Impact Factor (SJIF): 6.092 E-ISSN: 2583-1615

International Journal of Research in Academic World

Received: 23/June/2024 IJRAW: 2024; 3(7):169-173 Accepted: 26/July/2024

Adaptogenic Effect of *Terminalia chebula* on *Drosophila melanogaster*

[1]Noorain Fathima, [2]Damini CS and [*3]V Shakunthala

[1, 2, *3]Department of Zoology, University of Mysore, Manasagangothri, Mysuru, Karnataka, India.

Abstract

Objective: This study aims to evaluate the adaptogenic effect of *Terminalia chebula* (fruit powder) on *Drosophila melanogaster* and understand the dose-dependent effect of this medicinal plant on the development of stress resistance in both sexes of *D. melanogaster*.

Methods: The stress tolerance of flies maintained on standard wheat cream agar media and flies maintained on standard wheat cream agar media supplemented with 250mg and 300mg of *Terminalia chebula* fruit powder was studied by subjecting same-aged male and female flies to heat and cold stress. For evaluating heat resistance, the number of deaths after heat shock was recorded in each test group. In the case of cold resistance, the recovery time taken by different groups after cold stress was noted.

Key Findings: *T. chebula*-fed flies showed greater survivability in heat and cold stress compared to the control flies fed with standard wheat cream agar media. In males, *T. chebula*-treated groups showed a significant increase in heat and cold resistance compared to the control group. In females, *T. chebula* showed a significant increase in heat resistance. However, the tolerance capacity decreased with an increase in the dosage of *T. chebula* in female flies subjected to cold stress.

Conclusion: Overall results indicate that *T. chebula* has the potential to be used as an adaptogen to develop resistance against various stresses.

Keywords: *Drosophila melanogaster, Terminalia chebula, Heat* shock *resistance, Cold* shock resistance

Introduction

Medicinal plants have served as natural healers for many problems since ages. Once discovered, their constituents have been used in the development of drugs for various diseases (Sarasa *et al*., 2012; Agarwal *et al*., 2011; Gupta, 2012) [38, 1, 11]. Countries like India and China are the largest users of these valuable medicinal plants (Naik *et al.,* 2004) [28]. The demand for and use of these plants is rising rapidly due to their natural origin, minimal or no side effects, higher safety margins, and affordability (Ayyanar and Ignacimuthu, 2011) [3]. Additionally, there have been many instances where synthetic chemicals used for treatment have not been as effective as these natural medicinal plants (Gupta *et al*.,2012) [11]. Therefore, there is always significant importance and scope for these remarkable plants.

Terminalia chebula is a deciduous tree belonging to the family Combretaceae. It is highly cultivated in Taiwan and is native to Southeast Asian countries (Pulliah, 1934) [30]. Its fruit, in dried and ripe form, is used for various medicinal purposes both externally and internally. In India, *Terminalia chebula* is extensively used in Ayurveda, Siddha, Unani, and homeopathic medicines. It is a highly preferred plant for the treatment of asthma, bleeding, piles, sore throat, vomiting, and gout (Aneja and Joshi, 2009) [2]. The fruits of *Terminalia chebula* are used both internally and externally. Externally, the paste of the fruit is used to promote healing, reduce swelling, clean wounds, and prevent the accumulation of pus in skin diseases. Due to its anti-inflammatory properties, it is used to treat conjunctivitis. It can also be used as a tooth powder to cure bleeding gums and strengthen them (Usha *et al*., 2007) [48].

Internally, it is used with rock salt to treat 'Kapha' diseases, with salt to treat 'Pitta' diseases, and with ghee to treat 'Vata' diseases. When consumed with ghee, it promotes longevity and boosts energy. It is also effective against an enlarged liver and spleen, gastrointestinal ailments, tumors, worms, digestive disorders, diabetes (when mixed with Triphala and Haridra), hepatitis (when Triphala decoction is mixed with honey), obesity, anemia, urinary stones, and more (Kirtikar and Basu, 1935) [16]. *Terminalia chebula* possesses many biological and pharmacological properties such as: Antibacterial, antioxidant, antifungal, anticancer, wound healing etc. Other activities such as hypolipidemic/hypocholesterolemic activity, antiplasmodial activity, anti-ulcerogenic activity, antidiabetic and retinoprotective activity, have also been recorded (Gupta *et al.,*2012) [11]

Despite these remarkable properties, individuals with mental depression, severe weakness, pitta conditions, and pregnant women should take special care and precautions before using this plant (Gupta *et al*., 2012) [11].

