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Impact of environmental stress on biochemical profile and fitness traits in *Drosophila malerkotliana*

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***Abstract*: In natural condition, climatic changes affect the internal state of an organism. Effect of different environmental conditions on energy metabolites level can reveal the survival and fitness of organisms. In ectothermic insects, temperature plays a crucial role in influencing a number of fitness traits like duration of life cycle, survival, longevity and reproductive efficiency. Changes in temperature may lead to alteration in the levels of different biochemical profiles like body lipid, protein and glucose, as a measure to cope up with the changed environmental conditions. In the present study, mass culture stock of fruit fly, *Drosophila malerkotliana*, collected from Bilaspur, Chhattisgarh was examined for the effect of temperature on the body lipid (triglyceride), protein and reproductive ability, particularly, fertility and productivity in this species. The experimental results have indicated that transient thermal stress caused a significant effect on body triglyceride and protein content on age-dependent manner. Fitness traits, i.e., fertility and productivity were observed to be severely influenced by fluctuating temperature in the young as well as old age flies. Therefore, transient temperature stress leaves its distinct impact on the biochemical profile, fertility and productivity in *D. malerkotliana*.**

***Index terms*: *D. malerkotliana*, thermal stress, fertility, productivity, biochemical profile.**

I. INTRODUCTION

Every species on the planet can only tolerate a certain amount of environmental change. Environmental stress is described as any difference in environmental conditions from the species' optimal range, such as changes in pH, salinity, or temperature. It is the result of a series of alterations including both abiotic and biotic causes. Changing temperature and humidity are abiotic obstacles, while biotic problems include changes in available habitat, the presence of infections, parasites, rivals, and intruders (Markow, 2015). Environmental stress disrupts physiological homeostasis, lowering organism function and fitness (Pigliucci, 1996). Stress is characterised as acute or chronic depending on how long it lasts.

Exposure to a variety of stressors is inevitable in natural environments. Acute stressors are more common than chronic stressors.The overall effect of stress on an organism depends on its intensity and duration. Long-term exposure to relatively mild stress can have significant effects on the organisms (Kingsolver & Wood, 2016; Krebs &Loeschcke, 1994; Pigliucci, 1996) that can lead to several macromolecular damages. In response to macromolecular damage, the cellular stress responses are triggered, which is a universal defence mechanism.Thisdefence mechanism in turn restores cellular homeostasis (Kingsolver & Wood, 2016). As part ofthe cellular response to stress, heat-shock proteins (hsp90) are activated, which play a role inpreventing cellular changes that result from stress (Bhumika, 2019; Debat& David, 2001).Upon binding to unfolded proteins, heat shock proteins either assist in refolding those proteins, inhibit their nonspecific aggregation, or degrade them (Denlinger et al. 1991; Overgaard et al. 2008). But, thecellular defence mechanism can be damaged irreversibly if the environmental stress overwhelms it, in such a case, apoptosis occurs. (Klepsatel et al. 2016; Overgaard et al. 2008).

In ecology, temperature is the most important factor affecting organism physiology, especially for ectotherms (Atkinson, 1994;Berger et al. 2017; Hoffman & Parson, 1991; Terada et al. 2019). It is a key determinant of species geographical distribution, abundance and migration (Clarke, 2003; Cossins& Bowler, 1987; Hoffmann et al. 2003; Jorgensen et al. 2006). Therefore, the anthropogenic global warming, which is occurring at an unprecedented rate, poses a considerable threat to many species. In addition to the rising mean temperatures, organisms must also adapt to greater short-term fluctuations in temperature (Jorgensen et al. 2006;Krebs &Loeschcke, 1994; Overgaard et al. 2008; Pigliucci, 1996; Sisodia& Singh, 2006).It is therefore crucial to understand how thermal stress affects animal physiology in order to estimate biotic changes due to climate change (Pigliucci, 1996; Klepsatel et al. 2016). Assessing the effects of climate change on organisms requires an understanding of how environmental temperature affects metabolic and physiological functions.

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The present work has been done on *Drosophila malerkotliana*, a species thatbelongs to *D. bipectinata*species complex (Banerjee & Singh, 2017; Singh et al. 2015; Singh & Banerjee, 2016;Singh & Singh, 2001). Among the *Drosophila* species that occur most commonly in the Indian subcontinent, this species is highly prevalent. A large number of studieshave been conducted in *D. malerkotliana* related to inversion and allozyme polymorphisms to observe genetic characteristics of its populations (Parkash& Gill, 1980; Singh, 2019; Singh & Singh, 2020; 2022; Trehan& Gill, 1985; 1986; 1987).Due to its differential distribution between the northern and southern parts of our country, environmental factors, especially temperature fluctuations, are thought to play a role in that distribution (Singh, 2019; Singh & Singh, 2001). In a study conducted by Singh & Singh (2022), it has been found that the level ofheterozygosity increases from north India to south India.In one of the studies, Singh & Singh (2020) haveshown that there exists high level of genetic variation (0.603) in the same population (being used for this experiment).

