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Effect of fruit body extract of *Pleurotus ostreatus* (oyster) mushroom enhances heat resistance in *D. melanogaster*

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Abstract
The amount and quality of nutrients taken by organisms have a significant impact on stress resistance, life-history features, and reproduction. The balance between energy uptake and expenditure is critical to animal survival and reproductive success. However the present study the flies of *D. melanogaster* was cultured in wheat cream agar media and *Pleurotus ostreatus* (oyster) mushroom diet media. To understand the effect of *P. ostreatus* (oyster) mushroom diet on the heat resistance of *D. melanogaster*. The result revealed that the flies raised in *P. ostreatus* (oyster) mushroom had greater heat tolerance than compared to control wheat cream agar media treated flies. Furthermore also our result revealed that the female flies had capacity to tolerate the higher heat temperature (36°C), than compared to male flies treated in *P. ostreatus* mushroom diet of *D. melanogaster*. And the mated female flies had greater heat resistance than those of mated female flies. But in unmated male flies had greater heat resistance than compared to mated male flies of *D. melanogaster* treated in *P. ostreatus* (oyster) mushroom and control media flies. Thus these suggest that the *Pleurotus ostreatus* (oyster) mushroom in the diet increases the heat resistance in *D. melanogaster* due to the mushroom contain minerals protein and other nutrients.

Keywords: metaverse, meta influencers, human influencer, like

Introduction

The importance of nutritional status on physiological and biochemical processes in insects, any change in dietary regime is likely to affect all parts of their life, including reproduction as well as stress tolerance (such as temperature tolerance). However, only a few research have looked at the effect of nutritional resources on environmental stress tolerance, specifically temperature tolerance (Andersen *et al.*, 2010) [3]. There has been no clear-cut response in this regard since nutritional effects on temperature tolerance appear to be fairly complex and include numerous interacting components. Carbohydrate-rich diets boost *Drosophila* cold tolerance but protein-rich diets have the reverse effect on heat resistance (Andersen *et al*., 2010; Sisodia and Singh, 2012) [3, 34]. Nutritional effects on heat tolerance appear to be influenced by a number of interacting factors, including gender, mating status, and age. Although the physiological and biochemical bases of thermal responses are becoming clearer through metabolic and physiological studies (Overgaard *et al.*, 2007; Doucet *et al.*, 2009; Colinet *et al.*, 2012a; Kostál *et al*., 2012; Teets and Denlinger, 2013) [11, 9, 38], Nutrition, like rapid hardening, is known to play a role in how a species survives at different high or low temperature extremes (Rosenblatt and Schmitz, 2016; Andersen *et al.*, 2010; Sisodia and Singh, 2012; Kristensen *et al*., 2016; Mitchell *et al*., 2017) [31, 3, 34, 17, 24]. Andersen *et al*., discovered that *D. melanogaster* (Meigen) (Diptera: *Drosophilidae*) raised on a protein-rich larval diet outlived those raised on a carbohydrate-rich larval diet.

Temperature and nutrition are two of the most important factors in determining environmental quality, and animals are constantly confronted with combined stresses caused by insufficient temperature and nutrient imbalance under natural conditions (Cross *et al.*, 2015; Rosenblatt and Schmitz, 2016) [31]. Because animals receive energy and nutrients from food, nutrition can be regarded as a major factor that may influence all life-history components (Sterner and Schulz, 1988; Taylor *et al.*, 2005) [36, 37]. Animal feeding experiments have been crucial in understanding how organisms change their energy allocation (Chown and Nicolson, 2004; Cruz-Neto and Bozinovic, 2004) [8, 10]. The quantity and quality of nutrients consumed by organisms have a significant impact on life-history features such as disease vulnerability, fertility, reproduction, longevity, and stress resistance (Hoffmann and Parsons, 1991; Rion and Kawecki, 2007; Lee *et al.*, 2008) [14, 30, 19] Nutritional impact studies frequently test the physiological and morphological reactions of individuals exposed to varying quality and amount of foods. Deficiency or imbalance of fat, carbohydrate or protein can affect characters such as growth and reproduction. Protein deficiency reduces fecundity and growth in *Drosophila melanogaster* (Wang and Clark, 1995) [39] and in fruit-feeders protein is often limiting macronutrients (Mattson, 1980; Adams and Gerst, 1991; Hendrichs *et al.*, 1991; Markow *et al.*, 1999; Markow *et al.*, 2001) [22, 1, 12, 21, 20]. In contrast diet restriction on mild starvation can increase longevity as well as tolerance to stressors such as heat stress (Wenzel, 2006; Smith *et al.*, 2007) [40, 35] demonstrating the complexity of organismal nutrient acquisition and utilization. (Hallman and Denlinger, 1998; Chown and Nicolson, 2004; Nyamukondiwa and Terblanche, 2009; Andersen *et al.*, 2010; Colinet and Boivin, 2011; Sisodia and Singh, 2012) [8, 25, 3, 34].