Stress is a state of mental or emotional strain in an individual due to external environmental factors or internal metabolic alterations. Consuming herbal resources with stress-resistant

***Corresponding Author:** V Shakunthala

properties can enhance an organism's stress resistance. Plants rich in antioxidants and other bioactive compounds can increase survivability and stress tolerance in many organisms (Renuka Prasad *et al*., 2018) [33]. The quality of food given to an organism can influence its body condition and fecundity (Sisodia and Singh.2012) [42]. The intake of different amounts and qualities of nutrients directly affects life history traits such as disease vulnerability, fertility, reproduction, longevity, and stress resistance (Hoffmann *et al*.,1991; Rions *et al*.,2007; Lee *et al*.,2008) [13, 36, 20]. A plant derivative that increases resistance to stress, trauma, anxiety, and fatigue is called an adaptogen. Herbalists refer to them as rejuvenating herbs, tonics, rasayanas, or restoratives. Adaptogens help the body return to a balanced state by maintaining homeostasis, balancing the endocrine hormones and immune system (Winston D *et al*., 2007; Debnath *et al*., 2011) [50, 9].

Terminalia chebula is a constituent of the popular traditional herbal formulation called Triphala, used to treat chronic disorders (Sukhdev *et al*.,1999;Chattopadhyay *et al*.,2007) [46, 4]. It is also one of the ingredients in the polyherbal formulation "Geriforte," an Ayurvedic rasayana that promotes physical and mental health and improves the immune power of an organism, helping it resist any type of stress (Kokate *et al*.,2001; Singh *et al*.,1978) [17, 41]. However, many studies have not been conducted to investigate the adaptogenic effect of *Terminalia chebula* against heat and cold stress. Hence, the present study evaluates the adaptogenic effect of *Terminalia chebula in vivo* using *Drosophila melanogaster*.

Materials and Methods

***Drosophila* Culture:** *Drosophila melanogaster* wild fly stocks of Oregon-K flies (OK flies) were obtained from the *Drosophila* Stock Centre, Department of Zoology, University of Mysore, Mysore, Karnataka, India. These flies were maintained on standard wheat cream agar media seeded with dry yeast granules at 22±1ºC to establish the experimental stock. After obtaining enough flies for the experiment, they were divided into three groups and transferred into stock bottles containing different media.

The first group of flies was the control group, maintained on standard wheat cream agar media. The second group, test group 1, was maintained on wheat cream agar media supplemented with 250mg of *Terminalia chebula* (fruit powder) per liter of the media. The third group, test group 2, was maintained on wheat cream agar media supplemented with 300mg of *Terminalia chebula* (fruit powder) per liter of the media.

Heat Stress: To test for heat resistance, adult male and female flies of the same age were used. A total of 5 empty vials containing 5 flies each were used for all the test groups and both sexes (5 replicates each). The flies were then exposed to a temperature of 40ºC for about 75 minutes using a water bath (care was taken to ensure that the cotton plugs did not get wet and that water did not enter the vials). After heat shock, the vials were left undisturbed for about 2-3 hours, and then the number of dead flies was counted and recorded. The percentage of deaths in different test groups was calculated and compared.

Cold Stress: To test for cold resistance, adult male and female flies of the same age were used. A total of 5 empty vials containing 5 flies each were used for all the test groups and both sexes (5 replicates each). The flies were then exposed to a temperature of 0±5ºC for about 10 minutes by placing the vials in a freezer. After removing the vials, the recovery time of different flies was scored at intervals of 50 seconds. The number of flies that recovered in the first 100 seconds for males and females of different test groups was then calculated and compared.

Statistical Analysis

The statistical analysis for stress tolerance between different treatment groups and control was performed by using IBM SPSS version 29. The mean, standard deviation and level of significance was measured by one way ANOVA and two way ANOVA followed by Tukey's post hoc test with $p<0.05$ being statistically significant.