Two main objectives were established based on this concept. First, to study the effect of transient thermal exposure on the biochemical parameters of 8 days and 25 days aged flies and second, to study the effect of transient thermal exposure on the fertility and productivity of 8 days and 25 days aged female flies.

II. MATERIALS AND METHODS

A. Drosophila malerkotliana

Mass culture stock of *D. malerkotliana* was used for the present study. This stock was raised from the flies collected from Bilaspur, Chhattisgarh during July, 2015 and is being maintained in laboratory condition at 25±1ºC temperature, 65±2 RH and 12:12 dark and light cycle.For rearing flies yeast-agar culture medium was used which contained ingredient like maize powder 108g, brown sugar 96g, yeast powder 36g, agar agar 36g, Nipagin 8g and propionic acid 8ml in 2400ml water constituting one unit of food.

B. Collection of flies and treatments

In half pint food bottle 20 pairs of 10day aged flies were transferred. After three days, parental flies were discarded. From the bottles newly emerged virgin male and female flies were collected and aged for 8±1 days and 25±1 days. 8 days and 25 days aged virgin male and female flies in the group of 30 each were treated at 35ºC, 40ºC and 35/40ºC for 60 minutes separately. In case of 35/40ºC, flies were pre-treated for 90 minutes at 35ºC at 16 hours prior to treatment at 40ºC for 60 minutes. Flies reared at 25ºC were treated as control. Temperature treatment was given in BOD incubator (MAC, Macro Scientific works, New Delhi). After incubation at experimental temperature, flies were kept at room temperature for 60 minutes and were subjected to biochemical estimation.

C. Biochemical estimations

1) Triglyceride

Triglyceride estimation was performed using Triglyceride assay Kit (Becon's), For this; five flies in 6 sets were homogenised in 100µl PBST and centrifuged at 12000 rpm at 4ºC for 2 minutes to collect supernatant. Supernatant was then heated at 70ºC in water bath to inactivate lipase. 2µl of sample was then mixed with 200µl of reagent and incubated at 37ºC for 10 minutes; reading was taken at 505 nm (Tennessen et al. 2014).

2) Protein

Protein estimation was performed by Bradford's method (1976) using Bradford's reagent. For this; five flies in 6 sets were homogenised in 100µl PBS and centrifuged at 12000 rpm at 4ºC for 2 minutes to collect supernatant. 10µl of sample was then mixed with 200µl Bradford reagent and incubated at room temperature for 10 minutes; reading was taken at 595nm.

D. Fertility and Productivity

8 days and 25 days aged virgin female flies in the group of 15 were treated at 35ºC, 40ºC, and 35/40ºC for 60 minutes separately. In case of 35/40ºC, flies were pre-treated at 35ºC at 16 hours prior to treatment at 40ºC. Flies reared at 25ºC were considered as control. After incubation, each female was paired with same age untreated male in 15 separate vials. Fertility of flies was recorded by considering the number of vials in which progeny appeared. Each pair of flies was transferred to fresh food vials on third day for four consecutive times. To measure theproductivity, 4 sets of fifteen vials of each treatment group and control were observed daily at the same time for the total number of progeny produced.

E. Statistical analysis

Data of biochemical estimations and productivity were analyzed by applying one-way ANOVA followed by Tukey's post-hoc analysis. Age wise and sex wise biochemical comparisons were tested by MANOVA and unpaired t-test. All statistical analyses were performed by using Minitab18 and PAST3 (Paleontological Statistics, Version 3.21) (Hammer et al. 2001).

III. Results

A. Triglyceride

In 8 days aged male flies, the level of triglyceride content significantly decreased with transient heat treatment. The average triglyceride content at 25°C was 12.61 mg/dl, at 35°C the content decreased to 5.40 mg/dl which further decreased to 3.71 mg/dl at 40°C. In the case of flies pretreated at 35°C before treatment at 40°C, the triglyceride content was found to be 4.83mg/dl meaning that, pretreatment made the flies resistant to temperature fluctuation as compared to direct tretment at 40°C (Fig. 1). In the case of female of 8 days age, there was relatively constant triglyceride content in them at varying temperature except at 40ºC (Fig. 1). The average triglyceride content in 8 days aged female flies was 15.22mg/dl at 25ºC, 15.77mg/dl at 35ºC, 8.87mg/dl at 40°C and10.46mg/dlin pre-treated flies. The 8 days age females tend to have significantly higher triglyceride level with respect to their male counterpart (Fig.2A) and showed well resistance to temperature fluctuations (Fig. 1).