P. ostreatus is cholesterol-free and low in calories, carbohydrates, fat, and sodium, it is currently highly valued and recognized as a functional dietary element. When they are positioned close to one another, they supply critical nutrients such as riboflavin, selenium, potassium, niacin, proteins, and fiber (Akyuz *et al*., 2010; Sahoo *et al*., 2022)

[2, 33]. According to several studies (Zhang *et al*., 2012) [42], the consumption of mushrooms is rising quickly all over the world because they are a rich source of bioactive substances like laccase, functional protein glucans, ubiquinone-9, nebrodeolysin, and glycoprotein, proteoglycans, pleuran (-1, 3-glucan with galactose, and mannose), pleurostrin (peptide), minerals (Fe, Ca, K, P, and Na), dietary. Therefore the present study has been undertaken to study the effect of *P. ostreatus* (oyster) mushroom on heat resistance in *D. melanogaster*.

Materials method

Collection of fruit body extract of *Pleurotus ostreatus* (oyster) mushroom

The fruit body extract of oyster mushroom powder was purchased from the ROOTED
(Active naturals). Delivered by Amazon app by online.

Establishment of stock

Oregon K strain of *D. melanogaster* provided by *Drosophila* stock center was used in the investigation. Department of Zoology, University of Mysore, Mysuru. This stock was used in the present study, was cultured in bottles containing wheat cream agar medium (100g jaggery, 100g wheat powder, 10g agar, 1000ml distilled water, and 7.5 ml propionic acid were added). Flies were kept in a lab environment with 70% humidity, 12-hour cycles of darkness and light, and a temperature of 22°C ±1°C.

Experimental media preparations

To get experimental media, the different concentrations (2.5g, 5g, 10g) of *P. ostreatus* (oyster) mushroom powder was weighed and mixed thoroughly with 100ml of wheat cream agar media. To obtain 2.5g, 5g and 10g experimental media. Flies cultured in wheat cream agar media were considered as control. These experimental flies were also maintained in same laboratory conditions as described above. Flies obtained from control and experimental flies were used in the present experiment.

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5 days old virgins females and unmated male flies of control and treated mushroom media were used. To measure the heat resistance in *D. melanogaster*. Five replicates were carried out separately with respect to male and female of control and P. ostreatus (oyster) mushroom treated Twenty flies of same treated media were transferred to labelled vials respectively Plugged the each vial using cotton. Heat resistance labelled vials which contain flies were kept at 36°C using incubator and these flies were observed for 5 minutes intervals for note down the number of flies died in each vials respectively until all the flies had died. Separate experiment was run for both male and female flies.