Results and Discussion

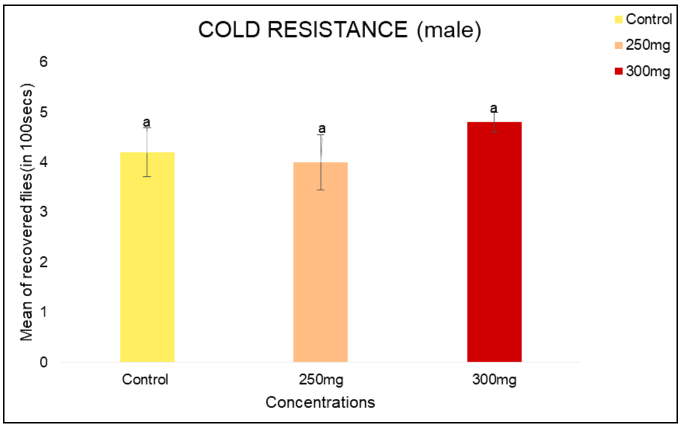

Fig 1: Effect of cold stress on recovery time in different test groups of *Drosophila melanogaster* (male)

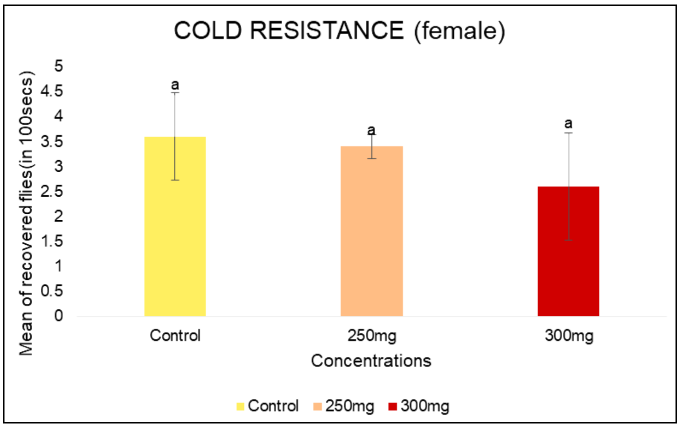

Fig 2: Effect of cold stress on recovery time in different test groups of *Drosophila melanogaster* (female)

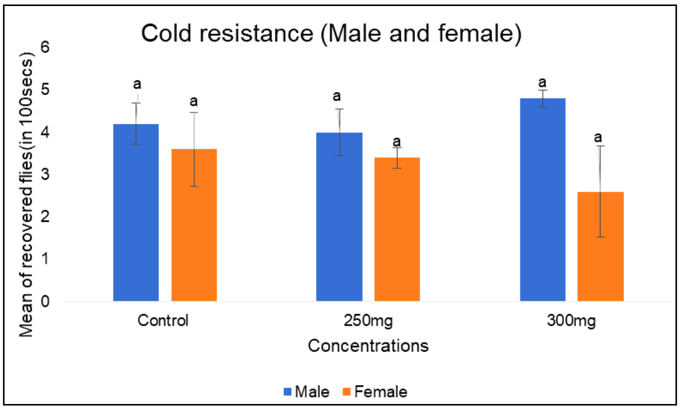

Fig 3: Comparison of the effect of cold stress on recovery time in different test groups of male and female *Drosophila melanogaster*.

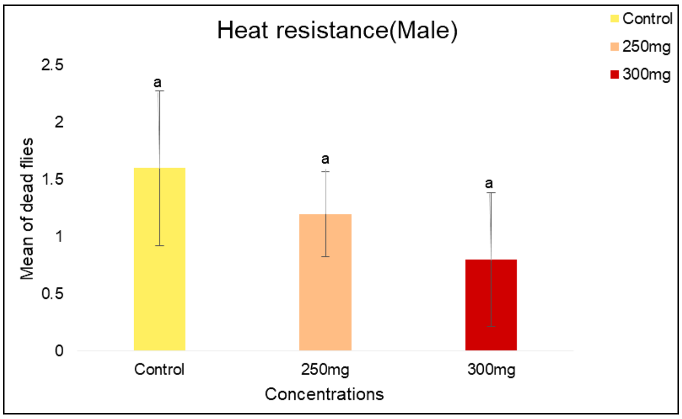

Fig 4: Effect of heat stress on the survival in different test groups of *Drosophila melanogaster* (Male).

