an organism tolerate to temperature extremes (Sørensen *et al.* 2007). Further diet restriction or mild starvation can also increase tolerance to stress. Sisodia and Singh, (2012)have also shown that amount and quality of nutrients intake by organisms have a strong impact on life history, traits such as disease vulnerability, fertility, reproduction, longevity and stress. The bulk of studies on physiological and evolutionary responses to nutrients deficiencies focus on reproduction and fecundity and environmental stress (Sisodia and Singh, 2012).

Drosophila melanogaster, a fruit fly, has been utilized for decades as the primary study organism for determining the root causes of Starvation resistance (reviewed in Hoffmann and Harshman 1999; Rion and Kawecki 2007; Gibbs and Reynolds 2012). Hoffmann and Parsons 1991; Hoffmann and Harshman 1999; Matzkin *et al.*2009, among others have explored the genetic or evolutionary adaptations of fruit flies to acute food shortages at species, population, and intra-population levels. However, the significant role of the environment in determining the phenotypic expression of Starvation resistance and its fitness consequences remains largely unexplored (Pijpe *et al.* 2007). Because animals' physiological ability to resist hunger depends on their nutritional state and dietary history, the nutrient composition of foods is the most significant environmental variable determining Starvation resistance (Jensen *et al.*2010; Laparie *et al* .2012).

Stress can be defined as any environmental factor that causes a potentially adverse change in an organism or biological system. According to Karan *et al.*(1998), stress resistance qualities in Drosophila frequently differ across latitudinal clines, suggesting that selection may either directly or indirectly alter these traits. Many species' individuals must endure famine or exposure to unsatisfactory nutrition. In areas where food is likely to be less available or unpredictable in the short term, positive selection for resistance to starving stress is anticipated. As is frequently the case when insects are restricted to foods deficient in protein relative to carbohydrate (P:C), compensatory feeding for the limiting component causes over intake of other nutrients when presented with nutritionally unbalanced diets (Raubenheimer and Simpson 1999). Due to this, lipid storage may rise and fitness may decrease (Simpson *et al.* 2004; Warbrick-Smith *et al.* 2006).

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). 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 starvation 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 Starvation resistance in the *Drosophila melanogaster*.

Materials and Methods

Material and methods:

The jeeni millet traditional mix was purchased from the Appollo pharmacy shop, Jayalakshmipuram, 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 $22^0 \pm 1^0$ 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.; **Jeen imillet 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 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 starvation resistance experiment in *D. melanogaster*.

Experimental procedure:

Starvation resistance: To study Starvation resistance five days old unmated (Virgins) and mated flies obtained from Jeeni millet, wheat cream agar, and mixed diet were used. Ten flies (male and female) obtained from Jeeni millet, wheat cream agar, and mixed diet were transferred to a new vial containing 5ml of 1% agar media and plugged with cotton. These vials were kept at 25° C under constant light condition and resistance to starvation of each fly was observed in 2 hour interval until its death. A total of three replicates were carried out for each of the Wheat cream agar, Jeeni millet and Mixed diet.