Result

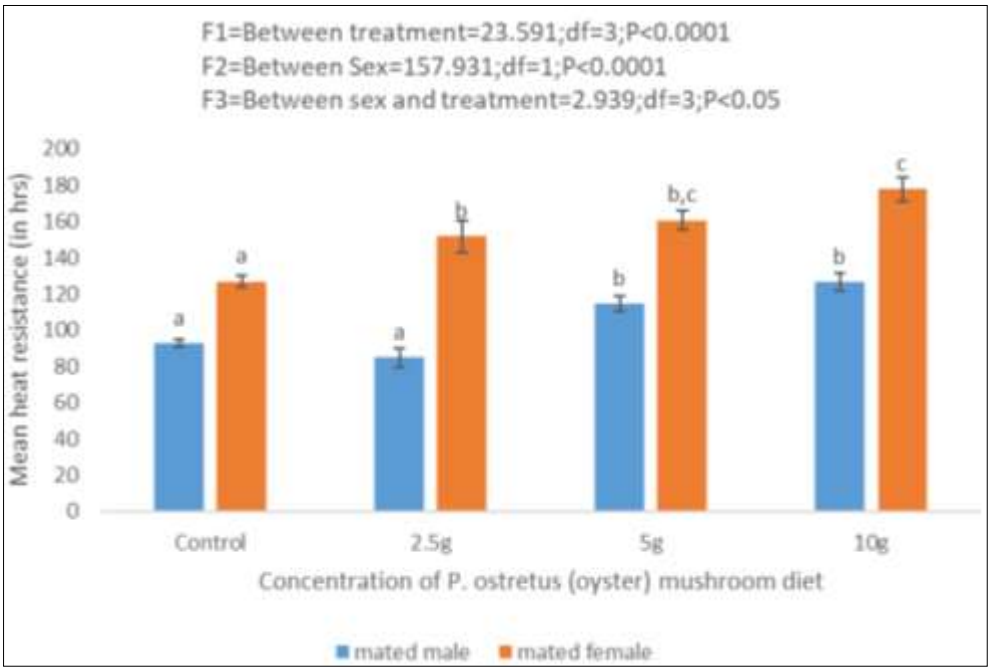

Fig 1: Effect of different concentration of oyster mushroom on heat resistance in mated male and female of *D. melanogaster*. [Control diet-wheat cream agar media; *P. ostreatus* (oyster) mushroom diet (2.5g, 5g, 10g)].

Different letters on the graph indicates significance at 0.05 levels by Tukey's post hoc test.

Figure (1) showed the effect of *P. ostreatus* (oyster) mushroom on heat resistance of mated male and female flies raised in control diet and oyster mushroom treated media. According to the data obtained the heat resistance was found high in *Drosophila* flies raised in oyster mushroom treated media compared to control diet flies. This result was found to be similar in both the sex studied. Further, heat resistance of female were significantly greater than those of male flies in both control diet flies and oyster mushroom treated flies. The above heat resistance data was subjected to Two way ANOVA followed by Tukey's post hoc test showed significant variation in heat resistance between control media flies and oyster mushroom treated flies, between sex. Tukey's post hoc test showed that there was a significant differences in heat resistance between control diet flies and flies raised in different concentration of oyster mushroom diet

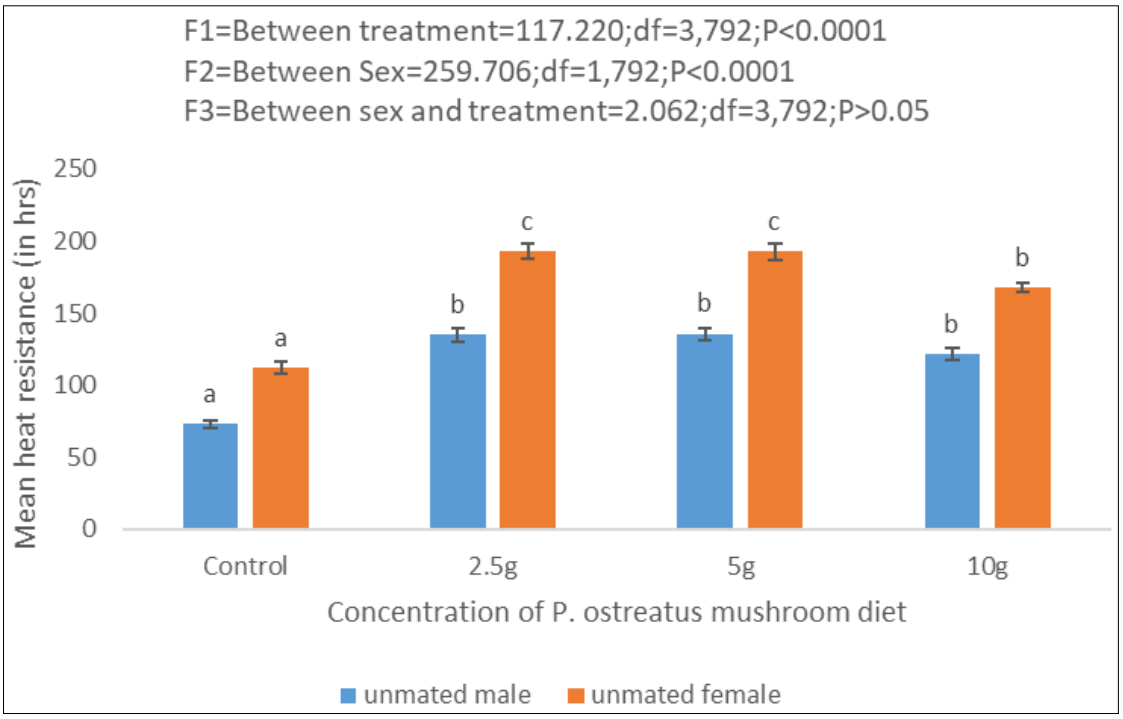

Fig 2: Effect of different concentration of oyster mushroom on heat resistance in unmated male and unmated female of *D. melanogaster*. [Control diet-wheat cream agar media; *P. ostreatus* (oyster) mushroom diet (2.5g, 5g, 10g)].