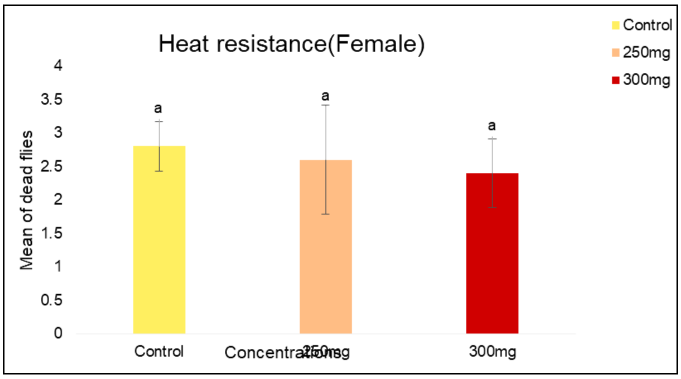

Fig 5: Effect of heat stress on the survival in different test groups of *Drosophila melanogaster* (Female).

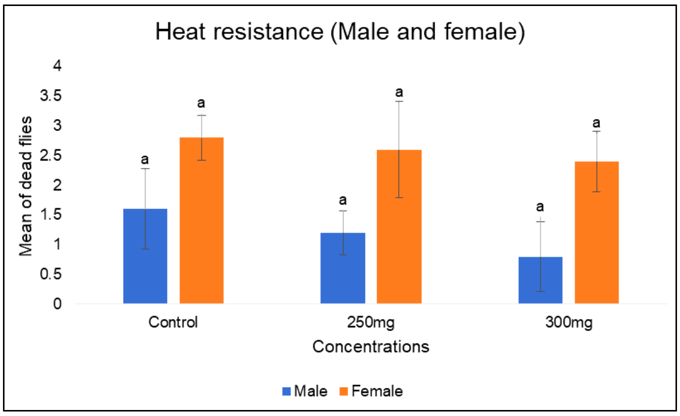

Fig 6: Comparison of the effect of heat stress on survival in different test groups of male and female *Drosophila melanogaster*.

Results

Figure 1-3 depicts the effect of cold stress on recovery time in different test groups of *Drosophila melanogaster* male and female. The recovery time from cold stress varied significantly across the different test groups of *Drosophila melanogaster*. In males, the group treated with 300mg of *Terminalia chebula* exhibited the fastest recovery time compared to the control group and the group treated with 250mg. Mean differences among the sex group and concentration group is statistically non-significant for all the groups.

However in females, the control group demonstrated improved recovery times compared to the 250mg group, and the 300mg group had an increased recovery time. This suggests that while a moderate concentration of *T. chebula* enhances cold stress tolerance, higher concentrations might have adverse effects.

Overall, males showed a better recovery from cold stress compared to females. However, at 300mg, decrease in recovery efficiency, was observed in females showing a more pronounced decline at higher concentrations compared to males.

Heat Stress Survival

The results of heat stress performed on *D. melanogaster* represented in the *figs 5 and 6* predict the effect of heat stress on survival in different test groups of *Drosophila melanogaster* male and females and Fig 6 depicts the comparison of the effect of heat stress on survival in different test groups of male and female *Drosophila melanogaster.* Survival under heat stress conditions varied among the different test groups. Male *Drosophila melanogaster* in the 300mg *T. chebula* group showed a significantly higher survival rate compared to the control and 250mg groups. This indicates that a higher concentration of *T. chebula* enhances heat stress tolerance in males.

In females, the 300mg group also exhibited increased survival rates compared to the control group. However, unlike to the cold stress results, the 250mg group showed a reduced survival rate, suggesting that there is varied effect of *T chebula* on survival in heat and cold stress in females. When comparing the survival rates between sexes, males generally had a higher survival rate under heat stress in all the groups compared to females.

In dipterans such as *Drosophila*, sensitivity to thermal shock is influenced by factors such as rearing temperature, short-term hardening, and long-term acclimation. The primary candidates responsible for thermal tolerance and adaptation in organisms are heat shock proteins (HSPs). Many HSPs play roles in response to both low temperature and diapause. The response to heat shock occurs in two stages: first, suppression, followed by the restoration of normal environmental conditions. However, the response to cold stress is more complex and involves many genes and proteins during the recovery phase (Colinet *et al*., 2010) [5].

Discussion

Insect physiology, development, metabolism, and reproduction are influenced by temperature changes (Colinet *et al*., 2015; Harrison *et al*., 2012; Sinclair *et al*., 2016) [7, 39]. The effects of thermal stress have been extensively quantified in *Drosophila* (David *et al*., 2005; Rivera-Rincon 2024) [8, 34], revealing adaptive responses to heat (Hoffmann *et al*., 1997; Loeschcke and Krebs, 1996; Morrison and Milkman, 1978) [12, 22, 25] and cold stress (Chen and Walker, 1993; Watson and Hoffmann, 1996) [49], exhibiting genetic variability for heat and cold tolerance.

