***Original Research Article***

# Effects of temperature on biochemical parameters of fall armyworm, *Spodoptera frugiperda* (J.E. Smith)


## ABSTRACT

Fall Armyworm *Spodoptera frugiperda* (J.E. Smith) is the major invasive pest of maize and spread to all agro climatic zones in Tamil Nadu. The ability of fall armyworm to adapt to varied climatic conditions may be due to their ability to modify biochemical characters. Among the different environmental parameters, temperature is one of the important components, which influences the level of carbohydrate, protein and lipid present in the caterpillars. After first notice during 2018, the fall armyworm presence has observed throughout India that shows their adaptation to different climatic conditions. The study on biochemical properties of fall armyworm under different temperature regime will provide insights about their preference to temperature regimes. In the present study, the biochemical parameters of fourth late instar fall armyworm larvae were studied at five different constant temperatures (15, 20, 25, 30 and 35 °C) in complete randomized design. The biochemical studies revealed that the amount of total proteins, total carbohydrates, total lipids are high in the larvae grown at 15 and 20°C. Amount of amino acids increased gradually with rising temperatures. The total proteins, total carbohydrates, total lipids are very less at 25°C while gradually increases at 30 and 35°C. The level of amino acids is very less in the larvae grown in 35°C while other biochemical parameters (proteins, carbohydrates, lipids) are comparatively more in 35°C.



*Keywords: Temperature, Spodoptera frugiperda, Carbohydrate, Protein & Lipids*


## 1. INTRODUCTION

Insects are exposed to a variety of stress, thermal stresses, which can affect their fitness and metabolism. Parmesan (2006) reported that climate change might cause increase spread of insect species at a rate of 6.1 kilometers a decade. It can affect the extent of the harm produced by insect pests in two ways: directly through their development, reproduction, and spread, and indirectly through changes in host physiology and defence mechanisms[1]. Temperature is one of the important abiotic factors which can cause physiological and metabolic changes in insects and results in changes in their life cycle [2]. Insects rely upon outside source to regulate their temperature [3]. The thermoregulation is important to maintain optimum integral temperature in insects. In general caterpillars prefer warmer temperature which allow them to minimize the amount of energy they have to spread to maintain their body temperature [4]. Thermal stress raises the body temperatures of insects to fatal levels**.** Berggren et al. (2009) revealed that 10°C increase in temperature doubles the metabolic activity of the insects[5]. The accelerated metabolic activity may lead to higher consumption, growth and developmental rates. Lee et al. (2007) revealed that

temperature affects diffusion, membrane fluidity, nucleic acid stability, gas and salt solubility and behaviour of enzymes[6].

The invasive alien species threatens the cultivation of important crops in developing countries. The increased transboundary movement of agricultural commodities, anthropogenic activists, climate change and other related factors paired the way for invasion of above 1300 species of invasive pests in 124 countries [7]. The tropical and subtropical native noctuid *Spodoptera frugiperda* threatened the maize cultivation in Africa after its invasion during 2015 and subsequently its presence on maize was noticed in 2018 at Shivamogga, Karnataka[8] . After its introduction into India, with in a short span of time the presence of fall armyworm has been recorded in most parts of India. This shows the ability of fall armyworm to thrive in varied climatic conditions. The fall armyworm can survive in 353 different plant species belonging to 76 botanical families [9]. Maize, rice, sorghum, cabbage, cotton, peanut, soybean and alfalfa are the crops predominantly preferred by FAW.

In Tamil Nadu fall armyworm infestation was noticed in 2018 since then its presence has been felt in all maize growing regions of the state because of its strong damage potential in maize. The newly hatched larvae move vertically within the plant and horizontally to the adjacent plants. High dispersal ability of moths favours wide geographical dispersion. The ability of fall armyworm to adapt to varied climatic conditions may also due to their ability to modify biochemical characters. Understanding the biochemical change at different temperature regimes will give insights about the preferred temperature regime and changes take place at the extreme temperature regimes in the fall armyworm. The changes in metabolic activities in insects helps them to survive in different temperature regimes [10]. The climatic change influences the insect pests *viz*., damage to crop plants, reproduction, distribution, change in host physiology and defence mechanisms. In this context, it is necessary to know the biochemical changes that takes place in fall armyworm under different temperature regimes.

