

शॉर्ट और लॉन्ग टर्म थर्मल स्ट्रेस के संपर्क में आने वाले स्पोडोप्टेरा
लिटुरा के शारीरिक प्रतिक्रियाओं पर अध्ययन

**Studies on physiological responses of *Spodoptera
litura* exposed to short and long-term thermal stress**

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**Studies on physiological responses of *Spodoptera litura* exposed to
short and long-term thermal stress**

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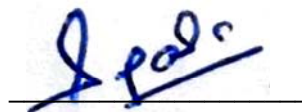
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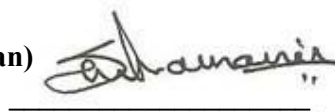
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CERTIFICATE

This is to certify that the thesis entitled, “**Studies on physiological responses of *Spodoptera litura* exposed to short and long-term thermal stress**” submitted to the Faculty of the Post-Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, in partial fulfilment of **Master of Science in Entomology**, embodies the results of *bona fide* research work carried out by **Ms. Sujatha G S, Roll No. 21274**, under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

It is further certified that all the assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by her.

Date: 14.09.2021

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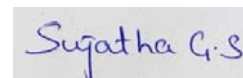
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LIST OF ABBREVIATIONS

%	Percent
°C	Degree Celsius
ANOVA	Analysis of variance
BSA	Bovine serum albumin
cm	centimeter
CaCl ₂	Calcium chloride
CRD	Completely randomized design
DDW	Double distilled water
DTT	Dithiothreitol
DW	Dry weight
EDTA	Ethylenediamine tetra acetic acid
ELISA	Enzyme linked immune sorbent assay
F ₁	First generation
F ₄	Fourth generation
FW	Fresh weight
g	gram
GSI	Gonadosomatic index
h	hour
HSD	Honestly significant difference
H ₂ SO ₄	Sulfuric acid
I ₂	Iodine
IARI	Indian Agricultural Research Institute
ICAR	Indian Council of Agricultural Research
Inc	Incorporated
KCl	Potassium chloride
KH ₂ PO ₄	Mono-potassium phosphate
KI	Potassium iodide
KOH	Potassium hydroxide
l	litre
LAO	Lacto-aceto-orcein
L:D	Light: Dark
mg	Milligram
ml	Milliliter

mM	Milli molar
nm	Nano meter
NaCl	Sodium chloride
Na ₂ CO ₃	Sodium carbonate
Na ₂ SO ₄	Sodium sulphate
P	Probability
RH	Relative humidity
rpm	Revolutions per minute
WC	Water content
USA	The United States of America

I. INTRODUCTION

Tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is one of the most destructive polyphagous insect pest damaging economically important 112 species of host plants (60 species from India) like tobacco, castor, cotton, soybean and groundnut throughout tropical and temperate world (Fand *et al.*, 2015). *S. litura* damages tobacco nurseries to the extent of 80 to 100 percent and field crop up to 10 to 25 percent, reducing tobacco production by 23 to 50 percent (Patil *et al.*, 2014). As an adult, this insect has a tremendous reproductive potential and may travel considerable distances (Armes *et al.*, 1997). This species is a migratory species that may be found on all continents and is a major problem because of its wide host range, nocturnal nature, high mobility of adult moths and reproductive capability (Feng *et al.*, 2005). Larvae cause significant losses in agricultural production during outbreaks by feeding on the leaves, buds, flowers and fruits of field crops (Sun *et al.*, 2016). With a changing climate, there is the potential for this insect to become an increasingly severe pest in certain locations due to increased habitat suitability and consequently it affects the local agricultural economy which may lead to the introduction of increased pest control efforts to manage this pest (Fand *et al.*, 2015).

One of the most significant abiotic factors influencing organism's nature, physiology, life history, distribution and abundance is temperature (Howe, 1967; Andrewartha, 1970; Hallman and Denlinger, 1998). Diverse insect species have a temperature range that is ideal for their survival, growth, and reproduction (Huffaker *et al.*, 1999). All living organisms consider temperature over and above the optimum range as heat stress (Paul and Keshan, 2016). Insects have an ideal temperature range in which their biological functions are best adapted, while insects may incur physiological costs and suffer damage that reduces their effectiveness at temperatures more than optimum levels (Chen *et al.*, 2018).

Climate change is taking place and the global-average surface temperatures have risen by around 0.6 degrees Celsius in the last 100 years (the highest rise of any century in the last 1000 years), with the 1990s being the warmest decade and 1998 being the warmest year since instrumental records started (Houghton *et al.* 2001: Bale *et al.*, 2002). The change in global temperature between 1850-1900 and period 2003-

2012 is 0.78°C. Environment is continuously changing to varying degree, this is creating stressful conditions for different species. Stress is described as "any environmental change that reduces an organism's fitness" (Koehn and Bayne, 1989), exposure to extreme environmental conditions such as cold or heat, desiccation, starvation, pesticides, and insecticides induce stress (Djawdan *et al.*, 1997). Insect has the potential to become a more serious pest in many areas due to increased habitat suitability as a result of a changing climate (Freudenberger *et al.*, 2016).

Shorter periods of extremely high temperature, known as heat stress and these heat stresses are expected to become extensive and severe in the near future as a result of global warming (Zhang *et al.*, 2013). In the months of April to June the north western parts of India usually experience the extreme high temperatures which are more than the optimum levels; this period may extend up to July. If the maximum temperature of the plains reaches more than 40°C and in hilly areas more than 30°C, then it will be called as heat stress (Guleria and Gupta, 2018). Warmer temperatures related with climate change and global warming have been proven to have direct consequences on insect population dynamics by affecting survival, generation time, fecundity, and dispersal.

Based on species and life stage of an insect the lethal temperature varies between 40 to 50°C, most of the insects can able to tolerate only some levels of variation in temperature (Chen *et al.*, 2018). Effects of the heat stress vary according to the life stage of the insect exposed because changes in the physiological responses to the temperature are subject to alter during development (Moran, 1994). Temperature impacts are typically studied by exposing organisms to repeated heat stress that has been shown to affect the life time fecundity and longevity of *Drosophila melanogaster* (Hercus *et al.*, 2003). Thermal stress improves insect thermo tolerance, but at the same time it also causes poor reproduction and other physiological problems (Feder *et al.*, 1996; Silbermann and Tatar, 2000; Shen *et al.*, 2014).

Generally, insects are susceptible to the high temperatures (Denlinger and Hallman, 1998), as a result high temperatures acts as negative climatic factor that inhibits insect population expansion in the field (Denlinger and Hallman, 1998; Bale *et al.*, 2002). In agro-ecosystems the climate is determined by the interconnection between biotic (crop plants, insects, microbes, weeds, etc.) and abiotic factors (temperature, rainfall, soil, relative humidity, pollutants, etc.). The effects of the biotic stress are

influenced by the abiotic factors and can become more problematic in combination (Mittler, 2006) which extraordinarily impacts on the growth and productivity of the crop up to 80% (Oerke *et al.*, 1994).

Temperature variations that modify the composition of the agro-ecosystem cause a shift in plant and animal diversity (NACCAP, 2008). As a result, multiple stresses that affect the resilience and sustainability of agro-ecosystems must be addressed in the context of climate change. One of the factors which limit the production of the agro-ecosystems is the risk of pest in various crop growth stages (Oerke *et al.*, 1994). The pest destruction varies greatly throughout agro climatic zones, due to the differing effects of many abiotic elements including temperature, humidity, rainfall (Sharma, 2005). This has serious implications for increased yield reductions in the face of looming climate change, as crop diversity may change and insect pests may become more prevalent.

When it comes to determining animal distribution and abundance, temperature is crucial. In insects and other ectotherms, temperature affects physiological processes, biophysical structures, and metabolic activities, along with development rates and growth. Many insect species are exposed to inadequate or excess temperatures throughout the year, which has aided the development of defensive biochemical and physiological mechanisms such as heat shock proteins (Hsps), which are thought to play a key role in environmental stress tolerance and temperature adjustment. The development of the insects take place under the minimum temperature scale and insect's reproduction, abundance, traits are highly influenced by the temperature (Andrade and Nascimento, 2012).

Thermal demands will be valuable in determining the effect of temperature on *S. litura's* growth, survival, and reproduction, as well as determining its prospective range and abundance in India. The hypothetical ideal temperature for different growth stages, such as eggs, young instar larvae, last instar larvae, and pupae, are 26.7, 24.7, 24.9, and 23.9°C, respectively (Zhu *et al.*, 2000). Whereas the minimum and maximum threshold temperatures for developmental life stages are 10.2 and 36.3°C (eggs), 9.9 and 38.7°C (larvae) and 9.8 and 38.2°C (pupae). Severe hot weather is projected to become more common in tropical areas, the impact of such occurrences on insect population projection is frequently underestimated as they have less influence on mortality, but they can alter reproduction.

Since 19th century, global mean surface temperature has increased, and the last 30 years have indeed been warmer than any other decade in the temperature record, 21st century being the warmest. Once the atmosphere is hot, insects can operate quicker and more effectively, and they can feed, evolve, multiply, and disperse more quickly (Drake, 1994). Temperature has a profound impact on insect development and growth because it directly impacts the rate of metabolic reactions (Davidowitz *et al.*, 2004). Thermal stress causes a variety of physiological stress reactions in insects (Livingstone, 2001).

Recently the studies have shown that the insects can change their metabolic profiles through adaptive acclimatization under thermal stress conditions (Hariharan *et al.*, 2014). In thermally changing habitats these plastic reactions are thought to be significant for insect fitness. Both cold and heat stress results in production of polyols and expression of heat shock proteins and high temperature pre-treatment improves the cold shock resistance (Segerkilde *et al.*, 2003; Sinclair and Chown, 2003). The responses to the higher and lower temperatures may vary depending upon the species. Under heat stress conditions sugars and alcohols are accumulated by the insects to overcome the degradation of cell membrane and stability of the protein (Salvucci, 2000). In the course of diapause, insects need lipid reserves to encounter the energy requirements (Hahn and Denlinger, 2007) and it also supply energy for the embryo development (Ziegler and Van Antwerpen, 2006) and it also helps in the flying (Beenackers *et al.*, 1984).

The typical pattern of protein synthesis is restricted at high temperatures, meanwhile the mechanism for heat stress situations is activated in insects. The function of the normal proteins altered while new proteins (heat shock proteins) are created. At high temperature the function of acetylcholinesterase is inhibited as a result the conduction of the impulses disrupted which causes the insects to lose their capacity to flee dangerous situations. Furthermore, high temperature influences the metabolism of lipids and sugars. Intense heat causes the cuticle wax to break down, melting of the lipid and water permeability to increase considerably (Du *et al.*, 2007).

Besides these, due to heat stress some of the metabolites in insects also get reduced which are responsible for the insect lifecycle. Considering the predicted rising temperatures under current climate change and heat wave scenarios, organisms are expected to suffer more intense and frequent thermal stress. Induced heat is

accumulated by organisms and can cause a variety of physiological stress responses. On thermal stress, there will be a significant decrease of water and lipid contents and increase of glycerol and glycogen content in adult insects. Results have shown that there was significant increase in sugar content in females than males of *Ophraella communa*, when females were exposed to long term phasic thermal stress at 40⁰C (Chen *et al.*, 2019).

Insect metabolism is highly dependent on ambient temperature due to their exothermic nature. Temperatures that allow insects to grow and develop optimally fall within a fairly wide range (Neven, *et al.*, 2000) For example, for codling moth the range is between 10 and 30°C (Rock and Shaffer, 1983). India being a tropical country, maximum day temperatures in the northern parts of the country may reach 42°C or higher for a few hours to several hours during the peak summer season. Increased temperature above the optimal range has a negative impact on the biology of tropical insects like *S. litura*. Since each developmental stage has a different physiological response to temperature, the impact of hot events varies depending on the stage of insect exposed. Despite the fact that many studies on the impact of temperature on population have been performed in the past, there is limited information available on the short and long-term consequences of thermal stress on subsequent biology, reproduction or physiology after exposure to short and long thermal stress and there are no studies related to metabolic responses of *S. litura* exposed to short term and long-term stress. So, present investigation was carried out with the following objectives.

OBJECTIVES

- i) To study the effects of short and long-term thermal stress on reproductive fitness in *Spodoptera litura*
- ii) To study the metabolic responses of *Spodoptera litura* exposed to thermal stress.

2. REVIEW OF LITERATURE

India being a vast country with varied geographical conditions which makes the country to experience wide range of temperatures from cold Himalayas in the north to the hottest Thar Desert in west. In northern parts of India the climate is characterised as most variable in nature like minimum temperatures in winter and maximum temperatures in summer months. But in peninsular part of India the temperature is stable all around the year. In summer months many parts of the country experiences scorching heat i.e. more than 40°C. The temperature in north western part of India exceeds 45°C in the summer months which posing a challenge for the livelihood of the people. The increase in temperature not only affecting the humans but it is also other organisms. Temperature above the normal levels might cause a serious impact on insect's abundance, distribution reproduction and the biology. Temperature above the optimum levels may be a day-to-day risk to the survival of insects. The effects of temperature stress will differ according to the species and stage of the insect due to differences in the physiological response to the stress.

Tobacco cutworm (*Spodoptera litura*) is an economically important pest in the agro-ecosystems throughout the world and causing humungous crop loss due to polyphagous nature of feeding. It is a serious threat to the agricultural economy because of diverse host range. It feeds on 112 species of plants belonging to 44 families over the southern and eastern parts of the world, with 40 species documented from India (Chari and Patel, 1983). However, it is primarily a tobacco pest, it also targets corn, chilli, cotton, sunflower, peanut, legumes, etc (Yadav *et al.*, 2012). This insect pest is more challenging because of the characteristics of facultative diapause during its development and seasonal migration to escape from adverse conditions (Devanand and Rani, 2008). It is migrating throughout the world and considered to be the major pest in all the continents of the world (Feng *et al.*, 2005).

2.1 Damage on host plants

Voracious feeding by the larval stage of insects on crops results in loss to the tune of 26-100 percent (Dhir *et al.*, 1992). The incidence of this larva occurs in the months of kharif (August and September mostly), when the crop ends its vegetative growth stage and entering into the reproductive growth stage. Scrapping of chlorophyll content and prodigious feeding on the leaves by the newly hatched larvae results in the appearance of web on leaves. As it matures the caterpillar entirely defoliates the leaves

with only the mid rib and stalks. As the larva grows older they become more active in their feeding. The soft pods eaten by larva wholly whereas, the hard pods become bored and grains will be eaten. This insect is a nocturnal feeder and in the day hours it used to hide beneath the plants, soil cracks and debris. The occurrence of this pest damage can be traced by the presence of faecal pellets on the leaves (Punithavalli *et al.*, 2014). In cotton crop, the larva feed voraciously on the leaves and they form big holes on the bolls which lead to the formation of yellow green to dark green larval excrements. In Maize, stems are frequently bored by the larva and the grains of the cob are also damaged (www.cabi.org). Egg masses underneath the leaves, presence of larva on the plant parts, presence of bored holes on the surface of the stem, leaves and skeletonization of leaves are the most prominent damage symptoms of this pest.

2.2 Biology and ecology of tobacco cut worm (*Spodoptera litura*)

Life cycle

According to the location and climatic conditions the total life period of *S. litura* differs, but the insect completes twelve generations in a year ordinarily which indicates it is completing a generation in a month. Temperature variations cause changes in the length of the life cycle. In winters, the life cycle of the insect is larger than a month and in summer less than a month. The total life period of the *S. litura* ranges from 43-48 days for male and 45-52 days for female (Latha *et al.*, 2014).

Egg

Female adults lay eggs on upper surface of the leaves in large clusters, covered with brown hairs which aid in protection against desiccation. The oviposition period ranges between 6 to 8 days, the shape of the eggs are flat and hemispherical in nature (Latha *et al.*, 2014). The eggs are pale green when they are laid freshly, pearly white after 24 hours and turn into yellow in the next day (Nataraj and Balikai, 2017). The size (diameter) of the egg is 0.6 mm, the eggs masses are arranged in 3 layers in rows which contain 300-350 eggs. The average fecundity ranges from 2000 to 2600 eggs and it is influenced adversely by the higher temperature and less humid conditions. The incubation period varies from 2 to 3 days.

Larva

Caterpillar in the course of development go through five moults with six larval instars. The overall larval period varies from 15 to 23 days. The length of the larva

ranges from 2.3 to 33 mm based on the stage of its development. The shape of the larva is cylindrical when it hatched newly.

First instar larva

Freshly hatched larvae are sluggish, green in colour. The larva's length in first instar ranges between 1.1 to 1.7 mm. It usually takes 3 to 4 days to complete first instar (Cardona *et al.*, 2007; Latha *et al.*, 2014).

Second instar larva

After completion of first instar, the body of the larva starts growing faster which results in the formation of wider body than head. The abdomen of the larva lined with four longitudinal stripes which are brown in colour. The length of the body ranges between 2.4 to 3.9 mm in the second instar larva. It will take 2 to 3 days to complete this stage (Cardona *et al.*, 2007; Latha *et al.*, 2014).

Third instar larva

Third instar is characterized as elongated with yellowish green colour. The body of this instar larva have scattered black spots on it. On the surface of the body three yellow lines are present (One in middle, two in lateral sides). It requires 2 to 3 days to complete the third instar stage. The larval length of this stage ranges from 7.6 to 8.5 mm. Three pairs of prothoracic legs and abdominal legs on the 6th, 7th and 9th segments are present (Cardona *et al.*, 2007; Latha *et al.*, 2014).

Fourth instar larva

The larva is dark green in colour and the abdomen lined with the longitudinal stripes which are yellow and green in colour. Normally, the fourth instar stage lasts between 2 to 3 days. Length of the larva ranges from 20 to 24 mm in the fourth instar stage (Cardona *et al.*, 2007; Latha *et al.*, 2014).

Fifth instar larva

The length of the fifth instar larva ranges between 46 to 48 mm. Head is reddish brown in colour and legs are black. In the abdomen prominent spiracles are seen. This instar larva is characterized as a voracious feeder. During day time it hide inside the soil and in night time it will start feed. Before moulting the larva stops feeding on the host (Cardona *et al.*, 2007).

Sixth instar larva

Head is black, body is greenish black or brown in colour. Complete fading of black patches on the abdominal segments was seen in the sixth instar stage. Y shaped shield is present on the head or thorax of the larva. Larva weigh up to 800mg. This larva usually consumes about 4g of the fresh foliage. In this stage the larva feeds nearly 80% of the total food consumed (www.cabi.org).

Pupa

Pupation takes place inside the soil close to the bottom of the plants. Pupa began as a pale yellowish colour and evolved into a dark reddish- brown colour. Two tiny spine like structures seen on the tip of the pupa's abdomen. In the 9th abdominal segment of the pupa of male insect have an 'x' shaped genital slit on the ventral side of the abdomen. Similarly, in female pupa inverted 'v' shaped genital slit present on the ventral side of 8th abdominal segment (Cardona *et al.*, 2007; Mogili and Uma, 2018). This character helps in differentiating the male insect pupa from the female. Normally the size of the pupa ranges from 15 to 20 mm. Generally, the pupal duration ranges from 7 to 10 days.

Adult

The male and female adults are 15-17 mm and 17-20 mm in length respectively. The total wingspan of adults varies from 30-38 mm. Both the male and female moths are grey brown in colour and their body covered with hairs. The male forewings prominently having oval shaped orbital spot with fainted reniform spot and a row of small horse glass shaped markings near the border. In female insects the above said character is less prominent. The male and female adults hind wings are greyish-white and bordered with grey colour. On an average the female moths take 8.3 days to complete their adult stage whereas, for male moths it is 10.4 days (Etman *et al.*, 1980).

2.3 Distribution

S. litura is one of the important pests in the tropics of the continent Asia. In south Asia the incidence of *S. litura* is very common. However, its presence ranges from the tropic and temperate regions of Asia, Australasia and Pacific islands (Feakin, 1973; Kranz *et al.*, 1977). China, Indonesia, India, Japan and Malaysia are the countries which having the largest population of this insect. Due to international trade this insect population also reached to the non-indigenous regions. All the developmental stages of

the insect can be found in the soil or plant parts and this can be easily fetched to the far-flung areas. Among the developmental stages the pupa can transfer to the far-flung areas due to its longer pupation time comparatively than the other stages (www.cabi.org).

2.4 Temperature

Temperature plays a key role in the insect distribution and multiplication, and temperature stress is one of the major problems in the field of insect ecology. Temperature stress, both the low and high temperature stresses elicit physiological and behavioural responses in organisms. Based on the temperature stress we can able to predict the occurrence of the pests, to instigate the natural enemies in the crop field and the area of distribution. It is also helpful in the tracing of origin and population dynamics of the insects (Wang *et al.*, 2010). Rate of growth and development and the rate of metabolism in the insects is greatly influenced by the temperature (Amarasekare, 2014). Temperature being a vital and key factor which has a serious effect on the insect's biology (Huffaker *et al.*, 1999), temperature more than the optimum levels have a detrimental effect on the biology of the tropical insects. Fluctuations in the ambient temperatures with shorter duration of higher temperature (Plain regions - 40°C or more and Hilly regions- 30°C or more) referred as the heat stress (Guleria and Gupta, 2018). Each developmental stage's physiological response to temperature differs because the consequences of thermal stress on the population of insects are specific to the particular stage (Zhang *et al.*, 2015).

2.5 *Spodoptera litura* reproductive fitness and thermal stress

Simmons (1993) carried out the experiment on the effect of constant and fluctuating temperatures on the survival of *Spodoptera frugiperda* pupae. At either lower temperature (10°C) or at higher temperature (20°C), no eclosion was detected (40°C). Pupae held at 15 and 35°C had lower survival rates and more deformity, ranging from 39 to 62 percent. The optimum rates of development were found at 30 and 35°C, but a large percentage of pupae held at 35°C emerged as deformed. Similarly, Neven (2000) opined insects are heat sensitive and they can adapt to thermally challenging conditions by altering their metabolism and respiration rates. The effects of heat on the nervous and endocrine systems are devastating.

Ma *et al.* (2004) examined the impact of the high temperature (27, 28, 29, 30, 31, 31.5, 32.5, 33, and 34°C), exposure time (2, 3, 4, 6 and 8 hours a day for 1, 2, 4, 6

days), and developmental stage (2nd, 3rd, 4th instar nymph and adult) on the aphid's survival. Temperatures of more than 29°C for 8 hours greatly reduced survival, which decreased as the temperature rose. The duration of exposure had an inverse relationship with survival. Aphid survival was drastically decreased when they were exposed to 32.5°C for 4 hours or longer. The resistance of mature aphids to high temperatures was lower than that of nymphs. High temperatures faced by 4th instar and adult aphids can have a significant impact on their survival.

To find out the consequences of high temperature (26, 29°C, 32°C and 35°C) Chen *et al.*, (2006) conducted an experiment on *Trichogramma ostriniae* under laboratory conditions. The findings of this study revealed that great reduction in the development of the insects were seen at higher temperature stress (32°C and 35°C). The egg-larval and pupal survival rates were decreased gradually with the increase in temperature. They also found that the temperatures of 26°C and 29°C the female insects were completing their oviposition and coupling normally however at 32°C and 35°C the adults were unable to reproduce.

Similarly, Booth and Kiddell (2007) studied the effects of temperature in the development of *Acheta domesticus* (House crickets). The insects reared under 25°C were grown at slower pace and they took many days to develop. But the weight of the body was more in the insects than that of insects which reared at 28°C. Total energy consumed for the metabolic needs was double the rate of 28°C in the insects reared under 25°C. Likewise, the impact of cold stress (-18°C for 1 hour) in pupa of the male wasp, *Dinarmus basalis* (a parasitoid wasp) was studied by Lacoume *et al.* (2007). After the stress, the reduction in the developmental success was observed in the male insects and producing a reduced levels of sperm stock than the control insects.

Cui *et al.* (2008) investigated the consequences of temperature stress (37, 39, 41, 43, and 45 °C for 1 hour later recovered and reared at 26°C) on *Bemisia tabaci* (*Gennadius*) biotype B and *Trialeurodes vaporariorum* reproduction and survival. Survivability of adults was substantially reduced when the temperature approached 41°C, according to the findings. At 45°C, all male *T. vaporariorum* died. Females of both white fly species were more resistant to high temperatures of 39°C. The fecundity of *B. tabaci* did not change with temperature, while the fecundity of *T. vaporariorum* decreased.

Likewise, Xu *et al.* (2009) conducted an experiment to find out the outcome of temperature stress on development of the ovary in the red flour beetle insect (*Tribolium castaneum*). In their study, they maintained two different lines (H and C lines). They gave 40°C for 1 hour in the H line insects and 30°C in C line insects (control temperature). They identified longer pre oviposition period and smaller size of the ovarioles in the treated lines (H line) compared to that of control insects (C line).

In addition, Mironidis and Soultani (2010) examined the impact of higher temperatures and exposure patterns on *H. armigera* adult survival and reproductive parameters (40, 42.5, 45, and 46.5°C for time periods ranging from 1.5 to 540 minutes). At all the higher temperatures studied the survival rate of both sexes decreased as the time spent increased. With the rise in temperature from 40 to 46.5°C, the longevity of the insects decreased rapidly. Exposure period to high temperatures was found to be inversely related to fecundity.

In the same way, reproductive success of male and female *Bicyclus anynana* insects under temperature stress examined by Janowitz and Fischer (2011). They gave the short-term stress of 40°C for 1 hour on insects. Due to temperature stress the female insects showed a reduction in the longevity, fecundity and egg size. In male insects the heat stress reduced the longevity but it had a very little impact reproductive behaviour.

Similarly, the short-term effects of high-temperature exposure on *Bactrocera oleae* egg development was experimented by Pappas *et al.* (2011). For two hours, larvae inside fruits, adults, and pupae were exposed to high constant temperatures ranging from 34°C to 42°C. After heat stress, female longevity and egg production were significantly reduced.

In the laboratory, Zhou *et al.* (2011) investigated the impact of short-term high temperature stress on the survival and growth of different stages, adult lifespan, and fecundity of *Ophraella communa* (Coleoptera: Chrysomelidae). When compared to the control (28°C), egg, larval, pupal, and adult survival rates were significantly affected in the 2-hour short duration stress at high temperatures (35 to 47°C). Females had a slightly higher survival rate than males after short-stress at high temperature, with the exception of the control and 35°C stress. They found that with an increase in short-term stress temperature, the survival rates of different stages, as well as adult longevity and fecundity of the beetle, decreased significantly.

Population dynamics of leaf roller (*Diaphania pulverulentalis*) was studied by Rahmathulla *et al.* (2012) throughout the year and they found that the rate of infestation was varied according to the month. In the summer months of the year the rate of infestation was least whereas in the rainy season the rate of infestation was maximum.

Guo *et al.* (2013) investigated the effects of high temperature on the fecundity and development of *Bemisia tabaci*. They compared the effects of temperature stress on five generations of insects at 27, 31 and 35 °C and one generation at 37 °C. At 31°C the maximum survival rate was recorded in the fifth generation but the fecundity of the female white flies reduced in lesser generations. At 37°C the rate of survival was not affected adversely but the female fecundities were reduced compared with that of 27°C. In the same way, the reproduction of male parasitoid wasp (*Anisopteromalus calandrae*) under different heat stress treatments (30°C, 40°C and 42°C) was studied by Nguyen *et al.*, (2013). As a result of heat stress mortality of the insects occurred above 42°C, sterility in the male insects at 40°C and sub-fertility at 38°C was observed.

Solangi *et al.* (2013) carried out the research on the effect of high temperatures on female *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) adult survival and longevity. Regular exposure of the beetles to 35°C in the laboratory followed by thermal treatment at 38, 40, 42, and 44°C was done. The longevity of the predator was greatly reduced as the temperature rose from 38° to 44°C. Similarly Zhang *et al.* (2013) reported that temperature stress (maximum 40°C for 3, 4, or 5 hours) had no immediate impact on mortality, copulation period, mating success, survival, or life time fecundity in diamondback moth, *Plutella xylostella*, but stressed females developed fewer hatched eggs after 3 or 4 hours. They came to the conclusion that a single hot day can harm reproduction, especially through maternal effects on egg hatching, and thus affect the population dynamics of the diamondback moth.

Blanckenhorn *et al.* (2014) conducted an experiment on the heat tolerance in yellow dung fly (*Scathophaga stercoraria*) at different stages and sex. According to them, early developmental stages when exposed temperature stress had increased mortality. Similarly, *O. communa* eggs, larvae, and pupae were exposed to high temperatures, their adult body lengths were drastically reduced and little impact on the body weights of females and males. High temperatures can affect the reproduction of *O. communa* in the field because adult insect fecundities are often proportional to their body sizes (Chen *et al.*, 2014).

According to Fand *et al.* (2014) the temperature stress (15-40 °C) reduced survival and reproductive potential of mealy bug, *Phenacoccus solenopsis* under laboratory conditions. For the potential growth and development, survival and reproduction rate the favourable temperature was predicted as 25 to 35°C. In butterfly, *Bicyclus anynana* exposure of short-term heat stress resulted in the reduction in the survival of female adults than the male adults. From this study Karl *et al.*, (2014) concluded that female insects were heat stress tolerant than their male counterparts.

In the Mediterranean whitefly, *Bemisia tabaci* significant reduction in the longevity and survival was recorded after temperature stress. In subsequent generations the survival rates increased even after past generations affected by stress and the fertility percent was not affected by the temperature stress within five generations (Lu *et al.*, 2014). Likewise, Rao *et al.* (2014) looked into the direct effects of rising temperature on *S. litura*. The average development time (days) of each stage decreased as the temperature increased from 20 to 35°C and concluded that temperature had a major impact on *S. litura*'s growth and reproduction.

