International Journal of Zoology Studies
www.zoologyjournals.com
ISSN: 2455-7269
Received: 02-08-2023, Accepted: 18-08-2023, Published: 04-09-2023
Volume 8, Issue 3, 2023, Page No. 44-49

Spirulina supplement increases heat resistance in *Drosophila melanogaster*

Shreeraksha, Shreejani H K, Krishna M S*
Department of studies in Zoology, University of Mysore, Manasagangothri, Mysore, Karnataka, India

Abstract
The quantity and quality of nutrients an organism consumes greatly impact the ability to withstand stress, its life history characteristics, and its capacity for reproduction. For an animal to survive and experience minimum reproductive stress, a healthy balance between energy intake and expenditure is essential. The capacity of organisms to change their physiology, behavior, or development in response to external factors. For organisms like flies or other living things that must deal with various forms of environmental stress, the availability of food and nutrients is an essential component. In the present study the flies of *D. melanogaster* flies are cultured in wheat cream agar media and Spirulina treated media to understand the effect of spirulina on the heat resistance. Our result revealed that the flies which fed spirulina treated media had greater heat resistance than control flies which had least resistance to heat. Further females were greater resistant to heat resistant than males. Also in the present study, further among mated and unmated flies, mated males and females were more resistant to the heat than those of unmated males and females in both control media and spirulina treated media. Hence this experiment suggests that spirulina treated media enhance the heat resistance in *D. melanogaster*.

Keywords: *Drosophila melanogaster*, heat resistance, mated flies, unmated flies, spirulina

Introduction
The simplest way that environmental change can impact on stress resistance, fecundity and physical condition is through nutritional impacts brought on by changes in the availability of different food types. In general, diet can be categorized as either quantitative (i.e., food availability) or qualitative (i.e., food consumption). Out of these two, the quantitative effects are evident since animals obtain their energy and other nutritional needs from food; consequently, under a wide range of natural conditions, there is a positive relation between food availability and fitness of an organism. On the other hand, qualitative effects are frequently divided into two categories, namely, non-nutritive effects and nutritive effects two broad categories that can be used to categorize diet effects are food availability and food composition (Pough, 1989; Sibly, 1991) [15, 20].

Both intrinsic and extrinsic factors known to affect all biochemical, physiological, and developmental changes that take place in an organism have an impact on the overall growth, development, and reproduction of an organism (Sterner and Schulz, 1998; Taylor *et al*., 2005) [27, 29]. Environmental stress is defined as the lack of acceptable or sufficient food supplies, which deprives a population of normal nutrients (White, 1993) [32]. Many species' individuals must endure times of famine or exposure to unsatisfactory nutrition. In areas where food is likely to be less plentiful or momentarily less consistent, positive selection for resistance to famine stress is anticipated. As is frequently observed when insects are restricted to food low in protein relative to carbohydrate (Raubenheimer and Simpson, 1999) [16] compensatory feeding for the limiting nutrients results in the over ingestion of other nutrients. This may lead to increased lipid storage and decreased fitness (Simpson *et al*., 2004, Warbrick-Smith *et al*., 2006) [21, 31]. Several variables can affect an organism's ability to withstand stress.

It has been stated that stress related to few resources has an impact on the populations of most species. Selection probably influences resistance traits either directly or indirectly since stress resistance traits in *Drosophila* frequently differ across latitudinal clines (Sisodia and Singh, 2010) [24]. In response to selection for stressful heat (Morrison and Milkman, 1978; Loeschcke and Krebs, 1996; Hoffmann *et al*., 1997); and cold (Chen and Walker, 1993; Watson and Hoffmann, 1996) events, *Drosophila* alters its tolerance to high or low temperatures.

In response to climatic shifts, organisms may go into coma, undergo physiological stiffening, or produce metabolites that enable them to withstand temperature extremes (Sorensen *et al*., 2003; Sorensen *et al*., 2005; Lalouette *et al*., 2007) [11, 25, 26]. Additionally, an organism may adjust its growth cycle or change how much energy it devotes to growth to make up for nutritional stress and lower body size, delaying the reproductive phase (Reichling and German, 2000; Lobe *et al*., 2006) [12,17].