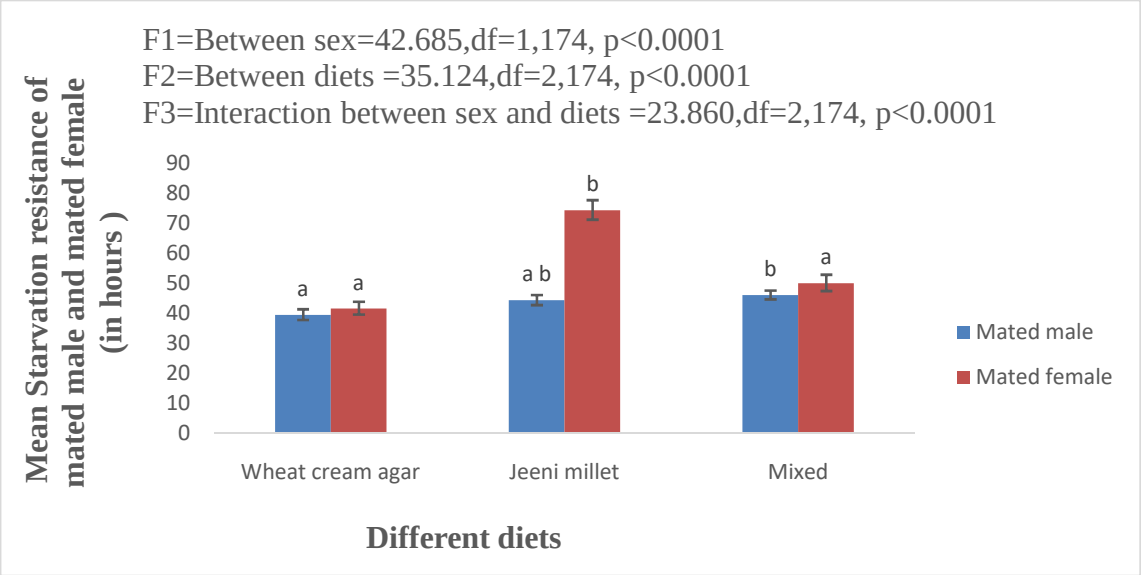
Results

Effect of the Jeeni millet traditional mix on the Starvation resistance in the mated male and female of *Drosophila melanogaster*

The mean and standard error value of the starvation 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 starvation resistance was greater in the jeeni millet media compared to the wheat cream agar and mixed diet. The result was found that the mated female had the greater starvation resistance than mated males in different diet.

The above data were subjected to the Two way ANOVA followed by the Tukey's post hoctest showed the significant variation between the diets, sex and interaction between the diets and sexes. However, non significant variation observed between the male and female of wheat cream agar media compared to the mixed diet which had the significant variation between the male and female as well as the jeeni millet flies had the non significant with wheat cream agar and mixed diet

Figure 1:Effect of the Jeeni millet traditional mix on the Starvation resistance in the mated male and female of *Drosophila melanogaster*.



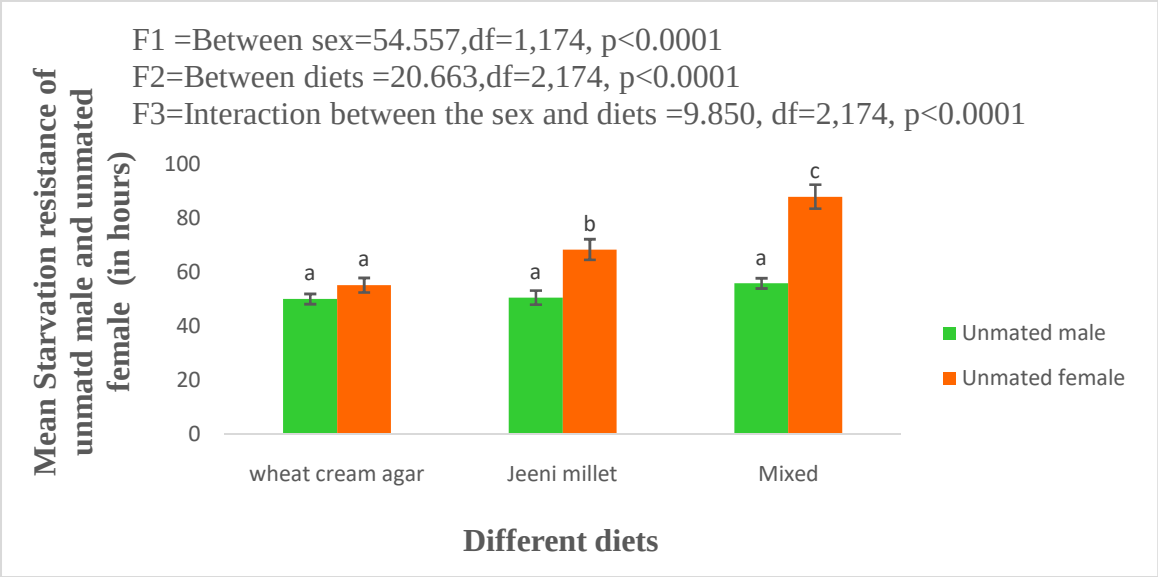
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

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

The mean and standard error value of the starvation 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 starvation resistance was greater in the mixed media compared to the wheat cream agar and Jeeni millet diet. The result was found that the unmated male had the greater starvation resistance than mated males in different diet.

The above data were subjected to the Two way ANOVA followed by the Tukey's post hoctest showed the significant variation between the diets, sexes and also interaction between the sexes and diets. Non significant variation observed between the males of different diet and females had the significant variation between the different diets.