In older age flies of 25 days, the triglyceride content in male showed significant variation at 35ºC (14.06mg/dl) and 35/40ºC (17.75mg/dl) with respect to control i.e. 25ºC (7.93mg/dl)

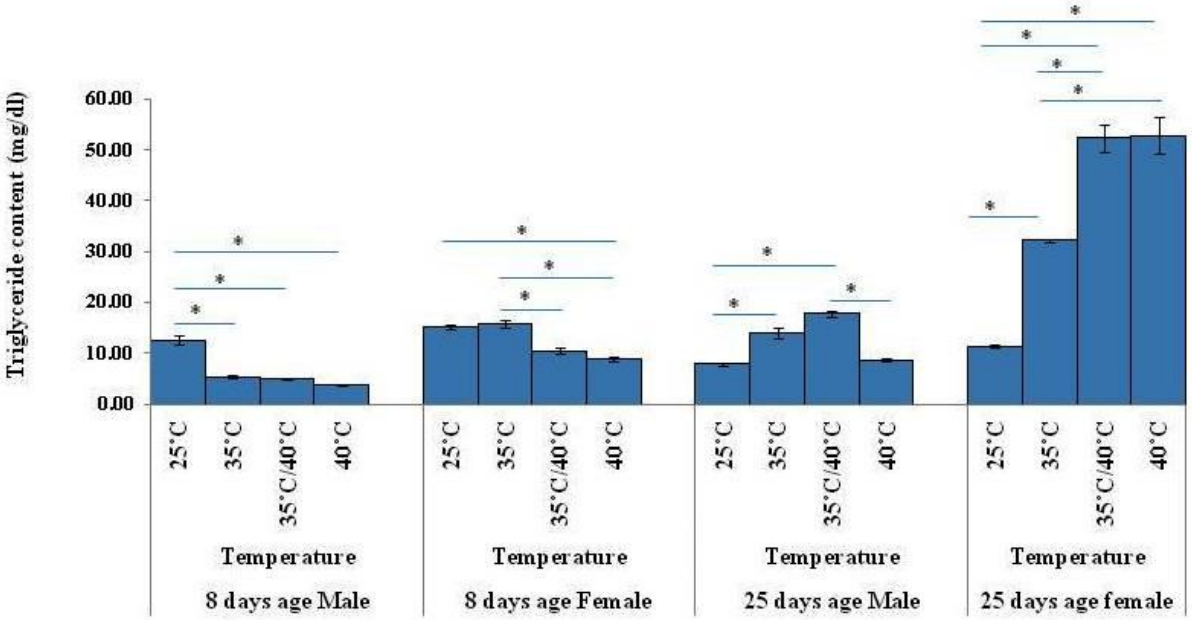


Fig. 1:- Bar graph showing mean±se triglyceride content (mg/dl) of male and female flies of 8 days and 25 days age groups. Significance was analyzed through One-way ANOVA followed by Tukey's post-hoc analysis (* $p<0.05$).