Spirulina (*Arthrospira platensis*) is a blue-green algae with a small, filamentous spiral shape. This cyanobacterium contains proteins, anti-inflammatory substances, antioxidants, including phycocyanin and phycocyanobilin, and proteins. Due to its numerous nutritional benefits and pharmacological effects, spirulina is taken by both humans and other animals (Kumar *et al*., 2017) [10]. Spirulina is regarded as a "superfood." Because of its purported health benefits, spirulina is becoming more popular as a food supplement; it can be consumed as a powder or in capsule form (Grosshagauer *et al*., 2020) [6]. Its plentiful supply of anti-inflammatory and antioxidant substances helps to support healthy brain function, the development of the neurological system, and it compensates for dietary deficiencies. Furthermore, it promotes a beneficial immunological response and decreases the adverse effects of an overactive immune system (Trotta *et al*., 2022) [30].

For a long time, the Aztec and Maya civilizations relied heavily on spirulina as food. Currently, it is thought that ingesting it is safe for humans due to its highly low toxicity. An excellent source of phenolics and fatty acids with

powerful anti-inflammatory capabilities is spirulina. Because of this, it might be utilized as a different supply of natural antioxidants in the manufacturing of pharmaceutical drugs (Bellahcen *et al*., 2020) [2]. Between 55 and 70 percent of the dry weight of spirulina is made up of protein. Spirulina is used as an animal supplement and in animal feed. Animals such as chickens, pigs, ruminants, and rabbits have participated in spirulina feeding tests (Holman and Malau-Aduli 2013) [8].

Nowadays, people consume spirulina algae in the form of spirulina chilli crunchy, chikki's, capsules, tablets, powders, smoothies, baked goods, salads, etc... It helps to control inflammation, blood sugar, cholesterol, blood pressures, muscle strength, supports anemia, may have anti-cancer properties, controls diabetes, reduces allergies, supports metabolism, improves digestive health, improves mental health, and also helps to promote the growth of healthy gut bacteria. Therefore present study has been under taken to understand the effects of Spirulina on Starvation resistance in *D. melanogaster*.

Materials and Method

The Urban Platter Store, an online distributor, supplied the spirulina powder.

Establishment of stock

The Oregon K strain of *D. melanogaster* used in our experiment was obtained from *Drosophila* stock centre, Department of studies in Zoology, University of Mysore, Manasagangothri, Mysuru. The flies were cultured by preparing wheat cream agar media (100g of jaggary, 100g of wheat powder, 10g of Agar was boiled in 1000ml of distilled water and 7.5 ml of propionic acid was added to avoid the fungal growth).

Establishment of experimental stocks

To get experimental media, the different concentration of spirulina powder i.e, 2.5g, 5g and 10gwas weighed and mixed thoroughly with 100ml of wheat cream agar media. The flies were cultured in 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.

Effect of Spirulina on Heat resistance

For heat resistance experiment, control and treated mated and unmated flies were used. Twenty flies (control and Spirulina treated flies) in a vial were kept in incubator for 37ºC.

The flies were observed for every 5 minutes of interval until the death of each fly. The heat resistance was observed in minutes, total replicates of five were run for each of control and spirulina treated flies. Separate experiment was run for both male and female.

