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Impact of Carbohydrate and Amino Acid Enriched Diet on The Heat Tolerance of *Drosophila Ananassae* and *Drosophila Bipectinata*

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Abstract

Elevated temperatures promote the accumulation of damages, which can be countered by improving diet. So, it is critical to comprehend how environmental temperature influences the survival of an organism. Providing a diet with additional nutrient regimens accelerates the physiological and metabolic processes in organisms, which results in better survival. Present study aimed to understand the impact of carbohydrate and amino acid-enriched diet on the heat tolerance of Drosophila ananassae and D. bipectinata. Adult flies were transferred to the media composition enriched with carbohydrate and amino acid in three replicates and maintained at 22ºC. 20 flies of a particular stage in three replicates were exposed to 24ºC, 28ºC, and 32ºC for 5 days. The impact of thermal stress on their survival was noted for 5 following days. The results of this study show different stages of Drosophila ananassae and D. bipectinata can handle heat stress in a better manner when fed with a carbohydrate and amino acid-rich diet. D. bipectinata survival rates were higher than D. ananassae when compared at each temperature at different diet regimens. This study provided convincing evidence of the positive influence of the tryptophan (100mg) diet compared to the sucrose diet on the survival, quality of life, and stress tolerance of both species.

Keywords: *Sucrose, tryptophan, survival frequency, deformation, heat shock*

1. Introduction

Life history traits, resilience to stress, and reproduction are all strongly influenced by the quantity and quality of nutrients that organisms ingest (Slotsbo et al., 2016). Animal survival and reproductive success depend on maintaining a balance between energy intake and expenditure (Taylor et al., 2005). Phenotypic plasticity, the capacity of organisms to modify their physiology, behavior, or development in response to external stimuli, is a characteristic that makes life unique. The way that stress tolerance and reproduction adapt to variations in embryonic nutrition is among the most well-known and significant instances of phenotypic plasticity (Roff and Gelinas, 2003). Numerous distinct life-history features and an adult's ability to respond to environmental stressors can be influenced by the feeding of organisms at each stage of life (Sisodia and Singh, 2010 [i]).

It can be difficult for many creatures to obtain the optional nourishment they need for survival and reproductive development in natural conditions. Body tissues always need a certain amount and ratio of nutrients during development in order to achieve optimal growth and function (Bauerfeind and Fischer, 2005). Survival, development, and reproduction are three traits that can be impacted by an excess or deficiency of fat, carbohydrates, or amino acid (Sisodia and Singh, 2012). Protein synthesis and physiological functions depend on amino acids. Tryptophan (Trp) is one of these important amino acids, having a variety of uses (Hoseini et al. 2020). According to Hoseini et al. (2019), it has a significant role in melatonergic and serotonergic activities, which control behavior and stress reactions. In *Drosophila melanogaster,* an amino acid deficit decreases fertility and development, and in fruit-feeders, amino acid is frequently the limiting macronutrient. The intricacy of an organism's food intake and usage is demonstrated by the fact that, in contrast, diet restriction on mild hunger may promote longevity as well as resistance to stressors like heat stress (Bubliy and Loeschcke, 2005). Organismal stress tolerance may be influenced by a multitude of circumstances. These encompass alterations in both behavior and physiology (Wenzel, 2006).

Present study focused on two species of the melanogaster species group, Within the ananassae subgroup. *Drosophila ananassae* is a domestic and global species with a stenothermic and circumtropical range, whereas *D. bipectinata* specifically has the bipectinata complex. Wild in nature, *D. ananassae* and *D. bipectinata* are mostly found on the Indian subcontinent. India spans a wide range of latitudes and elevations and is a sizable tropical and subtropical continent. The seasonal thermal amplitude exhibits a consistent rise from south to north, with progressively more pronounced warm and cold seasons. Latitude significantly increases seasonal fluctuations (Sisodia and Singh, 2010 [ii]). Although, recent years have seen a surge in interest in this species among regional experts, leading to in-depth investigations on its genetic makeup and population dynamics, but impact of diet and temperature has not studied widely. The present study aimed to assess the effects of 72-hour high-temperature exposure in Drosophila species under varying nutrient regimens. Consequently, we have looked at the relative significance of two crucial macronutrients, sucrose and tryptophan under thermal stress conditions on life-history traits such as oviposition, egg viability, hatching, pupation, eclosion and morphometric traits of adults.