Comparing thermal stress indicators can help better understand the relationship between thermal stress responses and life history traits among species. These temperature changes increase the likelihood of species with short lifespans experiencing higher temperatures during one or more of their developmental stages (Zhang *et al*., 2015) [51]. Rivera-Rincon, 2024 [34] further states that, *D. melanogaster* females presented a CTmax of 40.88°C under heat treatment, the highest among species, treatments, and sexes, while males under the same treatment exhibited a CTmax of 37.76°C hence the sex difference is very clearly observed in *D. melanogaster* species, Similarly, our results also exhibit the differences in thermal tolerance compared to *T. chebula* treatment groups females were significantly different in the thermal tolerance for cold and Heat resistance when compared to the control. Several studies have also revealed that females under the thermal treatment had the highest CTmax (37.04°C) within treatments and sexes for the species under study. Several previous studies also evaluated the effect of thermal stress on fecundity and thermal stress effectively reduced the fecundity of the species understudy during either development or adulthood (Krebs and Loeschcke, 1994; Melicher *et al*., 2021; Sisodia and Singh, 2006; Stazione *et al*., 2021; Rivera-Rincon *et al*., 2024) [18, 23, 43, 34] they showed an overall decrease in fecundity for *D. melanogaster and D. pseudoobscura* species. Interestingly, *D. melanogaster* exhibited the lowest fecundity rates across all stages and temperature treatments. The differences in response between sexes could be explained by sex-specific patterns previously described in *Drosophila* for required genes in stress response (Moskalev *et al*., 2011; Tower *et al*., 2020) [26, 47]. Furthermore, in Drosophila, sex determination pathways seem to regulate sex-specific patterns in stress adaptation, where females have been described to preferentially require more genes for stress response than males (Moskalev *et al*., 2011; Moskalev *et al*., 2012) [26, 27]. Studies of *D. melanogaster* treated with *chebula* have reported similar findings to our results, with thermal maxima around 40°C reported for multiple stocks with sex specific differences (Jørgensen *et al*., 2020; Lecheta *et al*., 2020; Rolandi *et al*., 2018) [15, 19, 37]. The Present study preliminary attempt to understand the association of plant extracts with thermal tolerance. These findings indicate that while *Terminalia chebula* can enhance stress tolerance, its concentration needs to be carefully optimized to avoid potential negative effects, particularly in female organisms. Further studies are needed to explore the mechanisms underlying these sex-specific responses and to determine the optimal dosage for various applications.

Important Findings

Optimal Concentration: The 250mg concentration of *Terminalia chebula* significantly improved both cold and heat stress tolerance in both the sexes of *Drosophila melanogaster*, while the 300mg concentration had detrimental effects, in females.

Sex Differences: Males generally exhibited better stress tolerance compared to females at the 300mg concentration of T. *chebula*. However, at higher concentrations, both sexes showed different rates of tolerance, with females being more adversely affected.

Adaptogenic Potential: The results suggest that *Terminalia chebula* has potential as an adaptogen, enhancing stress resistance in *Drosophila melanogaster*, but its efficacy is dose-dependent and varies between sexes.