Results

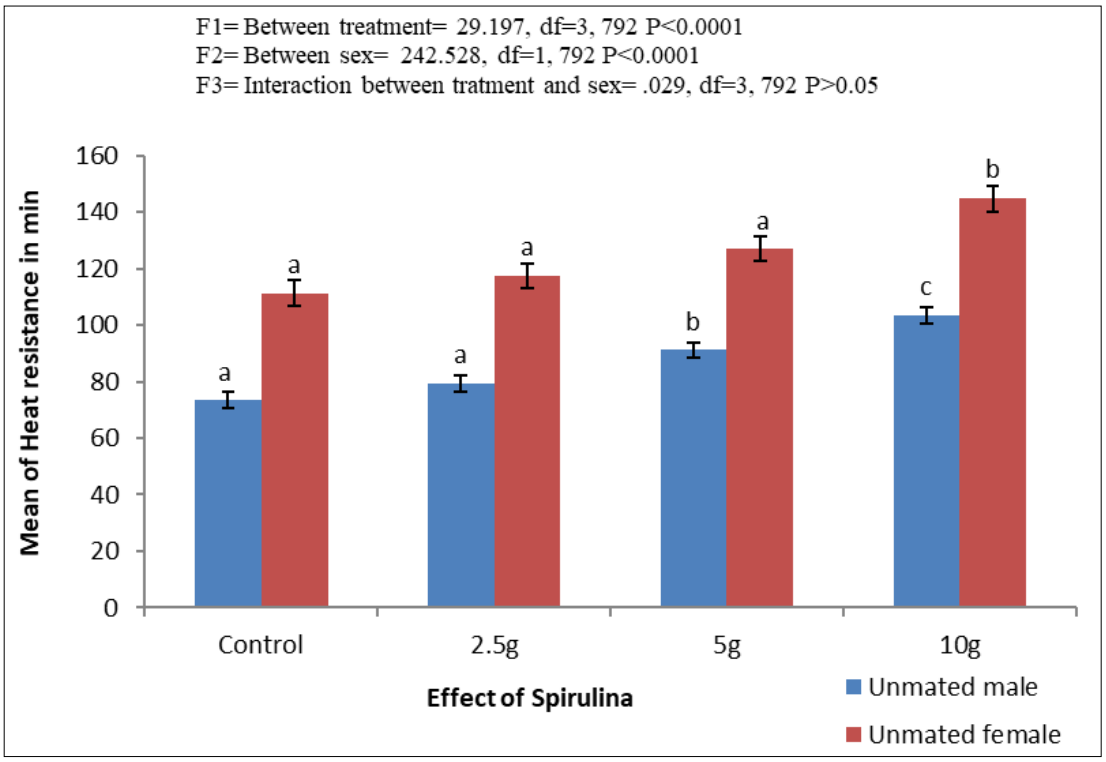

Fig 1: Effect of Spirulina on Heat resistance of unmated male and unmated female in *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration]

Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Fig 1 showed the effect of Spirulina on heat resistance unmated male and unmated female flies raised in control and spirulina treated media. According to the data obtained the heat resistance was found high in *Drosophila* flies raised in spirulina treated media compared to control flies. This result was found to be similar in both sexes studied. Further, heat resistance of female were significantly greater than those flies in both control and spirulina treated flies. As this above data obtained that the resistance increases as spirulina concentration increased found that flies raised in 10g spirulina treated flies were more reistance than compared to control and other two concentration i.e., 2.5g and 5g.

The above heat resistance data subjected to two way ANOVA fallowed by Tukey's Post hoc test showed significant variation in heat resistance between control and treated flies, between sexes. However non significant variation in heat resistance was noticed interaction between treatment and sex. Tukey's post hoc test showed significant difference in heat resistance between control and flies raised in different concentration of Spirulina.