When exposed to the temperature stress the larval stages of African armyworm (*Spodoptera exempta*), survival of the larval and subsequent stages was decreased (Aguilon and Velasco 2015). Due to heat stress (for 24 hours) spermatogenesis was delayed in the development and reduction of sperm stock in *Nasonia vitripennis* male. The mated females with these male insects had less sperm count in their spermatheca and laid less eggs (Chirault *et al.*, 2015). Similarly, Fand *et al.* (2015) found that the larval and pupal stages of *S. litura* were found to be lethal at a temperature of 38°C. At the extreme low (15°C) and maximum (> 35°C) test temperatures, females were unable to lay eggs. Temperature stress (exposure to 40°C) at later developmental stages had a greater negative impact on subsequent survival, and reproductive success in the *Plutella xylostella* than early stages (Zhang *et al.*, 2015).

Paul and Keshan (2016) studied the effects of heat stress on ovarian growth, fecundity in silkworm. The thermal treatment of larvae and pupae at 39°C (1 h and 2 h) had no effect on the reproductive performance, at 42°C or higher it has reduced fecundity. Likewise, the mating behaviour of irradiated *Spodoptera litura* was studied by Seth *et al.* (2016). According to their findings mating performance, remating tendency and fecundity were all significantly affected by irradiation.

Temperature is the most significant factor limiting the population of *Bradysia odoriphaga* and *B. difformis*. The survival rate of *B. odoriphaga* decreased rapidly as the temperature rose above 36°C and even longevity and reproductive ability were severely hampered (Zhu *et al.*, 2017). Similarly, Kim *et al.* (2017) conducted an experiment in which they studied the heat tolerance and assessed the susceptibility of the Indian meal moth, *Plodia interpunctella*. At a heat stress of 44 °C for one hour increased the mortalities significantly in all the stages of development except the larvae of late- instar (they shown highest tolerance). In the same way, Schou *et al.* (2017) examined the potential effects of developmental temperature (12, 15.5, 18, 20, 22, 25, 27, 29.5,31 and 32°C) in *Drosophila melanogaster*. They analysed for the parameters like egg to adult viability, developmental time and tolerance to both heat and cold temperatures. They found that the female insects were more tolerant than the male individuals.

Chen *et al.* (2018) investigated the effect of temperature on *Ophraella communa* life history parameters. *O. communa* was exposed to high temperatures (40, 42, and 44°C) at various stages of growth. The larval stage was found to be the most vulnerable to heat stress, with high temperatures having a positive impact on female reproduction but no stage-specific response.

Chevrier *et al.* (2019) conducted an experiment on influence of thermal stress on fertility of *Anisoperomalus calandreae* male insects. The early pupal stages of male insects were given the treatment of thermal stress at 48°C for 10 minutes. As a result, exposure to temperature stress reduced the sperm count. Likewise, Green *et al.*, (2019) found that the heat stress (at 30°C) reduced the egg to adult viability as well as the life span and reproductive potential of the surviving adults by reduction in ovary size and less sperm production in fruit fly (*Drosophila suzukii*). Denlinger *et al.* (2019) opined that the survivability of insects reduced in the subsequent stages when they were exposed to the higher temperature for shorter duration and thermal injury in the lower temperatures. Jung *et al.* (2019) predicted that in South Korea due to climate change by 2100, 98 percent of the farms will be infested with *S. litura* when compared to 63% in 2019.

Similarly, Chidhananda (2020) conducted an experiment on the consequences of thermal stress (42 and 46°C) for varying periods of time on developmental stages of *S. litura*. At both 42 and 46°C, there was no hatching success, and the larval survival

rate was lowest at 20 hours and 3 hours of exposure at 42 and 46°C, respectively. After 20 hours of exposure at 42°C, there was no adult emergence from stressed pupae and no adult survival. At both 42 and 46°C, the success of egg and larval maturation decreased as duration of exposure increased. At both higher temperatures, increased duration of thermal exposure had a negative impact on fecundity of *S. litura*.

Sales *et al.* (2021) reported that under laboratory conditions the temperature stress (40°C and 42°C for 5 days) caused most deaths in the juveniles of *Tribolium castaneum*. When they exposed pupal and immature life stage of the insects in thermal stress the reproductive potential of male insects were greatly affected. The exposure of larval stage in the heat stress did not affect the reproductive potential of male insects whereas the pupal and juvenile exposure drastically reduced the reproductive potential.

2.6 Metabolic response of insects exposed to thermal stress

Ahearn *et al.* (1969) studied the water losses in two different desert tenebrionid beetles (*Eleodes armata* and *Cryptoglossa verrucosa*) under different temperature (25°C, 30°C, 35°C, 40°C, 45°C and 50 °C for 12 hours duration) and humidity levels (0% RH). At lower temperature the water loss was less. Water loss through transpiration was prevailed at low temperature and humidity and it was more when the temperature increased above the optimum levels. The loss of water at 40°C and 45°C was significant as a result of the release of quinones in higher temperature levels.

Churchill *et al.* (1989) compared the metabolic responses of a freeze-tolerant (*Eurosta solidaginis*) and a freeze-avoidant (*Epiblema scudderiana*) insects to repeated temperature changes and reported that under cold stress *E. solidaginis* larvae retained a high level of energy and increase in glucose-6-P, indicating that glycogen phosphorylase was activated. Similarly, Chen and Delinger (1990) investigated the response of flesh fly (*Sarcophaga crassipalpis*) to the heat stress and cold stress (40 °C and 0 °C for 2 hours) by activation of phosphorylase enzyme in the body. Under heat and cold stress there was an increase in phosphorylase enzymes than in the optimum temperature levels.

Wolfe *et al.* (1998) studied the function of sorbitol as a thermo-protectant in silver leaf whitefly, *Bemisia argentifolii*. When the insects were subjected to the high temperatures (42°C) the levels of sorbitol increased tenfold. The levels of sorbitol accumulation were directly related with the temperature. They concluded that accumulation of sorbitol was characterised to be the mechanism of regulation of

thermal stress in the silver leaf insects. Likewise, Salvucci (2000) studied the thermo protective property of sorbitol and opined that under heat stress (44°C and 46°C) the accumulated concentrations of sorbitol protected the soluble proteins in whiteflies.

Nestel *et al.* (2003) investigated the levels of glycogen, haemolymph carbohydrate, and protein contents in different stages of the medfly (*Ceratitis capitata*). Glycogen and haemolymph carbohydrates are heavily used from the time the larvae stopped feeding until the mid-pupal stage, after which glycogen levels rise until the mid-pharate-adult stage, then dropped to low levels at the time of adult emergence. Protein levels dropped during the prepupal stage (coinciding with an increase in acid proteinase activity), then raised again as the pharate-adult stage approached.

The high temperature stress resulted in the drastic reduction of water levels in two spider beetles *Mezium affine* (50°C) and *Gibbium aequinoctiale* (54°C) and lead to mortality in insects, this was striking evidence that higher temperatures affect the water content (Yoder *et al.*, 2009). Similarly, the insects can also be harmed by the extreme temperatures due to water loss through evaporation (EWL) and reduced metabolism because of denaturation of enzymes. Peak metabolic rate was observed in the Ichneumonid wasp at 35°C, but further increase in temperature (40°C) lead to the reduction of metabolism rate and water loss (Tomlinson and Phillips, 2012)

Dmochowska *et al.* (2013) investigated the effects of prolonged post diapause on the metabolism of red mason bee (*Osmia rufa*). In summer months the glucose levels were more when compared to that of spring months due to increased activity of trehalase and maltase whereas no changes in the sucrase and cellobiase activities and there was a considerable reduction in the amylase activity. In the same way, Lencioni *et al.* (2013) investigated the link between rise in temperature and expression of heat shock proteins (*HSP 70*) of the insect *Diamesa cinerella*. With the extended exposure time (LT- 32°C for 1.73 hours), the expression of heat shock proteins was prevalent but more the temperature, survival rate was reduced.

Ju *et al.* (2014) exposed the *Corythucha ciliata* adults to high temperature stress in field (10.00 to 14.00 hours at 33.5 °C to 37.2 °C) and laboratory (2 hours at 33-43°C with 2 °C intervals) conditions, results showed that the water content decreased but other metabolic attributes increased irrespective of the field and laboratory.

In the investigation of metabolic activities under cold stress (18 h and 38 h at 4°C), Chowanski *et al.* (2015) found that there was slight reduction in the glycogen

levels while, other metabolites like polyols, glucose and total protein increased in the Madagascar cockroach (*Gromphadorhina coquereliana*). Similarly, when the silkworm larvae infected with fungal spores (*Beauveria bassiana*) the levels of total carbohydrates, amino acids and other metabolites increased. This result clearly indicates that stress could alter the energy budget and nutrient metabolisms in insects (Xu *et al.*, 2015).

For determination of total protein, carbohydrates and lipids in the coleopteran insects Bozdogan *et al.* (2016) worked on adult insects in the lab conditions. They found that no correlation between wet weight and metabolites. Further, they quoted that the level of metabolites also varied between insects of same genera. Likewise, Klepsatel *et al.* (2016) focused on how metabolic and physiological processes change with temperature, as well as the importance of determining the effects of climate change on *Drosophila melanogaster* energy reserves. They discovered that a constant increase in temperature or intermittent thermal stress induces a significant loss of stored metabolites, which had a negative impact on organism energy reserves.

Ghaedi and Andrew (2017) conducted an experiment on single (38°C- 3 hours) and multiple (38°C- 1 hour; 3 times) thermal stress in the adult *Myzus persicae* insects and their effects on the physiology of the insects. They concluded that multiple stresses induced the increased levels of glucose, more protein expression and synthesis of osmolytes than that of the single stress. Also, reduction in the source of energy in the multiple temperature stress than the single stress. Similarly, in Indian meal moth *Plodia interpunctella* temperature stress of 44 °C for one hour increased the mortality and the level of polyols and heat shock proteins due to thermal stress (Kim *et al.*, 2017).

Under thermal stress at higher temperature there was an increase in the levels of amino acids in *Drosophila melanogaster*. The levels of sugars were less in the both lower temperature and higher temperatures but at the optimum temperatures sugar levels attained maximum (Schou *et al.*, 2017). In the same manner, Chen *et al.* (2018) conducted an experiment on the consequences of thermal stress (40, 42 and 44°C for 3 hours per day, for 3,5,5 and 5 days for egg, larva, pupa and adult respectively) on the total protein content of *Ophraella communa*. They concluded that in short term stress, though the higher temperature prevailed the total protein content was almost same that of the control but under long- term exposure to the thermal stress (44°C) the total protein content was increased.

3. MATERIAL AND METHODS

The experiments on physiological responses of *Spodoptera litura* exposed to short and long-term thermal stress were conducted in the Division of Entomology, ICAR- Indian Agricultural Research Institute, Pusa Campus, New Delhi, during the year 2020-2021. This chapter briefly covers the materials used for rearing, equipments, chemicals, as well as the methodology followed to perform experiments and statistical analysis for the mentioned objectives.

3.1. Materials

3.1.1. Glass and Plastic wares

1. For rearing of *Spodoptera litura* plastic and glass jars (16 cm×8 cm) were used
2. For storing chemical solutions various sizes reagent bottles were used
3. Plastic Petri dishes (5-6 cm in diameter) were used for culturing of *Spodoptera litura*
4. For keeping the insect containing Petri plates, pupal vials, plastic trays (45 ×15 cm and 30 ×15 cm size) were used
5. Micro centrifuge tubes (1.5 & 2 ml) and Falcon tubes (15 & 50 ml) were used for homogenization the samples
6. Micro pestle for homogenizing adult insects
7. Squeeze bottles used during ovary dissection (Ringer's solution kept inside those bottles)
8. Glass rods used for uniform mixing of reagents
9. Plastic micropipette tips
10. Glass slides used for eupyrene sperm count

3.1.2. Instruments

- ❖ Stereo zoom binocular microscope with light source used for egg and eupyrene sperm count
- ❖ Temperature and humidity controlled incubator capable of maintaining the necessary temperatures (27°C, 42°C, 46°C) and relative humidity (70±5 per

cent) to investigate the effects of thermal stress on *Spodoptera litura* biological parameters.

- ❖ Vortex machine
- ❖ Micro plate reader (Bio Tek Instruments, Inc/USA)
- ❖ 96 wells ELISA micro plates
- ❖ Hand held motorised homogenizer
- ❖ Refrigerated centrifuge (Eppendroff)
- ❖ Mixer grinder
- ❖ Hot air oven
- ❖ Weighing balance
- ❖ Auto montage fitted with a computer for taking photos of the insect developmental stages
- ❖ Humidifier to maintain the culture room relative humidity (RH)
- ❖ Mini cooler (-20°C)
- ❖ Refrigerator (4°C)
- ❖ Deep freezer (-80°C)
- ❖ Micro pipettes (1-10 µl, 2-20µl, 10-100µl, 20-200 µl and 100-1000µl)
- ❖ Water bath

3.1.3. Experimental material

The *Spodoptera litura* larvae collected from fields were mass reared in culture room facility of insect physiology laboratory in Division of Entomology, ICAR-IARI, New Delhi. For insects rearing semi-synthetic artificial diet (larvae) and honey solution (adults) were used. Insects were reared in the optimum temperature at 27±1°C with 65±5% relative humidity, and 14:10 h light: dark cycle.

3.1.4. Reagents

1. Ethanol (70%) used for preventing fungal or bacterial contamination in petri plates, rearing trays, mating jars through surface sterilization.
2. For Gonadosomatic index

- Ringer's solution (KCl- 0.24g, NaCl- 11.2g, CaCl₂-0.27g and NaHCO₃- 0.2g in 1 liter of DDW)
3. For sperm count
- Belar's saline – which comprised of 6 g NaCl, 0.2 g KCl, 0.2 g CaCl₂, 0.2 g Na₂CO₃ in 1 liter of double distilled water (Flint and Kressin, 1969).
 - Lacto-aceto-orcein (LAO) Stain (2%)
4. For protein estimation
- Bovine serum albumin (BSA) as standard
 - Bradford reagent
 - Aqueous lysis buffer
 - Dithiothreitol (DTT)
 - Potassium dihydrogen phosphate (KH₂PO₄)
 - Ethylene diamine tetra acetic acid (EDTA)
5. For Carbohydrate Assay
- Glucose as standard
 - Disodium sulphate
 - Chloroform
 - Methanol
 - Anthrone
 - Concentrated Sulfuric acid (Conc. H₂SO₄)
6. For Glycogen assay
1. Disodium sulphate (Na₂SO₄)
 2. Chloroform
 3. Methanol
 4. Potassium hydroxide (KOH)
 5. Ethanol
 6. Calcium chloride (CaCl₂)

7. Iodine reagent (130 ml of CaCl₂ mixed with 0.5 ml of Iodine iodide solution)
8. Potassium iodide (KI)
9. Iodine (I₂)

3.1.5. Other materials used

- ❖ Black and white muslin clothes and rubber bands to secure the rearing jars containing *S. litura* adults
- ❖ Cotton plugs, tissue papers, blotting paper, aluminium foil, etc.
- ❖ Needles, forceps, camel hair brushes of various sizes for handling different stages of *S. litura*
- ❖ Honey solution (10 %)
- ❖ Semi synthetic diet
- ❖ Thermocol sheet and insect pins for stretching adults and pinning

Table 3.1. Chemical composition of semi-synthetic Diet

Fractions	Ingredients	Quantity
Part-I	Bengal gram <i>Cicer arietinum</i> var. <i>kabuli</i> flour	90.0 g
	Yeast Extract Powder	11.0 g
	Casein fat free	5.0 g
	L-ascorbic acid	3.0 g
	Methyl-p-hydroxy benzoate	2.0 g
	Sorbic acid	1.0 g
	Cholesterol	0.2 g
	Streptomycin sulphate	0.4 g
	Formaldehyde solution (40%)	1.0 ml
	Multivitamin and multimineral drops	1.0 ml
	Vitamin E USP 400 mg (Evion)	2 capsules
	Double distilled water	300 ml
Part-II	Agar-agar powder	16.0 g
	Double distilled water	300 ml

Table 3.2. Chemical composition of honey Solution (10%)

Particulars	Quantity
L-ascorbic acid	1.2g
Methyl paraben	0.2 g
Honey	20 ml
Vitamin –E	2 capsules
Multi vitamin	1.0 ml
Distilled water	200 MI

3.2. Methodology

3.2.1. Plan of work

It includes following activities

1. Rearing of *S. litura* in culture room on artificial semi-synthetic diet
2. Studies on effect of short term and long-term temperature stress on reproductive fitness of *S. litura*
3. Studies on metabolic responses in *S. litura* due to thermal stress

3.2.2. Prerequisites for rearing *Spodoptera litura*

Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae), the tobacco caterpillar, is an economically important and polyphagous pest in India, and is one of the major threats to today's intensive farming and changing crop patterns worldwide. To generate healthy *S. litura* stages in enough numbers for laboratory studies, a cost-effective mass culturing system is needed. For laboratory and field studies, continuous supplies of test insects are needed for which mass culturing on natural or artificial diet is widely practiced.

To maintain the culture of *S. litura*, egg masses or larvae were collected from cabbage and castor crops cultivated in fields of ICAR-Indian Agricultural Research Institute, New Delhi. These collected egg masses were kept on moistened blotting paper in the small plastic jars covered with muslin cloth and the collected larvae were released individually into Petri-dishes containing semi synthetic diet and both were maintained under optimum conditions of $27\pm 1^{\circ}\text{C}$, $65\pm 5\%\text{RH}$ and photoperiod of 14:10(L:D) h in insect rearing laboratory at Division of Entomology, ICAR-IARI, New Delhi. Until

pupation the larvae were provided with artificial semi-synthetic diet, freshly formed pupa were sexed and kept in the individual vials. The filter paper kept inside the vials before the transfer of pupa to make emergence easy. After the emergence of adults, for the successful mating and oviposition the moths were released in the jars (male: female moths in ratio 7:4) top covered with the black muslin cloth. As a food supplement, adults were given honey solution dipped in cotton. To aid egg laying, blotting paper strips were placed in the mating jar.

The laid eggs were fetched from the paper strips on the day-to-day basis and kept in small Petri-plates and eggs were sprinkled with water to avoid drying. The newly laid eggs were covered with turf of hairs. The colour of the egg mass was brown and before hatching it transformed into black colour. The neonates of F₁ generation were reared together but after 3-4 days they were separated and reared individually in a small Petri dish (5-6cm) containing semi synthetic diet. To obtain the healthy culture of *S. litura* proper hygienic conditions optimum conditions were maintained. To study the short and long-term thermal stress on the reproductive fitness and metabolic responses, the F₄ generation insects were used.

3.2.3. To study the effects of short and long-term thermal stress on different developmental stages of *Spodoptera litura*

For studying the effects of thermal stress on the biological attributes of *S. litura* all the developmental stages of the insects were chosen *viz.*, freshly laid eggs, 4th instar larva, freshly formed pupa and adults (both male and female). To study the impacts of short and long-term thermal stress on the different developmental stages of *S. litura*, the insects were exposed to two higher temperatures 42°C and 46°C for 4 hour and 1 hour respectively in a short and long-term stress with a RH of 60±5%. The maximum temperature and relative humidity during summer months in Delhi will be around 42-46°C for shorter duration and humidity will be 55-65%, based on this temperature and exposure durations were decided to study the effect of thermal stress on reproductive physiology of *S. litura*. Different developmental stages of *S. litura viz.*, 100 eggs (≤18hrs old), 100 4th instar larvae (newly molt, ≤12hrs old), 100 pupae 100 (newly formed, ≤12hrs old), male and female adults 30 number (newly emerged, ≤12hrs old) were kept under two different temperatures *viz.*, 42°C for 4 hour one time exposure in single stress and 2, 4, 2 and 2 days for egg, larva, pupa and adult in multiple stress respectively, 46°C for 1 hour, one time exposure in single stress and 2,4,2 and 2 days

for egg, larva, pupa and adult in multiple stress respectively by using temperature and humidity controlled incubator. Control insects were maintained at $27\pm 1^{\circ}\text{C}$, the exposure periods were determined based on the biology of *S. litura* reared at $27\pm 1^{\circ}\text{C}$. To avoid the starvation stress in larva and adults during the thermal stress exposure they were given artificial diet and honey solution respectively. After the thermal stress the regular renewal of diet was practiced.

Emerged adults from thermal stressed eggs, larvae and pupae and surviving adults after stress treatments from both single and multiple stress were allowed to oviposit in a mating jar maintained at $27\pm 1^{\circ}\text{C}$, $65\pm 5\%$ relative humidity and 14:10 h light: dark cycle. The observations like survival percent, maturation success, female ratio, longevity of male and female adults, size and weight of male and female adults, gonadosomatic index, eupyrene sperm count, pre oviposition period, oviposition period, post oviposition period, total fecundity, mating success, mating frequency, fertility percent from the adults emerged from different stressed stages like egg, larva, pupa and surviving adults.

Estimation of survival rate

After thermal stress the developmental stages (egg, larvae, pupa, adult) of the insects were studied for the survival rate through exposing them to 42°C and 46°C for single and multiple times for 4 hours and 1 hour respectively. The immediate survival of the adult stage was calculated by percent survival measured following recovery period of 1 hour after thermal stress at $27\pm 1^{\circ}\text{C}$. In egg, larva and pupal stages, it was calculated by percent hatching success from stressed eggs, percent pupa formed from stressed larva and percent adult emergence from stressed pupa respectively, the survival of stressed egg, larva and pupa was calculated using formula

$$= \frac{\text{No. of emerged individuals in the next stage after treatment}}{\text{No. of individuals in treatment stage}} \times 100$$

For calculating the adult survival rate the following formula was used,

$$= \frac{\text{No. of survived adults}}{\text{Total no. of adults exposed}} \times 100$$

Estimation of maturation Success

The impact of temperature stress on successive survival i.e., maturation success for treated eggs, 4th instar larva was investigated as an effect of temperature stress.

Maturation success is defined by the proportion of healthy eclosed adults from hatched eggs, surviving larva after heat exposure at 42 and 46°C for 4 hours and 1 hour respectively for single and multiple times. In the evaluation of maturation success healthy adult insects were taken into account, the deformed adults were excluded. The formula used for maturation success (Chen *et al.*, 2018) is given below,

$$= \frac{\text{No. of healthy emerged adults from treated stage}}{\text{Total no. of insects in the treated stage}} \times 100$$

Estimation of female ratio

Egg and larval stages were exposed to short and long-term temperature stress for estimation of female ratio. Pupa formed from the stressed egg and larva were sexed manually and separated as male and female individuals. Then the number of female and male pupa were counted and recorded. The female ratio was worked out using formula;

$$= \frac{\text{No. of female individuals}}{\text{Total no. of females and males}} \times 100$$

Estimation of total longevity of male and female adult insects

The male and female adult insects emerged from different developmental stages from various temperature stress treatments were released in the mating jar at the ratio of 2:2. Survival of the male and female adults was monitored in the mating jar on day-to-day basis. Until the death of the adults in the mating jar this monitoring process was continued. The duration taken from the emergence of the adult individual insects to the death was measured and this duration was considered as the longevity of the adults. The total longevity of the adult insects was calculated as the time interval between the emergence to the death of the adult insect.

Measurement of size and weight of the adult insects

The freshly emerged adults from the different stress treatments were taken into account for measuring the weight and size. To avoid the movement of the insects in weighing balance and measuring scale, they were kept inside the refrigerator for 10-15 minutes. Using digital weighing-balance the weight of the insects from different treatments and control were recorded. The observations were recorded in the unit of grams (g) for adult insect weight using an analytical balance. For measuring the size, male and female adults were killed, stretched and pinned on the surface of the thermocol

and by using measuring scale the wingspan of the adult insects was measured and recorded in centimetre (cm).

Estimation of gonadosomatic index (GSI)

To calculate the GSI six female adults were used per temperature stress treatment. Different developmental stages of insects treated with temperature stress, emerged adults from the stressed stages (less than or equal to 12 hours of eclosion) were initially weighed and dissected for the ovaries. Insect ringer's solution was used for removal of fat bodies and isolated ovaries were observed under the microscope. The rinsed ovaries kept for drying with the use of blotting paper. Before measuring the weight of wet ovaries empty blotting paper weight was noted. To measure the weight of the ovaries digital weighing balance was used. The calculation of GSI was done by using the formula (Paul and Keshan, 2016),

$$GSI = \frac{\text{Weight of the wet ovary (g)}}{\text{Total body weight (g)}} \times 100$$

Enumeration of eupyrene sperm

Lepidopterans sperm production is dimorphic in nature, they produce two type of sperms *viz.*, nucleated eupyrene sperms and anucleated non-fertile apyrene sperm (Meves, 1903), out of two types of sperms only eupyrene sperms are known to fertilise eggs (Cook and Wedell, 1996). Eupyrene sperm bundles will take up the stain while apyrene sperm bundles do not take up the stain upon staining with lacto-aceto-orcein (LAO) stain. For counting eupyrene sperms the freshly emerged adult males were used. Further, they were dissected using Belar's saline solution in 11 AM in the morning. Testes was removed from the dissected male reproductive tract and placed inside 0.5 ml micro centrifuge tube containing 60µl of LAO stain (2%) solution. The maceration process was done manually on the separated testes and thorough mixing of the contents was done by vortexing. From the micro centrifuge tube, 10µl aliquot was taken on the glass slides and used for counting of the eupyrene sperm. For this experiment, we used 6 aliquots per individual. Manual counting of the eupyrene sperm was done with the help of stereo zoom binocular microscope.

Assessment of reproductive fitness

Adults emerged from the thermal stress treatment of different developmental stages (egg, larva, pupa) and survived adults after thermal stress were used for

assessment of the effects of temperature stress (short and long- term exposure at 42°C and 46°C) on their reproductive fitness. Adults reared in the temperature of 27±1°C were considered as control for different thermal stress treatments. The freshly emerged male and female adults were kept inside the mating jar (16×8 cm) in 2:2 ratio along with this 10% honey solution was provided in the cotton swab as food. Five mating jars were maintained per treatment, the bottom jars were filled with water to maintain humidity at micro environment level. Mating jars were lined with butter paper and to aid in oviposition paper strips were kept inside the mating jar. The mating jars were covered with black muslin cloth and jars were maintained at temperature of 27±1°C and RH of 65±5%. The jars were checked daily for the oviposition and regular replacement of the paper strips was done for egg count. The jars were closely monitored for pre-oviposition, oviposition and post-oviposition periods. Dead female adults were used for calculating mating success and mating frequency.

Pre-oviposition period

This was calculated as the time interval between the day of release of female adult insects to the mating jar and the day of female started egg laying and it was recorded in days.

Oviposition period

It was calculated as the duration between starting of oviposition to end of oviposition by female adults and it is denoted in days.

Post oviposition period

It was measured as the duration between the end of oviposition to the death of female adults and it was recorded in days.

Mating success and mating frequency

For calculating the mating success, male and female adults from the stressed stages (egg, larva, pupa and adults) were released to the mating jar with honey solution in 2:2 ratio. After death of the insects, female individuals separated and their abdomen was dissected to count the number of spermatophores based on treatment. Mating success was expressed as percentage and it was calculated using the formula (Seth *et al.*, 2016),

$$= \frac{\text{No. of females with one or more spermatophores}}{\text{Total no. of females}} \times 100$$

The mating frequency of the insects was calculated using the formula (Seth *et al.*, 2016)

$$= \frac{\text{Total no. of spermatophores in mated females}}{\text{Total no. of mated females}}$$

Egg count/ Total Fecundity

During oviposition period, the eggs on the butter paper strips and black muslin cloth were collected regularly on day-to-day basis. After collection of the paper strips from the mating jar they were counted with the help of stereo zoom binocular microscope manually. The total number of eggs laid by the female adults during oviposition period referred as the fecundity/egg laying capacity of insect.

Fertility percent

To estimate the F₁ hatching/fertility percent, 100 eggs were collected from each jar of different thermal stress treatments and placed in the Petri plates lined with blotting paper and eggs were sprinkled with water to avoid drying. After successful hatching the neonates were counted under the microscope and the fertility percent was calculated using the formula,

$$= \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs}} \times 100$$

Statistical analysis

The collected information on different parameters (survival rate, maturation success, female ratio, adult male and female longevity, body size, body weight, gonadosomatic index, eupyrene sperm count, pre-oviposition, oviposition, post-oviposition period, total fecundity, mating success, mating frequency, fertility percent) were analysed statistically. Initially data was checked for normality before performing analysis of variance and all percent values were arcsine transformed before statistical analysis. Individual parameters were considered separately for the statistical analysis and the data was subjected to two-way analysis of variance (ANOVA) in the completely randomized design (CRD) to test the effect of temperature treatments, stage and their interactions on the biological attributes of *S. litura*. The means were separated stage and temperatures wise using Tukey's honestly significant difference (HSD), when

significant differences were observed ($p < 0.05$) and they were represented as mean \pm SE (standard error) using SPSS v 16.0 statistical package.

3.2.4. To study the metabolic responses in adult *Spodoptera litura* exposed to temperature stress at egg and larval stages

One of the important abiotic factors which have considerable impact on physiology, habit and competence of insects is temperature (Hance *et al.*, 2007). Due to change in climatic conditions and recurrent heat wave events the organisms at different levels anticipated to experience temperature stress. This abnormal increase in temperature may result in different stress responses physiologically. Insects being ectotherms, their dependence to the surrounding temperature for the metabolic activities is high. Insects can also be affected from this extreme temperature stress which might lead to irreparable changes in their physiology. Insects have composite life cycle and various life stages and they present in different environments at different stage and time. Due to this variability the insects may encounter different levels of stress. Because of this stress differences they react physiologically in various forms and rates (Coyne *et al.*, 1983; Kingsolver *et al.*, 2011). Hence, the rate of impact varies from one stage to another (Chiu *et al.*, 2015).