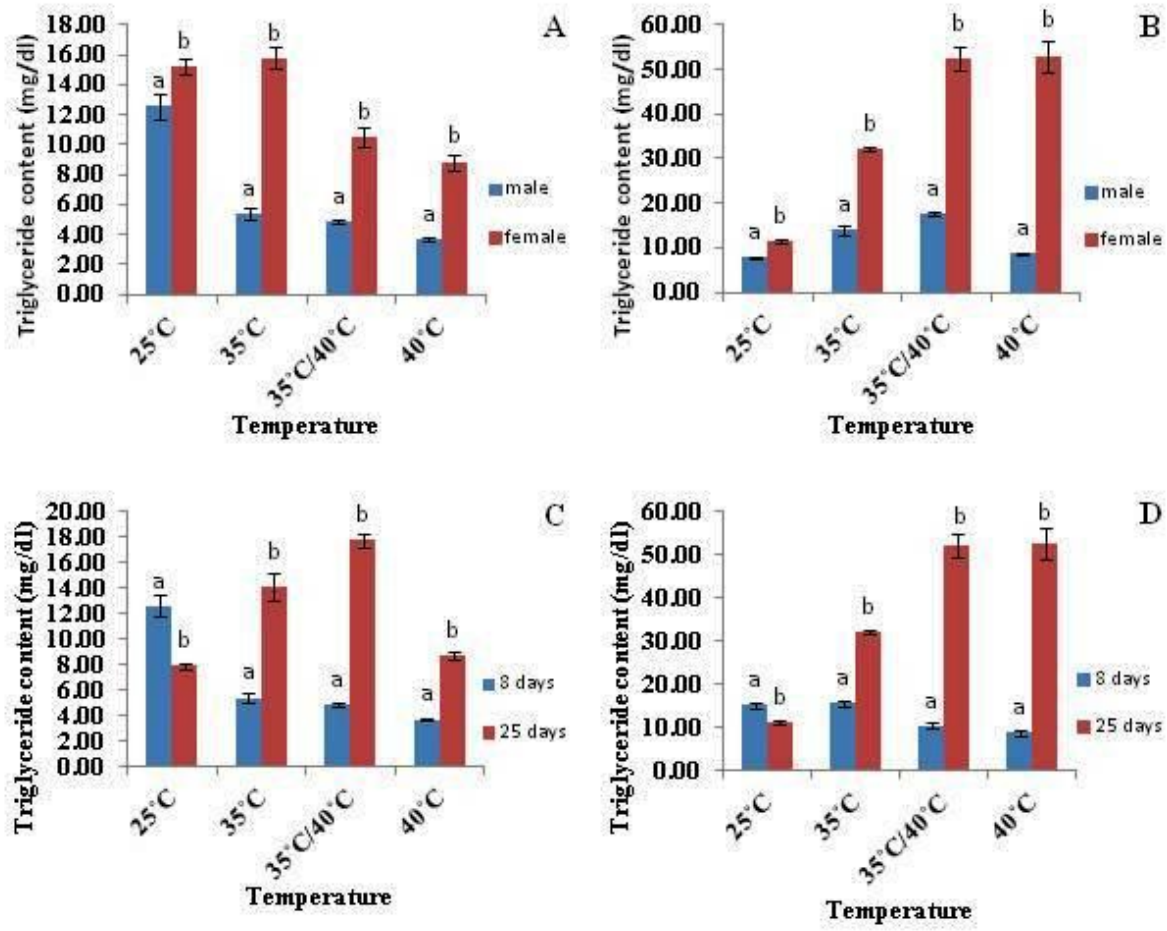
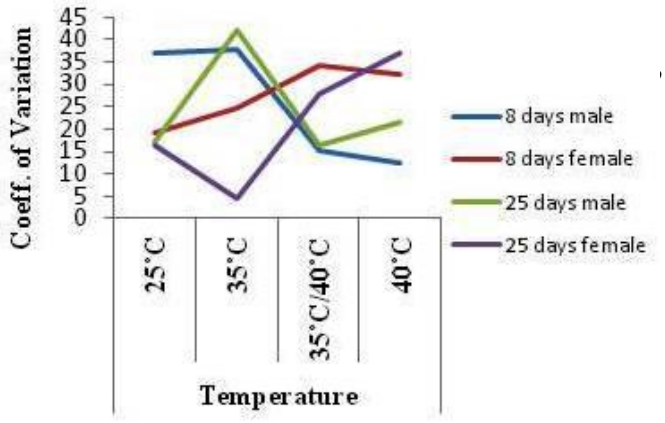


Fig. 2:- Comparative analysis of triglyceride content: Graph representing average triglyceride content. Vertical bars represent standard error. Significance was tested using student t-test (bar with different alphabet represent significant difference at $p<0.05$). **A**: comparison between 8



days age males and females; **B**: comparison between 25 days age males and females; **C**: comparison between 8 days and 25 days age males; **D**: comparison between 8 days and 25 days age females.

(Fig. 1). In the case of females of advanced age, significant variation in triglyceride content was observed at a temperature above 35ºC. The triglyceride content of 25 days aged female flies was 11.41mg/dl at 25ºC which significantly increased to 32.21mg/dl at 35ºC,52.80mg/dland 50.45mg/dl at 40ºCand 35/40ºC respectively (Fig. 1). In 25 days aged males and females, the females tend to have higher triglyceride content than males (Fig. 2B), but the higher aged females were not as resistant to temperature fluctuations as younger females (Fig.2D). There is loss in resistance as the age increases in the females. On comparing 8 days and 25 days aged male flies for their body triglyceride content, it was found that triglyceride content of young age male flies decreased with varying temperature treatment whereas in advance age male flies the triglyceride level increased with varying transient temperature (Fig. 2C) except at 40°C. Similar kind of result was obtained on comparing 8 days and 25 days aged female flies (Fig. 2D).At higher age, in both males and females, there is about a two-fold increase in triglyceride level at every temperature treatment (except at 25°C) with respect to their younger counterpart (Fig.2C and 2D).

Graph pertaining to coefficient of variation showed that in case of females of 8 days age, the coefficient of variation (CV) was found to be increasing with increasing temperature treatment (19% at 25ºC, 24.8% at 35ºC, 32.41% at 40ºC and 34.29% at 35/40ºC) whereas in case of young male CV was highest at 25ºC (36.95%) and 35ºC (37.95%) (Fig.3). In case of 25 days aged male and female flies, CV was highest at 35ºC (42%) in case of male whereas its female counterpart had the lowest CV (4%) at the same temperature (Fig.3).