2. Materials And Methods

Flies' samples:

Thermal tolerance experiments were performed on *Drosophila ananassae* and *D. bipectinata* collected from the Drosophila stock Center Manasa Gangotri University of Mysore, Mysore, Karnataka. The stock flies were maintained in a 22°C incubator on a 12:12 h light: Dark cycle at 60% humidity. A culture medium containing agar-agar, rava, jaggery, propionic acid, and distilled water was used to maintain flies of both species.

Bioassay conditions:

Drosophila ananassae adult flies were transferred to the media composition enriched with (1) carbohydrate (Sucrose 30 gm and 70 gm) and (2) amino acid (tryptophan 100 gm and 400 mg) in three replicates. Similarly, *D. bipectinata* were transferred to the media composition enriched with (1) carbohydrate (Sucrose 30 gm and 70 gm) and (2) amino acid (tryptophan 100 gm and 500 mg). Flies were maintained in a 22°C-incubator considering it a control temperature. Flies were exposed to 24ºC, 28ºC, and 32ºC for 5 days. The impact of thermal stress on their survival was noted (Table 1).

Oviposition:

Adult flies were maintained at experimental conditions for 8 days. The number of eggs laid and hatched into larvae was counted and recorded.

Hatching of eggs:

The eggs were kept on experimental media at temperature 22^{0}C, 24^{0}C, 28^{0}C and 32^{0}C for 5 days. The number of larvae that hatched was counted under the microscope. Survival frequency was calculated by counting the number of larvae.

Effect on pupation

Drosophila ananassae and *D. bipectinata* larvae transferred individually to carbohydrate and amino acid-enriched medium within 24 h of hatching. They were immediately exposed to heat stress at 24 ºC, 28ºC and 32ºC for three days then transferred to control conditions. The insect larvae were observed daily for development of larvae, pupation, and death up to 5 days after the treatment.

Effect on adult eclosion:

Pupa from the standard culture medium at 22^{0}C were transferred to carbohydrate and amino acid enriched medium within 24 h of attaining the pupa stage. They were immediately exposed to heat at 22^{0}C, 24ºC, 28ºC and 32ºC for five days then transferred to control condition. The insect pupa was observed daily for development of adults and death up to 5 days after the treatment.

Deformation in new adults' wings:

Immediately after attaining the adult stage, adults from the control condition (22^{0}C) were exposed to heat at 24ºC, 28ºC and 32ºC for five days. Males and females both were observed for deformation of wings and death for 5 consecutive days after being given conditions for 5 days.

Statistical analysis: All the experimental data were compared with control data. We took 22^{0}C as control temperature and heat stress condition was exposure to 24ºC, 28ºC and 32ºC for five days. Mean and standard deviation were calculated and ANOVA was used to find significant differences due to experimental conditions.