Thermoregulation mechanisms are needed for the insects to regulate their habitual and metabolic activities under temperature stress (Kim *et al.*, 2015). Ectotherms capacity to maintain performance and fitness despite variations in the environment is determined by their ability to dynamically alter physiological systems in reaction to variations in environmental temperatures (Kern *et al.*, 2015). Under increased heat stress insect individuals might acquire evolutionary response or plastic response or the combination of both these mechanisms (Esperk *et al.*, 2016). Insects can able to alter their metabolites based on the temperature stress for the adaptation and acclimation (Hariharan *et al.*, 2014; Schou *et al.*, 2017). Sugar alcohols and heat shock proteins are released to overcome the stress events at both higher and lower temperatures. Some species of insects exhibit almost identical responses for both higher and lower temperature stresses (Sejerkilde *et al.*, 2003; Sinclair and Chown, 2003). To protect the cell membrane and protein from denaturation, insect will accumulate sugar alcohols in their body under temperature stress (Salvucci, 2000).

In this experiment, we assessed the effects of temperature stress on the metabolic responses in the adult insects of *Spodoptera litura*, when egg and 4th instar larvae was given thermal stress. To identify the effects of temperature stress the insect eggs and 4th instar larvae were exposed to 42°C for 4 hours and 46°C for 1 hour (one time stress in egg and 4th instar larva- short term stress). Multiple stress treatments were given to the insects by exposing them to 42°C for 4 hours and 46°C for 1 hour per day (2 days for egg, 4 days for larvae), in control the insects were maintained at 27±1°C. Emerged adult male and females from each treatment were taken for the estimation of water content, protein, carbohydrates and glycogen contents by using the standard procedures.

A. Estimation of water content (%)

Materials used

1. Hot air oven
2. Digital weighing balance
3. Petri plates
4. Plastic trays
5. Thermometer
6. Refrigerator

Methodology

Freshly emerged adults (less than 12 hours from emergence) of 10 male and 10 female insects from each temperature stress treatment and control were taken and kept individually in a separate vial and labelled. Later these vials were frozen for 2 hours in deep freezer (-80°C). The insects kept inside the deep freezer were taken out and the fresh weight (g) of the individual adult male and female insects was recorded with the help of digital weighing balance. After measuring the fresh weight, the individual insects were placed in glass Petri-plates and labelled. These labelled glass Petri-plates were placed inside the hot air oven for drying. In hot air oven, the temperature was maintained at 60°C and they were kept for 48 hours for drying. After 48 hours, the individual insects were taken from the hot air oven and they were again weighed with the help of electronic weighing balance. This recorded weight after oven drying was considered as dry weight (in g) of the insect. With the recorded fresh and dry weights of the insects, the water content of different treatments was calculated using the formula,

$$WC (\%) = \frac{FW - DW}{FW} \times 100$$

Where,

WC- Water content in percentage

FW- Fresh weight in g

DW- Dry weight in g

B. Estimation of protein

Materials used

1. Micro centrifuge tubes (2ml)
2. Electronic weighing balance
3. Hand held homogenizer
4. Micro-pipettes
5. Plastic micro-pipette tips
6. Micro pestle
7. Mini cooler (-20°C)
8. Centrifuge
9. Incubator
10. ELISA microplates
11. ELISA reader

Reagents used

1. Aqueous lysis buffer (100 mM KH₂PO₄, 1mM DTT and 1mM EDTA)
2. Bradford reagent
3. Bovine serum albumin (BSA) standard

Preparation of standard graph

In our experiment, we used the methodology given by Bradford (1976) for the estimation of protein content in the extract of insect samples. In this method, as a standard we used Bovine serum albumin (BSA). For the preparation of stock solution of Bovine serum albumin (BSA) 5 mg of BSA was taken and dissolved in 5 ml of

aqueous homogenizing buffer. For the purpose of preparing standard curve, stock solution of BSA was pipetted out in different aliquots (0 μ l, 60 μ l, 80 μ l, 100 μ l, 200 μ l, 400 μ l, 600 μ l, 800 μ l and 1000 μ l) into micro centrifuge tubes and total final volume was made into 1ml with the addition of aqueous lysis buffer. From these serially diluted BSA standard solutions 10 μ l was taken and mixed with 500 μ l of Bradford reagent. After this the standard solutions were incubated in the dark place for 25 to 30 minutes (under room temperature). After incubation period the absorbance was read at 595 nm in micro plate reader. From the obtained readings the standard curve was prepared.

Extraction

Adult insects (5 male and female insects in each treatment) from different temperature stress treatments were taken and placed in deep freezer (-80°C) for 2 hours. After two hours the insects were taken out from the refrigerator and 50 mg body tissue was taken with the help of electronic weighing balance. Weighed insect tissues from different treatments were placed inside 2ml micro centrifuge tubes. After this 300 μ l of aqueous lysis buffer (100 mM of KH_2PO_4 -1.368g, 1mM DTT-15.412mg and 1mM EDTA-37.224mg in 100 ml of distilled water) was added into the tubes. The samples were homogenized manually after adding the buffer solution for 2 minutes. After the manual homogenization the remaining 700 μ l of the aqueous lysis buffer was added to the tubes. The contents in the tubes were completely homogenized using the handheld homogenizer for 2 minutes. Homogenized extract was then centrifuged at 10000 rpm for 10 minutes at 4°C, then supernatant was transferred into fresh micro centrifuge tubes.

Estimation

After centrifugation, the supernatant of the samples was collected at the rate of 10 μ l in the separate micro centrifuge tubes. The Bradford reagent was added to those sample solutions at 500 μ l. Later these samples were incubated for 25-30 minutes at room temperature in dark conditions. The samples were taken in the ELISA micro plates and the micro plates were kept inside the ELISA micro plate reader at 595 nm for taking readings. The obtained readings from ELISA reader were compared with the standard graph of Bovine serum albumin (BSA) standard to estimate the values of protein content in the insect tissues. The protein content was expressed as μ g/mg of tissue.

C. Estimation of Carbohydrate content

Materials used

1. Refrigerator
2. Weighing balance
3. Micro centrifuge tubes (2 ml) and rack
4. Micro-pipettes
5. Plastic micro-pipette tips
6. Micro pestle
7. Hand held homogeniser
8. Mini cooler (20°C)
9. Refrigerated Centrifuge
10. Water bath
11. Plastic float rack
12. ELISA plates
13. Micro plate ELISA reader (Biotek instruments Inc.)

Reagents used

1. 2% Na₂SO₄
2. Chloroform
3. Methanol
4. Anthrone reagent (500 mg anthrone in 500 ml Conc.H₂SO₄)
5. Glucose as a standard

Preparation of standard graph

Carbohydrate contents were determined by using the glucose as a standard. The stock solution of glucose standard was prepared by dissolving 5 mg of glucose in 5 ml 2% Na₂SO₄ buffer. For preparing standard curve, the volume of 0, 50, 100, 200, 300, 400 and 500 µl of glucose solution was pipetted in the different micro centrifuge tubes and the 1ml final volume was made up by adding 2% Na₂SO₄ buffer solution to the

tubes. From these dilutions 300µl of each glucose standard was taken and 200µl of water was added to it along with 1ml of anthrone reagent [Anthrone (500mg) and H₂SO₄ (500ml) in 1:1 ratio]. After addition of reagents the aliquot was thoroughly mixed manually and also by the help of vortexer. These micro centrifuge tubes were kept in the water bath at 90°C for 10 minutes with the help of plastic float rack. For the blank solution 0µl of glucose and 1000µl of 2% Na₂SO₄ were added. The different standard solutions of 200µl pipetted into ELISA micro plates and placed inside the ELISA reader and absorbance was read at 630nm. From the obtained readings, the standard graph was prepared for estimation of carbohydrates.

Extraction

After exposing freshly laid egg and 4th instar larval stage to the temperature stress, the adults emerged (5 male and 5 female) from them were kept in deep freezer (-80°C) for freezing for 2 hours. After two hours, the frozen insects were collected from the refrigerator. From each insect 50 mg of the body tissue was taken with the help of weighing balance. The collected tissue was placed in the 2 ml micro centrifuge tube, to that 200 µl of 2% Na₂SO₄ was added and homogenised manually for 2 minutes. After manual homogenisation each tube contents was completely homogenised using hand held homogeniser for 2 minutes. Then 1300 µl of chloroform and methanol solution in the ratio of 1:2 was added to the homogenized samples. This was thoroughly mixed with the help of vortex mixer. Later these samples were centrifuged individually for 10 minutes at 7600 rpm at 4°C. The supernatant from the centrifuged samples were taken in separate micro centrifuge tubes of 300µl.

Estimation

To the supernatant, 200µl of water and 1ml anthrone reagent (500mg anthrone in 500 ml of Conc.H₂SO₄) was added and vortexed. These micro centrifuge tubes were kept in the water bath at 90°C for 10 minutes with the help of plastic float rack. After this the samples were kept inside the ELISA micro plate reader using ELISA micro plates (150µl of sample kept in each well) and absorbance was read at 630nm. Estimation of carbohydrates was done by comparing the obtained readings of samples to that of the standards (Glucose as a standard) in the standard graph (Bemani *et al.*, 2012). The carbohydrate content was expressed as µg/mg of tissue.

D. Estimation of glycogen

Materials used

1. Falcon tubes
2. Micro centrifuge tubes (2 ml volume) and rack
3. Water bath
4. Hand held homogeniser
5. Refrigerated Centrifuge
6. ELISA micro plates
7. ELISA micro plate reader (Biotek)
8. Mini cooler
9. Refrigerator
10. Micro-pipettes (500, 200 and 20 μ l)
11. Plastic micro-pipette tips
12. Micro pestle

Reagents used

1. Disodium sulphate (Na_2SO_4)
2. Chloroform
3. Methanol
4. 33% Potassium hydroxide (KOH)
5. Ethanol (96%)
6. Calcium chloride (CaCl_2)
7. Potassium iodide (KI)
8. Iodine (I_2)
9. Iodine-iodide solution (0.26g of I_2 and 2.6g of KI dissolved in 10ml of distilled water) (Krisman, 1962)
10. Iodine reagent (130 ml of CaCl_2 mixed with 0.5 ml of Iodine iodide solution) (Krisman, 1962)

Preparation of standard graph

Glycogen content of the insect tissues was estimated by using glycogen as standard. The stock solution was prepared by adding 5 mg of glycogen in 5 ml of 2% Na₂SO₄. From this stock solution 100µl, 200µl, 400µl, 800µl, 1600µl, 2000µl, 2400µl, 2800µl and 3200µl were taken and the volumes were made to 5 ml each by adding Na₂SO₄ to it and the respective concentrations were 0.5, 1, 2, 4, 8, 10, 12, 14 and 16 µg/ml. From these standard solutions 50µl was taken and 250µl of I₂ reagent (130 ml of CaCl₂ mixed with 0.5 ml of Iodine iodide solution) was added (Krisman, 1962). From the above solution 100µl of aliquot was taken in the ELISA micro plates (3 replications with each 100µl per well). These micro plates were placed inside the ELISA micro plate reader for readings at 450 nm. The readings were taken and the standard graph was prepared.

Extraction

After exposing to the temperature stress (egg and 4th instar larval stage), the adults male (5 individuals) and female (5 individuals) emerged were kept in deep freezer (-80°C) for freezing for 2 hours. After two hours, the frozen insects were taken back from deep freezer. From each insect 50 mg of the body tissue was taken with the help of weighing balance. The collected tissue was placed in the 2 ml micro centrifuge tube, to that the reagents like 200 µl of 2% Na₂SO₄ was added and homogenised manually for 2 minutes. After manual homogenisation each tube was thoroughly homogenised using hand held homogeniser for 2 minutes. In the tube 1300 µl of chloroform and methanol in the ratio of 1:2 was added. Later samples were centrifuged individually for 10 minutes at 7600 rpm at 4°C in the centrifuge. From the centrifuged tubes the supernatant were discarded and the pellet was used for the estimation of glycogen. The pellets from the centrifuged tubes were transferred to the falcon tubes. After transferring 900µl of 33% KOH was added to the individual falcon tubes. Then, the tubes were kept inside the water bath for 15 minutes at 90°C with the help of plastic float rack. The falcon tubes were taken out from the water bath after 15 minutes and these tubes were added with 96% ethanol three times that of the volume of KOH for glycogen precipitation. After addition of ethanol the tubes were transferred carefully to the refrigerator at 4°C for overnight to allow the contents to settle. On the next day the tubes were centrifuged at 5600 rpm for 15 minutes. After centrifuge, the supernatant

was discarded and pellet was washed with distilled water for 2 or 3 times. The pellets were resuspended and mixed with 100µl of distilled water.

Estimation

To 50µl of the sample, 250µl of I₂ reagent (Krisman, 1962) was added and thoroughly mixed and from each sample 100µl of the aliquot was transferred to the ELISA micro plate wells. Three such replications were taken per sample. These samples in micro plates were placed in ELISA micro plate reader and the readings were taken at the wavelength of 450 nm. The obtained readings were compared with the standard graph to estimate the value of glycogen in the insect tissues (Nestel *et al.*, 2003). The glycogen content was expressed as µg/mg of tissue.

Statistical analysis

The collected information on different parameters (water content, protein, carbohydrates and glycogen content) of different temperature stress treatments were analysed statistically. Individual parameters were considered separately for the statistical analysis and the data was subjected to two-way analysis of variance (ANOVA) in the completely randomized design (CRD) to test the effect of temperature treatments (27°C, 42°C SS, 42°C MS, 46°C SS and 46°C MS), stage (male and female adults emerged from stressed eggs and larvae) and their interactions on the metabolic contents of *S. litura*. The means were separated temperature and stage wise using Tukey's honestly significant difference (HSD), when significant differences were observed ($p < 0.05$) and they were represented as mean \pm SE (standard error) using SPSS v 16.0 statistical package.



Egg patch of *Spodoptera litura*



Larva on diet hill



Rearing larva on artificial diet



Changing of diet



Pupal vials



Mating jars

Plate 1. Culturing of Tobacco cutworm (*Spodoptera litura*)



Plate 2. Incubator with temperature controlling facility for maintaining required temperatures for thermal stress



Plate 3. Thermal stress exposure of eggs



Plate 4. Thermal stress exposure of larva

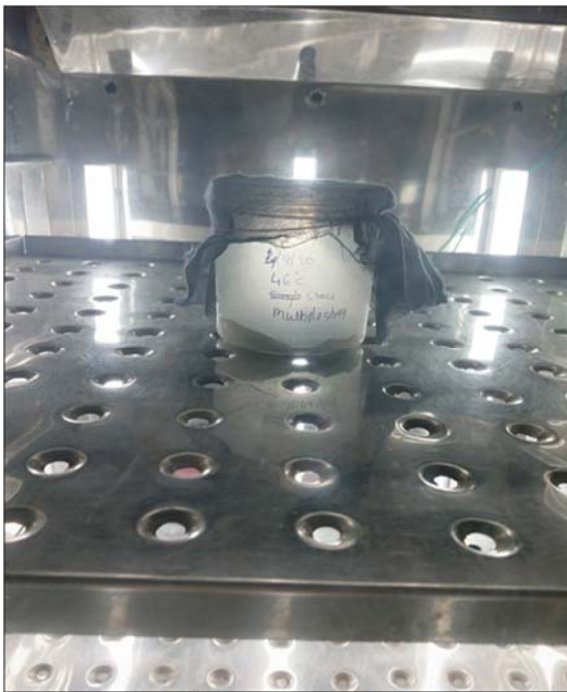


Plate 5. Thermal stress exposure of pupa



Plate 6. Measuring size of adult



Plate 7. Microscopic view of spermatophore



Plate 8. Dissected ovaries

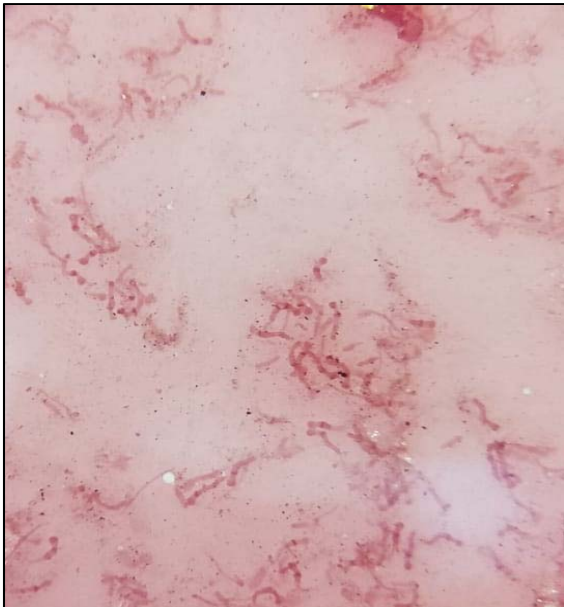


Plate 9. Eupyrene sperm bundles



Plate 10. Stereo zoom microscope



3rd instar larva



5th instar larva



6th instar larva



End of larval stage

Plate 11. Larval instars



Male



Female

Plate 12. Differentiation of Male and female pupa

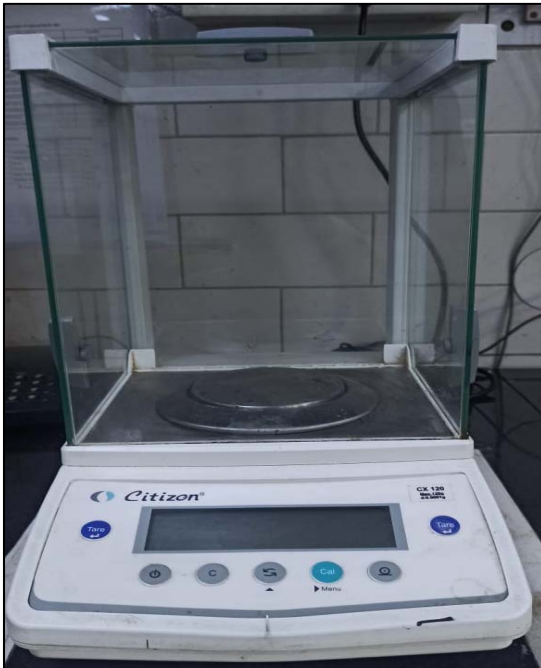


Plate 13. Weighing balance



Plate 14. Homogenizer and Homogenized samples



Plate 15. Water bath



Plate 16. Hot air oven



Plate 17. Centrifuge

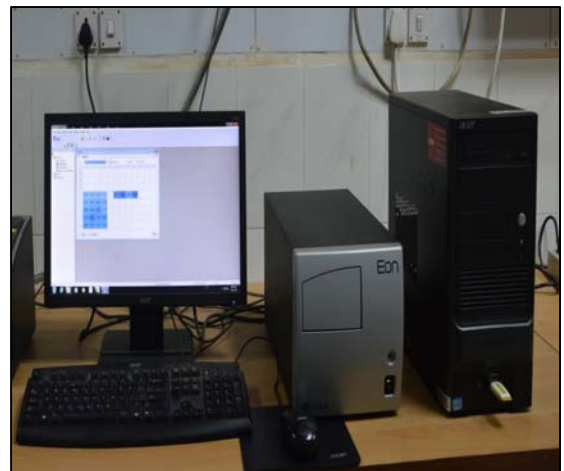


Plate 18. ELISA micro plate reader

IV. RESULTS

As a result of climate change the mean annual temperatures are in increasing trend. This increase in mean temperature may cause more intense and frequent thermal stress conditions all over the world. India being a tropical country, the temperature extremities are most common, the extreme day temperature often exceeds 40°C or more for several hours during peak summer season particularly in northern parts of the country. Temperature is one of the important abiotic factors which influence behaviour, physiology, life history, distribution and abundance of insects. Each developmental stage has a different physiological response to temperature; the impact of hot events varies depending on the stage of insect exposed. Insects may experience either single time or may experience multiple times the thermal stress and their effects also varies with the stage of exposure. So, it is highly important to study the effects of thermal stress with regard to current scenario. In the context of climate change, a research work was carried out on “**Physiological responses of *Spodoptera litura* exposed to short and long-term thermal stress**” at insect physiology laboratory, entomology division, IARI, New Delhi. Under biological and reproductive fitness, parameters viz., survival rate, maturation success, female ratio, size, weight, longevity of male and female adults, gonadosomatic index, eupyrene sperm count, pre-oviposition, oviposition, post-oviposition period, total fecundity, mating success and frequency, fertility percent were studied by exposing different developmental stages (egg, 4th instar larva, pupae and adults) to 27°C (control), 42°C- short and long term and 46°C- short and long-term thermal stress. Short term refers to single time exposure and long term refers to multiple time exposures i.e., 2, 4, 2 and 2 days for egg, larva, pupa and adults respectively. Apart from biological and reproductive attributes, we have also studied water content, protein, carbohydrate, glycogen levels in male and female adults emerged from the exposed eggs and larvae to different thermal stress treatments (27°C, 42°C- short and long term and 46°C- short and long-term thermal stress). The results of these parameters are presented in this chapter.

4.1 To study the effects of short and long-term thermal stress on the reproductive fitness of *Spodoptera litura*

The reproductive fitness of *Spodoptera litura* was investigated by exposing various developmental stages to short and long-term thermal stress. The recorded

values of individual parameters were then analysed separately for the significance using one way and two-way ANOVA in completely randomized design.

4.1.1. Survival rate

The survival rate of the *Spodoptera litura* was significantly influenced by temperature treatments ($F_{4,1625}= 29.36$; $P<0.0001$), the developmental stages of exposure ($F_{4,1625}= 66.39$; $P<0.0001$) and the interaction between stages and temperature ($F_{16,1625}= 3.42$; $P<0.0001$) (Table 1). Among the temperature treatments, the survival rate of the *S. litura* was lowest in the 46°C 1-hour multiple stress treatment ($F_{4,325}= 20.541$; $P<0.0001$) in egg, larva, pupa, male and female adults compared to control ($F_{4,325}= 3.582$; $P=0.007$). Compared to all the stages, the least survival rate was observed in the larval stage ($F_{4,495}= 44.853$; $P<0.0001$) with survival rate of 16.2% and the most tolerant stages to the thermal stress were pupae ($F_{4,495}= 10.335$; $P<0.0001$) and adult female ($F_{4,70}= 2.696$; $P=0.038$) with survival rate of 64.8% and 54% at 46°C 1-hour multiple stress treatment. Compared to pupa and female adult, relatively lower survival rates were observed in egg ($F_{4,495}= 10.156$; $P<0.0001$) and male adults ($F_{4,70}= 2.962$; $P=0.025$) with survival rate of 55.8% and 48% respectively at 46°C 1-hour multiple stress treatment. More or less similar trend of survival rates in different developmental stages were noticed for other temperature treatments (Table 2).

4.1.2. Maturation success

Maturation success was measured as the percent of healthy adult insects emerged from the developmental stages of egg and larva exposed to thermal stress. The developmental stage ($F_{1,990}= 10.13$; $P=0.002$) and temperature ($F_{4,990}= 71.299$; $P<0.0001$) had a significant effect on the maturation success of *S. litura*. The interaction of both developmental stages and temperature treatments did not had significant ($F_{4,990}= 0.345$; $P=0.847$) influence on the maturation success of the insects (Table 1). The maturation success of both egg and larval stress stages decreased with increase in temperature and frequency of exposure (single and multiple stress). The maturation success was observed to be least in larval stressed stage ($F_{4,495}= 38.234$; $P<0.0001$) compared to egg stressed stage ($F_{4,495}= 33.585$; $P<0.0001$). In both the stressed stages, the maximum effect was seen in the treatment, 46°C 1-hour multiple stress ($F_{1,198}= 1.976$; $P=0.161$) as compared to control ($F_{1,198}= 1.557$; $P=0.214$) with maturation success of 16.2% and 75.6% in egg stress, 9.9% and 69.3% in larval stress respectively (Table 3).

Table 1. Two- way ANOVA of the effects of temperature, developmental stage and their interactions on the fitness of *Spodoptera litura* under thermal stress

Parameters	Source^a	df	Mean square	F	P
Survival rate (%)	Stage	4, 1625	82563.79	66.39	<0.0001
	Temperature	4,1625	36520.09	29.36	<0.0001
	Stage × Temperature	16,1625	4261.33	3.42	<0.0001
Maturation Success (%)	Stage	1, 990	15681.6	10.13	0.002
	Temperature	4, 990	110370.6	71.299	<0.0001
	Stage × Temperature	4, 990	534.6	0.345	0.847
Female ratio (%)	Stage	1, 20	268.362	57.61	<0.0001
	Temperature	4, 20	270.225	58.01	<0.0001
	Stage × Temperature	4, 20	23.515	5.048	0.006
Female adult longevity	Stage	3, 180	81.698	82.942	<0.0001
	Temperature	4, 180	9.520	9.665	<0.0001
	Stage × Temperature	12, 180	7.457	7.57	<0.0001
Male adult longevity	Stage	3, 180	54.407	39.875	<0.0001
	Temperature	4, 180	29.062	21.3	<0.0001
	Stage × Temperature	12, 180	10.869	7.966	<0.0001
Size of female (cm)	Stage	3, 80	0.292	32.655	<0.0001
	Temperature	4, 80	0.444	49.665	<0.0001
	Stage × Temperature	12, 80	0.029	3.251	0.001
Size of male (cm)	Stage	3, 80	0.304	73.189	<0.0001
	Temperature	4, 80	0.139	33.578	<0.0001
	Stage × Temperature	12, 80	0.037	8.952	<0.0001
Weight of female (g)	Stage	3, 80	0.036	51.167	<0.0001
	Temperature	4, 80	0.024	34.23	<0.0001

	Stage × Temperature	12, 80	0.002	2.832	0.003
Weight of male (g)	Stage	3, 80	0.001	3.626	0.016
	Temperature	4, 80	0.002	13.868	<0.0001
	Stage × Temperature	12, 80	0.0000889	0.533	0.887
Gonadosomatic index (GSI)	Stage	3, 100	364.093	49.636	<0.0001
	Temperature	4, 100	345.47	47.097	<0.0001
	Stage × Temperature	12, 100	33.826	4.611	<0.0001
Eupyrene sperm	Stage	3, 80	615075.48	31.487	<0.0001
	Temperature	4, 80	3645093.015	186.597	<0.0001
	Stage × Temperature	12, 80	65685.088	3.363	0.001
Pre-oviposition	Stage	3, 80	1.347	3.793	0.013
	Temperature	4, 80	0.515	1.451	0.225
	Stage × Temperature	12, 80	0.722	2.033	0.032
Oviposition	Stage	3, 80	39.893	40.501	<0.0001
	Temperature	4, 80	24.085	24.452	<0.0001
	Stage × Temperature	12, 80	4.352	4.418	<0.0001
Post-oviposition	Stage	3, 80	8.490	8.844	<0.0001
	Temperature	4, 80	1.790	1.865	0.125
	Stage × Temperature	12, 80	0.923	0.962	0.492
Total fecundity	Stage	3, 80	736495.389	10.333	<0.0001
	Temperature	4, 80	2653845.39	37.234	<0.0001
	Stage × Temperature	12, 80	155184.702	2.177	0.021
Mating success	Stage	3, 80	4124.25	8.147	<0.0001
	Temperature	4, 80	5386.5	10.64	<0.0001
	Stage × Temperature	12, 80	378	0.747	0.702

Mating frequency	Stage	3, 166	7.02	5.492	0.001
	Temperature	4, 166	32.388	25.340	<0.0001
	Stage × Temperature	12, 166	1.290	1.009	0.443
Fertility per cent	Stage	3, 81	865.598	19.016	<0.0001
	Temperature	4, 81	3504.377	76.987	<0.0001
	Stage × Temperature	12, 81	40.9	0.899	0.552

^a Temperature levels are 27 (Control), 42 – 4 hours (Single stress), 42 – 4 hours (Multiple stress), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress); the stages are Egg, Larva, Pupa, Male adult and Female adult (For survival rate, longevity, size, weight); Egg and larva (for maturation success, female adult ratio); Egg, Larva, Pupa and Adult (for GSI, eupyrene sperm count, pre-oviposition period, oviposition period, post-oviposition period, total fecundity, mating success, mating frequency and F₁ hatching or Fertility per cent).

Table 2. Effects of short and long-term temperature stress on different developmental stages on the survival rate of *Spodoptera litura*

Treatments	Egg	Larva	Pupa	Adult (Male)	Adult (Female)
Control	86.4±1.77 ^{aA}	81±2.71 ^{aA}	90±0 ^{aA}	90±0 ^{aA}	90±0 ^{aA}
42°C- 4 hours Single stress	73.8±3.47 ^{abAB}	50.4±4.48 ^{bbB}	82.8±2.45 ^{abA}	72±9.62 ^{abAB}	84±6 ^{abA}
42°C- 4 hours Multiple stress	70.2±3.74 ^{abAB}	46.8±4.51 ^{bbB}	77.4±3.13 ^{ba}	72±9.62 ^{abAB}	72±9.62 ^{abAB}
46°C- 1 hour Single stress	63±4.14 ^{bcA}	21.6±3.84 ^{cbB}	72±3.61 ^{bcA}	54±11.78 ^{abA}	66±10.64 ^{abA}
46°C- 1 hour Multiple stress	55.8±4.39 ^{ca}	16.2±3.47 ^{cbB}	64.8±4.06 ^{ca}	48±12 ^{ba}	54±11.784 ^{ba}

Mean± Standard Error (SE) values of survival rate (%) of *Spodoptera litura*, after exposure of egg, larva, pupa, male and female adults to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa male and female adults respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa male

and female adults respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, $P < 0.05$).

Table 3. Effect of short and long-term temperature stress on egg and larval stages of *Spodoptera litura* on maturation success (%)

Treatments	Egg	Larva
Control	75.6±3.31 ^a	69.3±3.81 ^a
42°C- 4hours Single stress	55.8±4.39 ^b	45.9±4.52 ^b
42°C- 4 hours Multiple stress	39.6±4.49 ^c	35.1±4.41 ^b
46°C - 1hour Single stress	28.8±4.22 ^{cd}	16.2±3.48 ^c
46°C - 1hour Multiple stress	16.2±3.48 ^d	9.9±2.83 ^c

Mean± Standard Error (SE) values of *Spodoptera litura* maturation success (%), after exposure of egg, larva, to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2, 4 days for egg, larva respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4 days for egg, larva respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage (Tukey's HSD test, $P < 0.05$), absence of uppercases due to minimum number (less than 3) of stages.

