



The effect of Jeeni millet traditional mix reduces reproductive fitness in the *Drosophila melanogaster*

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Abstract

One of the most significant environmental factors affecting *Drosophila* growth and development is nutrition. Quantity and quality of nutrients in the food has a significant impact on the growth development, reproduction, health, and survival of the organism. Jeeni millet is a nutriceals which has the mixture of nine different millet and small amount cerals. It is rich in carbohydrates and protein contents and has high calorific value. In the present study we demonstrates the effect of jeeni millet on the reproductive fitness such as mating latency, copulation duration, fertility and thorax body size in the *Drosophila melanogaster* The *D.melanogaster* flies were obtained from different cultured media such as wheat cream agar media, Jeeni Millet media and Mixed media (Millet and wheat cream agar media in equal proportions). These flies were used to study it's effects on the reproductive fitness i.e., mating latency, Copulation duration and fertility in *D. melanogaster* in pairwise mating experiment. The experimental results signifies that the flies fed with Jenni millet diet had taken shortest time for mating while flies fed with wheat cream agar diet took longer duration to initiate the mating and mixed diet flies had taken the intermediate time for mating latency.

Copulation duration studies also showed that the flies fed with mixed diet copulated significantly longer time compared to jeeni millet diet and wheat cream agar diet. The Jeeni millet fed flies copulated in shortest time where as flies raised in wheat cream agar media had taken intermediate time for copulation.The Copulation duration times indicates the male transfer the greater quantity of sperm to the female.

The study of the fertility, also revealed that significant varition in fertility was noticed between the wheat cream and other treated media. The progeny production was least in the flies fed with the jeeni miilet and highest progeny production was observed in flies fed with the wheat cream agar media. where as average progeny production was noticed in the mixed media.

The study of Thorax size showed that significant variation between flies fed with the wheat cream agar and other treated media. The thorax size is larger in the flies fed with the wheatcream agar media and average is observed in the mixed media as well as jeeni millet fed flies had the smaller size.

Thus in *D.melanogaster* flies fed with jeeni millet mated faster, copulated shortest, produced least number of progeny and had smaller thorax size where as flies raised in the wheat cream agar had the mated slower, copulated greater compared to jeeni millet and produced highest number of progeny,and had the larger thorax size. Mixed diet flies mated intermediately but copulated longer and produced average number of progeny, and had the average thorax size. Thus the reproductive fitness of threes diets were as follows Jeeni millet <mixed diet< wheat cream agar diets in *D.melanogaster*, suggesting that Jeeni millet had the negative effect on the *D.melanogaster*

Keywords: Nutrition, wheat cream agar, Jeeni millet, mixed diets, reproductive fitness, mating latency, copulation duration, fertility, thorax body size

Introduction

Fitness is determined in large part by all biological processes that are directly related to reproduction. Which includes viability, longevity, production, fecundity, viability latency, and time between mating. According to Turner and Andersson(1983) ^[45], these mechanisms as a whole determined the fertility component of fitness, where fertility is interpreted broadly. Numerous studies have been done on the sexual behaviors of different species of *Drosophila*, with a focus on the fundamental courtship patterns, genetic control, function of stimuli, and contributions of the 2 sexes to variations in mating activity and repeated mating (Parsons, 1973; Banerjee and Singh; 1998) ^[27, 31] Numerous *Drosophila* species have been used to illustrate the contribution of fertility differences to selection (Turner and Andersson, 1983) ^[45]

Factors such as male size, age are known to affects reproductive fitness in different species of *Drosophila*. Diet is another important factors Known to affects on

reproductive fitness. Further nutritional availability has been demonstrated to influence sexual selection and mate preference (Janicke *et al.*, 2015; Kunz and Uhl, 2015;) ^[13, 16]. Studies on diets have shown that an ideal diet includes life time fecundity (Lee *et al.*, 2008; Maklakov *et al.*, 2009; Pirk, Boodhoo, Human and Nicolson, 2010; Solon-Biet *et al.*, 2015) ^[17, 19, 31, 41]. However, the optimal ratio of macronutrients (fat, protein, and sugar) varies by sex and species. *D. melanogaster* offers a useful animal model to under-stand nutritional basis of reproductive fitness.As animals develop the ability to perceive the features that suggest high fitness and reproductive potential in their possible mates (Andersson, 1994; Maynard-Smith and Harper, 2003) ^[1, 21]. Recent research reveals that the reported benefits are not caused by caloric intake per se but rather diet composition, such as the protein to carbohydrate ratio (Piper *et al.*, 2011; Fanson and Taylor, 2011; Simpson and Raubenheimer, 2009) ^[30, 6, 39].This may not come as a surprise given that different physiological and sex-specific