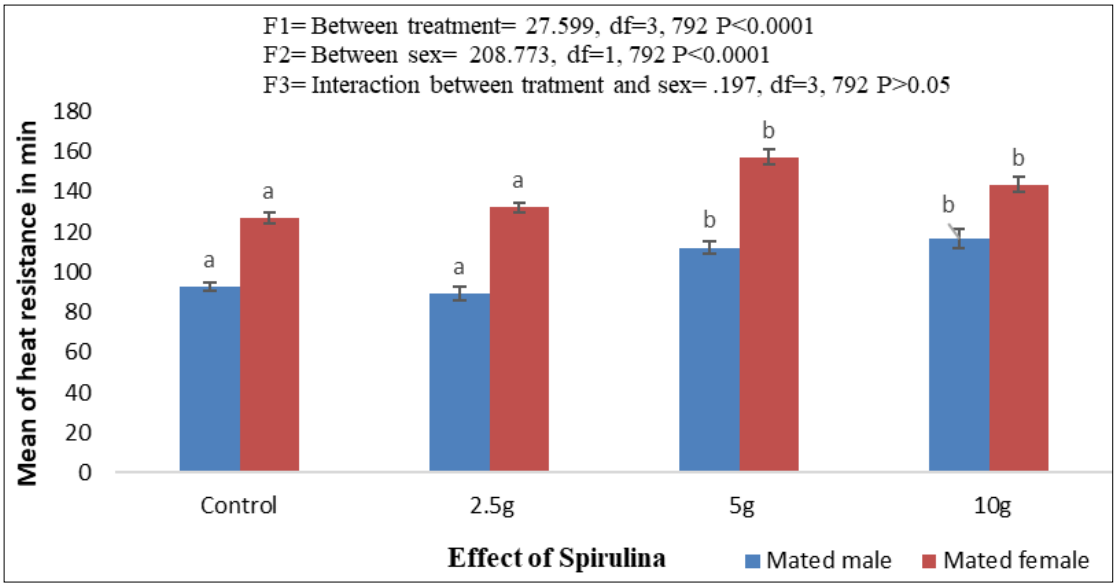

Fig 2: Effect of Spirulina on Heat resistance of mated male and mated female in *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration]

Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Heat resistance data of mated male and female flies raised in control and spirulina treated media was provided in figure 2. It was noticed that heat resistance was greater in 5g Spirulina treated, whereas flies treated with 2.5g spirulina treated flies showed lowest heat resistance. In both control and Spirulina treated media females were significantly greater resistance to heat compared to males. Two way ANOVA followed by Tukey's Post hoc test carried out on above data revealed significant variation in heat resistance between control and spirulina treated flies, between sexes and also interaction between treatment and sexes. In both male and female non significant variation was noticed in heat resistance between control and 2.5g spirulina treated flies. However significant variation in heat resistance was noticed between control flies compared to 5g and 10g spirulina treated flies by Tukey's Post hoc test.

Tukey's post hoc test showed the significance between control flies and flies raised in 5g and 10g of Spirulina treated media and there was no significant difference between control flies and flies raised in 2.5g of Spirulina. There was also significance difference between flies raised in 2.5g of Spirulina 5g and 10g of Spirulina treated media.