Figure 2: Effect of the Jeeni millet traditional mix on the Starvation resistance on the unmated male and female of *Drosophila melanogaster.*



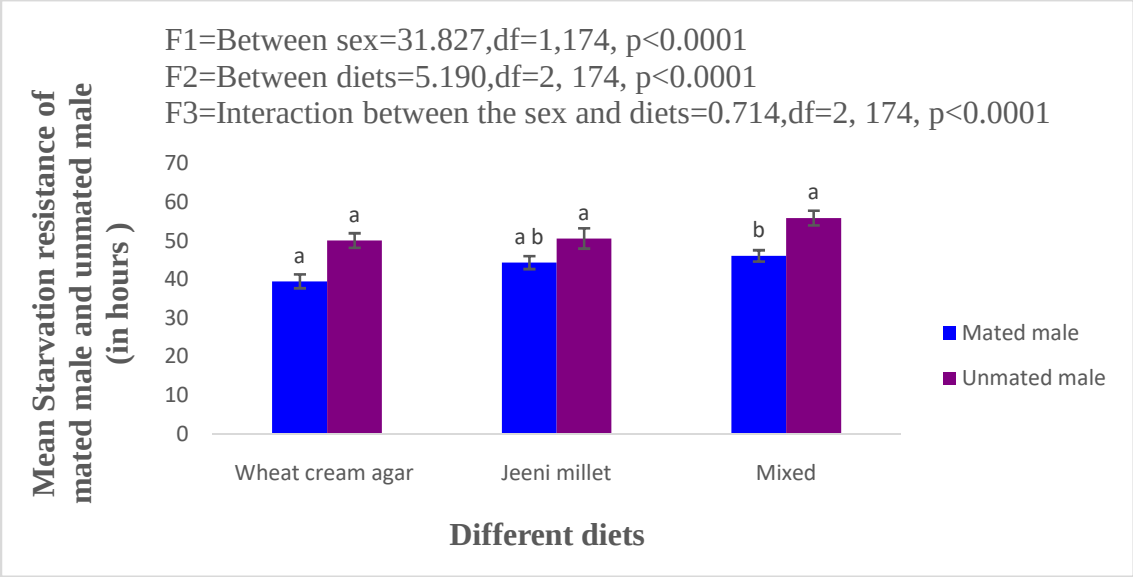
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Effect of the Jeeni millet traditional mix on the Starvation resistance on the mated male and unmated male of *Drosophila melanogaster.*

The mean and standard error value of the starvation resistance of mated male and un mated male flies raised with Jeeni millet, Mixed and Wheat cream agar media are provided in the Figure3. According to data it was noticed that starvation resistance was greater in the mixed diet compared to the wheat cream agar and Jeeni millet diet. The result was found that the unmated male had the greater starvation 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 variation between the sexes, diets and also interaction between the diets and sexes. Significant variation observed between the mated males of the Mixed and wheat cream agar diet whereas Jeeni millet diet had the non significant variation with mixed and wheat cream agar diet. The unmated males had the non significant variation in the different diets

Figure 3: Effect of the Jeeni millet traditional mix on the Starvation resistance of the mated male and unmated male in *Drosophila melanogaster.*



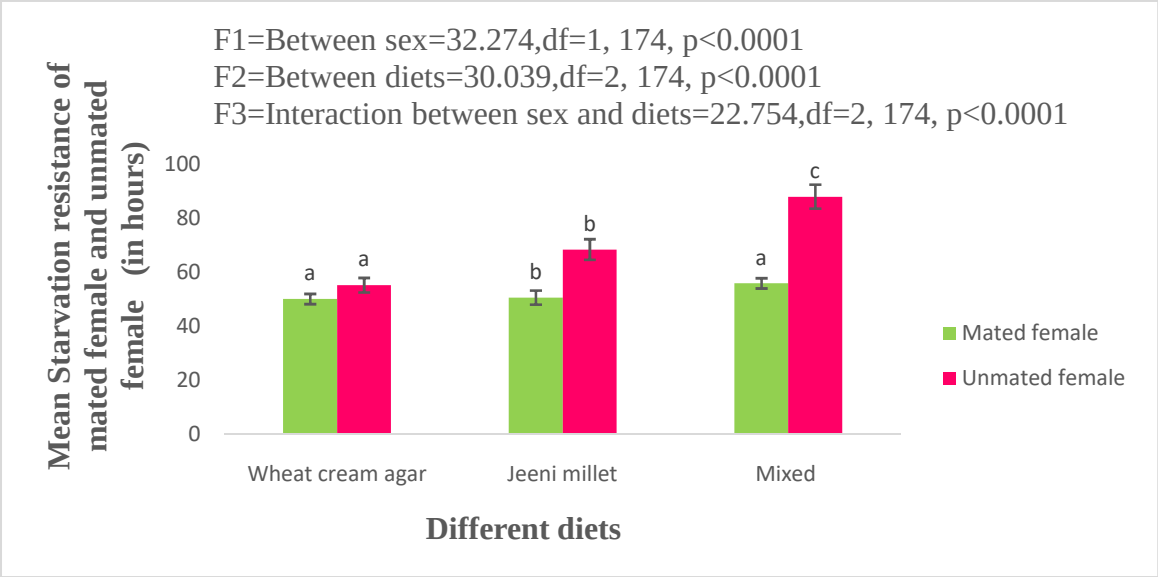
The different letters on the bar graph indicate the significant variation between the different diet by Tukey's post hoc test at 0.05 level.