tasks necessitate certain nutrients and that different diets (Fricke *et al.*, 2008; Maklakov *et al.*, 2008; Vargas *et al.*, 2010, Gosden and Chenoweth, 2011) [7, 19, 46, 9], frequently maximize certain allocation decisions, such as those focused on survival vs reproduction. Natural selection is likely to favor biological mechanisms that quickly alter allocation decisions in response to nutrient availability as well as mechanisms in individuals of the opposite sex to assess such decisions in their potential mates because diet composition can vary greatly over the course of an individual's lifetime.

Millets are nutri cereals with a high nutrient content, including protein, 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) [23]. In addition to minerals and vitamins, it contains phenolic components such phenolic acids, flavonoids, and tannins as well as insoluble fibers and peptides, carbs, and protein-rich foods.

Millets have potential health benefits, and epidemiological studies have shown that consumption of millets lowers the risk of heart disease, guards against diabetes, improves the digestive system, lowers the risk of cancer, detoxifies the body, boosts respiratory immunity, boosts energy, and improves the muscular and neural systems, as well as being protective against several degenerative diseases like Parkinson's disease and metabolic syndrome (Manach *et al.*, 2005; Scalbert *et al.*, 2005; Chandrasekara and Shahidi, 2012) [20, 37, 4].

Jeeni millet is a mixture of different millets i.e Bajra, foxtail, pearl, finger, barnyard, Porso, little millets, brown top millets, corn, jowar, Red rice, Barley and small amount of ceerals such as greengram, Bengalgram, horsegram, Urad dal, Flax seeds, Toor dal with different concentration of nutrients. The jeeni millet health mix contains the nutritinal value per 100g i.e Carbohydrates (69.4g), protein (13.57g), calorific value (399Kcal), Fat (7.49g), calcium (110mg), Iron (4.5g), natural sugars (0.6g).

Now a days the people are enormously consuming the Jeeni Millet traditional health 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 sound 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 Jeeni millets effect on the reproductive fitness of the organism. Therefore present study has been undertaken in *D. melanogaster*.

Material and methods

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 $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

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 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+ Millet)

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 reproductive fitness experiment in *D. melanogaster*.

Reproductive fitness experiment

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 5 days. Virgin female and unmated males are individually aspirated into mating chamber and observed for one hour. If mating does not occurs within one hour, flies were discarded. If mating occurs we recorded their mating latency (time elapsed between introduction of male and female into the mating chamber until initiation of copulation) and copulation duration (time elapsed between the initiation to termination of copulation of each pair) and these mated pairs were transferred to vial containing their respective media, once in seven days until their death. Total number of progeny produced by each mated pair was recorded as fertility. A total of twenty pairs were made separately for each of the wheat cream agar and Jeeni millet and Mixed media.

Body size measurement

The thorax size measured as the index of body size. A total 20 male flies from each diet such as wheat cream agar, jeeni millet and mixed were etherized and individually used to measure the body size in mm by using the zoom stereo microscope in IOE, University of Mysore, Manasagangothri, Mysuru.

Results

Effects of the Jeeni millet traditional mix on the Mating Latency in the *Drosophila melanogaster*

The Figure 1 shows the mean value of the mating latency of *D. melanogaster* flies which were raised in different diet (Wheat cream agar, Jeeni millet and Mixed). According to

the data obtained showed that, the flies raised in the control media had the high mating latency compared to the flies fed with the Jeeni millet which showed the least mating latency and Mixed treated diet showed that the intermediate mating latency period. The experimental data was subjected to the one way ANOVA followed by the Tukey’s Post hoc test revealed the

significant variation in the mating latency between the flies of different diet. Tukey’s Post hoc test showed that the *D. melanogaster* flies fed with control media diet had significant longer time for mating compared to the mixed and Jeeni millet diet fed flies. And non significant variation in mating latency was noticed between the flies fed with Jeeni millet and mixed media by Tukey’s post hoc test.