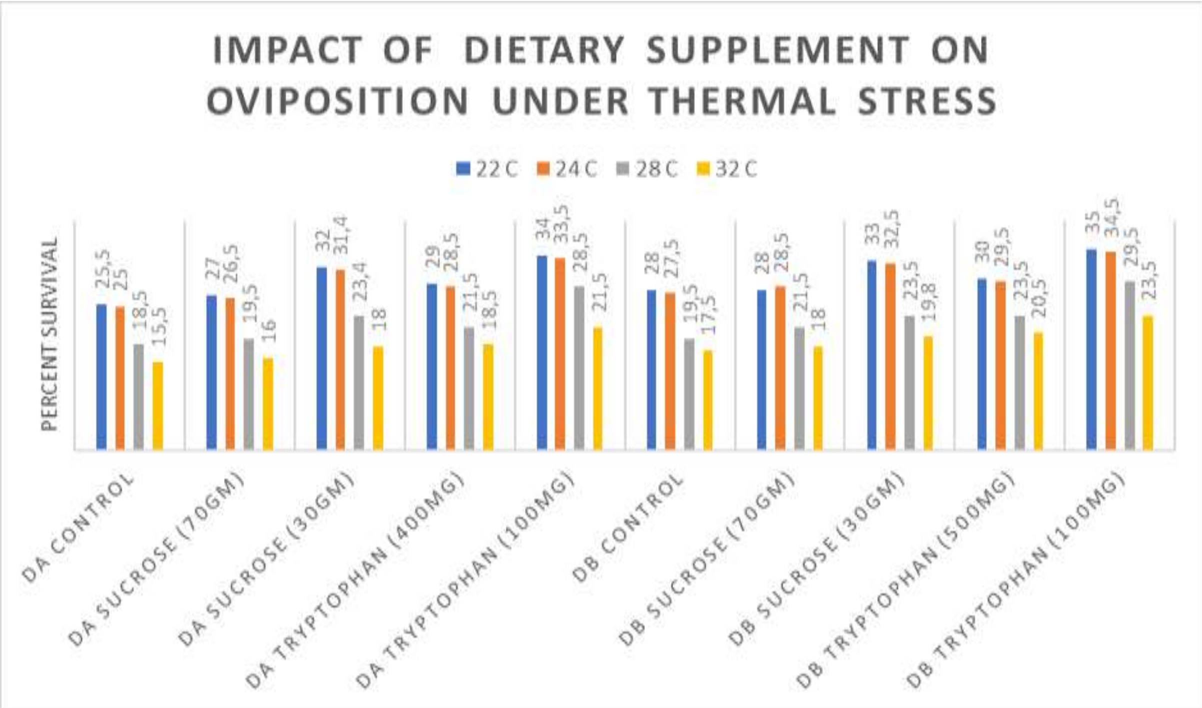
3. Results and Discussion

We discovered that dietary regimens had a major impact on heat shock survival. When exposed to heat shock, flies raised on amino acid-enriched medium survived better than those raised on carbohydrate-enriched media and standard media. Our results clearly support the studies of Andersen et al (2010) and Sisodia and Singh (2012).

Oviposition

We found a significant difference in the survival of eggs after oviposition of females on different dietary regimes when tested at different temperatures. 25.5% and 28% of 65 ±11 eggs produced/day were survived at 22^{0}C (control temperature) over unfortified media for *D. ananassae* and *D. bipectinata* respectively. It improved to 32% and 33% at carbohydrate–enriched (30gm) medium for respected species, while survival of amino acid-fed (100mg) egg improved to 33.5% and 34.5% for *D. ananassae* and *D. bipectinata* respectively. At 32ºC only 15.5% of eggs survived without enrichment of diet, which increased an additional 50% on feeding with tryptophan (100gm) for both species (Figure 1). Similar results were also noted by Sisodia and Singh (2012) in wild *D. ananassae* of India.