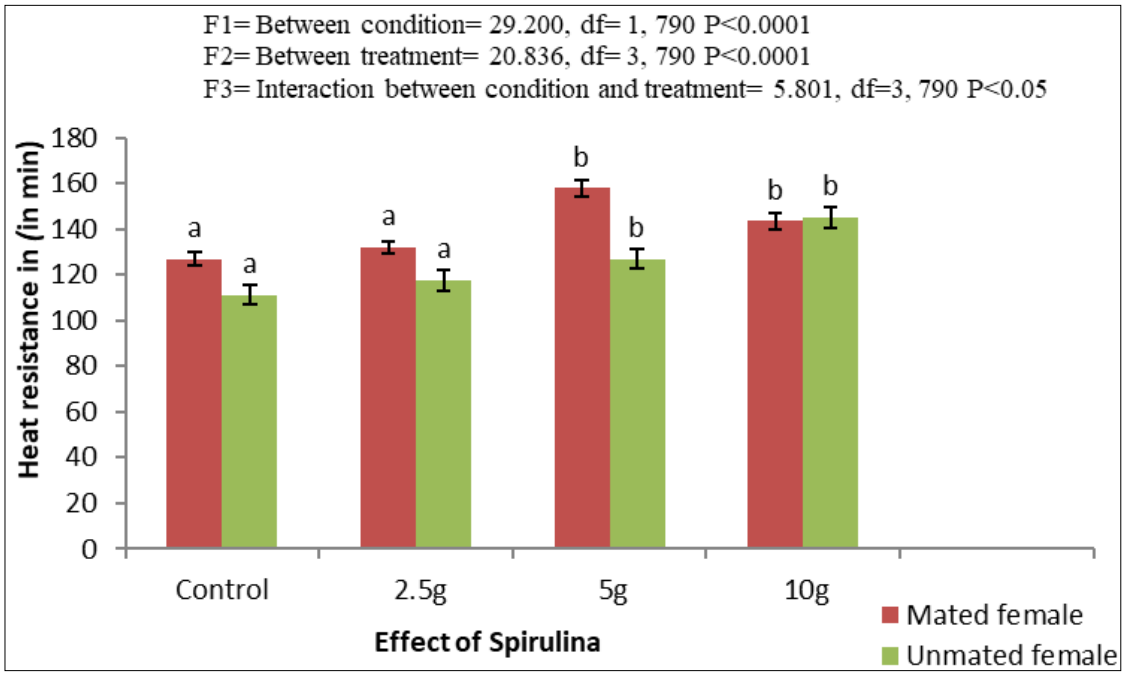

Fig 3: Effect of Spirulina on Heat resistance of mated female and unmated female in *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration]

Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

In Fig3 heat resistance data of mated and unmated female flies raised in control and spirulina treated media. It was noticed that heat resistance was greater in spirulina treated media and mated female in control media. And mated female flies treated in with 5g of spirulina had greater heat resistance than compare to other concentration and female flies raised in control media had lowest heat resistance compared to treated flies. In both control and spirulina treated media mated female flies were significantly greater resistance to heat compared to unmated female flies.

Two way ANOVA fallowed by Tukey's Post hoc test carried out on above data revealed significant variation in heat resistance between control and spirulina treated flies. There was non significant variation between control and flies raise in 2.5g spirulina treated media and there is a significant variation between control and 5g of spirulina and 10g of spirulina treated mated females.