Different letters on the graph indicates significance at 0.05 levels by Tukey's post hoc test.

Figure (2) showed the effect of *P. ostreatus* (oyster) mushroom on heat resistance of unmated male and unmated female flies raised in control diet and oyster mushroom treated media. According to the data obtained the heat resistance was found high in *Drosophila* flies raised in oyster mushroom treated media compared to control diet flies. This result was found to be similar in both the sex studied. Further, heat resistance of female were significantly greater than those of male flies in both control diet flies and oyster mushroom treated flies. The above heat resistance data was subjected to Two way ANOVA followed by Tukey's post hoc test showed significant variation in heat resistance between control media flies and oyster mushroom treated flies, between sex and also interaction between treatment and sex.

Tukey's post hoc test showed that there is a non significant differences in heat resistance between unmated male flies raised in different concentration of oyster mushroom diet.

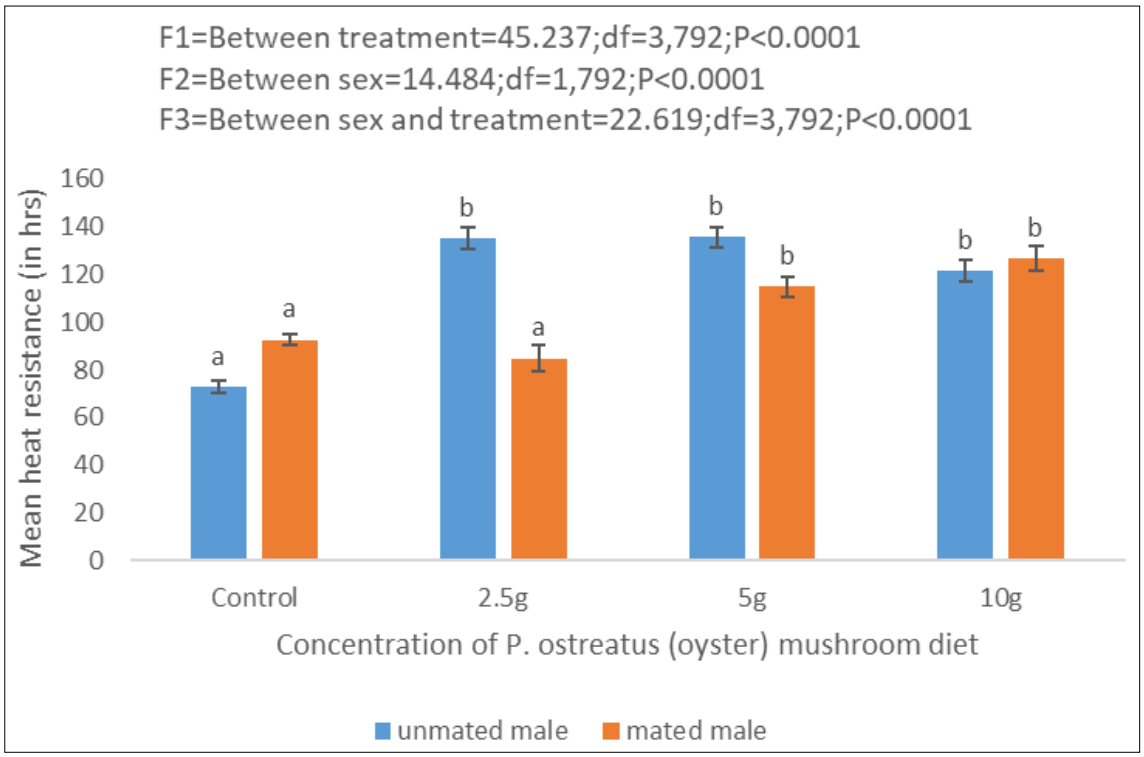

Fig 3: Effect of different concentration of oyster mushroom on heat resistance in unmated male and mated male of *D. melanogaster*. [Control diet-wheat cream agar media; *P. ostreatus* (oyster) mushroom diet (2.5g, 5g, 10g)].