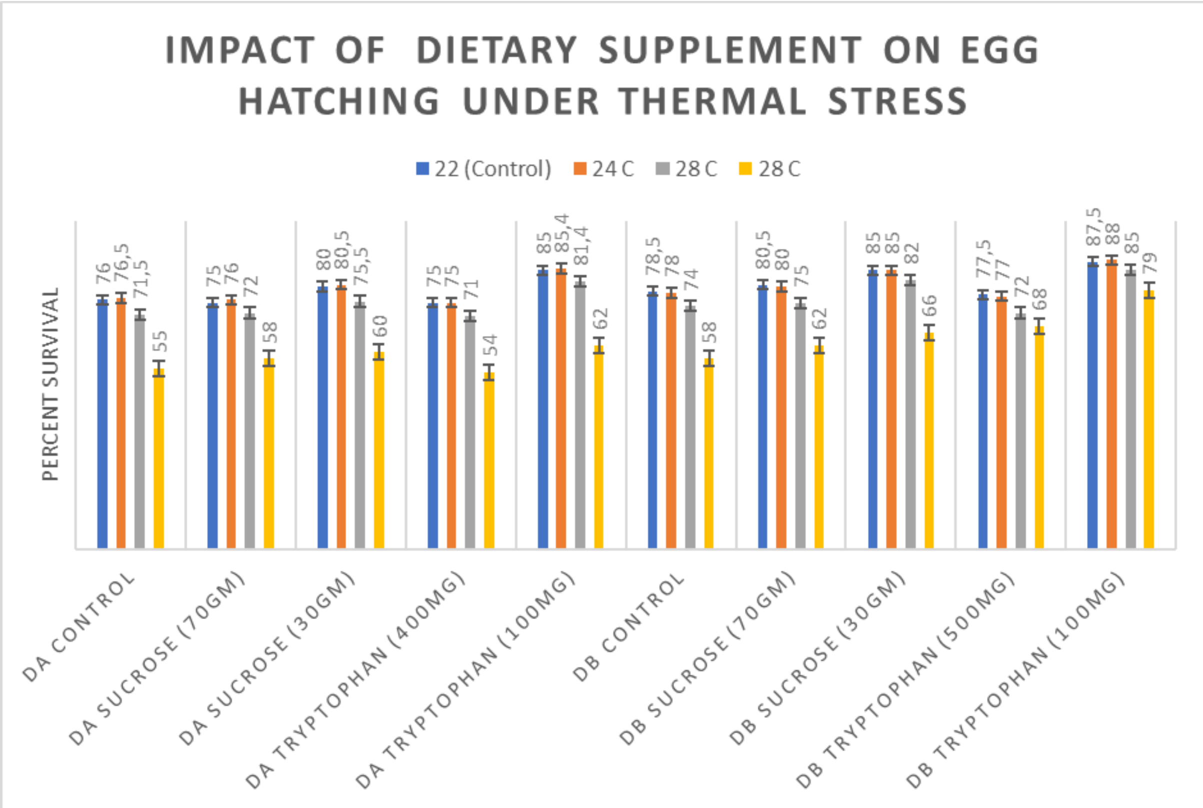
Figure 1: Impact of the carbohydrate and amino acid rich media on oviposition of adults under thermal stress



Hatching of eggs

The hatching of eggs to larva at various temperatures was affected by dietary regimes. Flies eggs developing on the tryptophan (100mg) enriched medium have higher survival rates than flies developed on sucrose–enriched and control medium ($P < 0.01$). It was 87.5, 88%, 85% and 79% at 22 ºC, 24 ºC, 28 ºC and 32ºC for *D. bipectinata* at tryptophan (100mg) whereas standard media eggs survival was 78.5%, 78%, 74% and 58% (Figure 2). Our results are supported *by* Andersen et al (2010) who noted the composition of larval food affects tolerance to thermal stress.

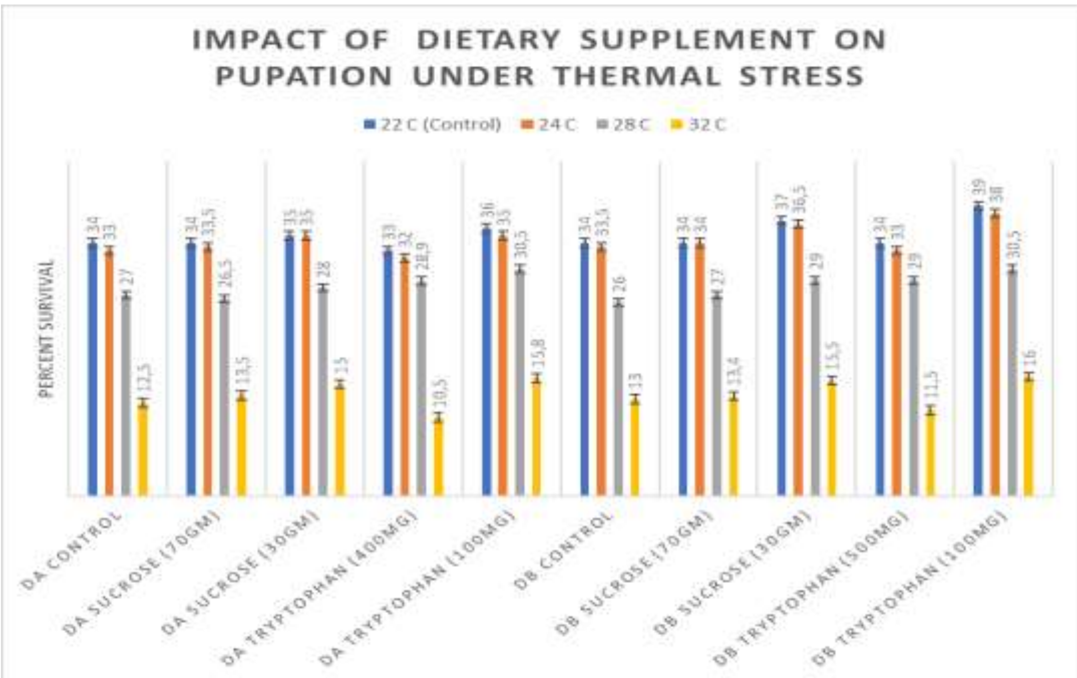
Figure 2: Impact of Dietary supplement on survival of larva after egg hatching under thermal stress