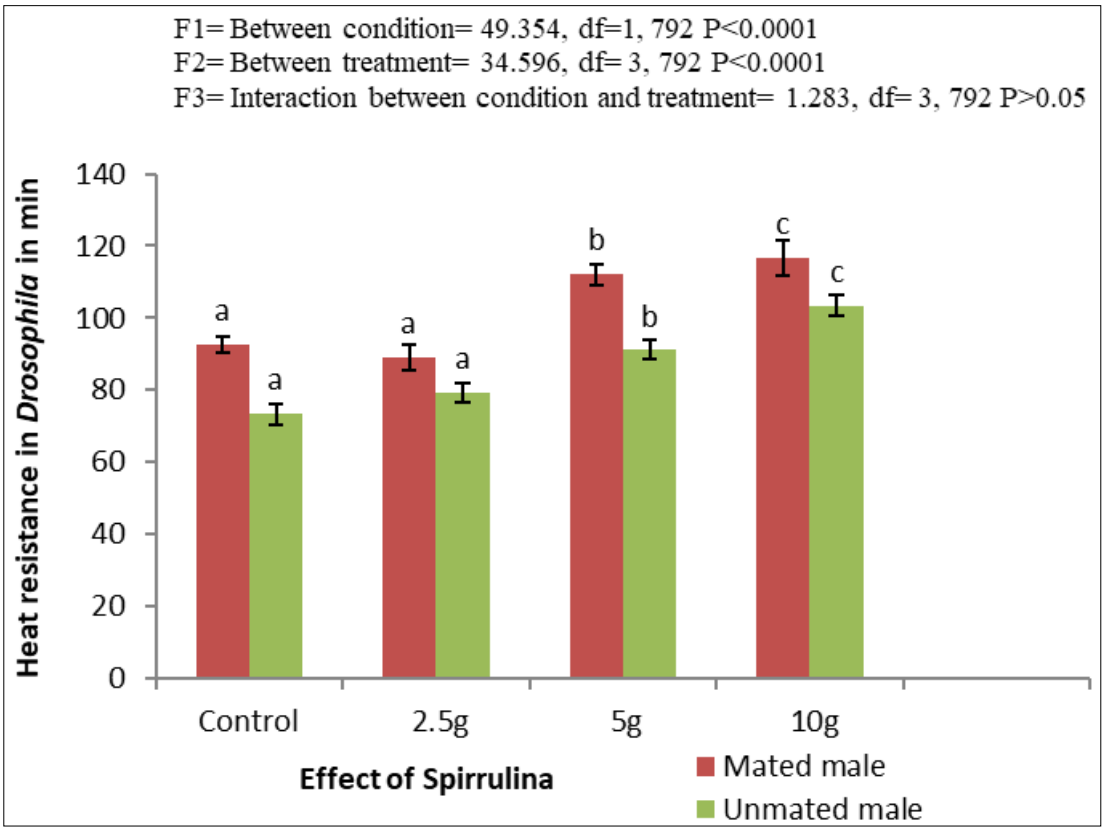

Fig 4: Effect of Spirulina on Heat resistance of mated male and unmated male in *D. melanogaster*. [Control diet- wheat cream agar media; spirulina diet (2.5g, 5g, 10g concentration]

Different letters on the bar graph indicates significance at 0.05 levels by Tukey's Post Hoc test.

Heat resistance data of mated flies were raised in control and Spirulina treated media was shown in fig 4. It was noticed that heat resistance was greater in mated males compared to unmated flies, whereas flies treated with control media had lowest heat resistance. In both control and Spirulina treated media mated male flies were significantly greater resistant to heat compared to unmated flies. Two way ANOVA followed by Tukey's Post hoc test carried out on above data revealed significant variation in heat resistance between control and spirulina treated flies. In both mated and unmated male flies non significant variation in mated male and unmated male was noticed in heat resistance between control and 2.5g spirulina treated flies. However the significant variation in starvation resistance was noticed between 5g and 10g spirulina treated flies as compared to control flies by Tukey's post hoc test.

Discussion

The availability of diet is known to influence on the various environmental stress such as thermal, starvation etc. In the present study, flies developed in spirulina was significantly greater Heat resistance compared to flies raised in control media Fig(1). This is because the spirulina contain significantly greater protein content compared to control media. These differences in the heat resistance of *D. melanogaster* were caused by differences in the ssquality and amount of dietary nutrients contained in the wheat cream agar media and spirulina treated media. A well-studied phenomena, especially in insects is the impact of physiological or morphological changes brought on by hardening or acclimation on temperature tolerance (Maness and Hutchison, 1980) [13].

Though the overall impact of them is uncertain a number of factors, including sex, physical condition, and diet, help the organism to resist the stress (Harshmann and Schmid, 1998). In the present study fig (1-2) revealed that females of both control and spirulina treated flies had significantly greater heat resistance than compared to the male flies. This may be due to females use the lipids and glycogen in their bodies as a source of energy. A heat resistant male, on the other hand, uses body lipids as a source of energy, which causes them to lose their source of stored energy and, as a result, exhibits less resistance to heat.

The result we obtained in fig (3) confirms Goenaga's research (Goenaga, 2011) [5], while studies on D. melanogaster has shown that mated females are more stress resistance than virgins are (Ballard, 2008; Hoffmann, 2005; Rion and Kawecki, 2007) [1, 7, 18]. This may be due to accessory gland proteins that males transfer to females during copulation, together with seminal gland proteins and release a complex protein mixture known as seminal fluid, which is delivered to the female along with sperm (Chen, 1984; Monsma and Wolfner, 1988; Wolfner, 2002) [3, 14, 34]. Accessory gland proteins (Acps) cause behavioral and physiological changes in mated females. They increase the rate of ovulation, increase egg production, help the female store sperm, and also shorten the lifespan of the mated female (Wolfner, 2002; Gillott, 2003) [4, 34]. Mated males were more resistant to heat than compared to unmated males this may be due to release of pheromones during copulation may responsible for resistance to withstand the stress.