## 2. MATERIAL AND METHODS

### 2.1 Maintenance of *Spodoptera frugiperda* Culture

Fall armyworm nucleus culture maintained at Department of Agricultural Entomology, TNAU, Coimbatore used in the present study. The Fall armyworm culture were maintained at this laboratory from the field-collected population for more than 15 generations. This homogenous population used to avoid any difference in their response in the experiments. To maintain the viability of the homogenous culture, field collected populations were introduced intermittently to the laboratory cultures.

The fall armyworm larvae reared in artificial diet (unpublished data). First three instar larvae were gregariously reared and from late third to fourth instar transferred to individual plastic containers (30 x 40 x 40 mm), in which holes were made in lids to provide aeration to the larvae. The artificial diet was changed periodically. The larval cultures were maintained at 28 ± 2°C and 70-80% relative humidity. The larvae after pupation kept in adult emergence cages. The adult diet consists of sugar and honey in 1:1 ratio along with mineral supplements were given. Twenty days old maize seedlings were placed inside the adult emergence cages as oviposition substrates. The eggs were collected and transferred to transparent plastic boxes (17 x 11 x 5 cm) containing the diet pieces.

## 2.2 Temperature studies

The maize leaves were kept inside the adult rearing cage for oviposition and the leaves were collected immediately after egg laying. The collected eggs were placed in the growth chamber (Make: Faithful Instrument Co., Ltd.) at different temperature regimes *viz*, 15, 20, 25, 30 and 35°C. Relative humidity of 80% and photoperiod of 14:8 maintained in the growth chamber throughout the study period. The experiment was conducted in complete randomized design and each temperature regime has been replicated three times. For individual temperature regime, the eggs were introduced in the growth chamber and maintained until pupation following the standard rearing methodology (Table 1). The fourth instar larvae from each temperature regime were collected and used for the biochemical analysis.

**Table 1: Details of *S. frugiperda* in growth chamber**

| Temperature | Date | | | |
|---|---|---|---|---|
| | Introduction of Eggs | 4th Instar | Pupa | Adult |
| 15°C | 15/12/2021 | 03/01/2022 | 12/01/2022 | 25/01/2022 |
| 20°C | 07/02/2022 | 25/02/2022 | 08/03/2022 | 21/03/2022 |
| 25°C | 23/03/2022 | 06/04/2022 | 16/04/2022 | 28/04/2022 |
| 30°C | 30/03/2022 | 10/04/2022 | 19/04/2022 | 29/04/2022 |
| 35°C | 09/05/2022 | 18/05/2022 | 25/05/2022 | 04/06/2022 |

## 2.3 Biochemical studies

### 2.3.1 Sample Preparation:

Twenty-five 4th instar *S. frugiperda* larvae of from each rearing temperature were collected in a chilled glass. The larvae were homogenized in distilled water (50 mg. /1 ml). The homogenates were centrifuged at 8000 rpm for 15 min at 5°C in refrigerated centrifuge. The deposits were discarded and the supernatant was kept in a deep freezer at -20°C for biochemical assays. Each biochemical assay was replicated three times.

### 2.3.2 Protein Concentration Determination

Protein concentrations were determined by the Bradford method[11] using bovine serum albumin (BSA) as a standard. Samples (10 µL) were mixed with 990 µL distal water in a test tube. To that 1 ml of Bradford reagent was added and kept in dark for 10 mins and the absorbance was measured at 595nm using UV-VIZ Spectrophotometer.

### 2.3.3 Carbohydrate concentration determination

Carbohydrate concentrations were estimated as described by Singh and Sinha[12]. Sample (0.5ml) was mixed with 0.5 ml of distal water and 4 ml of anthrone reagent was added and kept in boiling water bath for about 8 minutes, cooled and the absorbance was recorded at 630nm UV-VIZ Spectrophotometer.

### 2.3.4 Lipid concentration determination

Lipid concentration was determined as per the protocol of Knight *et al.* (1972) [13]. Sample (0.5ml) was taken in a test tube to which 5 ml of concentrated $H_2SO_4$ was added. The test tube was heated for 10 min in boiling water bath, cooled and from that 0.4 ml of aliquot was placed in dry, clean test tube labelled as unknown. Concentrated $H_2SO_4$. (0.4ml) was used a blank. Phosphor-vanillin reagent 6 ml was added to each test tube of sample and blank. Absorbances were measured at 525 nm after setting dark for 45 min UV-VIZ Spectrophotometer.