Different letters on the graph indicates significance at 0.05 levels by Tukey's post hoc test

Figure (3) showed the effect of *P. ostreatus* (oyster) mushroom on heat resistance of unmated male and mated male flies raised in control diet and oyster mushroom treated media. According to the data obtained the heat resistance was found high in *Drosophila* flies raised in oyster mushroom treated media compared to control diet flies. This result was found to be similar in both unmated and mated male flies. Further, heat resistance of unmated male were significantly greater than those of mated male flies in oyster mushroom treated flies and except control flies. The above heat resistance data was subjected to Two way ANOVA followed by Tukey's post hoc test showed significant variation in heat resistance between control media flies and oyster mushroom treated flies, between both unmated and mated male flies. However there is significant variation in heat resistance in different concentration of oyster mushroom treated media flies.

Tukey's post hoc test showed that there is a significant differences in heat resistance in mated and unmated control diet flies and flies raised in different concentration of oyster mushroom diet.

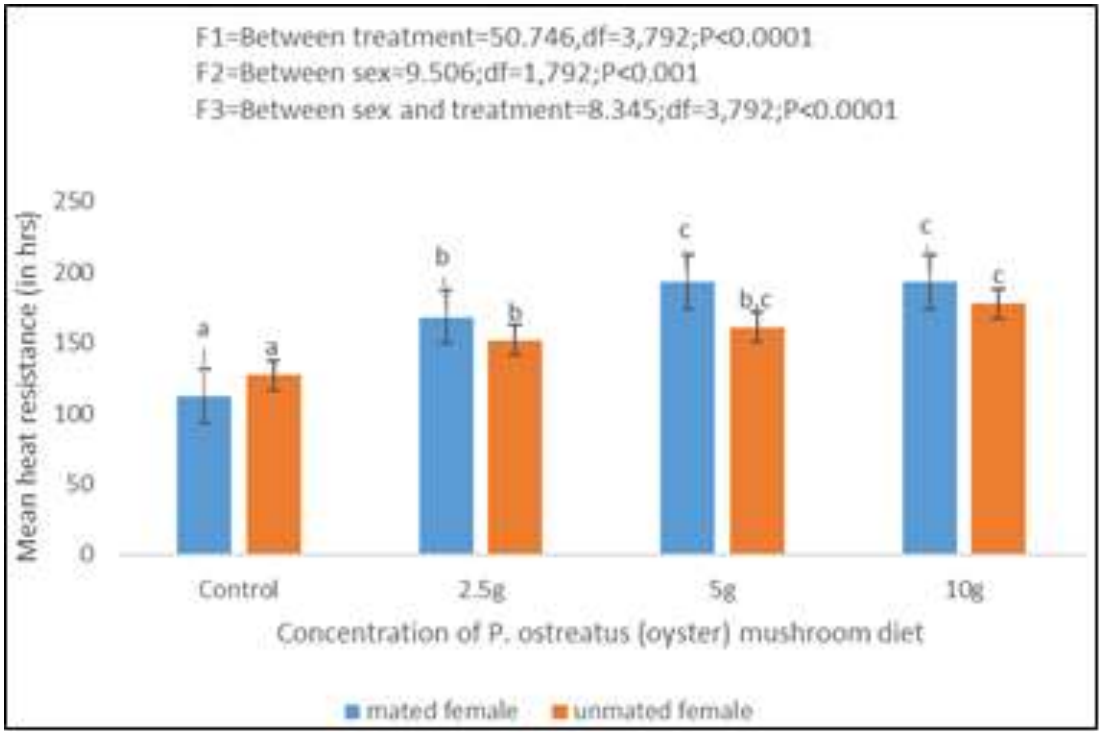

Fig 4: Effect of different concentration of oyster mushroom on heat resistance in mated female and unmated female of *D. melanogaster*. [Control diet-wheat cream agar media; *P. ostreatus* (oyster) mushroom diet (2.5g, 5g, 10g)].