4.1.3. Female ratio

The female ratio of the *Spodoptera litura* significantly increased with temperature ($F_{4,20} = 58.01$; $P < 0.0001$), developmental stage of exposure ($F_{1,20} = 57.61$; $P < 0.0001$) and interaction of both of them ($F_{4,20} = 5.048$; $P = 0.006$) (Table 1). Among the temperature treatments, the female ratio was maximum in the thermal stress treatment of 46°C- 1-hour multiple stress ($F_{1,4} = 14.625$; $P = 0.019$) 54.78% and 67.24% than control ($F_{1,4} = 25.632$; $P = 0.007$) 41.28% and 43.63% in both egg and larval stage respectively. Irrespective of thermal stress treatments, the female quotient was highest

when the larval stage ($F_{4,10}=39.514$; $P<0.0001$) was stressed as compared to egg stage ($F_{4,10}=19.168$; $P<0.0001$) (Table 4).

4.1.4. Adult female longevity

It is one of the parameters used to study the effect of short and long-term thermal stress on different developmental stages of *Spodoptera litura*. The longevity of female adults was significantly influenced by different developmental stages exposure ($F_{3,180}= 82.94$; $P<0.0001$), temperature treatments ($F_{4,180}= 9.66$; $P<0.0001$) and their interaction ($F_{12,180}= 7.57$; $P<0.0001$) (Table 1). The adult stage ($F_{4,45}= 0.316$; $P=0.866$) was less affected by thermal stress in which the longevity of the female adults was not changed significantly, whereas in the pupal stage ($F_{4,45}= 11.433$; $P<0.0001$) the longevity of female adults decreased significantly with increase in temperature and duration. In egg and larval stages the thermal stress had varied effects on the longevity of female adults with longest longevity noticed in the female adults emerged from thermal stressed larvae (Table 5).

4.1.5. Adult male longevity

The stage of exposure ($F_{3,180}= 39.875$; $P<0.0001$), temperature ($F_{4,180}= 21.3$; $P<0.0001$) and interaction of both ($F_{12,180}= 7.966$; $P<0.0001$) significantly influenced the longevity of male adults (Table 1). The adult longevity was not affected when the adults were exposed to the short and long-term thermal stress ($F_{4,45}= 2.938$; $P=0.031$) by recording no significant difference in the longevity of male adult of different treatments, whereas in the pupal stage ($F_{4,45}= 20.245$; $P<0.0001$) it was significantly affected in which the longevity of the male adults decreased significantly with increase in temperature and duration.

Table 4. The adult female ratio (%) of *Spodoptera litura* under short and long-term thermal stress on egg and larval stages

Treatments	Egg	Larva
Control	41.28±0.39 ^c	43.63±0 ^c

42°C- 4hours Single stress	47.16±0.31 ^b	52.01±1.01 ^b
42°C- 4 hours Multiple stress	47.61±0.76 ^b	51.17±0 ^b
46°C - 1hour Single stress	49.2±1.2 ^b	55.89±1.15 ^b
46°C - 1hour Multiple stress	54.78±1.96 ^a	67.24±2.6 ^a

Mean ± Standard Error (SE) values of adult female ratio (%) of *Spodoptera litura* after exposure of egg and larva to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4 days for egg, larva respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4 days for egg, larva respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage (Tukey's HSD test, P<0.05), absence of uppercases due to minimum number (less than 3) of stages.

Table 5. Effects of short and long-term temperature stress on all developmental stages of *Spodoptera litura* on adult female longevity

Treatments	Egg	Larva	Pupa	Adult female
Control	9.6±0.22 ^{aA}	9.7±0.34 ^{bc} A	9.1±0.38 ^a A	9.9±0.38 ^{aA}
42°C- 4hours Single stress	7.7±0.3 ^{bcB}	10±0.37 ^{bA}	7.1±0.18 ^b B	9.5±0.31 ^{aA}
42°C- 4 hours Multiple stress	7.3±0.21 ^{cC}	11.5±0.22 ^a A	6.9±0.31 ^b C	9.4±0.31 ^{aB}
46°C - 1hour Single stress	7.9±0.28 ^{bcB}	10.3±0.3 ^{ab} A	6.8±0.2 ^{bB}	9.5±0.45 ^{aA}
46°C - 1hour Multiple stress	8.7±0.26 ^{abA}	8.6±0.37 ^{cA}	6.2±0.47 ^b B	9.6±0.22 ^{aA}

Mean± Standard Error (SE) of *Spodoptera litura* adult female longevity after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

In both egg and larval stages the thermal stress had varied effect on the longevity of male adults. The maximum longevity of male adults was noticed in the treatment, 42°C- 4-hour multiple stress in larval stage (15 days), while minimum was recorded in the treatment of 46°C- 1-hour multiple stress in pupal stage (7.9 days) (Table 6).

4.1.6. Size of female adult

The female adult size of *S. litura* significantly influenced by the developmental stages of exposure ($F_{3,80}= 32.655$; $P<0.0001$), thermal stress treatment ($F_{4,80}= 49.665$; $P<0.0001$) and the interaction of both stages and treatment ($F_{12,80}= 3.2510$; $P=0.001$) (Table 1). The female size gradually decreased with increase in temperature in all the developmental stages exposed. Among all the developmental stages exposed, size of female adults emerged from the thermal stressed eggs ($F_{4,20}= 17.388$; $P<0.0001$) decreased significantly to minimum levels with increase in temperature, whereas in adults ($F_{4,20}= 3.588$; $P=0.023$) the reduction of size was less. The size of female adults emerged from pupal and larval stress stages was relatively higher than the adults emerged from stressed eggs. The female size was minimum in the treatment, 46°C- 1-hour multiple stress ($F_{3,16}= 16.872$; $P<0.0001$) compared to other temperature treatments in all the stressed stages (Table 7).

4.1.7. Size of male adult

The size of male adults was significantly influenced by the stage of exposure ($F_{3,80}= 73.189$; $P<0.0001$), temperature treatment ($F_{4,80}= 33.578$; $P<0.0001$) and the interaction of both developmental stages and treatments ($F_{12,80}= 8.952$; $P<0.0001$)

(Table 1). The adult male size decreased gradually with increase in temperature in all the developmental stages of exposure. Among different developmental stages exposure to thermal stress, adults ($F_{4,20}= 0.500$; $P=0.736$) and pupa ($F_{4,20}= 2.20$; $P=0.106$) were least affected by recording non significant differences in different temperature treatments. Whereas the most susceptible stage was larva ($F_{4,20}= 38.037$; $P=0.0001$) in which the reduction of size was more with increase in thermal stress exposure and duration. The size of male adults emerged from stressed eggs and pupae were higher than the larval stress stage. The minimum adult male size was noticed in the treatment, 46°C- 1-hour Multiple stress ($F_{3,16}=34.55$; $P=0.0001$) in all the developmental stages of exposure compared to all other treatments (Table 8).

Table 6. The adult male longevity of *Spodoptera litura* after exposing to short and long-term temperature stress on different developmental stages

Treatments	Egg	Larva	Pupa	Adult male
Control	11.6±0.34 ^{abA}	11.5±0.34 ^{bA}	11.6±0.34 ^{aA}	12.1±0.53 ^{aA}
42°C- 4 hours Single stress	10.4±0.27 ^{bB}	10.7±0.40 ^{bAB}	9.9±0.23 ^{bB}	11.9±0.35 ^{aA}
42°C- 4 hours Multiple stress	12.5±0.34 ^{abB}	15±0.39 ^{aA}	9.5±0.31 ^{bC}	11.7±0.42 ^{abB}
46°C - 1hour Single stress	10.9±0.43 ^{ba}	11.5±0.40 ^{bA}	8.8±0.29 ^{bcB}	10.8±0.60 ^{aA}
46°C - 1hour Multiple stress	11.4±0.31 ^{abA}	11±0.26 ^{bA}	7.9±0.35 ^{cB}	10.3±0.3 ^{aA}

Mean± Standard Error (SE) adult male longevity of *Spodoptera litura*, after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively)). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, $P<0.05$).

Table 7. Effects of exposing different developmental stages of *Spodoptera litura* to short and long-term thermal stress on the size of female adult (cm)

Treatments	Egg	Larva	Pupa	Adult
Control	3.8±0.03 ^{aA}	3.74±0.04 ^{aA}	3.84±0.03 ^{aA}	3.8±0.03 ^{aA}
42°C- 4hours Single stress	3.5±0.06 ^{bB}	3.68±0.04 ^{abA}	3.62±0.02 ^{bAB}	3.76±0.05 ^{abA}
42°C- 4 hours Multiple stress	3.42±0.06 ^{bcB}	3.58±0.04 ^{abcAB}	3.5±0.06 ^{bcB}	3.72±0.04 ^{abA}
46°C - 1hour Single stress	3.36±0.05 ^{bcC}	3.56±0.04 ^{bcAB}	3.46±0.04 ^{cBC}	3.68±0.04 ^{abA}
46°C - 1hour Multiple stress	3.22±0.06 ^{cB}	3.46±0.05 ^{cA}	3.28±0.04 ^{dB}	3.62±0.02 ^{bA}

Mean± Standard Error (SE) values of *Spodoptera litura* female adult size (cm) after exposure of egg, larva, pupa, adults to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 8. Effects of short and long-term thermal stress on different developmental stages on male adult size (cm) of *Spodoptera litura*

Treatments	Egg	Larva	Pupa	Adult
Control	3.52±0.02 ^{aA}	3.54±0.04 ^{aA}	3.52±0.02 ^{aA}	3.58±0.02 ^{aA}
42°C- 4hours Single stress	3.5±0.03 ^{aA}	3.46±0.04 ^{aA}	3.52±0.04 ^{aA}	3.56±0.03 ^{aA}
42°C- 4 hours Multiple stress	3.44±0.03 ^{abB}	3.28±0.04 ^{bC}	3.5±0 ^{aAB}	3.56±0.03 ^{aA}
46°C - 1hour Single stress	3.42±0.02 ^{abB}	3.12±0.02 ^{cC}	3.46±0.03 ^{aAB}	3.54±0.03 ^{aA}
46°C - 1hour Multiple stress	3.3±0.06 ^{bB}	3.08±0.02 ^{cC}	3.44±0.03 ^{aA}	3.54±0.03 ^{aA}

Mean± Standard Error (SE) values of *Spodoptera litura* male adult size (cm) after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

4.1.8. Weight of female adult

The stages of exposure ($F_{3,80}= 51.167$; $P<0.0001$), thermal stress treatments ($F_{4,80}= 34.23$; $P<0.0001$) and the interaction between stage and temperature ($F_{12,80}= 2.832$; $P=0.003$) had a significant effect on the weight of female adult (Table 1). In all the developmental stages the weight of female adults decreased significantly with increase in temperature and duration. Among the different developmental stages exposure, the female adults emerged from stressed eggs ($F_{4,20}= 6.553$; $P<0.002$) and larvae ($F_{4,20}= 33.871$; $P<0.0001$) recorded lower female weight as compared to adults ($F_{4,20}= 2.826$; $P=0.052$) and pupae ($F_{4,20}= 10.478$; $P=0.0001$). In the temperature treatments, 46°C- 1-hour Multiple stress ($F_{3,16}= 31.439$; $P<0.0001$) recorded lower weight of female adults irrespective of the exposure stages as compared to all temperature treatments (Table 9).

4.1.9. Weight of male adult

The developmental stage of exposure ($F_{3,80}= 3.626$; $P=0.016$), thermal stress treatment ($F_{4,80}= 13.868$; $P<0.0001$) had significant influence on the weight of male adults in *Spodoptera litura*. While the interaction between these two factors ($F_{12,80}= 0.533$; $P=0.887$) had no significant effect on the weight of male adult (Table 1). We observed gradual decrease in the weight of male adult with increase in temperature and duration. The pupal stage ($F_{4,20}= 2.88$; $P=0.049$) exposure to different temperature treatments did not showed any significant difference on weight of male adults, while others stages showed significant differences. Among egg, larvae and adults exposure, exposure of larvae ($F_{4,20}= 5.472$; $P=0.004$) to thermal stress had a greater impact on the weight of male adults. 46°C- 1-hour multiple stress treatment ($F_{3,16}= 1.977$; $P=0.158$) considered to be the harsh treatment where the weight of male adult weight recorded minimum in all the stressed stages compared to other thermal stress treatments (Table 10).

4.1.10. Gonadosomatic index

The gonadosomatic index (GSI), is the proportion of gonad mass to the total body mass. The gonadosomatic index of the *Spodoptera litura* was significantly influenced by the development stages exposed ($F_{3,100}= 49.636$; $P<0.0001$), thermal

stress treatments ($F_{4,100} = 47.097$; $P < 0.0001$) and the interaction of these two factors ($F_{12,100} = 4.611$; $P < 0.0001$) (Table 1).

Table 9. The female adult weight (g) of *Spodoptera litura* under short and long-term temperature stress

Treatments	Egg	Larva	Pupa	Adult
Control	0.2418±0.014 ^{aa}	0.2467±0.015 ^{aA}	0.2383±0.012 ^{aA}	0.2618±0.003 ^{aA}
42°C- 4hours Single stress	0.2018±0.016 ^{abB}	0.1882±0.011 ^{bB}	0.2193±0.01 ^{abAB}	0.2611±0.003 ^{aA}
42°C- 4 hours Multiple stress	0.1856±0.027 ^{abB}	0.1509±0.008 ^{bcB}	0.1859±0.009 ^{bcB}	0.2614±0.017 ^{aA}
46°C - 1hour Single stress	0.1467±0.014 ^{bc}	0.1231±0.002 ^{cC}	0.1815±0.007 ^{bcB}	0.2525±0.005 ^{aA}
46°C - 1hour Multiple stress	0.1316±0.009 ^{bcB}	0.1216±0.002 ^{cC}	0.1588±0.009 ^{cb}	0.2262±0.01 ^{aA}

Mean± Standard Error (SE) weight of female adult (g) of *Spodoptera litura*, after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, $P < 0.05$).

Table 10. Effects of short and long-term temperature stress on all developmental stages of *Spodoptera litura* on the adult male weight (g)

Treatments	Egg	Larva	Pupa	Adult
Control	0.153±0.005 ^{aA}	0.147±0.003 ^{aA}	0.1515±0.011 ^{aA}	0.1489±0.006 ^{aA}
42°C- 4hours Single stress	0.1419±0.003 ^{abA}	0.1395±0.006 ^{aA}	0.144±0.001 ^{aA}	0.1427±0.003 ^{abA}
42°C- 4 hours Multiple stress	0.1308±0.007 ^{abA}	0.1281±0.008 ^{abA}	0.1356±0.002 ^{aA}	0.1391±0.003 ^{abA}
46°C - 1hour Single stress	0.1232±0.009 ^{abA}	0.1203±0.003 ^{abA}	0.1315±0.001 ^{aA}	0.1355±0.001 ^{abA}
46°C - 1hour Multiple stress	0.1223±0.010 ^{bA}	0.1096±0.009 ^{bA}	0.1311±0.004 ^{aA}	0.1318±0.004 ^{bA}

Mean± Standard Error (SE) weight of male adult (g) of *Spodoptera litura*, after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42°C – 4 hours (single stress), 42°C – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46°C – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively)). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

The gonadosomatic index was decreased significantly with increase in temperature and duration in all the stages of exposure. The adult stage ($F_{4,25}= 1.281$; $P=0.304$) exposure to thermal stress did not had much effect on gonadosomatic index of *S. litura*, while the major impact was observed when the larvae ($F_{4,25}= 53.983$; $P<0.0001$) were exposed as GSI was lower than all the other stages exposed. Irrespective of the stages exposed, the GSI values were minimum in the treatment, 46°C- 1-hour multiple stress ($F_{3,116}= 33.860$; $P<0.0001$) than all other thermal stress treatments (Table 11).

4.1.11. Eupyrene sperm count

The stages of exposure ($F_{3,80}= 31.487$; $P<0.0001$), temperature ($F_{4,80}= 186.597$; $P<0.0001$) and interaction of both stage of exposure and temperature ($F_{12,80}= 3.363$; $P=0.001$) had significant influence of the eupyrene sperm count of *Spodoptera litura* (Table 1). In all the developmental stages of exposure the eupyrene sperm count decreased significantly with increase in temperature. The most tolerant stage to the short and long-term thermal stress was adult stage ($F_{4,20}= 45.341$; $P<0.0001$) in which the eupyrene sperm bundle count were comparatively more as compared to other stages in all temperature treatments. The most susceptible stage to the short and long-term thermal stress was egg stage ($F_{4,20}= 76.389$; $P<0.0001$) in which the eupyrene sperm count was least in all treatments than other developmental stages. The minimum values of eupyrene sperm count were recorded in the treatment, 46°C- 1-hour Multiple stress ($F_{3,16}= 1.995$; $P=0.155$) in all the developmental stages (Table 12).

4.1.12. Pre- oviposition period

The pre-oviposition period of *Spodoptera litura* was not significantly influenced by the temperature treatments ($F_{4,80}= 1.451$; $P=0.225$), whereas the developmental stage exposed ($F_{3,80}= 3.793$; $P=0.013$) and the interaction of stage and temperature ($F_{12,80}= 2.033$; $P=0.032$) had a significant influence on pre-oviposition period (Table 1). In all the developmental stages, the short and long-term thermal stress treatments and stages of exposure had varied effects on the pre-oviposition period. There was no clear trend in the pre-oviposition period in respect of thermal stress both in temperatures and frequency of exposure (Table 13).

Table 11. Effects of short and long-term temperature stress on different developmental stages on the Gonadosomatic index (%) of *Spodoptera litura*

Treatments	Egg	Larva	Pupa	Adult
Control	39.56±1.53 ^{aBC}	39.13±0.94 ^{aC}	39.46±0.41 ^{aAB}	38.21±0.63 ^{aA}
42°C- 4 hours Single stress	33.03±1.91 ^{bB}	27.21±1.04 ^{bB}	36.14±1.07 ^{abA}	37.46±0.67 ^{aA}
42°C- 4 hours Multiple stress	31.34±1.91 ^{bC}	26.54±0.43 ^{bC}	33.4±1.03 ^{bcB}	37±0.95 ^{aA}
46°C – 1 hour Single stress	28.42±1.14 ^{bC}	25.64±0.26 ^{bC}	31.17±0.99 ^{cB}	36.86±1.14 ^{aA}
46°C – 1 hour Multiple stress	27.12±0.84 ^{bC}	25.06±0.99 ^{bC}	29.84±1.43 ^{cB}	35.4±1.07 ^{aA}

Mean± Standard Error (SE) Gonadosomatic index (%) of *Spodoptera litura*, after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 12. Eupyrene sperm count under short and long-term temperature stress on different developmental stages in *Spodoptera litura*

Treatments	Egg	Larva	Pupa	Adult
Control	3114.4±25.36 ^{aAB}	3083.8±33.13 ^{aAB}	3046.6±41.02 ^{aB}	3201.6±26.31 ^{aA}
42°C- 4hours Single stress	2717±88.84 ^{bB}	2637.8±26.14 ^{bB}	2792±27.94 ^{bAB}	3032.2±79.33 ^{abA}
42°C- 4hours Multiple stress	2537.8±49.73 ^{bA}	2569.4±159.69 ^{bA}	2683.2±43.53 ^{bA}	2892.4±43.49 ^{bA}
46°C - 1hour Single stress	2108.4±50.48 ^{cB}	2144.8±40.73 ^{cB}	2228.4±50.73 ^{cB}	2816.6±84.86 ^{bA}
46°C - 1hour Multiple stress	1939.4±32.30 ^{cA}	1956±38.61 ^{cA}	2023.8±83.91 ^{cA}	2114±55.25 ^{cA}

Mean± Standard Error (SE) values of *Spodoptera litura* eupyrene sperm count after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42°C – 4 hours (single stress), 42°C – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46°C – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively)). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 13. Implications of short and long-term temperature stress on various developmental stages on pre-oviposition period (days) of *Spodoptera litura*

Treatments	Egg	Larva	Pupa	Adult
Control	2±0 ^{abA}	1.8±0.2 ^{aA}	1.6±0.25 ^{aA}	1.4±0.25 ^{aA}
42°C- 4hours Single stress	1.2±0.2 ^{bB}	2±0 ^{aA}	1.2±0.2 ^{aB}	1.8±0.2 ^{aAB}
42°C- 4 hours Multiple stress	2.2±0.37 ^{abA}	1.6±0.25 ^{aA}	2±0.32 ^{aA}	1.4±0.25 ^{aA}
46°C - 1hour Single stress	2.4±0.25 ^{aA}	1.6±0.25 ^{aA}	2±0.45 ^{aA}	1.8±0.37 ^{aA}
46°C - 1hour Multiple stress	2.8±0.37 ^{aA}	1.6±0.25 ^{aB}	1.6±0.25 ^{aB}	1.6±0.25 ^{aB}

Mean± Standard Error (SE) pre-oviposition period (days) of *Spodoptera litura* after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

4.1.13. Oviposition period

The developmental stages of exposure ($F_{3,80}= 40.501$; $P<0.0001$), thermal stress treatments ($F_{4,80}= 24.452$; $P<0.0001$) and interaction of both ($F_{12,80}= 4.418$; $P<0.0001$), all three had significant influence on the oviposition period (Table 1). With increase in temperature and exposure frequency the oviposition period was significantly reduced in adults emerged from stressed egg and pupa. In adult stage exposure ($F_{4,20}= 5.077$; $P=0.005$), the temperature treatments did not had a momentous influence on oviposition period of *S. litura*, wherein the oviposition period was more or less similar in all the thermal stress treatments. Whereas, in the adults emerged from stressed egg stage ($F_{4,20}= 13.531$; $P<0.0001$) the oviposition period was very less in all thermal treatments as compared to other developmental stages. The oviposition period was minimum in the treatment, 46°C- 1-hour multiple stress ($F_{3,16}= 21.537$; $P<0.0001$) in egg, larval and pupal stages (Table 14).

4.1.14. Post-oviposition period

The post-oviposition period of *Spodoptera litura* was significantly influenced by the developmental stage of exposure ($F_{3,80}= 8.844$; $P<0.0001$), whereas the thermal stress treatment ($F_{4,80}= 1.865$; $P=0.125$) and the interaction of stage and temperature ($F_{12,80}= 0.962$; $P=0.492$) did not had a significant influence on post-oviposition period (Table 1). In all the developmental stages, different short and long-term thermal stress treatments and stages of exposure had varied effects on the post-oviposition period. There was no clear trend in the post-oviposition period with increase in temperature and frequency of exposure (Table 15).

4.1.15. Total fecundity

The developmental stages exposure to thermal stress ($F_{3,80}= 10.333$; $P<0.0001$), thermal stress treatment ($F_{4,80}= 37.234$; $P<0.0001$) and the interaction of both stages and temperature stress treatments ($F_{12,80}= 2.177$; $P=0.021$) had significantly influenced the total fecundity of *Spodoptera litura* (Table 1). In all the developmental stages, egg ($F_{4,20}= 8.822$; $P<0.0001$), larva ($F_{4,20}= 7.442$; $P=0.001$), pupa ($F_{4,20}= 414.804$; $P<0.0001$) and adult ($F_{4,20}= 17.053$; $P<0.0001$) significant reduction in the total fecundity was observed with increase in temperature and frequency of exposure.

Table 14. The oviposition period (days) of *Spodoptera litura* under short and long-term temperature stress in different developmental stages

Treatments	Egg	Larva	Pupa	Adult
Control	5±0.32 ^{aB}	6.2±0.58 ^{aAB}	6.8±0.37 ^{aA}	6.8±0.2 ^{aA}
42°C- 4hours Single stress	4.8±0.37 ^{aA}	6±0.32 ^{abA}	4.8±0.37 ^{bA}	5.8±0.37 ^{abA}
42°C- 4 hours Multiple stress	1.8±0.37 ^{bC}	6.4±0.68 ^{aA}	3.6±0.51 ^{bcBC}	5±0.45 ^{bAB}
46°C - 1hour Single stress	2±0.55 ^{bC}	5.6±0.25 ^{abA}	3±0.63 ^{bcBC}	4.6±0.51 ^{bAB}
46°C - 1hour Multiple stress	2±0.55 ^{bC}	4.2±0.37 ^{bB}	2.2±0.37 ^{cC}	6.2±0.37 ^{abA}

Mean± Standard Error (SE) Oviposition period (days) of *Spodoptera litura* after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 15. Effects of exposing different developmental stages of *Spodoptera litura* to short and long-term temperature stress on post-oviposition period (days)

Treatments	Egg	Larva	Pupa	Adult
Control	3±0.32 ^{aA}	1.8±0.37 ^{aA}	1.6±0.25 ^{aA}	2.4±0.51 ^{aA}
42°C- 4hours Single stress	2.4±0.25 ^{aA}	2.6±0.51 ^{aA}	1.4±0.25 ^{aA}	2.4±0.4 ^{aA}
42°C- 4 hours Multiple stress	3.6±0.25 ^{aA}	3.6±0.68 ^{aA}	1.8±0.2 ^{aB}	2.6±0.25 ^{aAB}
46°C - 1hour Single stress	3±0.55 ^{aA}	3±0.32 ^{aA}	1.6±0.4 ^{aA}	2.6±0.25 ^{aA}
46°C - 1hour Multiple stress	3.4±0.93 ^{aA}	2±0.45 ^{aA}	2±0.55 ^{aA}	1.8±0.37 ^{aA}

Mean± Standard Error (SE) Post-oviposition period (days) of *Spodoptera litura* after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

The development stage, egg was most susceptible to the thermal stress in which the total fecundity of the adults emerged from stressed eggs saw a substantial reduction in fecundity than other stages. The most tolerant stage of development to the short and long-term thermal was adult stage in which the fecundity was reduced but to a minimum extent as compared to other stages. The thermal stress treatment of 46°C- 1-hour multiple stress ($F_{3,12}= 23.73$; $P<0.0001$) considered to be the most impactful treatment than other treatments in all the developmental stages (Table 16).

4.1.16. Mating success

The developmental stage of the insect exposed ($F_{3,80}= 8.147$; $P<0.0001$) and thermal stress treatment ($F_{4,80}= 10.64$; $P<0.0001$) had significant effect on the mating success of *Spodoptera litura*. But the interaction between stages of exposure and thermal stress treatments ($F_{3,80}= 0.747$; $P=0.702$) did not had significant influence on the mating success (Table 1). In all the developmental stages like egg ($F_{4,20}= 3.167$; $P=0.036$), larva ($F_{4,20}= 5$; $P=0.006$), pupa ($F_{4,20}= 2.2$; $P=0.106$) and adult ($F_{4,20}= 1$; $P=0.431$) there was a significant reduction in the mating success with increase in temperature and duration of exposure. The most susceptible stage to the short and long-term thermal stress was larval stage in which the mating success observed was lowest in all thermal stress treatments than other developmental stages. On the other hand, adult stage recorded maximum mating success per cent in all the treatments as compared to other developmental stages. Irrespective of the developmental stages, among temperature treatments 46°C- 1-hour multiple stress treatment ($F_{3,16}= 2.622$; $P=0.086$) resulted in the minimum mating success per cent (Table 17).

4.1.17. Mating frequency

The developmental stage of the insect exposed ($F_{3,166}= 5.492$; $P=0.001$) and thermal stress treatment ($F_{4,166}= 23.340$; $P<0.0001$) had significant effect on the mating frequency of *Spodoptera litura* whereas interaction between stages of exposure and thermal stress treatments ($F_{12,166}= 1.009$; $P=0.443$) had no significant influence on the mating frequency (Table 1). Irrespective of stages exposed to thermal stress the mating frequency showed decreasing trend with increase in temperature and duration in all the stages as compared to control.

Table 16. Effects of short and long-term temperature stress on the total fecundity of *Spodoptera litura*

Treatments	Egg	Larva	Pupa	Adult
Control	1692.1±26.38 ^{aA}	1751.3±37.07 ^{aA}	1842.1±19.65 ^{aA}	1749.3±68.04 ^{aA}
42°C- 4hours Single stress	1436±18.47 ^{abB}	1524.7±73.27 ^{aAB}	1396±29.35 ^{bB}	1662.3±7.14 ^{abA}
42°C- 4hours Multiple stress	1272.1±26.00 ^{abC}	1385.8±22.87 ^{abB}	1027.3±10.52 ^{cD}	1561.2±22.16 ^{bcA}
46°C - 1hour Single stress	910.9±230.10 ^{bcB}	711.4±291.27 ^{bB}	981.2±19.67 ^{cC}	1451.4±26.56 ^{cdA}
46°C - 1hour Multiple stress	574.3±234.99 ^{bcC}	656.9±268.45 ^{bB}	895.2±10.74 ^{dC}	1298.4±57.54 ^{dA}

Mean± Standard Error (SE) Total fecundity of *Spodoptera litura* adults after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 17. Implications of temperature stress on different developmental stages on mating success (%) of *Spodoptera litura* adults

Treatments	Egg	Larva	Pupa	Adult
Control	90±0 ^{aA}	90±0 ^{aA}	90±0 ^{aA}	90±0 ^{aA}
42°C- 4hours Single stress	72±11.02 ^{ab} A	63±11.02 ^{ab} A	81±9 ^{aA}	90±0 ^{aA}
42°C- 4 hours Multiple stress	63±11.02 ^{ab} A	54±9 ^{abA}	72±11.02 ^a A	81±9 ^{aA}
46°C - 1hour Single stress	45±14.23 ^{ab} A	36±16.84 ^b A	72±11.02 ^a A	81±9 ^{aA}
46°C - 1hour Multiple stress	36±16.84 ^b A	27±11.02 ^b A	54±9 ^{aA}	72±11.02 ^{aA}

Mean± Standard Error (SE) mating success (%) of *Spodoptera litura* adults after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

The most susceptible stage to the thermal stress was larva ($F_{4,37}= 16.098$; $P<0.0001$) in which the mating frequency reached to the minimum in all the temperature treatments as compared to other stages. Treatment of 46°C- 1-hour multiple stress ($F_{3,28}= 2.222$; $P=0.108$) is considered as alarming treatment because of minimum mating frequency observed in the adults emerged/survived from egg, larva, pupa and adult stages compared to other thermal stress treatments but in egg stage 46°C- 1-hour single stress caused minimum mating frequency (Table 18).