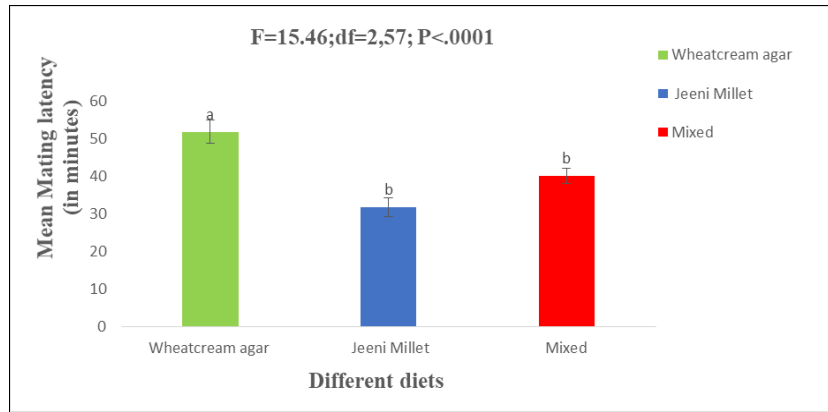


Fig 1: The effect Jeeni millet traditional mix on the Mating latency of *Drosophila melanogaster*

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.

Effects of the Jeeni traditional millet health mix on the copulation duration of *Drosophila melanogaster*.

Mean and stanrdad error value of the copulation duration of *Drosophila melanogaster* flies which are raised in different diet (Wheat cream agar, Jeeni millet and Mixed) were provided in Figure 2. According to the data obtained, the flies raised in the mixed media had longest copulation duration when compared to the flies fed with control media which showed the average duration of copulation as well as

the jeeni millet flies showed the shortest duration of copulation.

The experimental data was subjected to the one way ANOVA followed by the Tukey’s Post hoc test revealed the significant variation in the Copulation duration between the flies fed with different diet (Wheat cream agar, jeeni millet and mixed). Tuckey’s post hoc test showed that the *Drosophila melanogaster* flies fed with mixed media diet had significantly greater copulation duration compare to the Wheat cream agar and Jeeni millet diet fed flies. However, non significant variation in copulation duration was noticed between the flies fed with Jeeni millet and control diet, by Tuckey’post hoc test.

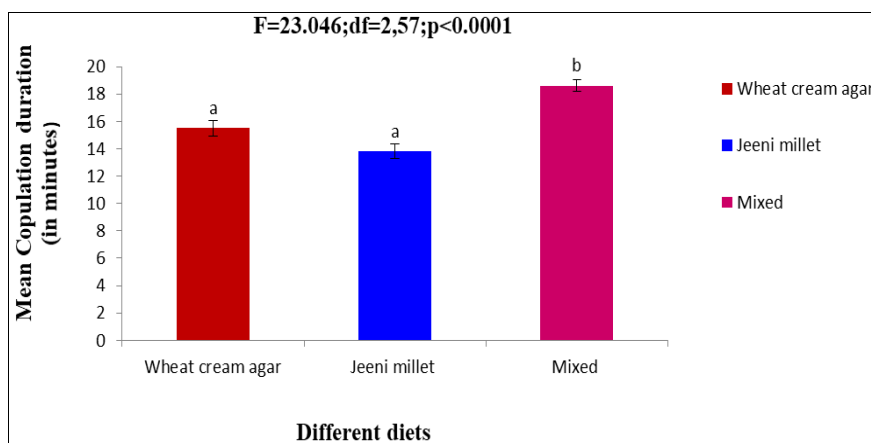


Fig 2: The effect Jeeni millet traditional mix on the Copulation duration of *Drosophila melanogaster*:

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

Effects of the Jeeni Millet Traditional health mix on the fertility the *D. melanogaster*

Figure 3 revealed the mean and Standard error value of the fertility of *D. melanogaster* flies which are cultured in different diets (wheat cream agar, Mixed and Jeeni millet). According to the data obtained, the flies raised in wheat cream agar media had the higher fertility rate compared to

the flies cultured in the mixed diet which showed the average fertility rate and the jeeni millet diet shows the least fertility rate..