Different letters on the graph indicates significance at 0.05 levels by tukey's post hoc test

Figure (4) showed the effect of *P. ostreatus* (oyster) mushroom on heat resistance of mated female and unmated female flies raised in control diet and oyster mushroom treated media. According to the data obtained the heat resistance was found high in *Drosophila* flies raised in oyster mushroom treated media compare to control diet flies. This result was found to be similar in both mated and unmated female flies. Further, heat resistance of mated female were significantly greater than those of unmated female flies in (*P. ostretus*) oyster mushroom treated flies. The above heat resistance data was subjected to Two way ANOVA followed by Tukey's post hoc test showed significant variation in heat resistance between control media flies and oyster mushroom treated flies, between both mated and unmated female flies. Tukey's post hoc test showed that there is a significant differences in heat resistance between control diet flies and flies raised in different concentration of oyster mushroom diet.

Discussion

Sisodia and Singh, 2012 [34] who while studying in *D. ananassae* flies developed on protein enriched medium have higher heat resistance than flies grown on carbohydrate enriched medium. Very few flies developed on carbohydrate rich diet have revived after heat shock. Flies developed on protein rich diet cope up with heat shock faster than flies developed on carbohydrate –rich diet. In the present study Figure (1 and 2) shows that both unmated and mated female flies have greater heat resistance than compared to unmated and mated males treated in *P. ostreatus* (oyster) mushroom diet of *D. melanogaster*. Many studies suggested that Hsp70 seemed to be merely an important part of a molecular cascade, which possibly involved other heat shock proteins being responsible for regulating heat stress resistance in adult *D. melanogaster*. Earlier study says that the indication of stress response, oxidative stress is usually generated when the organism experiences adverse conditions and indicates the ability of the organism to resist adversity. After thermal hardening, the antioxidant activity of female flies decreased to allow a better adaptation to high temperatures. Additionally, it was clear that some of the antioxidant indexes such as the activities of CAT and SOD, and the inhibition for OH· and $\cdot O_2^-$ in the HTE groups increased compared with those in the TH + NHTE group suggesting that HTE had the function of enhancing antioxidant activity *in vivo* in female flies. Females showed higher SR

than males throughout all treatments during the adult lifespan. This is consistent with the majority of other research on SR in insects. Females may be able to withstand more because of bigger energy reserves stored in their larger body size, as demonstrated by other insect species (Knapp, 2016; Matzkin *et al.,* 2009; Ballard *et al.,* 2008; Blanckenhorn *et al.,* 2007) [15, 23, 4, 5]. An earlier study found that the P:C ratio of the larval diet can have a minor but long-lasting effect on adult survival. *D. melanogaster* reared on a larval diet with the greatest P:C ratio of 8:1 had better adult survival than those raised on the other diets when the P:C ratio of the adult diet was 1:8. High protein intake during development may have resulted in an adult phenotype with enhanced somatic repair and maintenance mechanisms (Runagall Mc Naull *et al.,* 2015) or altered aging related nutrient sensing pathways (Pasco and Léopold, 2012; Pooraiiouby *et al.,* 2018) [27, 28]. In the present study (Figure 1and 2) also observed that both unmated and mated male and female flies reared in *P. ostreatus* (oyster) mushroom diet had greater heat resistance significant than those of control media treated flies. May be because of presence of rich protein and other nutrients content in the *P. ostreatus* (oyster) mushroom. Mating did not raise mean SR in mated males compared to virgins, but it had a significant genotype-specific effect on the characteristic. According to Himuro and Fujisaki (2010) [13], mating is costly for both females and males in the seed bug Togo hemipterus, as virgins survived longer than non-virgins when exposed to starving circumstances. In the present study (Figure 3) shows that the unmated male flies have greater heat resistance than mated male flies reared *in Pleurotus ostreatus* (oyster) mushroom diet. As a result, of the earlier study the benefit conferred by mating on females may be associated to additional substances that *D. melanogaster* males transport with sperm, such as male accessory glandproteins (Acps), which have been shown to trigger changes in female physiology and behavior (Wolfner, 2002; Kubli, 2003; Chapman and Davies, 2004) [41, 7]. Food intake increases after mating in *Drosophila* females (Carvalho *et al.,* 2006) [6], among other female post-mating reactions and these findings may provide a physiological explanation for the variations in SR between mated and virgin females. In the present study (Figure 4) observed that the mated female flies had greater heat resistance than compared to mated female flies reared in *P. ostreatus* (oyster) mushroom diet.

Thus these overall result finding that the female flies had greater heat resistance than compared to male flies. And also the flies treated in oyster mushroom diet had greater positive impact on heat resistance may be due to the presence of rich protein and other nutrients in *P. ostreatus* (oyster) mushroom diet.

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