4.1.18. Fertility percent

Fertility percent or hatching per cent of eggs laid by adults of *S. litura* emerged from short and long-term thermal stress was significantly influenced by developmental stage of exposure ($F_{3,81}= 19.016$; $P<0.0001$) and the temperature of exposure ($F_{4,81}= 76.987$; $P<0.0001$). The interaction of both stages and temperature ($F_{12,81}= 0.899$; $P=0.552$) had no significant effect on the fertility percent (Table 1). In all the developmental stages exposure to short and long-term thermal stress, the fertility percent showed decreasing trend with increase in temperature exposure and frequency. Among the different developmental stages thermal stress exposure, the adults emerged from stressed larvae ($F_{4,20}= 78.695$; $P<0.0001$) showed lower fertility percent, while adults ($F_{4,20}= 4.449$; $P=0.010$) survived after thermal stress showed maximum fertility percent in different temperature treatments as compared to control. The maximum intolerance was observed in the treatment, 46°C- 1-hour multiple stress ($F_{4,20}= 8.249$; $P<0.0001$) in which the fertility percent reached to minimum levels compared to other temperature treatments in all the developmental stages of insects (Table 19).

4.2. To study the metabolic responses of *Spodoptera litura* exposed to short and long-term thermal stress

Freshly laid egg and 4th instar larva were given short and long-term thermal stress at 42°C single (one time exposure for 4hours) and multiple stress (4hour exposure for 2 and 4 days in egg and larva respectively) and 46°C single (one time exposure for 1hour) and multiple stress (1 hour exposure for 2 and 4 days in egg and larva respectively). Later the stressed individuals were recovered at $27\pm 1^\circ\text{C}$. Eggs and larvae maintained at the temperature of $27\pm 1^\circ\text{C}$ were considered as control.

Table 18. Consequences of exposing different developmental stages to short and long-term thermal stress on mating frequency of *Spodoptera litura* adults

	Egg	Larva	Pupa	Adult
Control	4.5±0.27 ^{aA}	4.7±0.3 ^{aA}	4.4±0.48 ^{aA}	4.6±0.43 ^{aA}
42°C- 4hours Single stress	2.4±0.22 ^{bA} B	2.3±0.26 ^{bB}	3.2±0.36 ^{abA} B	3.7±0.47 ^{ab} A
42°C- 4 hours Multiple stress	2.2±0.29 ^{bA}	2.1±0.31 ^{bA}	3.4±0.48 ^{abA}	3.3±0.42 ^{ab} A
46°C - 1hour Single stress	2.1±0.23 ^{bA}	2±0.45 ^{bA}	2.8±0.56 ^{abA}	2.7±0.3 ^{bA}
46°C - 1hour Multiple stress	2.5±0.22 ^{bA}	1.5±0.34 ^{bA}	2.2±0.29 ^{bA}	2.6±0.31 ^{bA}

Mean± Standard Error (SE) mating frequency of *Spodoptera litura* adults after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 19. Effects of short and long-term temperature stress on various developmental stages on fertility per cent of *Spodoptera litura*

Treatments	Egg	Larva	Pupa	Adult
Control	83.96±2.74 ^{aA}	85.17±3.03 ^{aA}	88.37±1.63 ^{aA}	87.42±2.58 ^{aA}
42°C- 4hours Single stress	60.59±2.15 ^{bB}	56.07±0 ^{bBC}	64.9±1.65 ^{bAB}	71.61±4.85 ^{ab} A
42°C- 4 hours Multiple stress	56.82±1.01 ^{bAB}	53.20±1.80 ^{bA} B	61.05±1.59 ^{bA} B	69.96±8.66 ^{ab} A
46°C - 1hour Single stress	54.38±1.45 ^{bcBC}	49.61±0 ^{bcCD}	58.01±1.61 ^{bA} B	63.43±2.29 ^{bA}
46°C - 1hour Multiple stress	46.40±2.76 ^{cb}	45.34±1.28 ^{cb}	56.21±4.20 ^{bA} B	61.74±2.44 ^{bA}

Mean± Standard Error (SE) Fertility per cent of *Spodoptera litura* after exposure of egg, larva, pupa, adult to short and long-term temperature stress (27°C (Control), 42°C – 4 hours (single stress), 42°C – 4 hours (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively), 46°C – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4,2 and 2 days for egg, larva, pupa and adult respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Freshly emerged and survived male and female adults from the thermal stressed eggs and larvae were used for the estimation of metabolic responses. The metabolic responses such as water content, protein content, carbohydrates content and glycogen content were estimated in this experiment.

The levels of water content, protein, carbohydrates and glycogen content significantly varied with thermal stress treatment, frequency and sex of adults. The metabolic responses were increased with increase in temperature and duration in both male and female adults. The metabolites content were highest in the treatment of 46°C multiple stress (1 hour exposure for 2 and 4 days in egg and larva respectively) in both male and female adults. Compared with male adults, the metabolic response was more in female adults. For the estimation of protein, carbohydrates and glycogen we used different standards viz., Bovine serum albumin (BSA), Glucose and Glycogen (Fig. 1-3). The estimated values of metabolic responses were checked for normality and percent values were arcsine transformed for analysis. The data were subjected to one way and two-way analysis of variance (ANOVA) in the completely randomized design (CRD) to test the effect of temperature treatments (27°C, 42°C SS, 42°C MS, 46°C SS and 46°C MS), stage (male and female adults emerged from stressed eggs and larvae) and their interactions on the metabolic contents of *S. litura*.

4.2.1. Water content

The water content of both male and female adults significantly influenced by stage of exposure ($F_{3,180} = 3.388$; $P=0.019$) and thermal stress treatment ($F_{4,180} = 50.584$; $P<0.0001$). The interaction between stages and temperature ($F_{12,180} = 0.179$; $P=0.999$) had no significant influence on the water content of male and female adults (Table 20). The water content of both male and female adults emerged from stressed eggs and larvae decreased significantly with increase in temperature and frequency. Among egg and larva stress, the male ($F_{4,45} = 10.904$; $P<0.0001$) and female ($F_{4,45} = 18.349$; $P<0.0001$) adults emerged from the larva showed lower water content than the adults emerged from stressed eggs. Irrespective of egg and larval stress, female adults were most tolerant to the thermal stress compared to male adults. The minimum level of water content was seen in the treatment, 46°C- 1hour multiple stress ($F_{3,36} = 0.809$; $P=0.498$) in both male and female adults emerged from stressed eggs and larvae as compared to other thermal stress treatments (Table 21).

Table 20. Two- way ANOVA of the effects of temperature, developmental stage and their interactions on the metabolic responses of adult male and female *Spodoptera litura*

Parameters	Source^a	df	Mean square	F	P
Water Content (%)	Stage	3, 180	31.096	3.388	0.019
	Temperature	4,180	464.265	50.584	<0.0001
	Stage × Temperature	12,180	1.646	0.179	0.999
Protein Content (µg/mg)	Stage	3, 80	0.791	120.634	<0.0001
	Temperature	4, 80	7.278	1110	<0.0001
	Stage × Temperature	12, 80	0.033	5.040	<0.0001
Carbohydrate content (µg/mg)	Stage	3, 80	285.387	278.825	<0.0001
	Temperature	4, 80	366.434	358.008	<0.0001
	Stage × Temperature	12, 80	2.118	2.077	0.028
Glycogen (µg/mg)	Stage	3, 80	0.656	847.567	<0.0001
	Temperature	4, 80	1.751	2262	<0.0001
	Stage × Temperature	12, 80	0.098	126.995	<0.0001

^aTemperature levels are 27°C (Control), 42°C – 4 hours (single stress), 42°C – 4 hours (Multiple stress), 46°C – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress); the stages are male and female adults emerged from stressed eggs and larvae.

Table 21. Effects of short and long-term temperature stress at egg and larval stages in the water content (%) of male and female adult *Spodoptera litura*

Treatments	Male adult		Female adult	
	Egg stress	Larval stress	Egg stress	Larval stress
Control	54.01±0.57 ^{aA}	53.30±1.43 ^{aA}	55.53±0.97 ^{aA}	54.76±0.94 ^{aA}
42°C- 4hours Single stress	52.60±0.91 ^{abA}	52.14±1.16 ^{aA}	54.12±0.93 ^{abA}	52.67±0.83 ^{abA}
42°C- 4hours Multiple stress	50.84±0.54 ^{abA}	49.89±0.33 ^{abA}	50.91±0.47 ^{bcA}	51.61±0.44 ^{bcA}
46°C- 1hour Single stress	49.01±1.17 ^{bcA}	47.60±1.55 ^{bcA}	49.75±1.04 ^{cdA}	48.92±0.85 ^{cdA}
46°C- 1hour Multiple stress	45.98±1.27 ^{cA}	44.41±0.65 ^{cA}	46.35±1.22 ^{dA}	46.24±0.72 ^{dA}

Mean± standard error (SE) Water content (%) of male and female adults of *Spodoptera litura* when eggs (freshly laid) and larva (4th instar larva) were exposed to short and long-term thermal stress (27°C - Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4 days for egg, larva respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4 days for egg, larva respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05). Stages are male and female adults emerged from stressed eggs and larvae.

4.2.2. Protein content

Effect of temperature treatments (27°C, 42°C SS, 42°C MS, 46°C SS and 46°C MS) ($F_{4,80} = 1110$; $P < 0.0001$), stage (male and female adults emerged from stressed eggs and larvae) ($F_{3,80} = 120.634$; $P < 0.0001$) and their interactions ($F_{12,80} = 5.040$; $P < 0.0001$) had significant influence on the protein contents of *S. litura* (Table 20). The protein content of both male and female adults increased significantly with increase in temperature and duration of exposure in egg and larval stages. The most susceptible stage to the short and long-term thermal stress was larval stage in case of both male ($F_{4,20} = 200.898$; $P < 0.0001$) and female adults ($F_{4,20} = 280.528$; $P < 0.0001$). Compared to male adults the female adults had more protein levels as a response to thermal stress. The maximum effect of thermal stress was observed in the treatment, 46°C- 1hour multiple stress ($F_{3,16} = 28.858$; $P < 0.0001$) where the protein levels reached very high in both male and female adults emerged from stressed egg and larval stages in comparison with other treatments (Table 22).

4.2.3. Carbohydrate content

The carbohydrate content of both male and female adults emerged from stressed eggs and larvae were significantly influenced by stage of exposure ($F_{3,80} = 278.825$; $P < 0.0001$), thermal stress treatment ($F_{4,80} = 358.008$; $P < 0.0001$) and the interaction between stages of exposure and temperature ($F_{12,80} = 2.077$; $P = 0.028$) (Table 20). In both male and female adult insects the carbohydrate content increased significantly with increase in temperature and frequency of exposure in egg and larval stages. The carbohydrate levels recorded maximum in larval stage of both male ($F_{4,20} = 216.606$; $P < 0.0001$) and female adults ($F_{4,20} = 65.430$; $P < 0.0001$). The carbohydrate level was more in the female adults compared to male adults. In the treatment of 46°C- 1hour Multiple stress ($F_{3,16} = 55.995$; $P < 0.0001$) carbohydrate levels reached maximum in egg and larval stage exposure of male and female adults compared to other thermal stress treatments (Table 23).

4.2.4. Glycogen content

Effect of temperature treatments ($F_{4,80} = 2262$; $P < 0.0001$), stage (male and female adults emerged from stressed eggs and larvae) ($F_{3,80} = 847.567$; $P < 0.0001$) and

their interactions ($F_{12,80} = 126.995$; $P < 0.0001$) had significant influence on the glycogen contents of *S. litura* (Table 20). The glycogen content of both male and female adults increased significantly with increase in temperature and duration of exposure in egg and larval stages. The largest values of glycogen were seen in larval stage of both male ($F_{4,20} = 265.757$; $P < 0.0001$) and female adults ($F_{4,20} = 1560$; $P < 0.0001$). Compared to male adults the female adults had more glycogen levels in their body. The maximum effect of thermal stress observed in the treatment of 46°C- 1hour Multiple stress ($F_{3,16} = 307.548$; $P < 0.0001$) where the glycogen levels reached very high in egg and larval stage in both male and female adults compared to all other thermal stress treatments (Table 24).

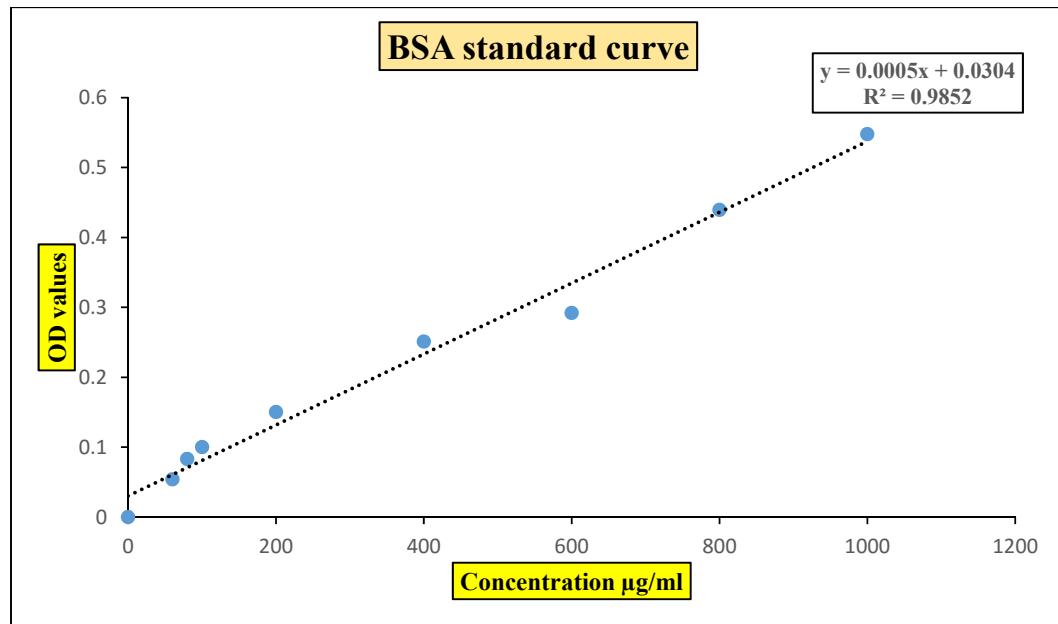


Figure 1. Bovine Serum Albumin (BSA) standard curve for protein estimation in male and female adults of *Spodoptera litura*

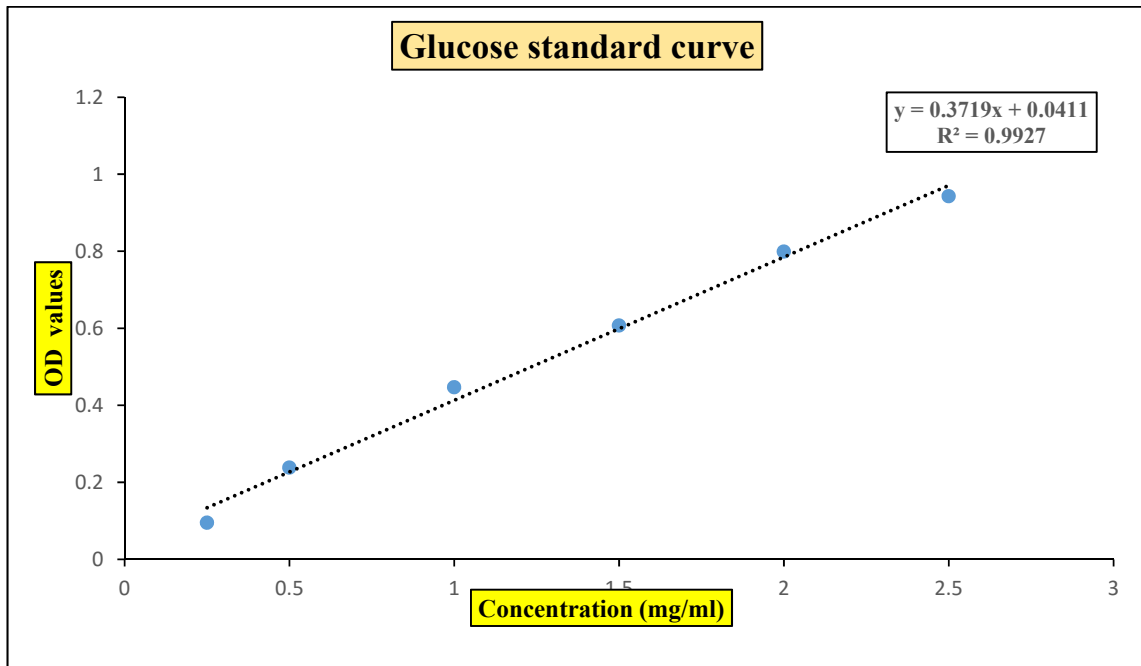


Figure 2. Glucose standard curve for estimation of carbohydrates in male and female adults of *Spodoptera litura*

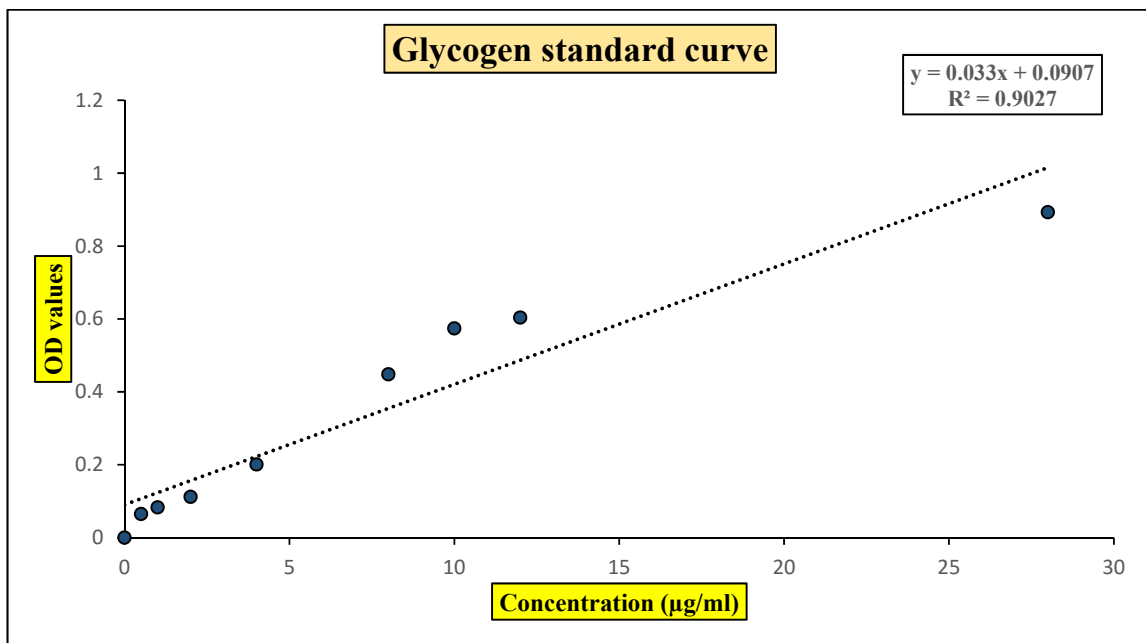


Figure 3. Glycogen standard curve for glycogen estimation in male and female adults of *Spodoptera litura*

Table 22. Protein content ($\mu\text{g}/\text{mg}$) of male and female adults of *Spodoptera litura* after exposing egg and larval stages to short and long-term temperature stress

Treatments	Male adult		Female adult	
	Egg stress	Larval stress	Egg stress	Larval stress
Control	1.65 \pm 0.018 ^{cb}	1.68 \pm 0.013 ^{cb}	1.85 \pm 0.020 ^{ea}	1.86 \pm 0.025 ^{ea}
42°C- 4hours Single stress	1.96 \pm 0.018 ^{db}	1.99 \pm 0.021 ^{db}	2.33 \pm 0.036 ^{da}	2.44 \pm 0.081 ^{da}
42°C- 4hours Multiple stress	2.37 \pm 0.014 ^{cc}	2.44 \pm 0.039 ^{cc}	2.57 \pm 0.021 ^{cb}	2.70 \pm 0.010 ^{ca}
46°C- 1hour Single stress	2.64 \pm 0.012 ^{bb}	2.70 \pm 0.033 ^{bb}	2.83 \pm 0.032 ^{ba}	2.90 \pm 0.023 ^{ba}
46°C- 1hour Multiple stress	3.06 \pm 0.026 ^{ac}	3.23 \pm 0.076 ^{ac}	3.45 \pm 0.036 ^{ab}	3.70 \pm 0.054 ^{aa}

Mean \pm standard error Protein content ($\mu\text{g}/\text{mg}$) of male and female adults of *Spodoptera litura* when eggs (freshly laid) and larva (4th instar larva) were exposed to short and long-term thermal stress (27°C - Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4 days for egg, larva respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4 days for egg, larva respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 23. Influence of short and long-term thermal stress (at egg and larval stage) on the levels of carbohydrates ($\mu\text{g}/\text{mg}$) of *Spodoptera litura* adults

Treatments	Male adult		Female adult	
	Egg stress	Larval stress	Egg stress	Larval stress
Control	23.66 \pm 0.39 ^{CB}	24.04 \pm 0.21 ^{CB}	30.14 \pm 0.32 ^{DA}	30.45 \pm 0.55 ^{DA}
42°C- 4hours Single stress	26.61 \pm 0.40 ^{DB}	27.47 \pm 0.22 ^{DB}	31.79 \pm 0.63 ^{CD A}	32.34 \pm 0.82 ^{DA}
42°C- 4hours Multiple stress	28.59 \pm 0.22 ^{CB}	29.04 \pm 0.30 ^{CB}	33.49 \pm 0.61 ^{CA}	35.24 \pm 0.53 ^{CA}
46°C- 1hour Single stress	30.50 \pm 0.42 ^{BD}	32.38 \pm 0.47 ^{BC}	36.36 \pm 0.28 ^{BB}	38.15 \pm 0.20 ^{BA}
46°C- 1hour Multiple stress	34.53 \pm 0.35 ^{AC}	36.05 \pm 0.30 ^{AC}	39.22 \pm 0.47 ^{AB}	42.68 \pm 0.71 ^{AA}

Mean \pm standard error Carbohydrates ($\mu\text{g}/\text{mg}$) of male and female adults of *Spodoptera litura* when eggs (freshly laid) and larva (4th instar larva) were exposed to short and long-term thermal stress (27°C - Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4 days for egg, larva respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4 days for egg, larva respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

Table 24. Glycogen content ($\mu\text{g}/\text{mg}$) in adults of *Spodoptera litura* with respect to short and long-term thermal stress in the egg and larval stages

Treatments	Male adult		Female adult	
	Egg stress	Larval stress	Egg stress	Larval stress
Control	0.184 \pm 0.007 ^{cA}	0.174 \pm 0.005 ^{dA}	0.195 \pm 0.007 ^{eA}	0.190 \pm 0.003 ^{eA}
42°C- 4hours Single stress	0.212 \pm 0.006 ^{cB}	0.219 \pm 0.012 ^{dB}	0.334 \pm 0.009 ^{dA}	0.313 \pm 0.008 ^{dA}
42°C- 4hours Multiple stress	0.241 \pm 0.012 ^{cD}	0.322 \pm 0.012 ^{cC}	0.521 \pm 0.012 ^{cB}	0.709 \pm 0.095 ^{cA}
46°C- 1hour Single stress	0.416 \pm 0.010 ^{bD}	0.460 \pm 0.010 ^{bC}	0.703 \pm 0.011 ^{bB}	0.772 \pm 0.011 ^{bA}
46°C- 1hour Multiple stress	0.596 \pm 0.025 ^{aC}	0.660 \pm 0.018 ^{aC}	1.162 \pm 0.020 ^{aB}	1.321 \pm 0.019 ^{aA}

Mean \pm standard error Glycogen content ($\mu\text{g}/\text{mg}$) of male and female adults of *Spodoptera litura* when eggs (freshly laid) and larva (4th instar larva) were exposed to short and long-term thermal stress (27°C - Control), 42 – 4 hours (single stress), 42 – 4 hours (Multiple stress- 2,4 days for egg, larva respectively), 46 – 1 hour (Single stress) and 46°C- 1 hour (Multiple stress- 2,4 days for egg, larva respectively). Different lowercase letters indicate significant differences among temperature treatments in a particular stage, uppercase letters indicate significant differences among different stages for a particular temperature treatment (Tukey's HSD test, P<0.05).

V. DISCUSSION

Investigations were conducted to determine the effects of thermal stress in different developmental stages on the reproductive fitness and metabolic responses of survived male and female adults from stressed stages of *Spodoptera litura* in the laboratory conditions. This chapter presents the brief discussion of the obtained results in said objectives.

5.1. Effects of short and long-term thermal stress on reproductive fitness of *Spodoptera litura*

The adverse effects of thermal stress on the biology and physiology of the insects are directly influenced by the magnitude and frequency of thermal stress and developmental stage and sex (Enriquez and Colinet, 2017). In summer months the north western and northern parts of India often experiences the higher levels of day temperature. The maximum temperature and relative humidity during summer months in Delhi reaches around $>42^{\circ}\text{C}$ for shorter durations and humidity would be 55-65%. Based on this prevailing conditions and the biology of test insect, the temperature and exposure durations were decided to study the effects of thermal stress on reproductive physiology of *Spodoptera litura*. With rapid multiplication rates and species with overlapping generations, *Spodoptera litura* may face thermal stress either in a single stage or in all stages of life. Insects may experience at any stage the thermal stress either once or multiple times. As a result of this thermal stress exposure the reproductive fitness of the insect may be affected (Rao *et al.*, 2015). The adverse effects on the reproductive fitness were studied in the heat susceptible developmental stages of the insect. Biological and reproductive fitness of *S. litura* was investigated by measuring rate of survival and maturation, female ratio, longevity of adults, size and weight of adults, gonadosomatic index, eupyrene sperm count, oviposition, pre and post-oviposition period, fecundity, mating success and frequency and fertility percent. Altogether our results indicating that the magnitude of temperature and duration, various stages and the interaction between the stages and temperature all had the significant effects on the reproductive fitness of *S. litura*.

5.1.1. Survival rate

Temperature is critical factor for the survival of insects as it may cause short and long-term thermal injury in the insects (Mironidis and Soutani, 2010). As a result of thermal injury insects may die suddenly when the temperature levels surpass the ideal temperature. In our study the response to short and long-term thermal stress in the temperature treatments of 42 and 46°C was revealed by the reduction of survival rates in egg, larva, pupa, male and female adults. The survival rate of *S. litura* was minimum in the larval stage stress at 46°C multiple stress for 1 hour (4 days stress) as compared to other developmental stages (Fig. 4), similar results were reported by Chen *et al.* (2018) in *O. communa*. Larger body size helps the female adults to survive under thermal stress due to less dehydration compared to male adults (Chen *et al.*, 2014; Wang *et al.*, 2016). Our results concurred with earlier researches where female adults of *T. vaporariorum* (Cui *et al.*, 2008) and *Aphidius avenae* (Roux *et al.*, 2010) survived more than male adults. Just like *S. litura*, survival rates of *Helicoverpa armigera* adults (male and female) decreased significantly with increase in temperature and duration (Mironidis and Soutani, 2010). In contrast to our results, Zhang *et al.* (2015) reported larval stage was most tolerant stage to the thermal stress when compared to other developmental stages in diamond back moth *Plutella xylostella* and Klok and Chown (2001) also remarked that in kelp fly (*Paractora dreuxi*) the late instar larva or nymphs survived than that of adults to the thermal stress.

5.1.2. Maturation success

The exposure to thermal stress may results in sudden death because of severe thermal injury or death in subsequent stages (Xie *et al.*, 2008). In our experiment we took egg and larval stages for thermal stress to compute maturation success. Both these stages were susceptible to the thermal stress as the maturation success decreased significantly with increase in temperature exposure and frequency. In larval stage, the thermal stress exposure at 46°C multiple stress for 1 hour (4 days stress) caused maximum reduction in the maturation success (9.9%) (Fig. 5). As a consequence of injury caused by thermal stress, the carry-over effects in the late stages of development reduced the maturation success (Blanckenhorn *et al.*, 2014). Comparably Chen *et al.* (2018) also reported that survival of *O. communa* significantly reduced in both egg (3 hours exposure to $\geq 40^{\circ}\text{C}$ for 3 days) and larval stage stress (3 hours exposure to $\geq 46^{\circ}\text{C}$ for 5 days). Unlike our results, Zhang *et al.* (2015) found that *P. xylostella* eggs exposed

to 40°C for 4 and 8 hours had no significant difference on the maturation success as compared to control insects. Whereas, the exposure more than 8 hours resulted in poorer maturation success. In larval stage it decreased first (up to 16 hours) and increased in prolonged exposure (16 and 24 hours at 40°C).