Our study also supported by Sisodea and Singh(2012) who also found that the flies raised on protein-enriched medium are more heat-resistant than those raised on carbohydrate-enriched medium. Fly development on a diet high in protein can withstand heat shock more quickly than fly development on a diet high in carbohydrates. Unknown physiological factors may be responsible for flies raised on protein-enriched feed having higher heat knockdown tolerance. One option might be connected to the induction of heat shock proteins, which are well known for helping the body deal with various types of stress (Sorensen *et al*, 2003; Sinclair *et al*, 2007; Jones and Candido, 1999; Schmidt and Paaby, 2008; Tammariello *et al*,1999; Wilder *et al*; 2010) [9, 19, 22, 25, 28, 33]. According to Anderson *et al*. (Singh and Singh, 2008) [23] Hsp 70 is increased in flies raised on protein-enriched medium as opposed to those raised on protein-deficient media.

Even though a broad spectrum of intrinsic and extrinsic factors, such as diet, social interaction, environmental temperature, and age, can affect stress resistance in an organism, in our study we used flies fed at the same temperature and same age with different amounts and types of nutrients, resulting in changes in the *Drosophila melanogaster* heat resistance.

From this experimental study we can conclude that, the flies which developed in spirulina treated media had greater heat resistance and withstand environmental stress than compared to flies which fed control media.

Acknowledgement

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

Reference

1. Ballard JWO, Melvin RG, Simpson SJ. Starvation resistance is positively correlated with body lipid proportion in five wild caught *Drosophila simulans* populations. Journal of Insect Physiology,2008:54:1371-1376.
2. Bellahcen TO, A Amiri A, Touam I, Hmimid F, Amrani AE, Cherif A, *et al*. Evaluation of Moroccan microalgae: Spirulina platensis as a potential source of natural antioxidants. Journal of Complementary and Integrative Medicine,2020:17(3):20190036.
3. Chen PS. The functional morphology and biochemistry of insect male accessory glands and their secretions. Annu Rev Entomol,1984:29:233-55.
4. Gillott C. Male accessory gland secretions: Modulators of Female Reproductive Physiology and Behavior. Annu Rev Entomol,2003:48:163-184.
5. Goenaga J, Mensch J, Fanara JJ, Hasson E. The effect of mating on starvation resistance in natural populations of *Drosophila melanogaster*. Evol Ecol, 2011:DOI 10.1007/s10682-011-3540-4.
6. Grosshagauer S, Kraemer K, Somoza V. The true value of Spirulina. Journal of agricultural and food chemistry,2020:68(14):4109-4115.
7. Hoffmann AA, Hallas R, Anderson AR, Telonis-Scott M. Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. J Evol Biol,2005:18:804-810.
8. Holman BWB, Malau-Aduli AEO. Spirulina as a livestock supplement and animal feed. Journal of animal physiology and animal nutrition,2013:97(4):615-623.
9. Jones D. Candido EPM Feeding is inhibited by sublethal concentrations of toxicants and by heat stress in the nematode Caenorhabditis elegans: relationship to the cellular stress response. J Exp Zool,1999:284:147-157.
10. Kumar A, Christian PK, Panchal K, Guruprasad BR, Tiwari AK. Supplementation of spirulina (*Arthrospira platensis*) improves lifespan and locomotor activity in paraquat-sensitive DJ-1β Δ93 flies, a Parkinson's Disease model in *Drosophila melanogaster*. Journal of Dietary Supplements,2017:14(5):573-588.
11. Lalouette L, Kostal V, Colinet H, Gagneul D. Renault D. Cold exposure and associated metabolic changes in adult tropical beetles exposed to fluctuating thermal regimes. FEBS Journal,2007:274:759-1767.
12. Lobe SL, Bernstein MC, German RZ. Life-long protein malnutrition in the rat (*Rattus norvegicus*) results in altered patterns of craniofacial growth and smaller individuals. J Anat,2006, 208-812.
13. Maness JD, Hutchison VH. Acute adjustment of thermal tolerance in vertebrate ectotherms following exposure to critical thermal maxima. *J. Therm. Biol*,1980:5:225-233.
14. Monsma SA, Wolfner MF. Structure and expression of a *Drosophila* male accessory gland gene whose product resembles a peptide pheromone precursor. Genes Dev,1988:2:1063-73.
15. Pough FH. Organismal performance and Darwinian fitness: approaches and interpretations. Physiol Zool,1989:62:199-236.
16. Raubenheimer D, Simpson SJ. Integrating Nutrition: A geometrical approach. Entomol Exp Appl,1999:91:67-82.
17. Reichling TD, German RZ. Bones, muscles and visceral organs of protein malnourished rats (*Rattus norvegicus*) grow more slowly but for longer durations to reach normal final size. J Nutri,2000:130:2326-2332.
18. Rion S, Kawecki TJ. Evolutionary biology of Starvation resistance; What we have learned from *Drosophila*. J evol Biol,2007:20:1655-1664.