References

1. Agarwal M, Sharma P, Kushwaha S. Antifertility efficacy of 50% ethanolic extract of *Calendula officinalis* in male rats. *Int J Pharmacy Pharmaceutical Sci* 2011; 3(5):192-196.
2. Aneja KR, Joshi R. Evaluation of antimicrobial properties of fruit extracts of *Terminalia chebula* against dental caries pathogens. *Jundishapur J Microbiol* 2009; 2(3):105-11.
3. Ayyanara M, Ignacimuthu S. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats in India. *J Ethnopharmacol*. 2011; 134:851-64.
4. Chattopadhyay RR, Bhattacharyya SK. Plant Review *Terminalia chebula*: An update. Phcog Rev. 2007; 1(1):151-6.
5. Colinet H and Hoffmann A. Gene and protein expression of *Drosophila* Starvin during cold stress and recovery from chill coma. Insect Biochem. Mol. Biol. 2010; 40:425-428.
6. Colinet H, Lee SF and Hoffmann A. Temporal expression of heat shock genes during cold stress and recovery from chill coma in adult *Drosophila melanogaster*. *FEBS J*. 2010a; 277:174-185.
7. Colinet H, Sinclair BJ, Vernon P and Renault D. Insects in Fluctuating Thermal Environments. Annu. Rev. Entomol. 2015; 60:123-40.
8. David *et al*., David JR, Araripe LO, Chakir M, Legout H, Lemos B, Petavy G, Moreteau B. Male sterility at extreme temperatures: A significant but neglected phenomenon for understanding *Drosophila* climatic adaptations. *J. Evol. Biol*. 2005; 18(4):838-846.
9. Debnath J *et al*. An experimental evaluation of anti-stress effects of *Terminalia chebula*. *Journal of Physiological and Biomedical Sciences*. 2011; 24(2):13-19.
10. Feder ME and Hofmann GE. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 1999; 61:243-282.
11. Gupta PC. *Withania coagulans* Dunal-An Overview. *Int J Pharmaceutical Sci Review Research*. 2012; 12(2):68-71.
12. Hoffmann *et al*., AA, Hoffmann H, Dagher MJ, Hercus D, Berrigan. Comparing different measures of heat resistance in selected lines of Drosophila melanogaster. *J. Insect Physiol*. 1997; 43(4):393-405.
13. Hoffmann AA, Parsons PA. Evolutionary genetics and environmental stress. Oxford University Press, Oxford, 1991.
14. Hoffmann AA, Sørensen JG and Loeschcke V. Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Therm. Biol*. 2003; 28:175-216.
15. Jørgensen *et al*., LB, Jørgensen RM, Robertson J, Overgaard. Neural dysfunction correlates with heat coma and ctmax in *Drosophila* but does not set the boundaries for heat stress survival. *J. Exp. Biol*., 2020, 223(13) p. jeb218750.
16. Kirtikar KR, Basu BD. *Terminalia chebula* In: Indian Medicinal Plants, 2nd eds, Allahabad, India: Lolit Mohan Basu Pub 1935, 1020-23.
17. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 12th ed. Pune, India: Nirali Prakashan, 2001, 216-7.
18. Krebs and Loeschcke. Effects of exposure to short-term heat stress on fitness components in *Drosophila melanogaster*. *J. Evol. Biol*. 1994; 7(1):39-49.
19. Lecheta *et al*. Integrating gwas and transcriptomics to identify the molecular underpinnings of thermal stress responses in Drosophila melanogaster. Front Genet. 2020; 11:658.
20. Lee KP, Simpson SJ, Wilson K. Dietary protein-quality influences melanization and immune function in an insect. Func Ecol. 2008; 22:1052-1061.
21. Li AQ, Popova-Butler A, Dean DH and Denlinger DL. Proteomics of the flesh fly brain reveals an abundance of upregulated heat shock proteins during pupal diapause. *J. Insect Physiol*. 2007; 53:385-391.
22. Loeschcke and Krebs. Selection for heat-shock resistance in larval and in adult *Drosophila buzzatii*: comparing