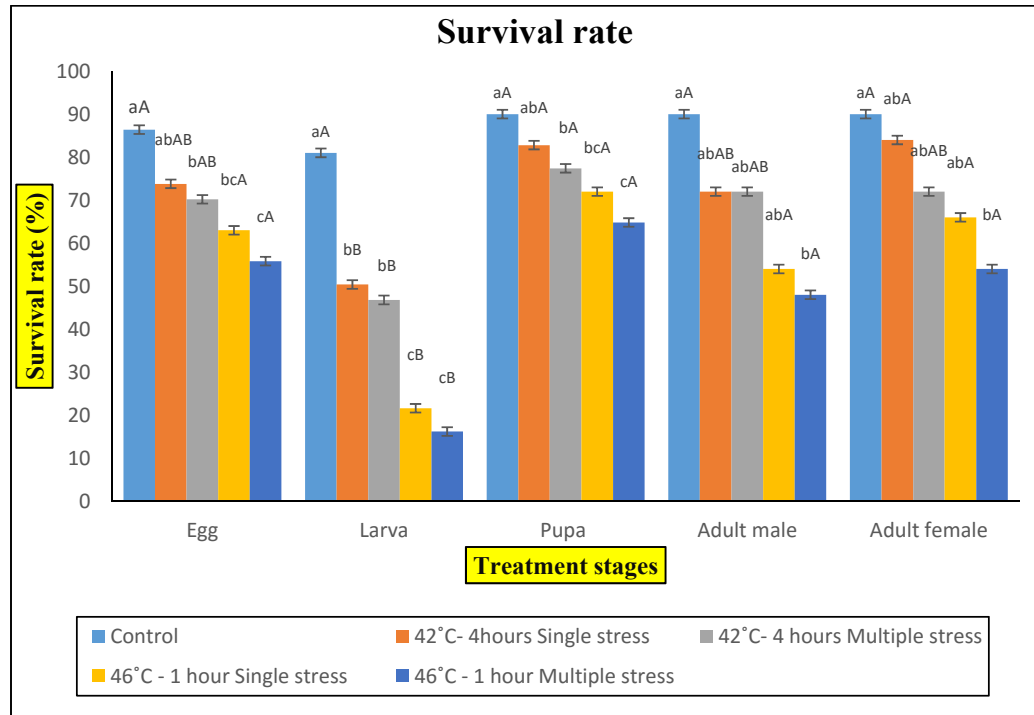


Figure 4. Effects of short and long-term temperature stress on different developmental stages on survival rate of *Spodoptera litura*

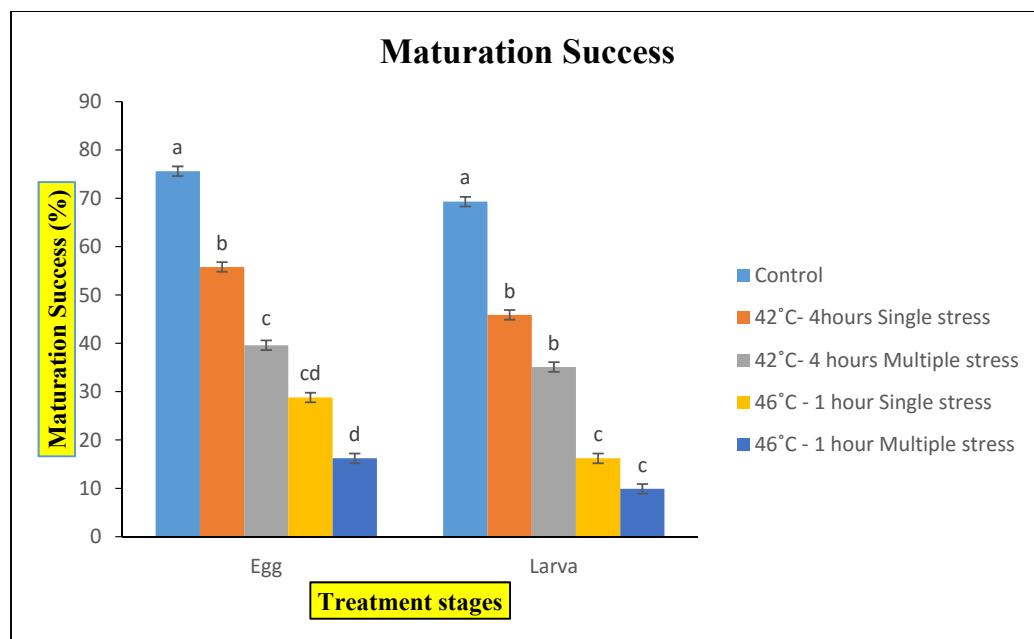


Figure 5. Effects of short and long-term temperature stress in egg and larval stages on maturation success of *Spodoptera litura*

5.1.3. Female ratio

Our study revealed that the female ratio of *S. litura* was more at higher temperature with multiple exposure [46°C multiple stress for 1 hour (4 days stress)] as compared to the control treatment (27°C) (Fig. 6). The reason for increased female ratio is thermotolerance and more survivability of female adults due to thermal stress than that of male adults. Dehydration losses were less in the female adults because of larger body size as compared to male adults which helps in increased thermotolerance in female adults (Chen *et al.*, 2014; Wang *et al.*, 2016). The mortality rates recorded was more in male adults of *Trichogramma euproctidis* than female adults. This biased sex ratio resulted from thermal stress were also seen in the subsequent generations of insects (Moiroux *et al.*, 2014). In the case of parasitoid, *Campoletis chlorideae* (Pandey *et al.*, 2008), *Aphidius gifuensis* (Kang *et al.*, 2017) and *Ophhraella communa* (Chen *et al.*, 2018) similar results of higher female ratio was observed under thermal stress. Contradictory to our results the female adults ratio decreased with increase in temperature in *Aphelinus asychis* (Wang *et al.*, 2016), *Pezothrips kellyanus* (Varikou *et al.*, 2012) and *Aphelinus varipes* (Yashima and Murai, 2013).

5.1.4. Longevity of female and male adults

Thermal stress injury will cause reduction in the longevity of insects by the mechanisms of damage repairing and maintaining normal functions under stressed conditions (Colinet *et al.*, 2015). In our research longevity of both male and female adults of *S. litura* decreased in adults emerged from pupal stress stage at all higher temperature exposures compared to the control treatment. Compared to female adults, longevity of male adults was more in all treatments (Fig. 7-8). Similar to our results Cheng *et al.* (2017) found that male and female adult longevity of *Bradysia odoriphaga* decreased significantly with increase in temperature and longevity of male adults was higher. Likewise, the longevity of *Helicoverpa armigera* decreased significantly due to thermal stress (Mironidis and Soutani, 2010). Zhu *et al.* (2017) reported that the longevity of *Bradysia* adults significantly reduced under short-term thermal stress. In egg, larva and adult stages of *S. litura* the short and long-term exposure of thermal stress had varied effects on the longevity of male and female adults. Similar to this Chen *et al.* (2018) reported that there were no significant differences among longevity of adults under thermal stress ($\geq 40^{\circ}\text{C}$ for 3 h). In *Drosophila melanogaster*, adult male longevity

increased with increase in temperature (Sorensen *et al.*, 2007). In oriental fruit moth, *Grapholita molesta* the longevity increased under thermal stress (Liang *et al.*, 2014).

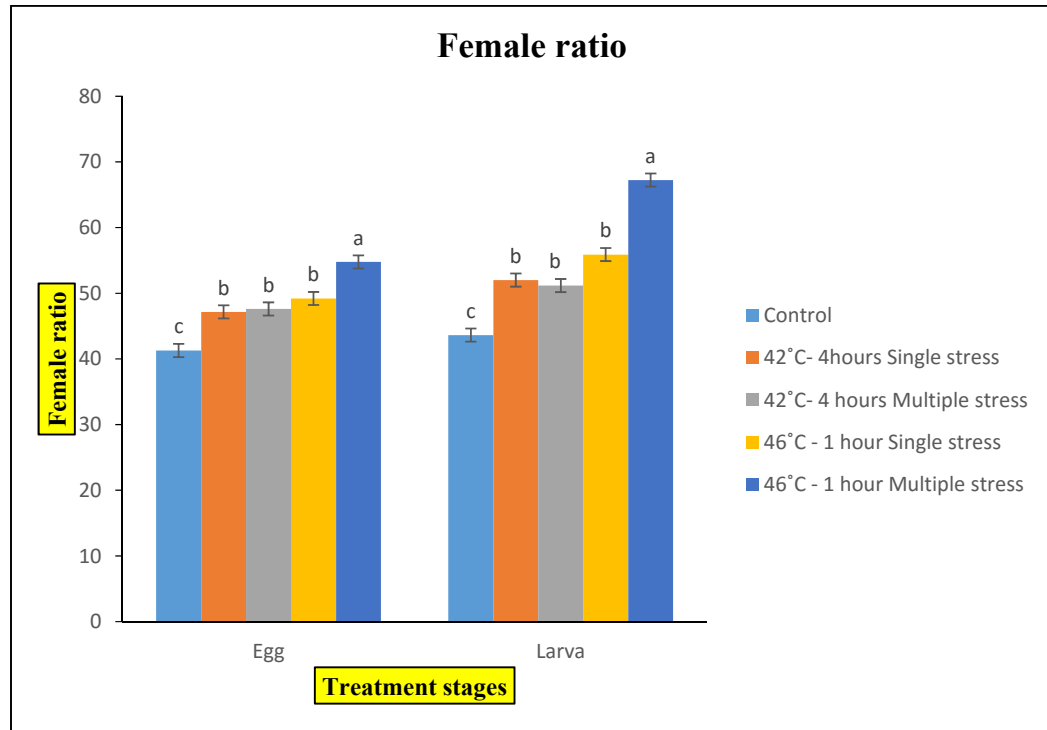


Figure 6. Female ratio of adult *Spodoptera litura* under short and long-term temperature stress exposed in different developmental stages

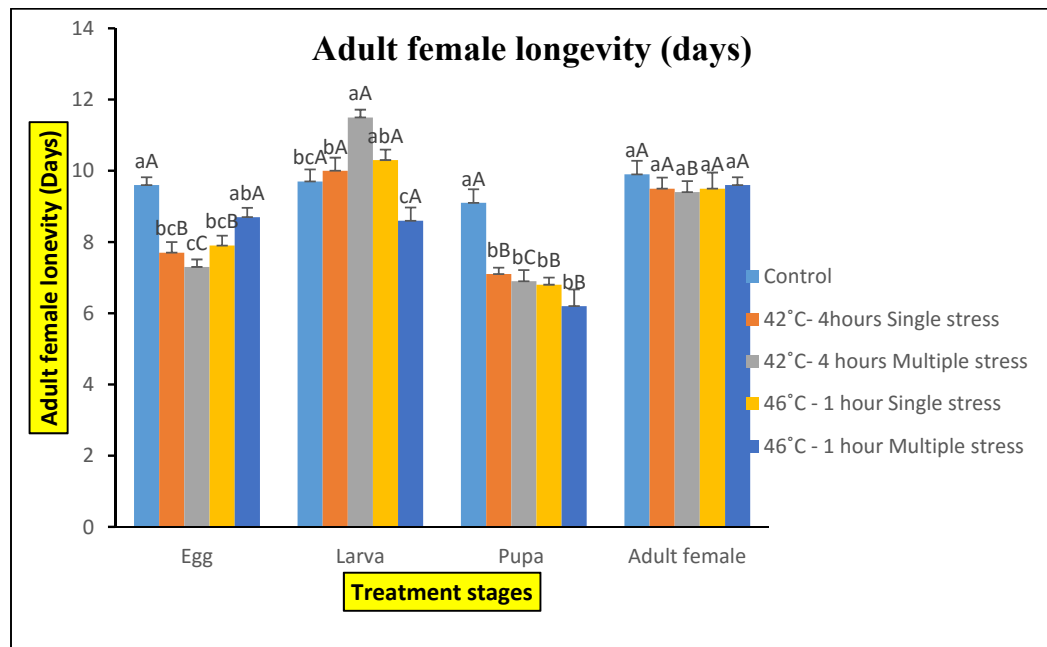


Figure 7. Longevity of female adult insects under single and multiple thermal stress in different stages

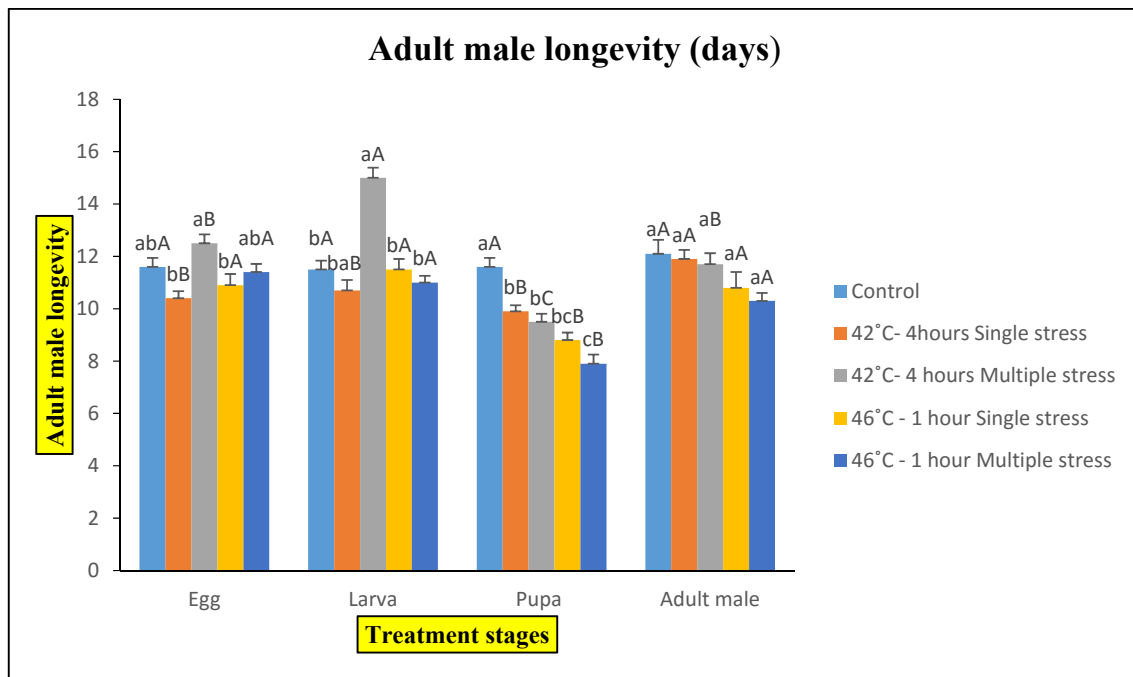


Figure 8. Consequences of single and multiple thermal stress on different developmental stages on adult male longevity of *Spodoptera litura*

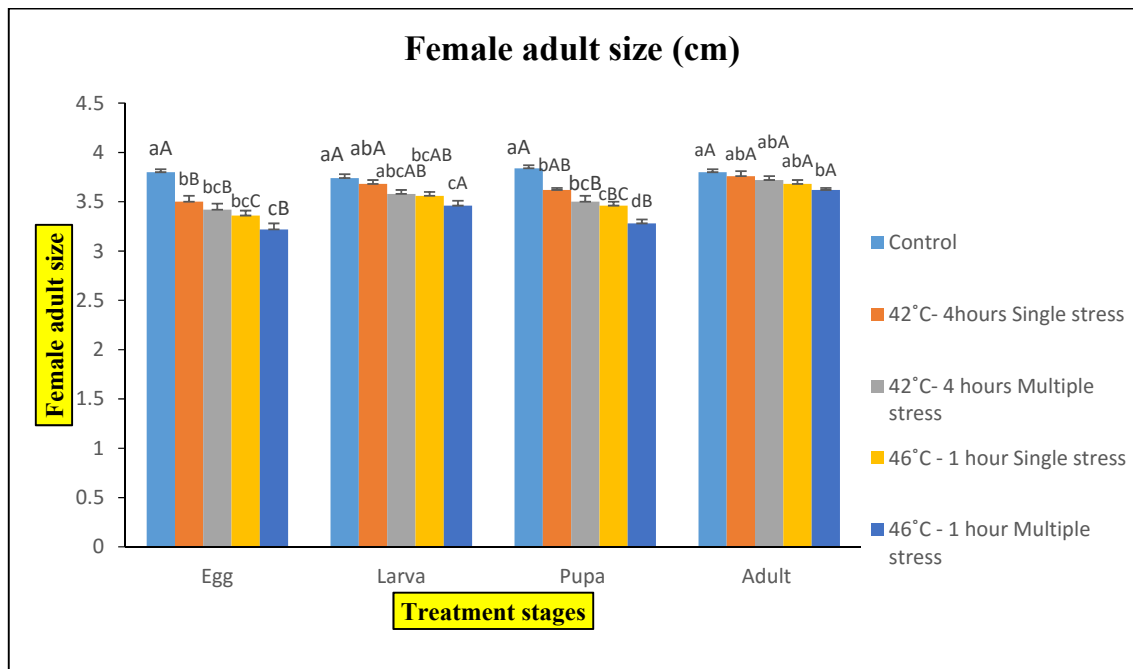


Figure 9. Effects of short and long-term temperature stress in various stages on female adult size (cm) of *Spodoptera litura*

Roux *et al.* (2010) quoted in their research that female adults of *Aphidius avenae* had increased longevity after thermal stress. The reason for reduced longevity in female adults compared to male adults related with size, i.e., the larger body size of female adults requires high amounts of energy for survival and reproduction (Cheng *et al.*, 2017). Under field conditions fluctuating temperatures throughout the day may result in improved longevity in *Spodoptera litura* compared to the constant temperatures of laboratory conditions.

5.1.5. Size and weight of male and female adults

The reaction to difference in temperature, the insects following a general principle of “hotter the condition smaller the size”, as a result of this the size of insects tend to decrease with increase in temperature (Atkinson, 1994; Kingslover and Huey, 2008). When stress is prevailing the life forms may have picked to allot majority of the stored reserves for survival and reproduction instead of growth, after attaining optimum size (Joerhn and Behmer, 1997). Thermal stress might affect different developmental stages such as eggs, larvae, pupae and adults in different manner (Loeschcke and Krebs, 1996). The results of our study revealed that the reduction in the size of both male and female adults decreased significantly with increase in temperature, stage of the insect exposed to thermal stress. Maximum size reduction was seen in the female adults emerged from stressed egg and pupa, whereas in males, the adults emerged from stressed larvae recorded maximum size reduction. The minimum size was observed in the treatment of 46°C multiple stress for 1 hour (4 days stress) exposure in all the stages exposed in both male and female adults (Fig. 9-10). Similar reduction in the female adult size was reported by Danks (1987) in *Nezara viridula* due to summer temperatures. In the same way size of *O. communa* adults were reduced significantly in the larval stage stress. Same kind of cases was reported in aphid, *Acyrtosiphon pisum* (Chen *et al.*, 2006) and parasitoid, *Trichogramma ostrinia* (Smith *et al.*, 1998).

Our findings indicated that reduction in the weight of female and male adults with increase in temperature and duration in all the stressed stages. The stress in larval stage significantly reduced the weight of male and female adults compared to other stages. The 46°C multiple stress for 1 hour (4 days stress) exposure in all the developmental stages decreased weight of female and male adults significantly by recording lowest weight in this treatment as compared to other treatments (Fig. 11-12). Supporting to our results, the thermal stress reduced the weight of *Aphis pisum* and

Aphis fabae (Dixon *et al.*, 1982), *Toxoptera aurantii* (Murdie, 1969) and females of *Sogatella furcifera* and *Nilaparvata lugens* emerged

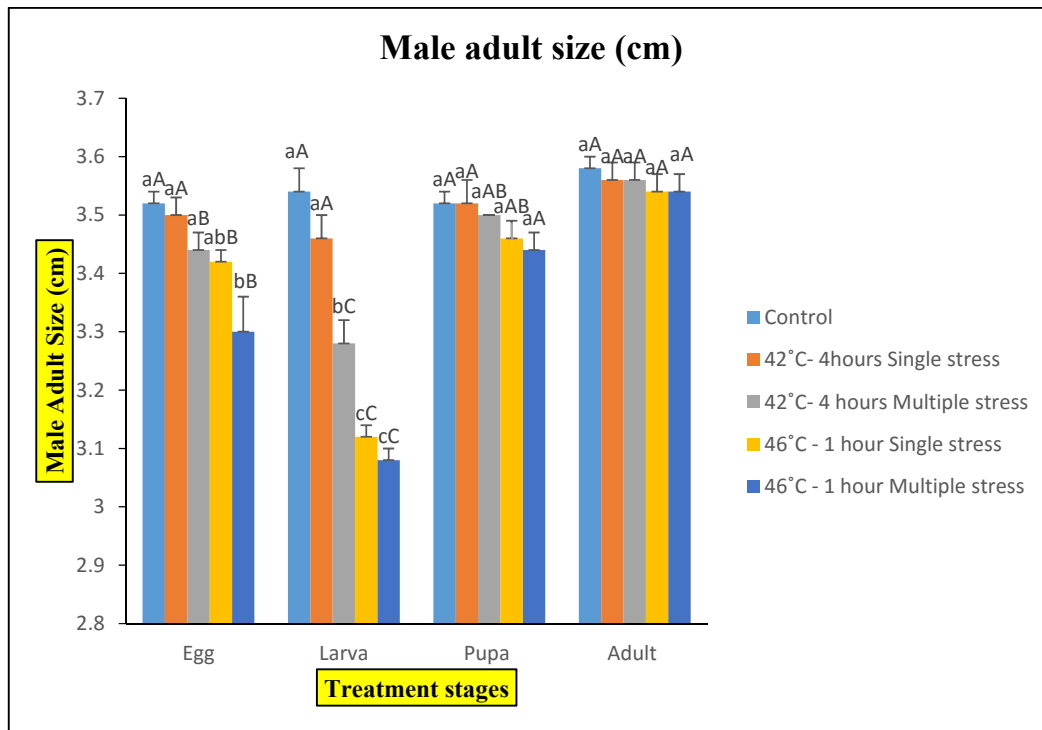


Figure 10. Exposure of different stages of *Spodoptera litura* to short and long-term temperature stress on male adult size (cm)

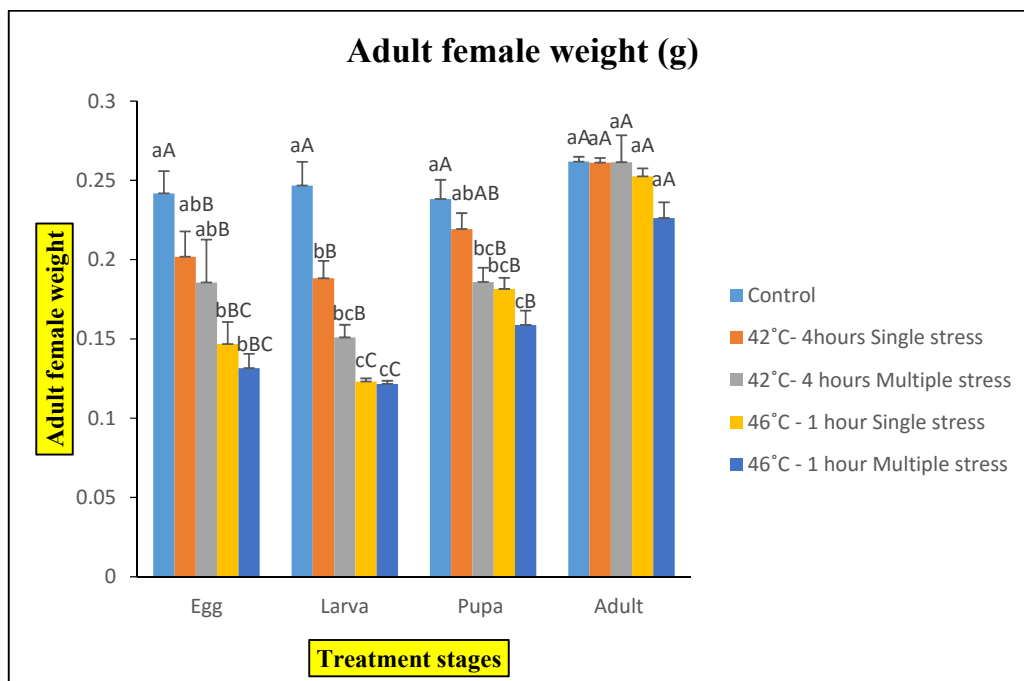


Figure 11. Changes in adult female weight in relation with short and long-term thermal stress on various stages of *Spodoptera litura*

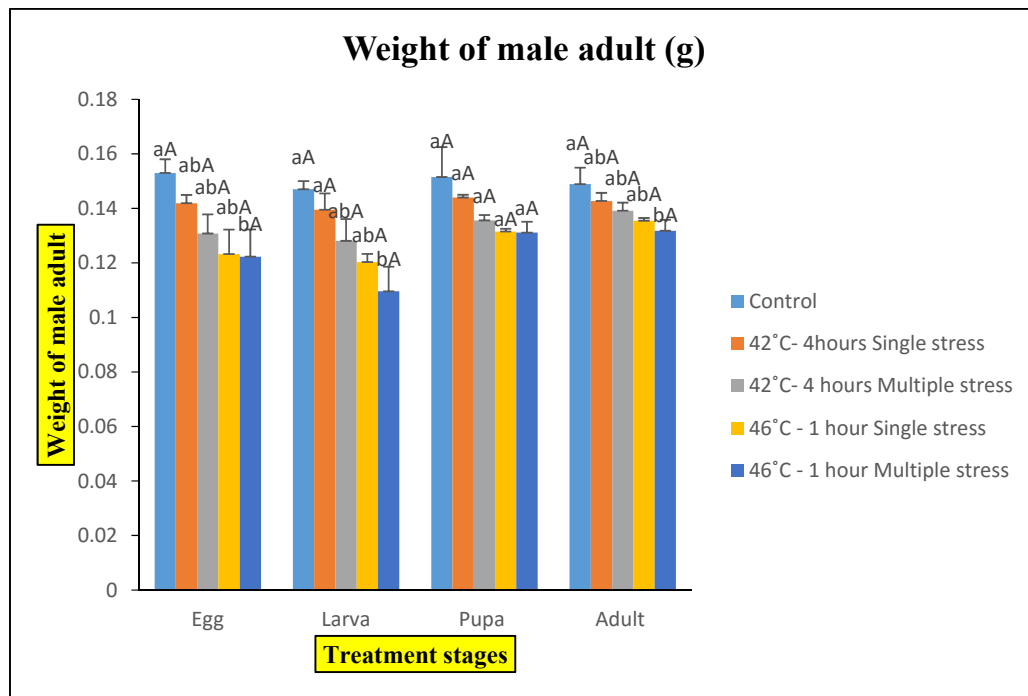


Figure 12. Effect of temperature stress both short and long-term exposure in different developmental stages on male adult weight of *Spodoptera litura*

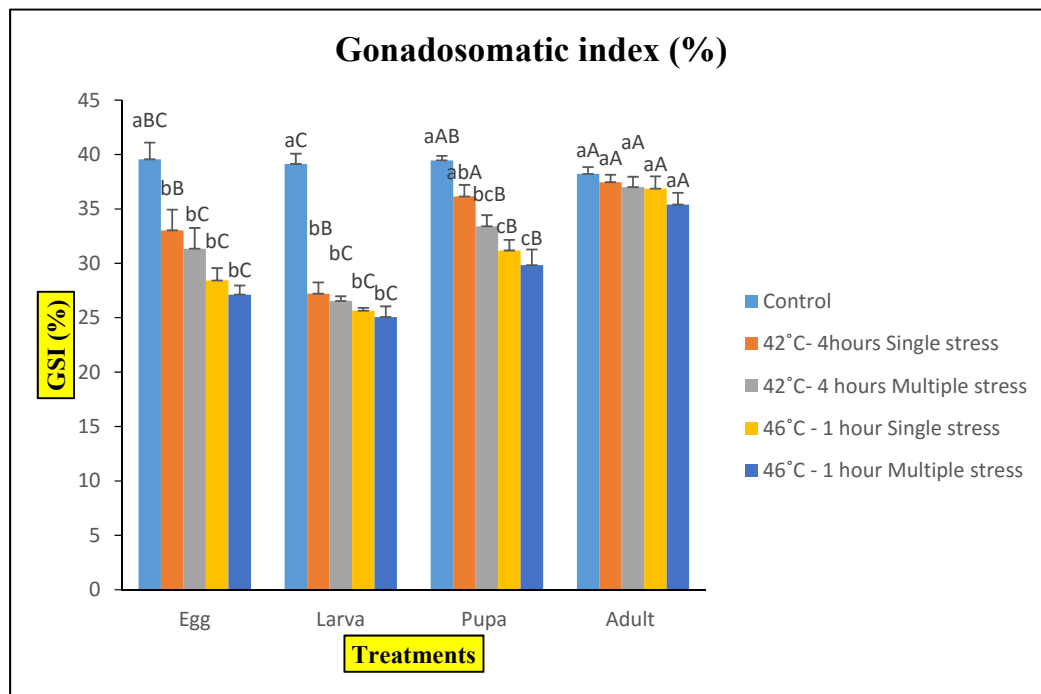


Figure 13. Influence of single and multiple thermal stress on various developmental stages on Gonadosomatic index of adult *Spodoptera litura*

from stressed nymphs (Ma *et al.*, 1998).

Smaller size of adult insects is due to reduced effect on the growth rate than rate of development (Berrigan and Charnov, 1994; Booth and Kiddell, 2007; Radmacher and Strohm, 2010; van der Have and de Jong, 1996). The size and weight were affected due to increased behavioural and physiological activities during thermal stress conditions (Chown and Terblanche, 2006). Higher amounts of energy spent by insects under thermal stress conditions resulted in reduced size and weight (Gillooly *et al.*, 2001; Clarke and Fraser, 2004). Generally, the larger body size is conducive for the multiplication of insect population (Chen *et al.*, 1988; Credland *et al.*, 1986). Smaller size of the adults may result in poor reproductive fitness in insects.

5.1.6. Gonadosomatic index

The thermal stress treatments often not results in the mortality of the insects and perform normal functions after stress, but their reproductive function may affect adversely due to thermal stress (Keshan *et al.*, 2014). In our study the obtained results of gonadosomatic index indicating that the reduction in the ovary weight of female adults emerged from different stressed stages. The maximum reduction was observed in 46°C multiple stress for 1 hour (4 days stress) exposure irrespective of stages. The stress in larval stage had the maximum effect on the gonadosomatic index compared to other stages (Fig. 13). The similar results reported in *B. mori* adults emerged from thermal stressed larva and pupa in which the decreased gonadosomatic index than control insects (Paul and Keshan, 2016). The reduced fertility in the *D. melanogaster* under mild thermal stress was reported by Hercus *et al.* (2003). As a result of thermal stress in insects delayed oocyte maturation, failed expression of proteins for the development of yolk and dissolving of egg chambers lead to poor ovarian development (Gruntenko *et al.*, 2003). The thermal stress severely affected the development of ovary in *Tribolium castaneum* (Xu *et al.*, 2009).