The experimental data was subjected to the one way ANOVA followed by the Tukey’s Post hoc test revealed significant variation in the fertility between the flies of different diet (control, jeeni millet, mixed). Tukey’s Post hoc test showed that the *D. melanogaster* flies fed with different diets showed the significant variation with each other.

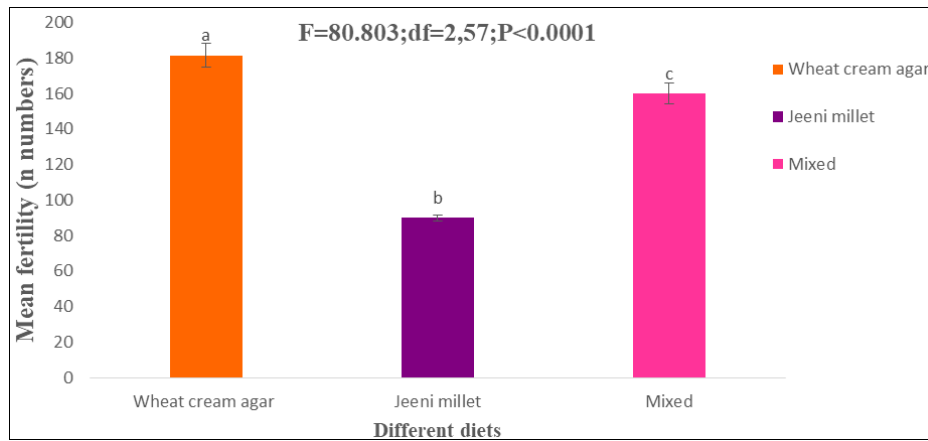


Fig 3: Effect of Jeeni millet traditional mix on the Fertility of *D. melanogaster*.

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

Effects of the Jeeni Millet Traditional health mix on the Body size in the *D. melanogaster*

The Mean and Standard error value of the thorax size of *D. melanogaster* flies which were cultured in different diets (wheat cream agar, Jeeni Millet and Mixrure of wheat cream agar and Jeeni millet) was given in the Figure 4. According to the data obtained, the, the flies raised in control media has

the longer thorax size compared to the flies cultured in the mixed diet which shown the average thorax size and the jeeni millet diet showed the smaller thorax size.

The experimental data was subjected to the one way ANOVA followed by the Tukey’s Post hoc test revealed significant variation in the thorax size between the flies of different diet (control, jeeni millet, mixed).Tuckey’s post hoc test showed that the *D. melanogaster* flies fed with wheat cream agar media had longer thorax compared to jeeni millet and mixed diet flies.

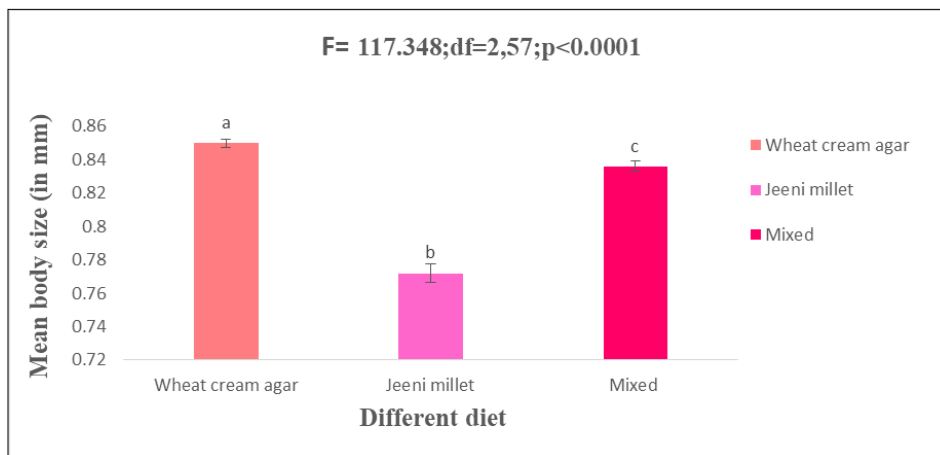


Fig 4: Effect of Jeeni millet traditional mix on the Body size in *Drosophila melanogaster*

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

In animals reproductive success, health maintenance, and energy balance depend on food and nutrition. The availability, quantity and quality food has an impact on reproduction in *Drosophila melanogaster*. In the present study the effect of jeeni millet on the reproductive fitness i.e Mating latency, copulation duration and fertility have been studied in *D.melanogaster*.