Pupation

We found a significant difference in pupation of larvae under the two different dietary regimes exposed to different temperatures ($P < 0.01$). Pupa developed on the low dose of tryptophan-enriched medium were 36% and 39% pupa for *D. ananassae* and *D. bipectinata* respectively at 22ºC compared to 34% pupation on standard media. This dose was also noted effective in improving the survival of pupa by 20% at higher temperatures 28ºC and 32ºC (Figure 3). Previous study by Krittika et al (2019) also evident that regulation of dietary amino acid restriction does regulate height and lifespan of pupation in *Drosophila melanogaster*.

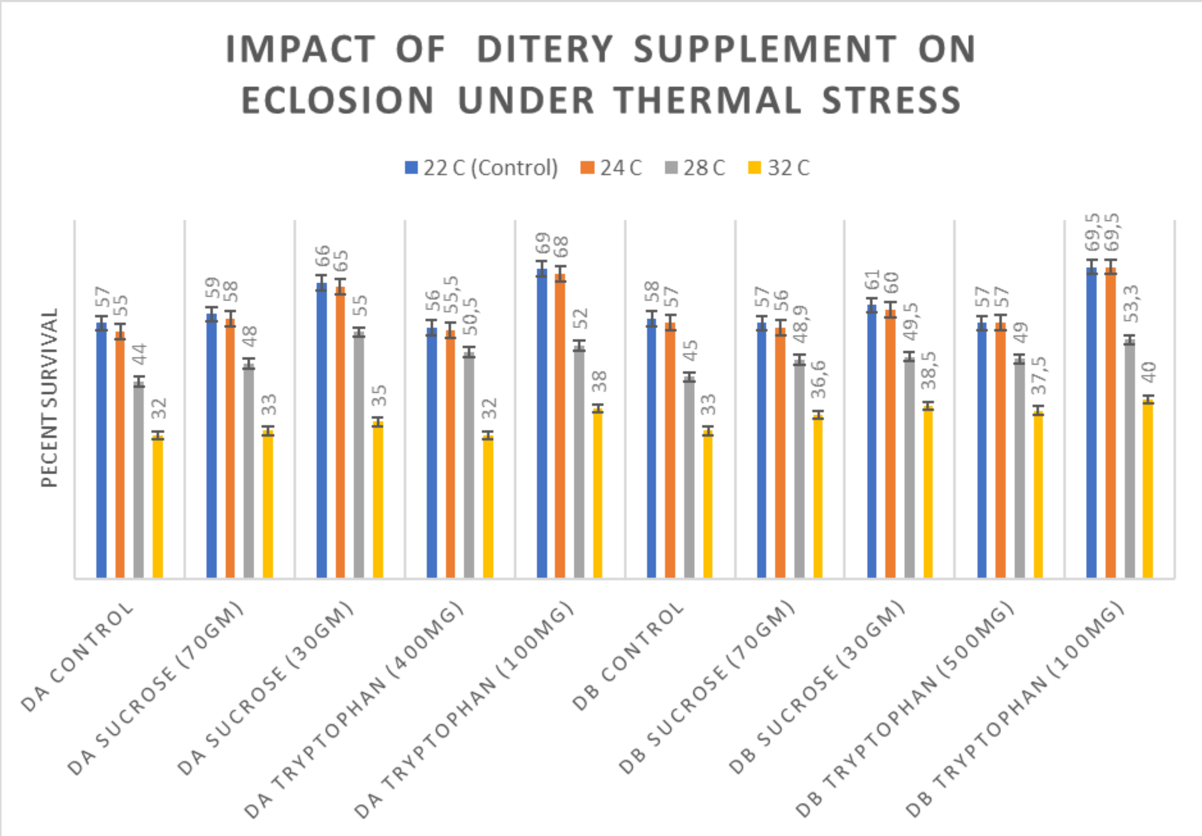
Figure 3: Impact of dietary supplement on survival of pupa under thermal stress