5.1.7. Eupyrene sperm count

Stress in the insects may results in the reduced production of both eupyrene and apyrene sperm bundles. Irradiation in the freshly emerged adults of male *S. litura* reduced the eupyrene sperm count drastically (Seth *et al.*, 2016). Eupyrene sperm count of *S. litura* in our study showed a decreasing trend with increase in temperature in the adults emerged from all the stressed developmental stages. The egg and larval stages

were most susceptible to the thermal stress. At higher temperature the sperm count reduced to the minimum in 46°C

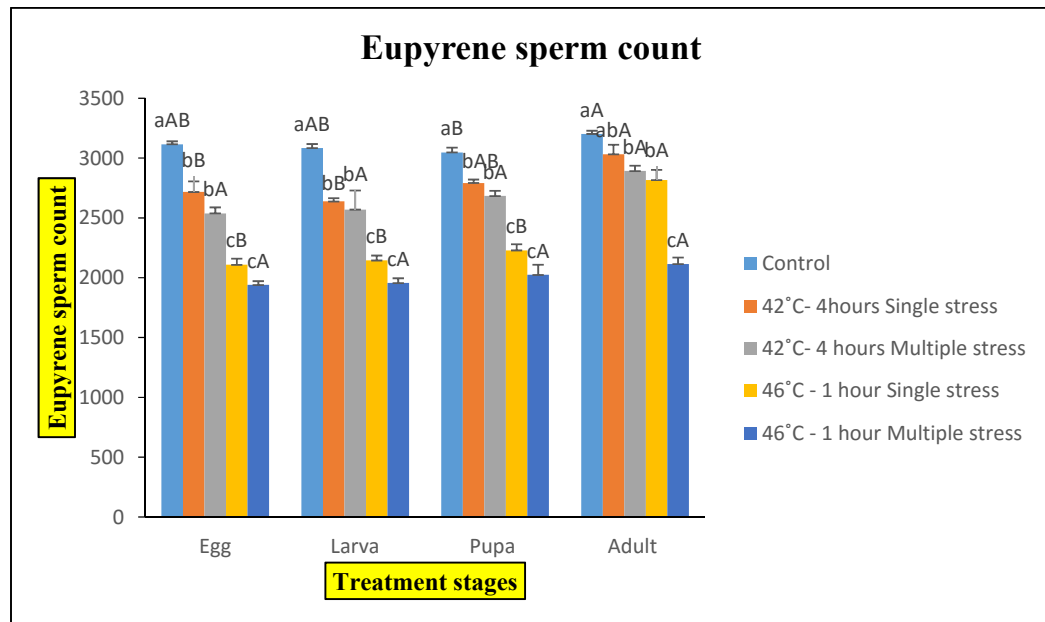


Figure 14. Levels of eupyrene sperm count in adult *Spodoptera litura* under short and long-term thermal stress on different developmental stages

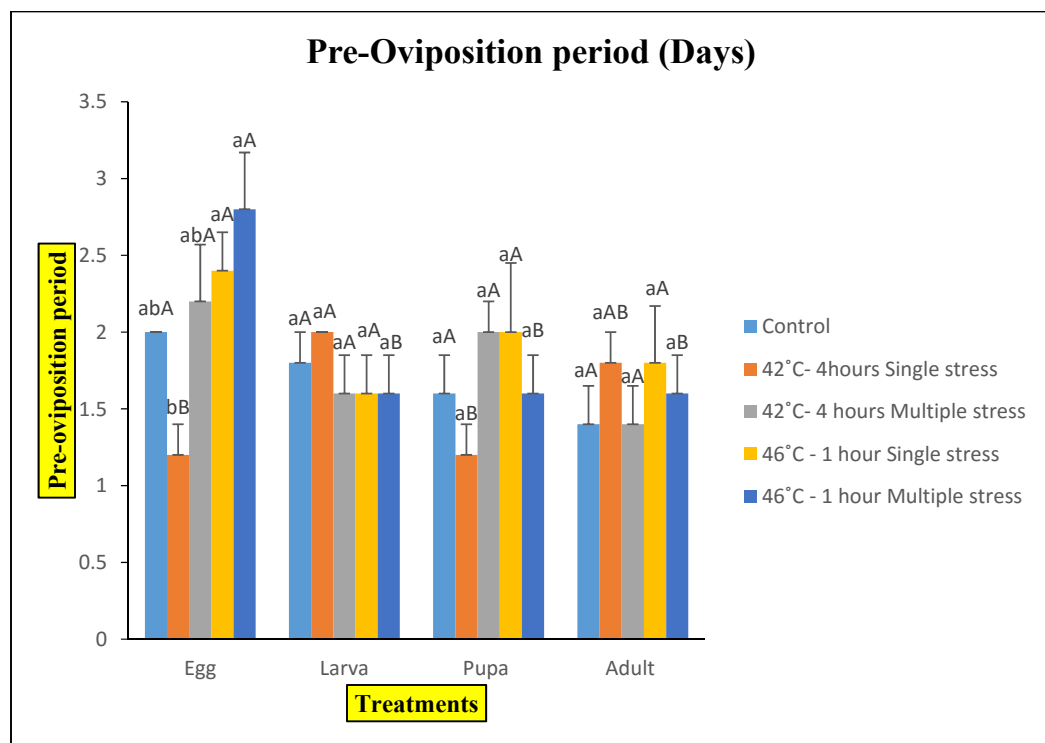


Figure 15. Pre-oviposition period of *Spodoptera litura* after exposing different developmental stages to short and long-term thermal stress

multiple stress for 1 hour (4 days stress) in all the stages (Fig. 14). Similar kind of results was reported by Helinski and Knols (2009) in the *Anopheles arabiensis*, where irradiation in the pupal stage negatively influenced the sperm production. Sales *et al.* (2021) reported that the thermal stress of 40°C and 42°C (5 days) exposure significantly damaged the male reproduction in *Tribolium castaneum* beetles and stress in pupal stage resulted in severe reduction in sperm count. Thermal stress mitigation capacity of sperms was lesser due to larger surface area of sperms as compared to ovaries (Hammerstedt *et al.*, 1990). Similar kind of damage to the sperm characteristics like sperm count, sperm motility were observed in the *Mytilus galloprovincialis* under thermal stress conditions (Boni *et al.*, 2016). The drop in sperm count associated with breakdown of cells or necrosis in testis of stressed male adults (Kroemer *et al.*, 2009).

5.1.8. Reproductive fitness

Under thermal stress conditions, thermotolerance is achieved by reducing the physiological functions (Shen *et al.*, 2014). The thermal injury in the earlier part of life results in the carry over effects in later part of the insects which leads to poor reproduction (Zhang *et al.*, 2015). Reproductive fitness often considered to be the most susceptible physiological trait than other parameters (Jorgensen *et al.*, 2006).

In the ambient temperature levels, the pre-oviposition period used to be shorter in duration than higher and lower temperatures. Nishi and Takahashi (2002) documented that the pre-oviposition period decreased with increase in temperature in *Amphibolus venator*. Chen *et al.* (2015) reported that the optimum levels of temperature facilitated the reduced pre-oviposition period in *H. oblita*. Laneesha (2016) found that there was a reduction in the pre-oviposition period at higher temperatures (>30°C) in papaya mealybug, *Paracoccus marginatus*. The short and long-term thermal stress in different developmental stages had a varied effect on pre-oviposition period of *S. litura*. There was no clear trend between increase in temperature and pre-oviposition period (Fig. 15). Zhang *et al.* (2016) reported that increased pre-oviposition period in *Neoseiulus barkeri* under short term thermal stress compared to control.

Oviposition period of insects is severely influenced by the thermal stress because temperature affecting the insect reproductive behaviour by allocating the resources to survival and metabolism. Temperature above the optimum levels reduced the oviposition period of insects. In our study the oviposition period of *Spodoptera litura* significantly decreased with increase in temperature in adults emerged from

stressed egg and pupal stages. At 46°C the oviposition period was least in adults emerged from stressed egg, larva and pupa (Fig. 16). Similarly, Marchioro and Foerster (2012) identified that the oviposition period of *Plutella xylostella* was reduced in both higher and lower temperatures. Laneesha (2016) found that increasing temperature reduced the oviposition period of papaya mealybug *P. marginatus*. Choi *et al.* (2020) found that in *Bactrocera dorsalis* high temperature at 35°C reduced the oviposition period to lower levels. Rao and Gowda (1961) reported that oviposition period in *Chelonus rufus* reduced with increase in temperature and decrease in humidity. Similarly, in *Thaumastocoris peregrinus* oviposition period decreased at higher temperatures when compared to optimum level temperature (Barbosa *et al.*, 2019). Zhang *et al.* (2016) also reported that *N. barkeri* had reduced oviposition period when exposed to thermal stress.

In our study the post-oviposition period of *S. litura* not affected by the short and long-term thermal stress because there was no clear trend in the post-oviposition period of different treatments (Fig. 17). Likewise, Zhang *et al.* (2016) also concluded that there was no effect on the post-oviposition period of *N. barkeri* due to thermal stress. Contrasting to our results, El-Aw *et al.* (2016) reported that increase in temperature results in increase in post-oviposition period in *Planococcus citri*, but they have used 20-30°C for their study.

The experience of thermal stress in the initial stages has significant influence on the reproduction of the insects (Jorgensen *et al.*, 2006). The fecundity of *S. litura* significantly reduced by exposure to different temperatures for different durations. Thermal stress exposure in the initial stages such as egg and larva recorded the lowest fecundity compared to the later stages of life. In all the stages, exposure at 46°C for 1hour multiple stress (4 days stress) resulted in the reduced fecundity (Fig. 18). In line with our results Zhou *et al.* (2011) reported that fecundity of *O. communa* reduced in a significant manner when exposed to higher temperatures (2h stress). Similarly, in insects like *O. communa* (Chen *et al.*, 2018), *Aphidius avenae* (Roux *et al.*, 2010), *Diarohabda carinulata* (Acharya *et al.*, 2013), *H. armigera* (Mironidis and Soutani 2010), *Liriomyza huidoberensis* (Huang *et al.*, 2007), *Sacrophaga crassipalpis* (Rinehart *et al.*, 2000), *Drosophila buzzatii* (Jorgensen *et al.*, 2006) and *Trialeurodes vaporariorum* (Cui *et al.*, 2008) also recorded reduced fecundity compared to the control which is almost parallel with our results. This reduction of fecundity may be due to two different phenomena like thermal stress cause damage to the sperm of male

adults (Moiroux *et al.*, 2014; Janowitz and Fischer 2011; Krebs and Loeschke, 1994) and in female adults it caused the poor ovarian and oocytes development (Paul and Keshan, 2016; Rinehart *et al.*, 2000). Due to the reduced fecundity the population of the *S. litura* might reduce in the subsequent generations. Contrastingly, fecundities of *T. turkestani* (Yang *et al.*, 2013) and *T. viennensis* (Li *et al.*, 2010) unchanged or improved with thermal stress.

5.1.9. Mating Success and mating frequency

In all the developmental stages exposure to short and long-term thermal stress, the mating success of *S. litura* was significantly reduced. Larval stage was most susceptible to the thermal stress since the mating success recorded was least in adults emerged from stressed larvae (Fig. 19). At 46°C multiple stress for 1hour (4 days stress), the mating success was least irrespective of the stages. In the same way irradiated *S. litura* had reduced mating success compared to the control (Seth *et al.*, 2016). Similar to our results in *Chilo partellus* the mating success reduced drastically when exposed to thermal stress (Unnithan and Paye, 1991).

Mating frequency of *S. litura* adults significantly reduced in adults emerged or survived from all stages with minimum mating frequency in adults emerged from stressed larvae (Fig. 20). Our results are closely related with the results of Zheng (2013) the mating frequency reduced linearly with increase in temperature in *O. communa*. Contrastingly to our findings, Seth *et al.* (2016) found that mating frequency increased rapidly with the irradiation treatments in *S. litura*. The potential reason for reduced mating frequency might be poor attractiveness of the adults of both male and female insects under thermal stress (Fasolo and Krebs, 2004; Zhang *et al.*, 2013). Because of poor attractiveness the frequency of mating and courtship reduced significantly.

5.1.10. Fertility percent

The parental generation exposure to thermal stress will have a carry-over effect in the subsequent generations (Zhang *et al.*, 2013). In our study the fertility percent was indirectly proportional to the increase in temperature. Thermal stress in early stages such as egg and larva resulted in poor fertility percent than the later stages (Fig. 21). This indicates that parental generation have transferring effects of thermal stress in early stages to the subsequent generations (Cheng *et al.*, 2017).

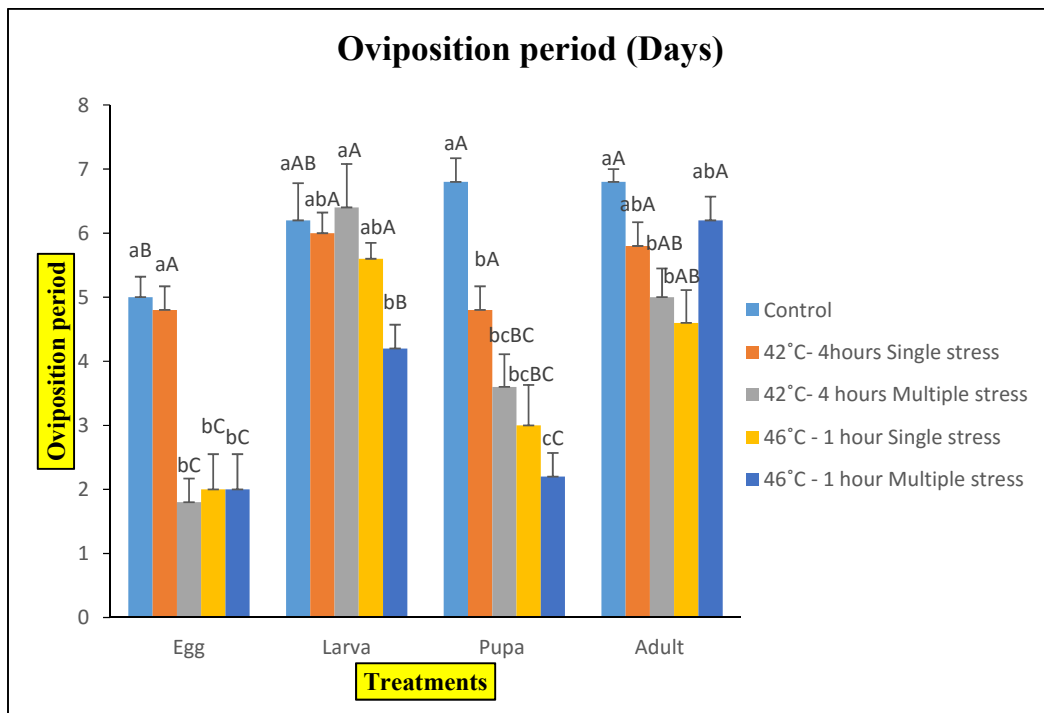


Figure 16. Effects of short and long-term temperature stress on different developmental stages on the oviposition period of *Spodoptera litura*

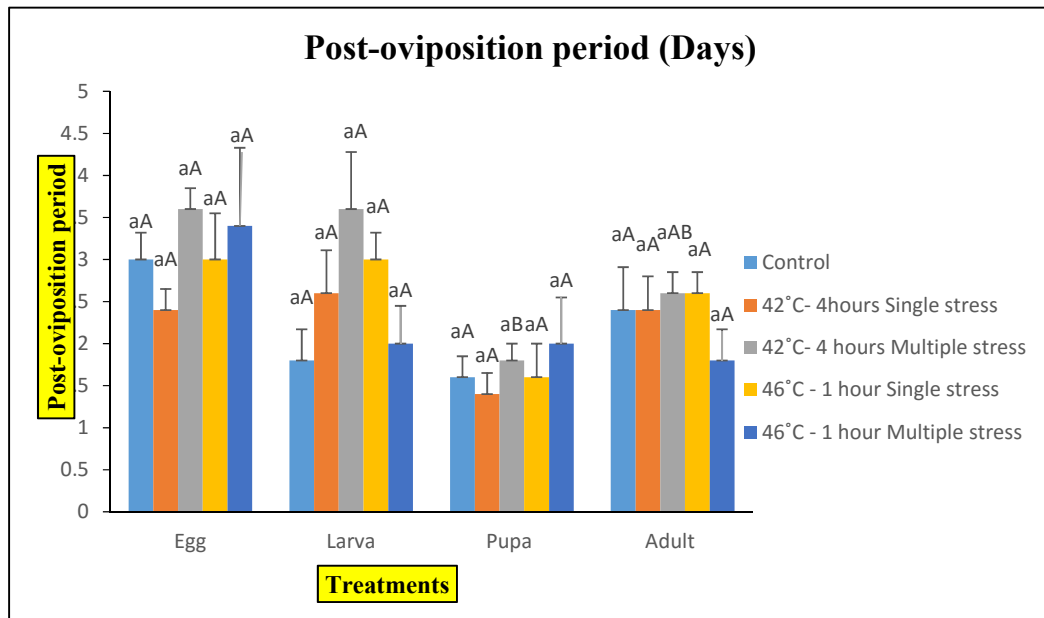


Figure 17. Post-oviposition period of *Spodoptera litura* after exposing various stages to short and long-term temperature stress

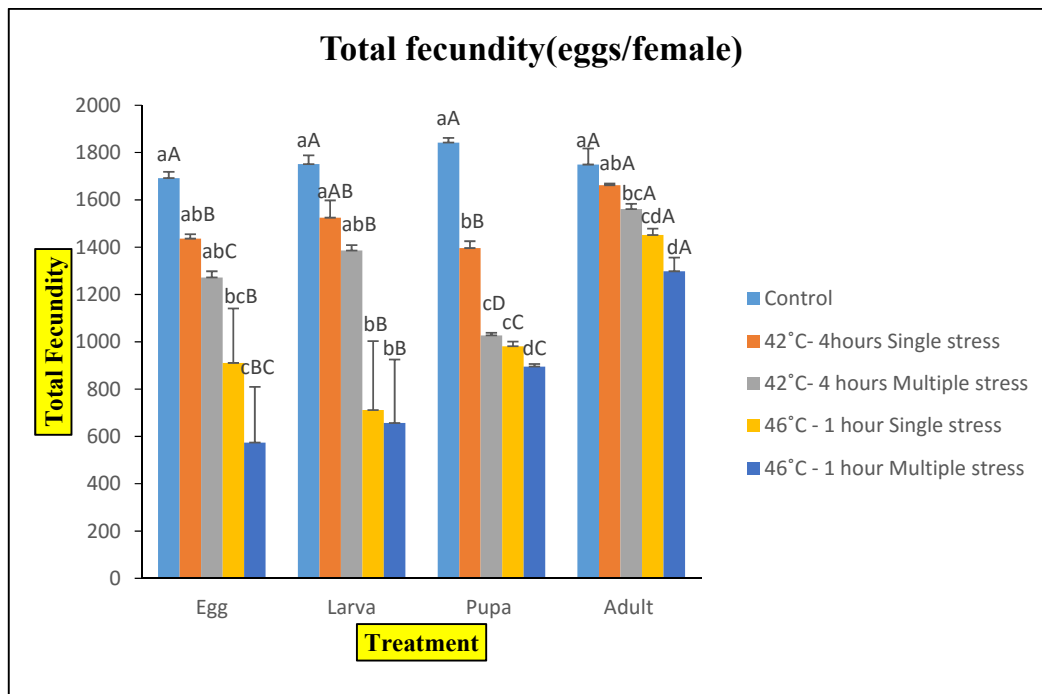


Figure 18. Impact of single and multiple temperature stress on different developmental stages on the total fecundity of *Spodoptera litura*

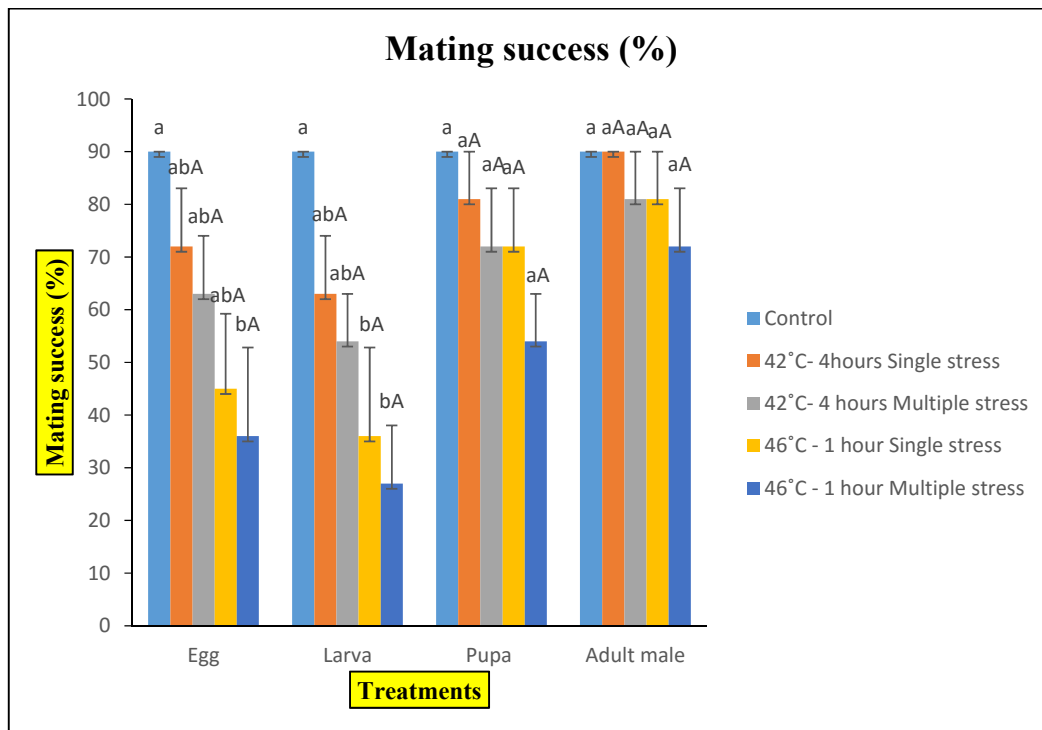


Figure 19. Mating success in *Spodoptera litura* adults when exposed to short and long-term temperature stress in different developmental stages

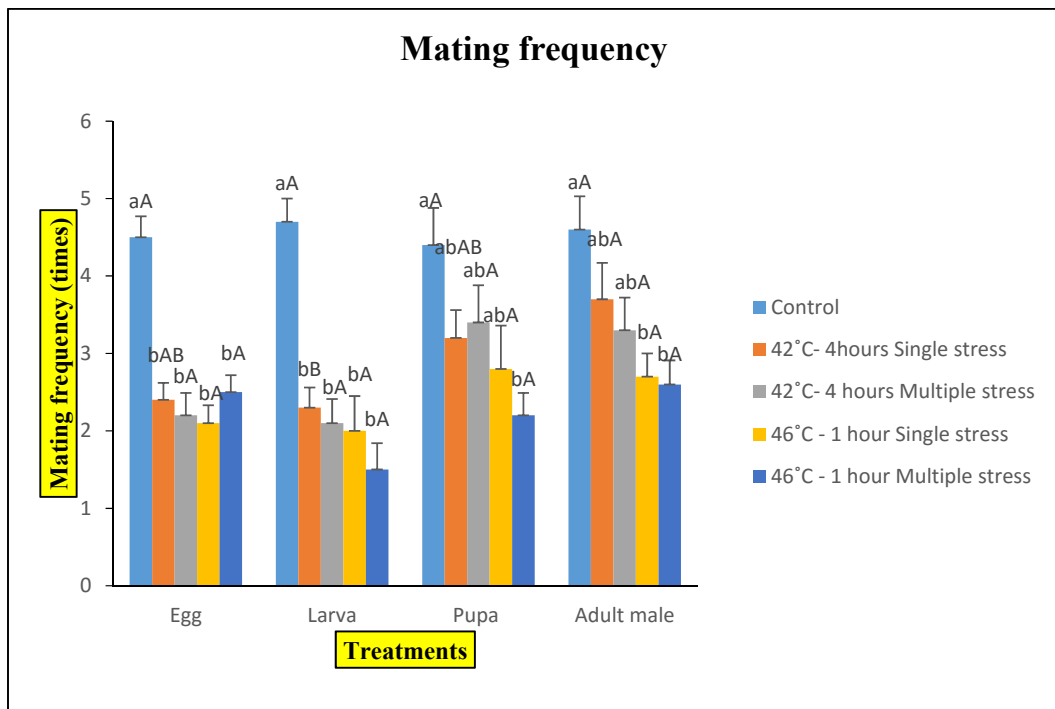


Figure 20. Consequences of short and long-term thermal stress on different developmental stages in the mating frequency of *Spodoptera litura* adults

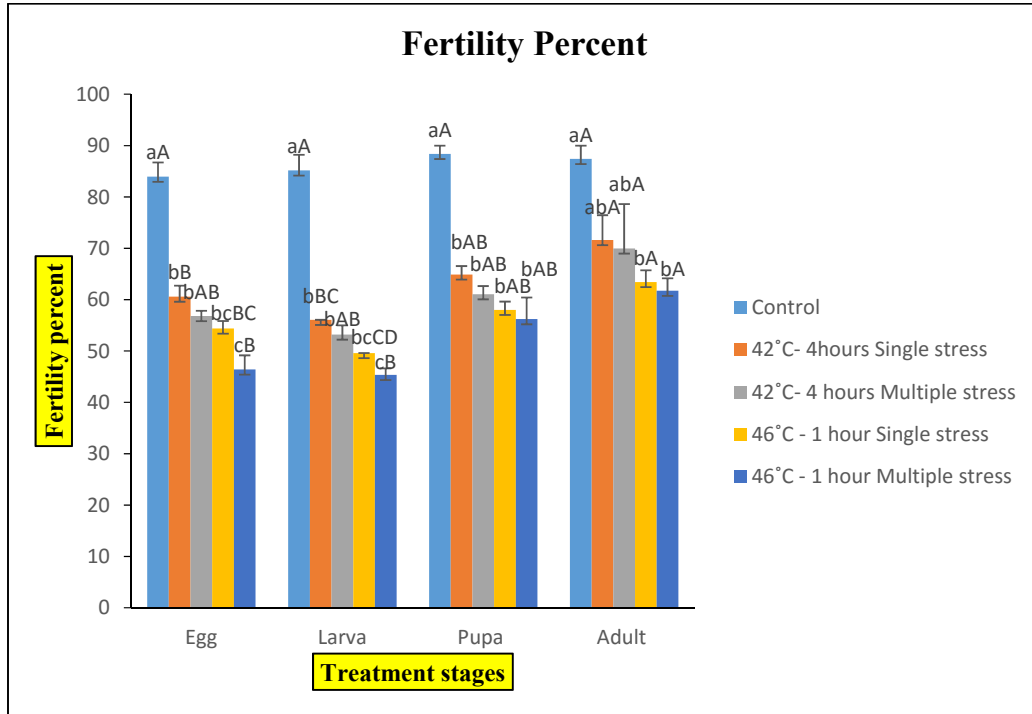


Figure 21. Impact on fertility percent when different developmental stages of *Spodoptera litura* exposed to short and long-term temperature stress

Similar to our results, earlier research in whitefly *T. vaporariorum* (Cui *et al.*, 2008), fruit fly *D. melanogaster* (Silbermann and Tatar, 2000) and flesh fly *S. crassipalpis* (Reinhart *et al.*, 2000) reported that the fertility percent was negatively influenced by the thermal stress treatments. Thermal stress exposure resulted in unviable sperm production in the male insects causing unfertilized eggs (Moiroux *et al.*, 2014; Reinhart *et al.*, 2000). The fecundity of the *S. litura* reduced under thermal stress treatments also causing the reduction in fertility percent of the stressed adults.

5.2. Effect of short and long-term thermal stress on metabolic responses of *Spodoptera litura*

Poikilothermic organisms existing in different kind of ecosystems, and they must encounter difficult obstacles for their existence and multiplication by reproduction. Major abiotic variable that influences the poikilothermic organisms is temperature (Cossins and Bowler, 1987; Bale *et al.*, 2002) and an organism's physiology can be changed by the temperature where it is present (Jia *et al.*, 2011). Insects must face the challenge of thermal stress during its existence. In the recent past and near future the thermal stress conditions will be more severe and recurrent which is attributed to climate change (Zhang *et al.*, 2013). Insects cope up with thermal stress by adopting different behavioural (like hiding, flight, etc.) as well as metabolic strategies (like synthesis of proteins, enzymes, sugars, etc.) to escape from the stress or to tolerate the stress events. To manage resulting consequences by thermal stress, synthesized metabolites like water (Ju *et al.*, 2014), sugars and sugar alcohols (Colinet *et al.*, 2015) and proteins play a major role in insect's thermo tolerance. The reproductive fitness of *Spodoptera litura* severely influenced by the exposure of short and long-term thermal stress. Likewise, *S. litura* developed various metabolic responses under thermal stress to overcome the risk of mortality. But limited information available regarding metabolic responses of *S. litura* under thermal stress. Particularly early life stages of an insect can be severely influenced by the thermal stress whereas the adults can be able to adapt different behavioural strategies to cope up with thermal stress (Hoffmann *et al.*, 2003). As an objective of our study, we quantified different metabolic responses like water content, protein level, carbohydrate level and glycogen level of adult *S. litura* emerged from stressed egg and larva exposed to short and long-term thermal stress. The obtained results of metabolic responses are discussed further.