Effect of the Jeeni millet traditional mix on the Starvation resistance of the matedfemale and unmated female in *Drosophila melanogaster.*

The mean and standard error value of the starvation 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 starvation resistance is greater in the mixed media compared to the wheat cream agar and jeeni millet diet. The result was found that the un mated (virgin) females had the greater starvation resistance than mated females in different diet.

The above data were subjected to the Two way ANOVA followed by the Tukey post hoc test showed the significant variation between the sexes, diets and also interaction between the diets and sexes. significant variation observed between the unmated females of the mixed, wheat cream agar and jeeni millet diet. The non significant variation is observed in the mated females of mixed and wheat cream agar diet. But the jeeni millet diet had the significant variation with mixed and wheat cream agar diet.

Figure 4: Effect of the Jeeni millet traditional mix on the Starvation resistance of the mated female and unmated female in *Drosophila melanogaster.*



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.

Discussion

There are several factors like diet, Sex and environmental conditions which contribute to starvation resistance although their general importance is uncertain (Sisodia and Singh 2012). Diet is the most important external factor affecting development, reproduction, health and survival of the organism further the quality and quantity of the nutrients present in the diet is the atmost important for resisting environmental stress, such as starvation resistance. 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 Starvation resistance in the *D. melanogaster.*

Three main strategies can be used to increases the starvation. *Drosophila* have the capacity to store more energy (lipids, carbohydrates, and protein), consume it more slowly (activity levels, metabolic rate), or tolerate the loss of a larger portion of their initial energy supply (lower energy stores needed to maintain life). These mechanisms are not mutually exclusive, and it is likely a combination of these tactics that helps a population resist starvation.

In the present study, the results (Fig.1, Fig2, Fig3, Fig 4) revealed that the starvation resistance was greater in the mixed diet than the jeeni millet which had the average starvation resistance and wheat cream agar media had the least starvation resistance suggests the amount, quantity, quality of the nutrient in the diet had the responsible for the variation in the starvation resistance.

Our Study also confirms the study of Sisodia and Singh(2012),that is dietary effect of the nutrients present in food affecting the Starvation resistance of *Drosophila ananassae*, showed that flies developed on carbohydrate enriched medium show higher starvation resistance than flies developed on protein enriched medium. Greater starvation resistance requires physiological changes which are likely to trade-off with other fitness-related traits.

By decreasing the amount of nutrition in particular protein (yeast) offered to adult flies (caloric restriction) increases their starvation resistance, with up to two fold difference between females previously fed ad labium yeast than those given no yeast (Chippindale *et al*. 1993). Starvation resistance is ultimately driven by the balance between the supply and demand of energy. The relative proportion of protein and carbohydrate an animal consumes determines not only the level of energy supply (lipid storage derived from ingested carbohydrates), but also that of energy demand from metabolically active tissues (muscles built primarily from ingested proteins).

To be more specific, Matzkin *et al.* (2009) conducted a thorough survey of the sex-specific patterns of starvation resistance in 15 different species of the genus *Drosophila* and discovered significant interspecific variation in the direction and magnitude of sexual dimorphism in starvation resistance among *Drosophila* species. Even within *D. melanogaster*, it has been demonstrated that the pattern of sexual dimorphism in starvation resistance varies significantly depending on strain, mating status, age, and assay condition (Service 1989; Huey *et al.* 2004; Vermeulen *et al.* 2006; Matzkin *et al.* 2009). Starvation resistance in many organisms is expressed in a sex-specific manner due to differences in nutritional demands between males and females. In the present study ,we also studied the starvation resistance of the male and female flies in wheat cream agar and Jeeni millet and mixed media. The results (Figure1, Figure 2, Figure3, Figure 4) revealed that female flies were significantly had greater starvation resistance than those of males flies in the three different diets i.e. jeeni millet, wheat cream agar and mixed diet. We can explains the results by some possible mechanisms i.e.1) Females have a relatively higher fat and/or protein content, 2) a higher portion of this content is available for energy metabolism (i.e., females with starvation have a lower residual fat and/or protein content than males), and 3) females use energy more effectively (Rion and Kawecki 2007).Insects provide ample evidence of sexual dimorphism in characteristics involved in starvation resistance. Mated females ingested more food and thus accumulated greater quantity of lipids compared with males (Carvalho *et al*. 2006; Lee*et al.* 2013).