Fig. 3:- Line graph showing the coefficient of variation for triglyceride content of males and females of 8 days and 25 days age groups.

B. Protein

In the level of protein content, significant variation occurred in 8 days aged male flies only at 35ºC (0.71mg/ml) with respect to control (1.02mg/ml). At other temperature treatment i.e. at 40ºC (0.98mg/ml) and 35/40ºC (0.82mg/ml) significant variation was not found (Fig.4). Whereas, in case of female counterpart, there occurred a significant drop in protein content at different temperature treatments (Fig.4). The protein content in 8 days females was 1.21mg/ml at 25ºC which decreased to 0.896mg/ml at 35ºC, 1.07mg/ml at 40ºC, 0.94mg/ml at 35/40ºC (Fig. 4). Here also, in the case of protein content in young age flies, the females tend to have significantly higher protein level with respect to males(Fig.5A). But in the case of protein in young age flies, the males seem to be resistant to fluctuation in protein level with respect to temperature fluctuation rather than females (Fig. 4).

At 25 days age, the females tend to have higher protein content at each temperature treatment with respect to male (Fig.5B). In 25 days aged male and female flies, it was observed that there is a slight increase in protein content with respect to temperature treatment. In case of 25 days aged male, significantly higher protein level was observed at 40ºC (1.1mg/ml) with respect to control (0.83mg/ml), increment in protein level was also observed at 35ºC (0.97mg/ml) and 35/40ºC (0.86mg/ml) but that was found to be not significant (Fig. 4). In the case of 25 days aged female flies, a significant increase in protein content was observed at 40ºC (1.28mg/ml) with respect to control (1.16mg/ml) (Fig. 4). The protein content was found to be 1.12mg/ml at 35ºC, 1.19 mg/ml at 35/40ºC (Fig. 4). On comparing 8 days and 25 days aged male flies for their body protein content, it was found that protein content of young age male flies decreased with varying temperature treatment, whereas in advance aged male flies the protein level increased with varying transient temperature except for pre-treated flies (Fig.5C). Similar kind of result was obtained on comparing 8 days and 25 days aged female flies (Fig.5D). Similar to the case of triglyceride content, protein content was also higher in both 25 days aged male and female flies with respect to their younger counterparts (Fig.5C and 5D).

In terms of coefficient of variation, it was found that in 8 days age males and females highest CV was observed in males at 25ºC (30%) whereas in other transient temperature treatment CV was found to be constant in both males and females (Fig.6). At 25 days age, in females, the CV was very less (up to 5% only) whereas in its male counterpart, CV was

higher than female at 25ºC, 35ºC and 35/40ºC (14.89%, 20.94% and 18.45% respectively) (Fig.6). On comparing male of 8 days and 25 days age, there was no specific trend in CV, whereas in case of the females of 8 days and 25 days age, higher CV was observed in younger age fly (around 15%) only but in other condition, CV was very less.

The result of MNOVA for triglyceride content showed that there was significant interaction between age-sex, age-temperature, sex-temperature and age-sex-temperature. In case of protein significant interaction was observed between interactions of age-sex and age-temperature only.

Fig. 4:- Bar graph showing mean±se protein content (mg/ml) of male and female flies of 8 days and 25 days age groups. Significance was analyzed through One-way ANOVA followed by Tukey's post-hoc analysis (* $p<0.05$).