19. Schmidt PS, Paaby AB. Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. Evolution,2008:62:1204-1215.
20. Sibly RM. The life-history approach to physiological ecology. Func Ecol,1991:5:184-191.
21. Simpson SJ, Sibly RM, Lee K, Raubenheimer D. Optimal foraging with multiple nutrient requirements. Anim Behav,2004:68:1299-1311. 28.
22. Sinclair BJ, Gibbs AG, Roberts SP. Gene transcription during exposure to and recovery from, cold and desiccation stress in *Drosophila melanogaster*. Insect Mol Biol,2007:16:435-443.
23. Singh P, Singh BN. Population genetics of *Drosophila ananassae*. Genet Res,2008:90:409-410.
24. Sisodia S, Singh BN. Resistance to environmental stress in *Drosophila ananassae*: latitudinal variation and adaptation among populations. J Evol Biol,2010:23:1979-1988.
25. Sorensen JG, Kristensen TN, Loeschcke V. The evolutionary and ecological role of heat shock proteins. Ecol Lett,2003:6:1025-1037.
26. Sorensen JG, Nielsen MM, Kruhoffer M, Justesen J, Loeschcke V. Full genome gene expression analysis of the heat stress response in *Drosophila melanogaster*. Cell Stress and Chap,2005:10:312-328.
27. Sterner RW, Schulz KL. Zooplankton nutrition: recent progress and a reality check. Aquatic Ecol,1998:32:261-279.
28. Tammariello SP, Rinehart JP, Denlinger DL. Desiccation elicits heat shock protein transcription in the flesh fly, *Sarcophaga crassipalpis*, but does not enhance tolerance to high or low temperatures. J Insect Physiol,1999:45:933-938.
29. Taylor EN, Malawy MA, Browning DM, Lemar SV, DeNardo DF. Effects of food supplementation on the physiological ecology of female Western diamond-backed rattlesnakes (*Crotalus atrox*). Oecologia,2005:144:206-213. doi: 10.1007/s00442-005-0056-x.
30. Trotta T, Porro C, Cianciulli A, Panaro MA. Beneficial effects of spirulina consumption on brain health. Nutrients,2022:14(3):676.
31. Warbrick-Smith JST, Behmer K, Lee P, Raubenheimer D, Simpson SJ. Evolving resistance to obesity in an insect. Proc. Natl. Acad. Sci. U.S.A,2006:103:14045-14049.
32. White TCR. The inadequate environment: nitrogen and the abundance of animals. Springer Verlag, Berlin, 1993.
33. Wilder SM, Mayntz D, Toft S, Rypstra AL, Pilati A, *et al*. Intraspecific variation in prey quality: a comparison of nutrient presence in prey and nutrient extraction by predators. Oikos,2010:119:350-358.
34. Wolfner MF. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. Heredity,2002:88:85-93.