Aggarwal (2014), who investigated the physiological implications of sex- and population-specific variations in *D. leontia's* starvation resistance. Under conditions of starvation stress, females stored higher amounts of body lipids and glycogen and used both of these energy sources, whereas starved males only digested body lipids as a source of energy. Increased energy storage or a slower rate of depletion of these reserves are related to improved starvation resistance (Hoffmann and Harshman 1999; Rion and Kawecki 2007; Gibbs and Reynolds, 2012).

Several studies have demonstrated that mating has significant effects on starvation resistance, particularly in *D. melanogaster* (Service 1989; Rush *et al.* 2007; Goenaga *et al.* 2012). This effect is primarily mediated by male seminal fluid peptides transferred to females. According to Rush *et al.* (2007), the main cause of the rise in starvation resistance in female *D. melanogaster* is the post-mating increase in food consumption and its associated increase in lipid storage. The energy needed for maintenance of the tissues necessary for insect life could be covered by sugar and lipid storage as well as by proteins via tissue resorption. Sex-specific starvation resistance could be not only a result of differences in the content of particular tissues/compounds that enable energy acquisition but also could be the result of sex-specific abilities to utilize particular tissues/compounds for energy acquisition (Aggarwal2014).

Further, in this study we also studied the variation in the starvation resistance in the mated and unmated (Virgin) flies. According to the obtained results (Figure 3), the unmated males have greater starvation resistance than those of mated males this is because the sexual activity in mated male transfer the sperms and accessory gland proteins Acp's during mating and looses it's energy which is not in the case unmated male they does not looses it's energy and sperms this may inturn results the increased starvation resistance in unmated males. mating status affected male starvation tolerance in a different way.

As well as, the study (Figure 4) also revealed variation in the starvation resistance among the mated female and the unmated females. That is unmated females had the greater significant starvation resistance than the mated females in the three different diets i.e., Jeeni millet, mixed and wheat cream agar media. we can explains this results by the possible reason that is according to the Chapman *et al*. 1996 seminal fluid products from the main cells of the male accessory gland are responsible for the cost of mating in females and that increasing exposure to these products increases female death rate, it may leads to early death during starvation than the unmated females. Alternatively, unmated Virgin female flies may use nutrients reabsorbed from eggs to withstand starvation, but a recent study revealed that oosorption (autophagic reabsorption of oocytes) this may suggests that increased starvation resistance than mated female. However, the Fowler and Partridge (1989) also shown that female flies exposure to the male flies, and mating and remating leads to the reduced the life span in the female of wild fruit flies so, this may be results the reducing the survivability of mated females during the starvation than the virgins female flies of *D. melanogaster*. Our study also supported by study of Himuro and Fujisaki (2010), who while studying the seed bug *Togo hemipterus*, also shown that as virgins survived longer than nonvirgins when exposed to starvation conditions. In contrast to our study, several studies also showed that the mated females exhibited an increased tolerance to starvation in comparison to virgins, whereas It has been shown that mating is costly for both females and males. Among other female post-mating responses (Ravi Ram and Wolfner 2007), it has been shown that food intake increases after mating in *Drosophila* females (Carvalho *et al.* 2006) and actually, these results may provide a physiological explanation for the differences in Starvation resistance between mated and virgin females.

Numerous intrinsic and extrinsic factors, including diet, social interactions, environmental temperature, and age, genetic variation also can affect starvation resistance and its physiological correlates (Service *et al.* 1985; Vermeulen *et al.* 2006; Pijpe *et al.* 2007; Rush *et al.* 2007; Lee and Jang 2014).From the our study we noticed that variation in the starvation resistance in the different diet, this suggests that the amount, quantity ,quality of the nutrients present in the diet was responsible for the variation in the starvation resistance in the *D. melanogaster* and also we observed the same aged unmated (Virgins) male and female flies had greater starvation resistant than the mated male and female with irrespective of diet.

Hence by the our study experiments we can conclude that the mixed media which had the mixture of both wheat cream agar and Jeeni millet in equal proportions which provide energy and helps for longer food storage to withstand the starvation resistance than the wheat cream agar media and Jeeni millet media. The consumption of the food along with jeeni millet which enhances the starvation resistance.

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