direct and indirect responses. Evolution. 1996; 50(6):2354-2359.
23. Melicher *et al*. Fluctuating thermal regimes extend longevity and maintain fecundity to increase shelf-life of *Drosophila melanogaster* cultures. Physiol. Entomol. 2021; 46(3-4):179-18.
24. Michaud MR and Denlinger DL. Genomics, proteomics and metabolomics: finding the other players in insect cold-tolerance. In Low temperature biology of insects (ed. D. L. Denlinger and R. E. Lee, Jr). Cambridge: Cambridge University Press, 2010, 91-116.
25. Morrison and Milkman. Modification of heat resistance in *Drosophila* by selection. Nature. 1978; 273(5657):9-50.
26. Moskalev *et al*. Radiation hormesis and radioadaptive response in *Drosophila melanogaster* flies with different genetic backgrounds: the role of cellular stress-resistance mechanisms. Biogerontology. 2011; 12:253-263.
27. Moskalev *et al*. The role of d-gadd45 in oxidative, thermal and genotoxic stress resistance. Cell Cycle. 2012; 11(22):4222-4241.
28. Naik GH, Priyadarsini KI, Naik DB, Gangabhagirathi R, Mohan H. Studies on the aqueous extract of *Terminalia chebula* as a potent antioxidant and a probable radioprotector. Phytomedicine. 2004; 11:530-38.
29. Parsell DA and Lindquist S. The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. Annu. Rev. Genet. 1993; 27:437-496.
30. Pulliah T. Encyclopedia of world medicinal plants. New Delhi, India: Regency Pub Vol 4, pp1931-1934.
31. Qin W, Neal S, Robertson R, Westwood J and Walker V. Cold hardening and transcriptional change in *Drosophila melanogaster*. Insect Mol. Biol. 2005; 14:607-613.
32. Rako L, Hoffmann AA. Complexity of the cold acclimation response in *Drosophila melanogaster*. *Journal of Insect Physiology*. 2006; 52:94-104.
33. Renuka Prasad BP, Ashadevi JS. Withania somnifera promotes stress resistant activity in *Drosophila melanogaster*. *Journal of drug delivery and therapeutics*. 2018; 8(6-8):83-88.
34. Rivera-Rincón N, Stevison LS. Data and code from: A comparison of thermal stress response between *Drosophila melanogaster* and *Drosophila pseudoobscura* reveals differences between species and sexes, 2024.
35. Rinehart JP, Li A, Yocum GD, Robich RM, Hayward SA and Denlinger DL. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proc. Natl. Acad. Sci. USA. 2007; 104:11130-11137.
36. Rion S, Kawecki TJ. Evolutionary biology of starvation resistance: what we have learned from *Drosophila*. *J Evol Biol*. 2007; 20:1655-1664.
37. Rolandi C, Lighton JRB, De la Vega GJ, Schilman PE, Mensch J. Genetic variation for tolerance to high temperatures in a population of *Drosophila melanogaster*. Ecol Evol. 2018; 8(21):10374-10383. doi: 10.1002/ece3.4409. PMID: 30464811; PMCID: PMC6238130.
38. Sarasa D, Sridhar S, Prabakaran E. Effect of an antidiabetic extract of *Trigonella foenum-graecum* on normal and alloxan induced diabetic mice. *Int J Pharmacy Pharmaceutical Sci*. 2012; 4(1):63-65.
39. Sinclair BJ, Marshall KE, Sewell MA, Levesque DL, Willett CS, Slotsbo S, Helmuth BS. Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures?. Ecol. Lett. 2016; 19(11):1372-1385.
40. Sinclair BJ, Roberts SP. Acclimation, shock and hardening in the cold. *Journal of Thermal Biology*. 2005; 30:557-562.
41. Singh N, Nath R, Mishra N, Kohli RP. An experimental evaluation of anti-stress effects of Geriforte. *Int J Crude Drug Res*. 1978; 3:125-32.
42. Sisodia S, Singh BN. Experimental evidence for nutrition regulated stress resistance in *Drosophila ananassae*. PLOS ONE. 2012; 7(10):e46131.
43. Sisodia and Singh. Effect of exposure to short-term heat stress on survival and fecundity in *Drosophila ananassae*. *Can. J. Zool*. 2006; 84(6):895-899.
44. Sørensen JG, Kristensen TN and Loeschcke V. The evolutionary and ecological role of heat shock proteins. Ecol. Lett. 2003; 6:1025-1037.
45. Stazione L, Norry FM, Sambucetti P. Do longevity and fecundity change by selection on mating success at elevated temperature? Correlated selection responses in *Drosophila buzzatii*. Evol. Biol. 2021; 48 (3):312-320.
46. Sukhdev SH, Deepak M, Joseph GVR, Joseph S, Nagar G. Indian herbal pharmacopoeia. IDM, Mumbai and RRL (CSIR) Jammu Tawi. 1999; II:154-9.
47. Tower J, Pomatto LC, Davies KJ. Sex differences in the response to oxidative and proteolytic stress. Redox Biol. 2020; 31:101488.
48. Usha C, Satyanarayanan R and Velmurugan A. Use of an aqueous extract of *Terminalia chebula* as an anticaries agent: A clinical study. *Indian J Dent Res* 2007; 18(4):152-56.
49. Watson and Hoffmann. Acclimation, cross-generation effects, and the response to selection for increased cold resistance in *Drosophila*. Evolution. 1996; 50(3):1182-1192.
50. Winston D, Maimes S. Adaptogens: Herbs for strength, stamina, and stress relief. Rochester, VT: Healing Arts Press, 2007.
51. Zhang W *et al.* Stage-specific heat effects: Timing and duration of heat waves alter demographic rates of a global insect pest. Oecologia. 2015; 179:947-957.