Eclosion

We observed higher eclosion and development of adult flies on tryptophan-enriched medium than flies developed on sucrose-enriched medium. The survival frequency varied in a highly significant way for two types of flies at various temperatures. Survival rate was improved from 32% to 38% and 33% to 40% at 32 ºC *D. ananassae* and *D. bipectinata* respectively (Figure 4). Higher eclosion rate and survival of adults can be explained with the fact that heat stress reduces energy reserves in Drosophila (Klepsatel et al., 2016).

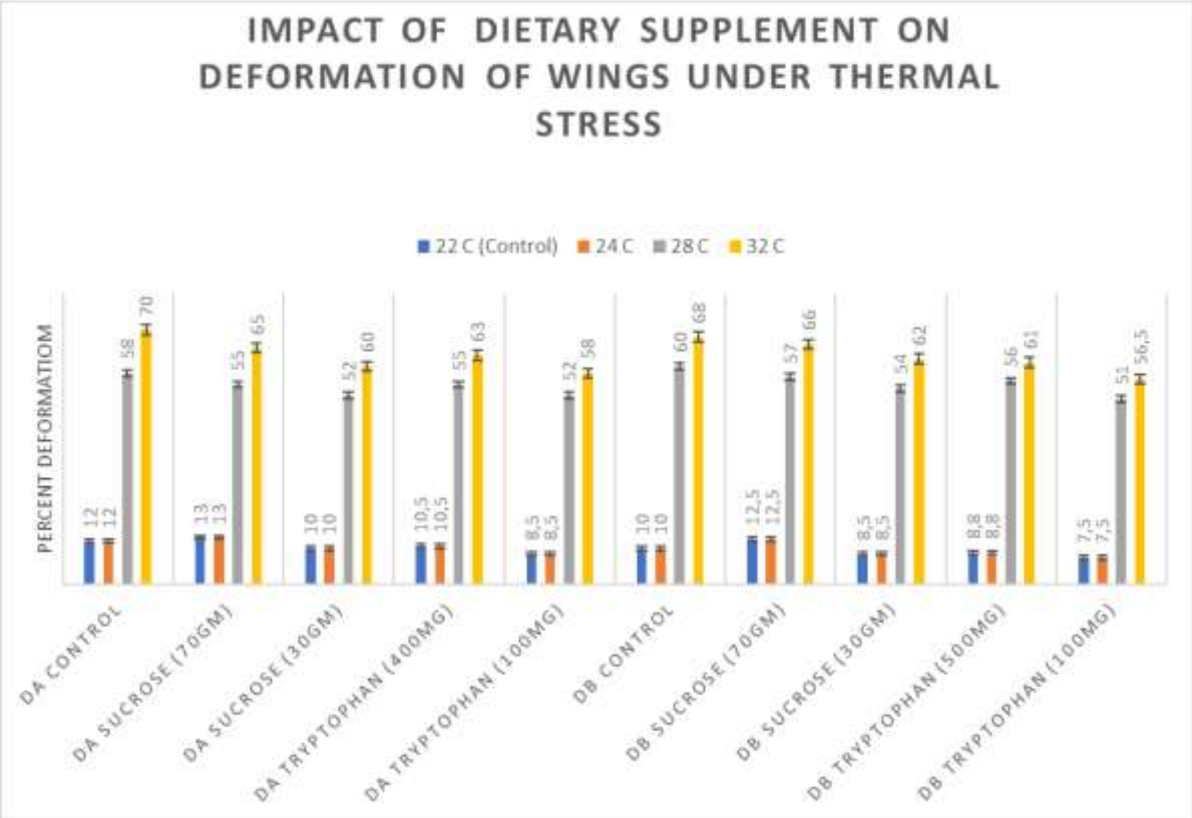
Figure 4: Impact of dietary supplement on survival of adults after eclosion under thermal stress