5.2.1. Water content

Water content in insects helps in preventing the protein from denaturation, DNA damage and prevents lipid peroxidation. If the insects facing dehydration means ultimately it will lead to death (Lopez- Martinez *et al.*, 2009). For preventing the insect cells from damage, the maintenance of water balance is necessary (Phillips and Hanrahan, 1986). Major portion of water is lost through the cuticular spaces based on the surrounding temperature (Hadley, 1994). As a result of higher temperatures, the hydrocarbons of cuticle layer start dissolves and the loss of water increases through evaporation (Menzel *et al.*, 2017). This mechanism differs with the developmental stage of the insect and surrounding temperature. The loss of water through evaporation helps in maintaining the body temperature of an insect by cooling effect (Heinrich, 1980; Hadley *et al.*, 1991; Prange, 1996). But after certain point, the insects can't tolerate the thermal stress as it fails to maintain the water balance. The findings of our study revealed that the significant reduction in the water content with increase in temperature and frequency in both male and female adults emerged from the thermal stress exposed eggs and larva. Compared to male adults, female adults had slightly higher levels of water content in their body. At 46°C multiple stress for 1hour (4 days stress), the water content was least as compared to other treatments (Fig. 22). Similar to our results, the loss of water due to elevated temperatures were reported in several other insects such as *O. communa* (Chen *et al.*, 2019); ichneumonid wasp, *Lissopimpla excelsa* (Tomlinson and Phillips, 2012); tenebrionid beetle, *Eleodes armata* (Ahearn and Hadley, 1969) and grasshopper, *Melanoplus sanguinipes* (Rourke, 2000). Some insects improved their thermotolerance to the higher temperatures by regulating the loss of water (Prange, 1996). Balanced water loss due to thermal stress is reported in some insects like *Myzus persicae* (Ghaedi and Andrew, 2016), *Corythuca ciliate* (Ju *et al.*, 2014) and *Paratarsotomus macropalpis* (Wu and Wright, 2015). To cope with thermal stress the insects maintain the water balance to reduce their body temperature under thermal stress.

5.2.2. Protein content

Proteins produced during high and low temperature stress helps insects to tolerate the effects of stress. These proteins are helpful in several other thermoregulation mechanisms and pathways. Proteins produced under thermal stress acting as a folding agent for cellular proteins (Hightower, 1991). The proteins

synthesized during thermal stress helpful in thermotolerance of insects up to a greater extent also helpful in preventing the stockpiling of degenerated proteins. Our findings indicating that thermal stress in egg and larval stages of *S. litura* resulted in significant increase in the protein levels in both male and female adults. The female adults recorded higher protein content than the male adults. Stress in larval stage induced higher protein synthesis in male and female adults. Increasing trend of protein levels with increase in exposure temperature and frequency of was observed in insects (Fig. 23). Similar results of increasing protein levels under short and long-term thermal stress in *O. communa* is being reported by Chen *et al.* (2018) in which the protein content in male and female adults significantly increased at 44°C compared to control. In adults of *Myzus persicae*, multiple exposure (3 times) to thermal stress (38°C) resulted in elevated levels of total protein (Ghaedi and Andrew, 2017). In the same way, Chowanski *et al.* (2015) reported that the increase in total protein in the Madagascar cockroach (*Gromphadorhina coquereliana*) with exposure to cold stress. Contrasting to our findings, Rauschenbach *et al.* (1994) reported that in *Drosophila* adults exposure to thermal stress resulted in the decreased level of total proteins. The total protein was higher in the multiple thermal stress treatment which clearly indicates that proteins act as a thermo-protectant during thermal stress (Jain and Roy, 2009).

5.2.3. Carbohydrates

To refrain from thermal stress damage the insects produce and stockpile up various sugars and sugar alcohols. Accumulation of these sugars and polyols assists in protecting the proteins from degradation due to thermal stress. Back *et al.* (1979) reported that aphids and whiteflies produced sugar alcohols under temperature stress to assist in stabilization of proteins. In our study the *S. litura* adults emerged from the short and long-term thermal stress produced more carbohydrates with increase in temperature and frequency of exposure. Compared to control insects the 46°C multiple stress exposure resulted in higher carbohydrates level in both male and female adults. Stress in both egg and larval stages resulted in more accumulation of sugars in insects (Fig. 24). Accumulation of sugar alcohols such as erythritol, galactitol, lyxitol and mannitol in aphids under thermal stress was reported to act as a thermo-protectant (Michaud *et al.*, 2008; Rangel *et al.*, 2008). Our results coincided with the results of Chen *et al.* (2019) in which carbohydrate content of *O. communa* male and female adults increased under thermal stress significantly. In *Aphis gossypii*, *Bemisia argentifolii* (Hendrix and Salvucci, 1998), *Myzus persicae* (Ghaedi and Andrew, 2016)

and *Plodia interpunctella* (Kim *et al.*, 2017) also similar increase in total carbohydrates content were reported.

5.2.4. Glycogen content

Under thermal stress conditions, glycogen acts as a source of energy and it also helps in translocation of glucose molecules to give assistance in higher temperatures (Pant and Jaiswal, 1982). In insects glycogen is primarily helpful in the production of sugar alcohols and sugars (Storey and Storey, 1983). Glycogen levels in insects help in flight by which insects can able to adopt behavioural strategy to manage thermal stress. In our study, we found that increase in glycogen levels of adult *S. litura* when exposed to short and long-term thermal stress in egg and larval stages. Compared to males, female insects produced higher levels of glycogen (nearly doubled glycogen content in female adults). The increasing trend in glycogen levels with increase in temperature observed in both the stressed stages. At 46°C multiple stress exposure the glycogen levels recorded to be maximum in both male and female adults (Fig. 25). Similar to our results, Chen *et al.* (2019) reported that increase in glycogen levels with increase in temperature in *O. communa*. Whereas, Klapsatel *et al.* (2016) reported that exposure to thermal stress had no effect on the glycogen levels in *Drosophila melanogaster*. Contrasting to our findings, Malmendel *et al.* (2006) reported decreased levels of glycogen under thermal stress in *Drosophila melanogaster*. This increase in glycogen levels might improve feeding behaviour after thermal stress in later developmental stages of insects.

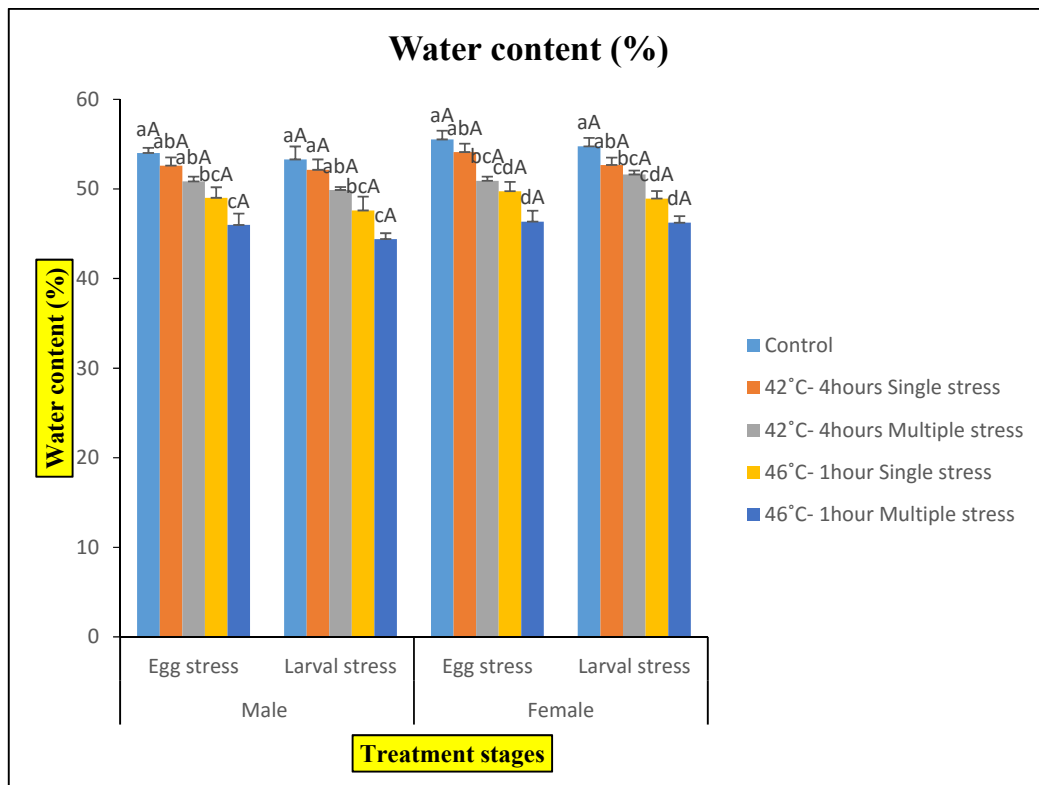


Figure 22. Effects of short and long-term temperature stress in egg and larval stages on water content (%) of adult male and female *Spodoptera litura*

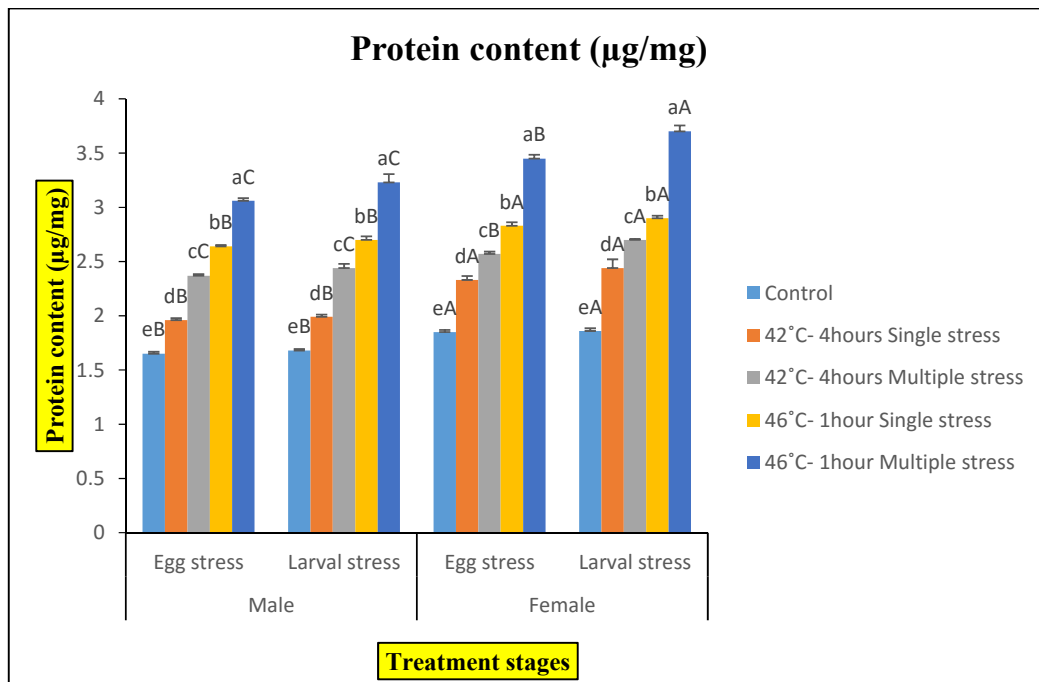


Figure 23. Consequences of single and multiple thermal stress in egg and larval stages on protein content (µg/mg) of adult male and female *Spodoptera litura*

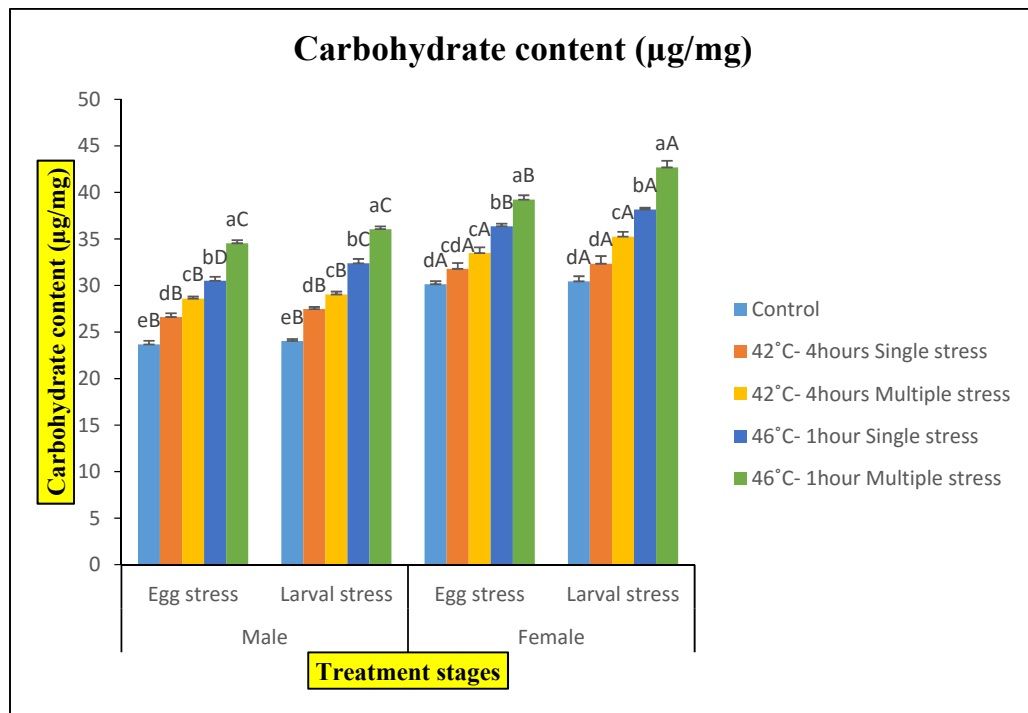


Figure 24. Carbohydrate content ($\mu\text{g}/\text{mg}$) of male and female adult insects of *Spodoptera litura* under single and multiple thermal stress in egg and larval stages

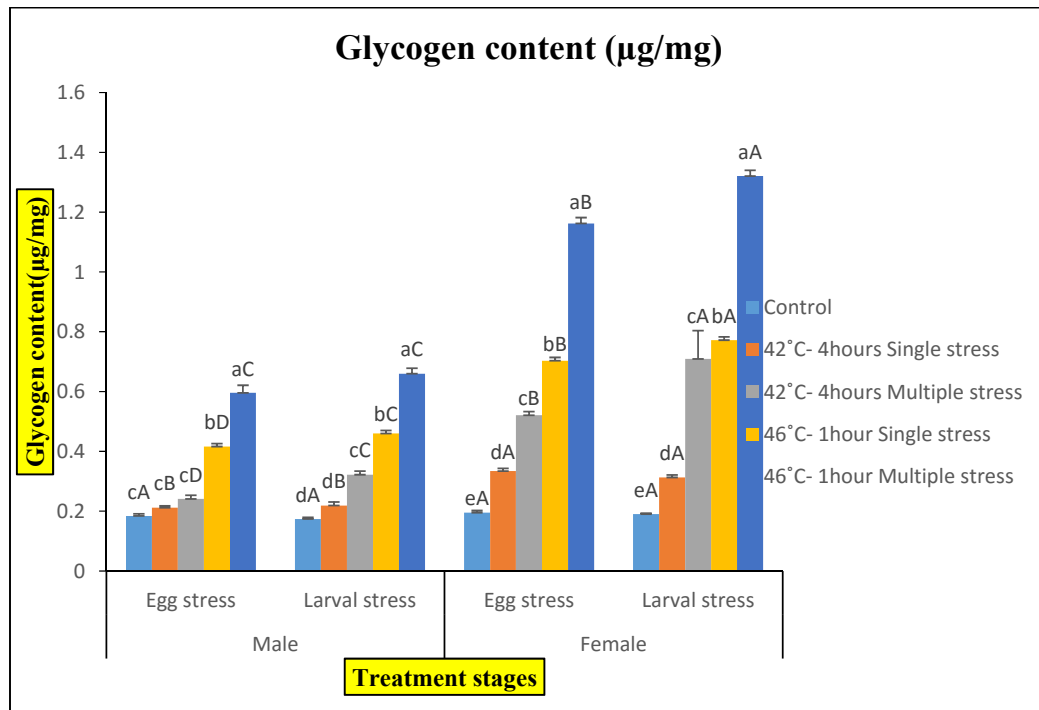


Figure 25. Influence of short and long-term temperature stress in egg and larval stages on the glycogen content ($\mu\text{g}/\text{mg}$) of adult male and female *Spodoptera litura*

VI. SUMMARY AND CONCLUSION

One of the important abiotic factor influencing behaviour, physiology, life history and abundance of insects is temperature. As a result of climate change, the hot events frequency had occurred in recent past and they tend to become more frequent in the near future, insects are also being affected by these thermal stress conditions. While this does not always result in death of the insects and it is rarely incorporated in population prediction. Earlier studies have focused either on the effects of constant exposure of extreme temperatures on the insects or thermal stress exposure on the immediate survival of insects. The information on the effects of short and long-term thermal stress exposure on the biology, reproduction and metabolic responses is scanty. With these limitations, experiments were performed to find out the effects of short and long-term thermal stress exposure in different developmental stages on the reproductive fitness and metabolic responses in *S. litura* at Division of Entomology, ICAR- Indian Agricultural Research Institute, New Delhi during the academic year of 2020-2021. The key results of the experiments are summarised briefly in this chapter.

6.1. Effects of short and long-term thermal stress on reproductive fitness of *S. litura*

The effects of short and long-term thermal stress exposure on different developmental stages of *S. litura* [100 eggs (≤ 18 hrs old), 100 4th instar larvae (newly molt, ≤ 12 hrs old), 100 pupae 100 (newly formed, ≤ 12 hrs old), male and female adults 30 number (newly emerged, ≤ 12 hrs old)] were investigated by exposing insects to different thermal stress treatments *viz.*, 27°C (control), 42°C- short and long term and 46°C- short and long-term thermal stress. Short term refers to single time exposure and long term refers to multiple time exposures *i.e.*, 2, 4, 2 and 2 days for egg, larva, pupa and adults respectively with the help of temperature-controlled incubator as described by Huang *et al.* (2007) and Chen *et al.* (2018). Exposure to thermal stress showed a significant influence on the different fitness parameters of *S. litura*. Biological attributes and reproductive fitness parameters included survival rate, maturation success, female ratio, size, weight, longevity of male and female adults, gonadosomatic index, eupyrene sperm count, pre-oviposition, oviposition, post-oviposition period, total fecundity, mating success and frequency, fertility percent. In response to higher temperature insects died rapidly due to thermal injury. Thermal stress in the larval stage had the

most severe effect on the survival rate than stress in other stages. Survival rate was minimum at 46°C multiple stress for 1 hour in all the stages. For calculating maturation success, egg and larval stages were stressed and maturation success decreased with increase in temperature and frequency of exposure. When compared to egg stage, stress in larval stage resulted in less maturation success. Maturation success was least in the treatment 46°C multiple stress for 1 hour exposure than control. Adults emerged from the stressed egg and larva was taken into account for computing female ratio. Female ratio was maximum in higher temperature with multiple exposures. The maximum female ratio was observed in the treatment of 46°C multiple stress for 1 hour exposure than control. The longevity of male and female adults emerged from stressed pupa recorded lower longevity as compared to other stages. Size and weight of female adults recorded was least in the adults emerged from stressed egg whereas in the male, adults emerged from stressed larva recorded lower size and weight. The stressed adults recorded larger size and more weight as compared to other stages. Stress in egg, larva and pupal stage had significant impact on gonadosomatic index than adult stage. The most susceptible stage was larva and the most deleterious treatment was 46°C – 1h multiple stress. Level of eupyrene sperm decreased with increase in temperature and duration. The most susceptible stage was adults emerged from stressed eggs in which the eupyrene sperm count recorded least as compared to other stages. Adults emerged from the stressed developmental stages like egg, larva, pupa and survived adults after thermal stress were released into mating jar at 2:2 ratio (Male: Female adults) to study the reproductive parameters. To assess the reproductive fitness, parameters such as pre-oviposition, oviposition, post-oviposition, total fecundity, mating success, mating frequency and fertility percent were measured. In all the developmental stages, different short and long-term thermal stress treatments and stages of exposure had varied effects on the pre-oviposition and post-oviposition period and there was no clear trend observed between treatments. The egg stage was most susceptible to the thermal stress in which the oviposition period, total fecundity of the adults emerged from stressed eggs saw a substantial reduction than other stages. Adult stage was most tolerant to the thermal stress in which the oviposition period and fecundity recorded maximum. Mating success and mating frequency of adults emerged from the stressed stages decreased significantly with increase in temperature. The larval stage was most susceptible to thermal stress as the mating success and mating frequency recorded least compared to other stages. The fertility percent showed decreasing trend with increase

in temperature exposure and frequency. Among the different developmental stages thermal stress exposure, the adults emerged from stressed larvae showed lower fertility percent at 46°C – Multiple stress 1 h, while adults survived after thermal stress showed maximum fertility percent.

6.2. Metabolic responses of *S. litura* exposed to thermal stress

For studying the metabolic responses of *S. litura* freshly emerged male and female adults from short and long-term thermal stress exposed eggs and 4th instar larva were used. The thermal stress treatments were 42°C for 4 hours and 46°C for 1 hour (one time stress in egg and 4th instar larva- single stress). Multiple stress treatments were given to the insects by exposing them to 42°C for 4 hours and 46°C for 1 hour per day (2 days for egg, 4 days for larvae), in control the insects were maintained at 27±1°C. Under metabolic responses we estimated water content, protein, carbohydrates and glycogen contents by using the standard procedures.

Water content of both male and female adults emerged from stressed egg and larva reduced significantly with increase in temperature and frequency of exposure. Stress in larval stage resulted in reduced water content in both male and female adults than stress in egg stage. The water content was least in 46°C for 1hour multiple stress treatment for both male and female adults. Compared to male adults, female adults were more tolerant to thermal stress in both the stages. Protein content of male and female adults gradually increased with increase in temperature and duration in both the egg and larval stress. The higher protein content was seen in larval stage in case of both male and female adults. Compared to male adults, the female adults had more protein content as a response to thermal stress. The maximum effect of thermal stress was observed in the treatment, 46°C- 1hour multiple stress, in comparison with control. The carbohydrate content of both male and female adults emerged from stressed egg and larva increased significantly with increase in temperature and frequency of exposure. The carbohydrate levels were maximum in the male and female adults emerged from stressed larva. Levels of carbohydrates were higher in female adults than the male adults under thermal stress. Compared to control the levels of carbohydrates increased significantly in 46°C- 1hour multiple stress treatment. Glycogen content of the adults increased multiple times with increase in temperature and duration. Adults from stressed larvae recorded maximum glycogen content in both male and female adults.

Female adults had more glycogen levels than the male adults. At 46°C- 1hour multiple stress the glycogen levels increased multiple times than control.

CONCLUSION

For survival and reproduction, ectothermic species must face significant challenges including biotic and abiotic stresses. Temperature is one of the most important abiotic variables that affects ectotherms. The temperature stress reduced the survivability of insects and fitness of the survived individuals were also affected. The reproductive fitness also decreased when the early stages experienced the thermal stress compared to later stages. Physiological metabolites like water, protein, carbohydrates and glycogen play an important role in organism's response to thermal stress. Physiological thermal tolerance differs depending on life stages exposed, this increase in physiological metabolites can provide possible protection against thermal stress. The present research study gives the holistic account on the reproductive fitness and metabolic responses of *Spodoptera litura* under single and multiple stress which will helps in predicting population dynamics and distribution of *S. litura* under changing climate scenario.

Studies on physiological responses of *Spodoptera litura* exposed to short and long-term thermal stress

ABSTRACT

Tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is one of the most destructive polyphagous insect pest damaging economically important 112 species of host plants. One of the most significant abiotic factors influencing organism's nature, physiology, life history, distribution and abundance is temperature. Shorter periods of extremely high temperature, known as thermal stress are expected to become extensive and severe in the near future as a result of global warming. Effects of the thermal stress vary according to the life stage of the insect exposed because changes in the physiological responses to the temperature are subject to alter during development. The physiological responses of short and long-term thermal stress on various developmental stages [27°C – control, 42°C 4hours single and multiple (2, 4, 2 and 2 days for egg, larva, pupa and adults respectively) stress, 46°C 1hour single stress and multiple (2, 4, 2 and 2 days for egg, larva, pupa and adults respectively) stress] of *S. litura* were evaluated by recording survival rate, maturation success, female ratio, longevity, size and weight of male and female adults, GSI, eupyrene sperm count and reproductive fitness parameters comprises pre-oviposition, oviposition, post-oviposition period, total fecundity, mating success, mating frequency and fertility percent in adults emerged from the stressed stages. In all the parameters the observed values varied between the treatments. Other than pre-oviposition and post-oviposition period the thermal stress exposure had significant influence on all parameters. The increase in temperature and frequency of exposure caused a reduction in most of the biological and reproductive fitness parameters. The female ratio increased due to thermal stress at higher temperatures compared to control. The metabolic responses like water content (%), protein, carbohydrates and glycogen contents ($\mu\text{g}/\text{mg}$) of male and female adults of *S. litura* emerged from stressed egg and larva were studied. The results revealed that the water content reduced significantly in both male and female adults under thermal stress. The protein, carbohydrates and glycogen content increased many times in the stressed adults compared to control. 46°C 1hour multiple stress considered to be the most impactful treatment as insects exhibited the least performance in all the parameters. The early developmental stages like egg and larva were the most susceptible stages to thermal stress.

Key words: *Spodoptera litura*, thermal stress, reproductive fitness, developmental stages, metabolic response, survival rate, eupyrene sperm, mating success, protein content, glycogen content

शॉर्ट और लॉन्ग टर्म थर्मल स्ट्रेस के संपर्क में आने वाले स्पोडोप्टेरा लिटुरा के शारीरिक

प्रतिक्रियाओं पर अध्ययन

सारांश

तंबाकू काटने वाला कीट, स्पोडोप्टेरा लिटुरा (फैब्रिकियस) (लेपिडोप्टेरा: नोक्टुइडे) सबसे विनाशकारी पॉलीफैगस कीटों में से एक है जो मेजबान पौधों की आर्थिक रूप से महत्वपूर्ण ११२ प्रजातियों को नुकसान पहुंचाता है। जीव की प्रकृति, शरीर क्रिया विज्ञान, जीवन इतिहास, वितरण और बहुतायत को प्रभावित करने वाले सबसे महत्वपूर्ण अजैविक कारकों में से तापमान एक है। अत्यधिक उच्च तापमान की छोटी अवधि, जिसे थर्मल स्ट्रेस के रूप में जाना जाता है और निकट भविष्य में ग्लोबल वार्मिंग के परिणामस्वरूप व्यापक और गंभीर होने की उम्मीद है। ऊष्मीय तनाव के प्रभाव कीट के जीवन स्तर के अनुसार भिन्न भिन्न होते हैं, क्योंकि तापमान के लिए शारीरिक प्रतिक्रियाओं में परिवर्तन विकास के दौरान परिवर्तन के अधीन होते हैं। विभिन्न विकास चरणों पर लघु और दीर्घकालिक थर्मल तनाव की शारीरिक प्रतिक्रियाएं [२७°C - नियंत्रण, ४२°C ४ घंटे एकल और एकाधिक (अंडे, लार्वा, प्यूपा और वयस्कों के लिए क्रमशः २, ४, २ और २ दिन) तनाव, ४६°C १ घंटा एकल तनाव और एस लिटुरा के मल्टीपल (अंडे, लार्वा, प्यूपा और वयस्कों के लिए क्रमशः २, ४, २ और २ दिन) तनाव] का मूल्यांकन जीवित रहने की दर, परिपक्वता सफलता, महिला अनुपात, लंबी उम्र, आकार और पुरुष और महिला वयस्कों के वजन को दर्ज करके किया गया था। , जीएसआई, यूपाइरीन स्पर्म काउंट और रिप्रोडक्टिव फिटनेस पैरामीटर्स में प्री-ओविपोजिशन, ओविपोजिशन, पोस्ट-ओविपोजिशन पीरियड, टोटल फीकुंडिटी, मेटिंग सक्सेस, मेटिंग फ्रीक्वेंसी और स्ट्रेस स्ट्रेज से निकले वयस्कों में फर्टिलिटी प्रतिशत शामिल हैं। सभी मापदंडों में देखे गए मान उपचारों के बीच भिन्न थे। प्री-ओविपोजिशन और पोस्ट-ओविपोजिशन अवधि के अलावा थर्मल स्ट्रेस एक्सपोजर का सभी मापदंडों पर महत्वपूर्ण प्रभाव पड़ा। तापमान में वृद्धि और जोखिम की आवृत्ति के कारण अधिकांश जैविक और प्रजनन फिटनेस मापदंडों में कमी आई है। नियंत्रण की तुलना में उच्च तापमान पर थर्मल तनाव के कारण महिला अनुपात में वृद्धि हुई। एस लिटुरा के पूर्व और बाद की ओविपोजिशन अवधि पर थर्मल तनाव का विविध प्रभाव पड़ा। एस लिटुरा के नर और महिला वयस्कों में पानी की मात्रा (%), प्रोटीन, कार्बोहाइड्रेट और ग्लाइकोजन सामग्री ($\mu\text{g}/\text{mg}$) जैसी चयापचय प्रतिक्रियाओं का अध्ययन तनावग्रस्त अंडे और लार्वा से किया गया था। परिणामों से पता चला कि थर्मल तनाव के तहत पुरुष और महिला दोनों वयस्कों में पानी की मात्रा में काफी कमी आई है। तनावग्रस्त वयस्कों में

नियंत्रण की तुलना में प्रोटीन, कार्बोहाइड्रेट और ग्लाइकोजन का स्तर कई गुना बढ़ जाता है। ४६°C १ घंटे के बहु तनाव को सबसे प्रभावशाली उपचार माना जाता है क्योंकि कीड़ों ने सभी मापदंडों में सबसे कम प्रदर्शन किया है। अंडे और लार्वा जैसे प्रारंभिक विकास चरण थर्मल तनाव के लिए अतिसंवेदनशील चरण थे।

मुख्य शब्द: स्पोजोप्टेरा लिटुरा, थर्मल स्ट्रेस, प्रजनन स्वास्थ्य, विकास के चरण, चयापचय प्रतिक्रियाएं, जीवित रहना अनुपात, यूपाइरीन शुक्राणु, प्रोटीन सामग्री, ग्लाइकोजन सामग्री

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