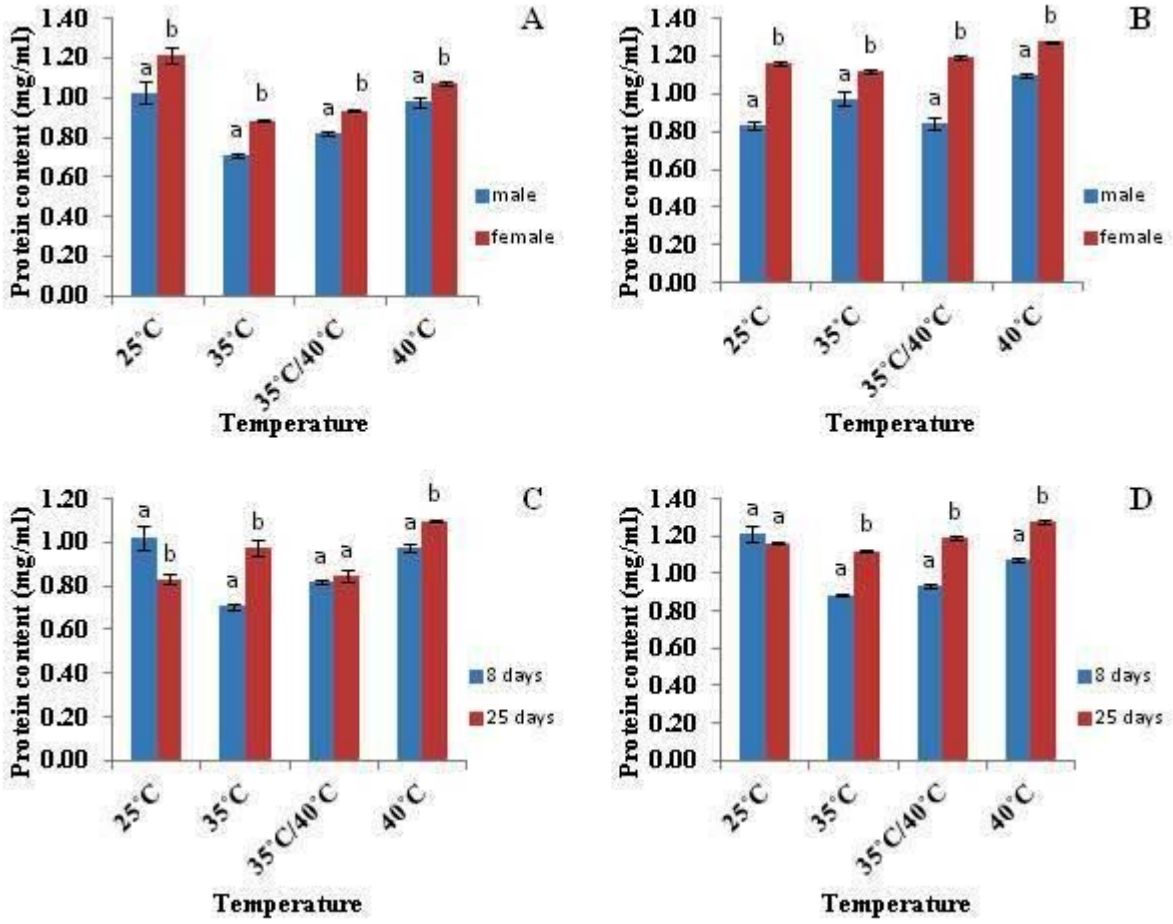
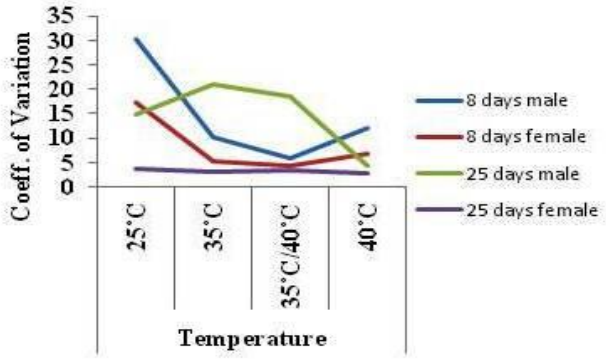


Fig. 5:-Comparative analysis of protein content: Graph representing average triglyceride content. Vertical bars represent standard error. Significance was tested using student t-test (bar with different alphabet represent significant difference at $p<0.05$). **A**: comparison between 8 days age males and females; **B**: comparison between 25 days age males and females; **C**: comparison between 8 days and 25 days age males; **D**: comparison between 8 days and 25 days age females.

Fig. 6:- Line graph showing the coefficient of variation for protein content of males and females of 8 days and 25 days age groups.

C. Fertility and Productivity

In the results of fertility and productivity, significant difference was not observed, but variation with individual treatment was visible. In 8 days age females, at 25ºC, fertility was observed to be 60% and productivity was found to be 101.22. On the other hand, at older age i.e. 25 days, fertility was 80%whereas the productivity was only 86.5. At the younger age, fertility and productivity were found to be affected if females were treated at transient temperatures. Treatment of males to transient temperature didn't affect much to the mating female (untreated) productivity and fertility. When both males and females were treated to transient temperature variations, it drastically influenced the productivity and fertility of females (Fig.7A). In older age flies, females treated at a temperature above 35°C (40°C and flies pre-treated at 35°C before treatment at 40°C) fertility and



productivity were severely affected. Even when males and females both were treated to a transient temperature, that resulted in a similar result.Whereas, when only males were treated, it resulted in to drastic change (productivity 36.75, fertility 53.33%) only at 35/40°C temperature(Fig. 7B). In 8 days aged females with increasing transient temperature treatment, a decrease in fertility but increase in productivity was recorded whereas, in case of 25 days aged females the fertility and productivity both decreased with increasing temperature treatment.