Deformation in adult's wings

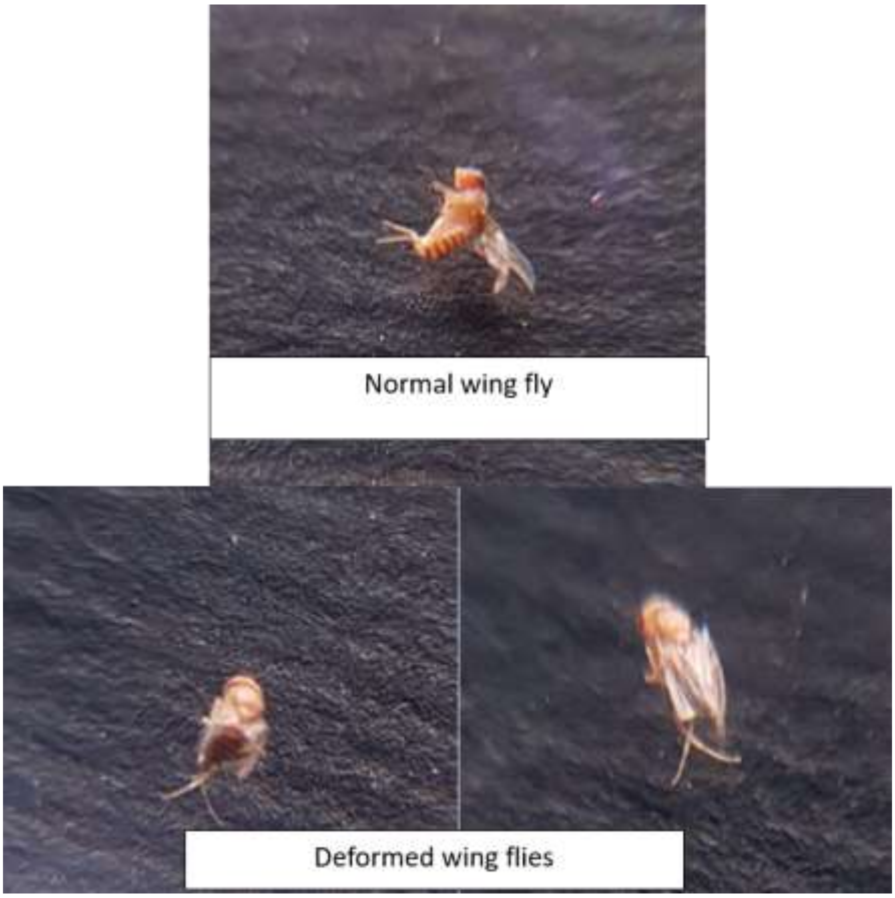
We discovered a strong correlation between dietary regimens on a reduction in the deformation of wings of adult flies after heat exposure. Flies fed with tryptophan-enriched medium have less deformation from heat exposure than flies developed on a sucrose–enriched medium. Tryptophan (100mg) was noted most effective in reducing deformation in both species at all three temperatures tested (Figure 5). Structural deformation of the wings of adults (figure 6) was noted very high at 28 ºC and 32 ºC compared to 22 ºC, however, it was significantly reduced at tryptophan (100mg) enriched medium when compared to control conditions for both the species tested.

Figure 5: Impact of dietary supplement on deformation of wings under thermal stress



Elevated temperatures promote the accumulation of damages, which can be countered by improving diet (Jacobson et al., 2010). Another research by Klepsatel et al., (2016) shows that heat stress significantly reduces an organism's energy reserves, which may have an effect on an individual's fitness. These studies explain cause of deformation of wings in present study.

Figure 6: Normal and deformed wings



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4. Conclusion

Tryptophan (100mg) enriched medium was noted highly effective for better survival of *D. ananassae* and *D. bipectinata* at 22 ^{o}C, 24 ^{o}C, 28 ^{o}C and 32 ^{o}C. Low dose of tryptophan positively influenced the survival, quality of life, and stress tolerance of both species.

Conflict Of Interest: None.

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