The mating latency is the one of the important components of the fitness character shown by Prakash (1967) [32] who found the positive correlationship between the mating speed and the fertility in *D.robusta*. During the mating period the courtship activity were performed by the males to increase the receptivity of the females to make the female sexually excited. The mating speed also indicates the vigourness of the male flies, receptivity of females and attractiveness for

mating. Shorter mating period indicates the higher vigourness. (Pathak *et al.*,2011) [29].

In the present study the significant variation in mating latency was noticed between different diet studied (fig.1). Flies fed with only Jenni millet diet mated faster as they took least time for mating where as the flies cultured with mixed diet took average time for mating.As well as the flies raised in the wheat cream agar diet becomes slower maters as they took longest time for mating. This is because as speed is the reverse of time, individuals which had the higher mating latency are considered to be slow maters while those populations with shorter mating latency were fast maters (Guruprasad *et al.*,2008) [10]. The mating speed also indicates the vigourness of the male flies. Our study showed that quality of nutrients available in the different diets was responsible for the variation observed in the mating latency between diet.The study data supports the works of Schultzhaus *et al.*, (2017) [38] and Anitha and Krishna, (2020) [2] who while studying *Drosophila aslo*

found that ratio of protein to carbohydrates had significant influence on mating latency. These studies in species of *Drosophila* suggest that quality of nutrients in the diet known to affect reproductive fitness.

According to Spiess (1970) ^[43], the male or female's courtship behavior culminates in copulation. The time of copulation is very important to animal's survival since it determines how many sperms from the male are delivered to the female reproductive system. Transfer of sperm to females during mating is its main purpose, providing the chance for males to have progeny. The courtship activities in copulation are known to be influenced by genotype, environmental factors, male size, female size, male and female age and strain (Guruprasad *et al.*, 2008) ^[10]. In *Drosophila* like other insects, the duration of copulation is male determined (Parsons, 1973) ^[27]. In the present study (fig.2) it was found that the flies raised in mixed diet had copulated longest. And flies raised in the wheat cream agar media which showed the average copulation duration, as well as the flies cultured in the Jeeni millet diet showed the least copulation duration. This suggests that nutrients present in the control, Jeeni millet and Mixed diet was responsible for the variation occurred in the duration of copulation. Our studies also confirm the studies of Anitha and Krishna, (2020) ^[2] who while studying in *D.melanogaster*. have also found that variation in protein and carbohydrates ratio in the diet affect the components of copulation duration. The studies in the species of *Drosophila* have also shown that duration of copulation affected by the many factors such as body size, male age and the diet (Guru Prasad *et al.*, 2008) ^[10]. Larger males copulated longer compared to the smaller males, in species of *Drosophila*. (Hegde and Krishna, 1997) ^[12].

Fertility is also another important character which have influence on the reproductive fitness of the organism. Further in the present study (fig.3) we also studied fertility in control and jeeni millet treated media to understand the effect of jeeni millet on fertility. It was found that wheat cream agar media had significantly greater fertility rate compared to flies fed with the jeeni millet diet which shown the least fertility rate and mixed diet which had the intermediate fertility rate. The fertility rate of all the three diet as follows control > Mixed > Jeeni millet. Suggesting dietary components of diet used in the present study had the effect on the fertility. The earlier studies in the *D.melanogaster* also shown that the variation in the ratio of the carbohydrate and protein in diet had the effect on the fecundity and fertility (Anitha and Krishna, 2020) ^[2]. The earlier studies in *D.melanogaster* also shown that the high sucrose diet reduced fertility and protein enriched diet results higher fertility rate (Ramesh *et al.*, 2014) ^[33].