In younger age flies, the fertility was found to decrease in females when both the mates were treated at 35ºC (53.33%) but the productivity was increased to 159.25. Similarly, at 40°C in female treated group, the fertility was found to decrease but productivity was increased (46.67%, 141.57). When both males and females were treated at 40°C, the fertility and productivity were 46.67% and 99.57 respectively. In case of pre-treatment (35/40°C) group, fertility and productivity were 60%, 80.33 when only females were treated; 33.33%, 155 when only males were treated and 33.33%, 126.4 when both males and females were treated(Fig.7A).

Transient heat treatment in 25 days aged flies adversely affected the fertility and productivity in females. Highest fertility was observed at 25ºC (80%) with corresponding productivity of 86.5. The fertility decreased to 60% but productivity increased to 160.56 when females were treated at 35°C. When males were treated at 35°C, no specific change was observed in fertility and productivity (73.33%, 83.45). When both males and females,were treated at 35°C, the fertility decreased to 53.33% and productivity increased to 115. At 40°C, there was a drastic change in both productivity and fertility in each experimental group. In the case of pre-treated groups, a significant decline for both the traits was observed (Fig.7B).

IV. DISCUSSION

Several abiotic factors are known to influence the survival and propagation of animals. Temperature plays a vital role in the distribution, growth, and reproduction of several animal species. Ectothermic animals experience fluctuating temperatures on both daily and seasonal scale. In some cases, these fluctuations may become severely stressful or even lethal, and so these animals possess several physiological, biochemical and behavioural adaptations that allow them to overcome such stressful situations (Kingsolver & Wood, 2016; Malmendal, 2006; Pigliucci, 1996). Due to the exothermic nature of a large number of animals, metabolic rate in them is principally dependent upon environmental temperature. Their optimal growth and development fall within a fairly broad range of temperatures (Neven, 2000). Temperature is, therefore, one of the most important environmental factors dictating the animal's survival and dispersal. The holometabolous insects, like *Drosophila* live in varying environmental conditions from the relatively constant tropics to the highly seasonal temperate latitudes. They show plastic nature and thus can adapt in such different areas with varying temperatures (Pigliucci, 1996;Sisodia& Singh, 2006). Organisms with fixed phenotype show specialized adaptation to their home place but are unable to adapt in different environmental conditions (Cooper et al. 2014;Krishnamoorti& Singh, 2017).

We have used a mass culture stock of the fruit fly, *Drosophila malerkotliana* raised from the flies collected from Bilaspur in 2015, to examine the effect of temperature on its some biochemical parameters and fitness traits. In the present work, we found that transient thermal stress causes depletion of body triglyceride in young flies (8 days) of both the sexes. Decrease in the stored body fat content could be caused by increased cellular stress and damage, which might eventually result in the apoptotic death of fat bodies, because at higher temperatures the energy demands (higher metabolic rate) exceed the energy acquisition, with consequent energy deficit being covered by utilization of lipid reserves (Arrese&Soulages, 2010).A decrease in protein content with an increase in temperature is observed in young flies which might be due to the breakdown of protein or denaturation of enzymes at the higher temperature. In these young flies, more energy is invested in reproduction, instead of cellular stress-related damage maintenance. So, increasing productivity with high temperature is seen although fertility was decreased.