From the results of the copulation duration (fig.2) and fertility (fig.3) we can explain that the flies fed with the jeeni millet media taken shorter copulation duration and produced the least number of progeny this is because Jeeni millet flies with its shorter copulation duration they could not able to transfer required amount of sperms to fertilize the eggs in turn resulting least fertility rate. Flies raised on the mixed diet although taken longest duration of copulation and produce the average number of progeny because during its copulation it could not able to transfer the enough accessory glands and progeny resulting the average fertility rate. Further flies fed with wheat cream agar had taken lesser time for copulation than those mixed media flies but produce greater number of the progeny this because during

its copulation they may be rapidly transfer greater amount of the sperms and seminal fluids and accessory glands hence resulting the greater fertility rate. These are the possible explanation, however in our study we have not quantify and measures the amount of the sperms and accessory glands transfer to the females during the copulation. Earlier studies in *Drosophila* have also found that the longer copulation durations are linked to the transmission of more sperm in many insects (e.g., Thornhill and Alcock, 1983) ^[44]. We can also say that prolonged copulation serve as a male's physical mate guard (Parker, 1970; McLain, 1989) ^[26,22].

Male insect reproduction, including intrasexual countersuccess, sperm priority, and female fecundity, may be influenced by body size. Size confers a reproductive benefit on several mating component behaviors, (Hedge and Krishna, 1999) ^[11]. In the present study we have measured the thorax size as the index of body size. It was found that wheat cream agar media had significantly larger thorax size compared to flies fed with the jeeni millet diet which showed the smaller thorax size and mixed diet which had the moderate thorax size. This suggest that amount, quality and quantity of nutrients present in Wheat cream agar, Jeeni millet and Mixed diet is affected the thorax size in *D. melanogaster*. Over all the thorax size greater in wheatcream agar media followed by the mixed media that follows the jeeni millet media. Our studies also confirm the earlier studies on the body size variation in species of *Drosophila*, several research also found that the moderate or high level of dietary sugars inhibit growth and reduced body size in contrast, dietary protein promotes the larger body size across a wide concentration range. And several studies in the *D.melanogaster*, also found that flies exposure to the different proteins concentration also affect on the body size. Body size also influences mating latency, fecundity, and other fitness components in *D. bipectinata* (Krishna and Hedge, 1999) ^[11] and they also hypothesised that male size had a positive correlation with aspects of mating success in populations of the same species individuals of different sizes in *Drosophila*. In our study (fig.4) we also found the effect of thorax size on mating components such as mating latency, copulation duration and fertility. The flies raised with the Jeeni millet media had the smaller thorax size, mated faster, taken shorter duration of copulation and produced the least number of progeny. Further the flies cultured in the wheat cream agar media had larger thorax size, mated slower, took average duration of copulation and produced greater number of progeny as well as the flies raised in the mixed media had showed average thorax size, mated intermediately, copulated longer, and produce the average number of progeny. Earlier studies also reveals that the male size also influence on mating components (Santos *et al.*, 1988, Hegde and Krishna, 1997). ^[36, 12]. This also confirms the earlier studies in *Drosophila* they also found that positive correlation between body size and fitness characters Such as mating latency, copulation duration, fertility, fecundity and body size (Santhos *et al.*, 1988) ^[36]. This also supports the 'Bigger is Better' hypothesis proposed by S.N.Hegde and Krishna, 1997 ^[12], while studying size assortive mating in *D.malerkotliana*.

In species of *Drosophila* studies have also revealed that variation male age (Prathiba *et al.*, 2011) ^[18], female age (Somashekar *et al.*, 2011) ^[42], and variation in the environmental factors such as temperature, (Santhosh *et al.*, 2015) ^[36], light also affect reproductive fitness of the flies.

However in the current study, flies of the same age were used and maintained under the same laboratory condition but reared in different diets therefore the observed variation in the mating components such as mating latency, copulation duration, fertility and thorax size in *D.melanogaster* was due to the influence of the nutrition availability, quantity, quality of dietary food.

From our study in *Drosophila melanogaster* we can conclude that reduced reproductive fitness is observed in the jeeni millet flies compared to the wheatcream agar and mixed media and The reproductive fitness of *Drosophila melanogaster* in our study follows that Wheatcream agar>mixed> Jeeni millet suggests that consumption of jeeni millet had negative effect on the reproductive fitness.

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