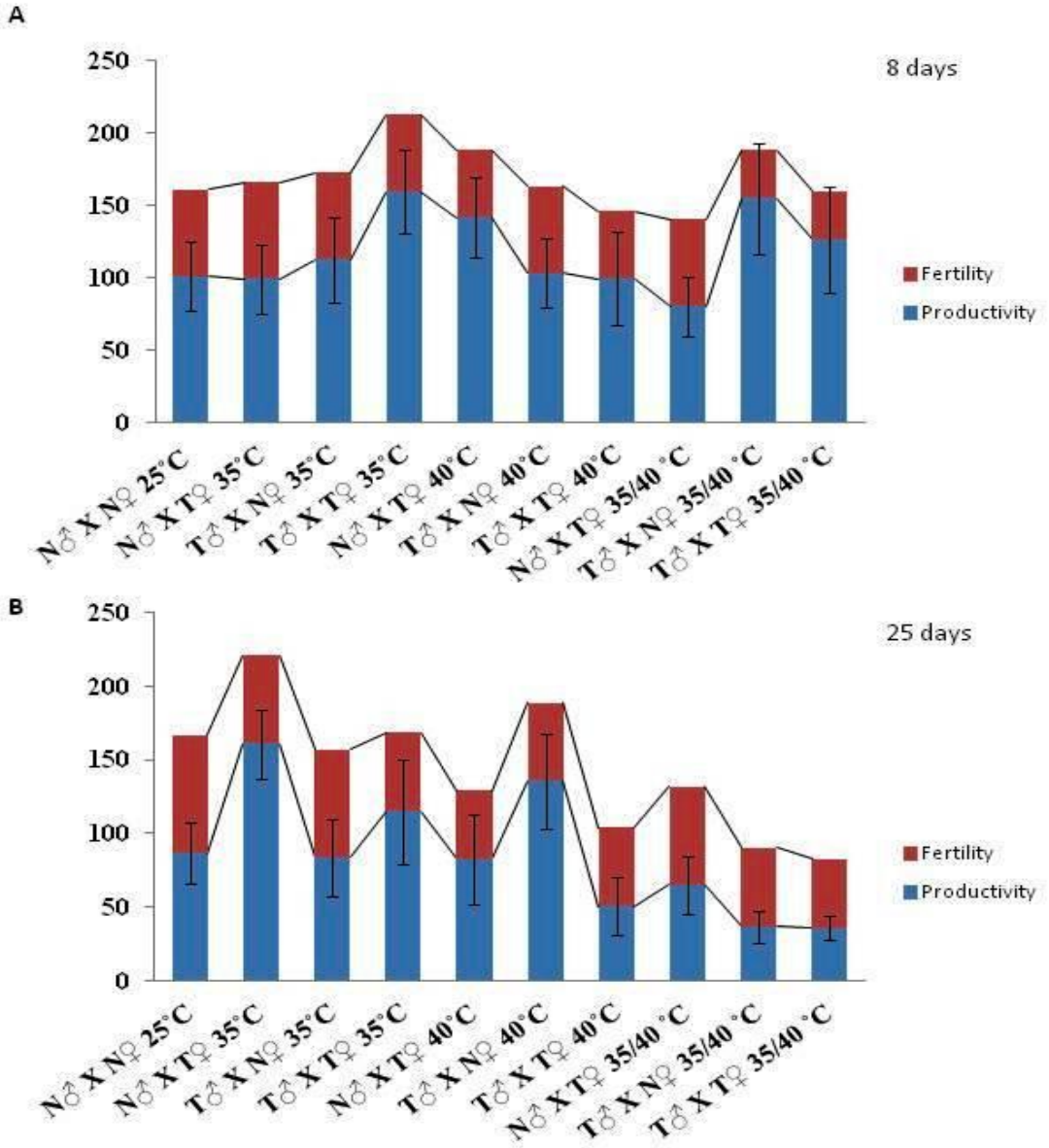


Fig. 7:- Productivity and fertility: Stacked graph showing the effect of transient heat treatment to male, female and to both male and female on average productivity and percentage fertility. Vertical bars represent standard error for average productivity, calculated from the total number of fertile females. **A**: 8 days aged flies; **B**: 25 days aged flies.

In older age, in both males and females of *D. malerkotliana*, with increasing temperature, significant elevation in protein and triglyceride content was observed but the fertility and productivity were significantly decreased. Refolding and degradation of damaged proteins, as well as the synthesis of new proteins that replace irreversibly damaged ones, are all costly processes that might considerably increase the energy requirements of the different metabolic processes, macromolecular maintenance, and repair (LeBourg et al. 2001;Klepsatel et al. 2016). Therefore energy directed towards reproduction decreases and thus a drop in productivity with increasing temperature is observed sin advanced aged flies.

Apart from age, concerning sex, females were found to be well adapted to temperature fluctuations. At a young age (8 days), males showed a drastic decrease in triglyceride content with increasing temperature but the female counterpart did not show any significant change in triglyceride level. Even in older age, where males showed an increase in triglyceride content with a slight increase in temperature but on the other hand female tolerated up to some extent. In the case of protein, both males and females showed similar kinds of results.

The experimental results have also indicated that the triglyceride content in females was significantly higher (more than double) than males at a varying temperature in both age groups. But in the case of protein, such a prominent difference was not observed except in certain conditions at a higher age. Variation in the range of triglyceride and protein content with increasing temperature was more prominent in the case of females than in males.

The major inference which can be drawn from this study is that the young age flies are more fertile and productive than their older counterpart; however, a marked fluctuation occurs in their biochemical profile due to transient thermal stress. The young age individuals show more variation (CV) in their triglyceride and protein content compared to older flies. This may be attributed to the varying physiology and reproductive involvement of young flies. Female flies of advance age are less productive and are better adapted to fluctuating temperatures. Old age flies of both sexes showed significantly higher biochemical profiles compared to their young age counterparts. Variation in the range of different biochemical parameters was less in older age flies. As an adaptive feature, older age flies use their triglycerides and protein mainly for survival rather than the reproductive activity.

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AUTHOR'S CONTRIBUTIONS

AKS and SS designed the experiment. SS and A performed the experiments. SS and AKS analyzed the results. SS,AKS and A did statistical analysis, SS wrote the manuscript and AKS helped in manuscript preparation.

COMPETING INTERESTS

Authors have no competing interests.

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