### 2.3.5 Amino acid concentration determination

Free Amino acid assayed by ninhydrin reagent according to Lee and Takahashi (1966) [14]. Sample (0.5ml) was taken and 0.5 ml of distal water was added and 4 ml of ninhydrin citrate glycerol reagent was added. The tubes were heated for 10 min in boiling water bath, cooled and absorbance were taken at 570 nm UV-VIZ Spectrophotometer.

## 2.4 Statistical Analysis

The statistical analysis were carried out using the statistical package SPSS 20.0 version for windows.

## 3. RESULTS

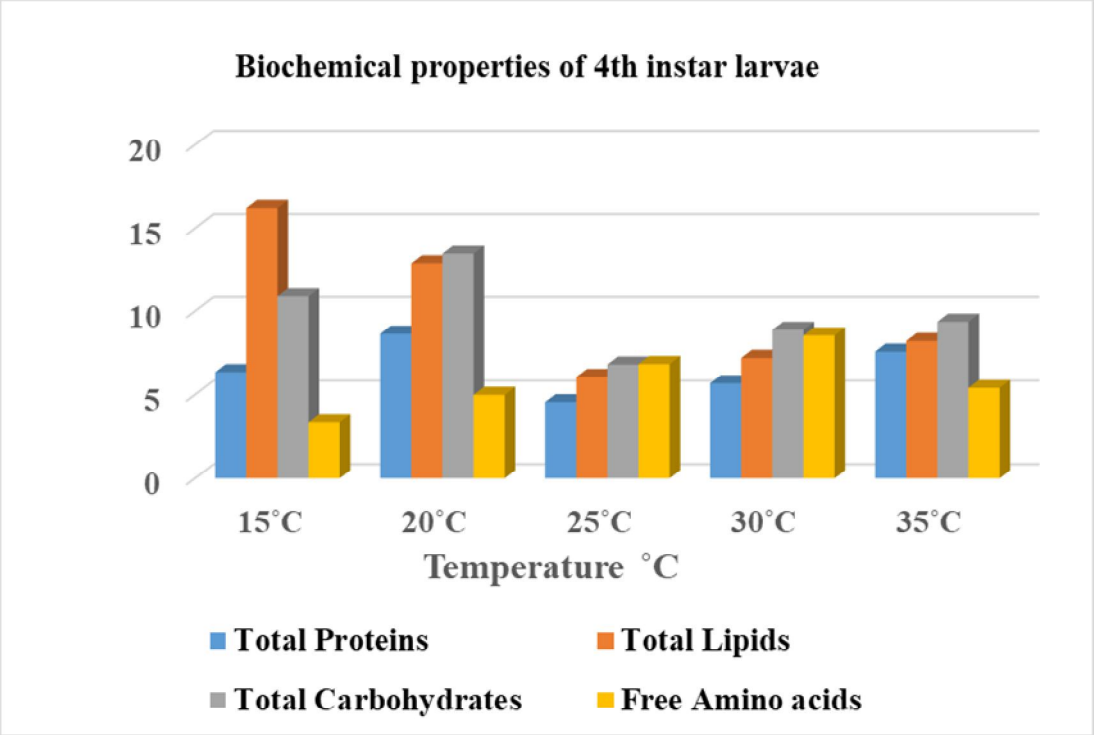
The effect of temperature on biochemical properties of *S. frugiperda* were presented in Table 2. The total protein, lipid and carbohydrates were expressed as mg/g body weight of the insect. The protein concentration was highest (8.63 mg/g body weight) in the *S. frugiperda* larvae reared at 20°C followed by larvae reared at 35°C (7.57 mg/g body weight). The optimum temperature 25 and 30°C recorded 4.55 and 5.69 mg/g body weight protein concentration respectively. The highest lipid concentration (16.94 mg/g. body weight) was recorded in *S. frugiperda* at 15°C rearing temperature. The total lipid concentration was gradually decreased from 20°C to 30°C and then again increased at 35°C. The lipid concentration at 20, 25, 30 and 35°C temperatures were 12.89, 6.07, 7.20 and 8.20 mg/g body weight respectively.

The maximum total carbohydrate concentration was 13.47 mg/g body weight at 20°C. The carbohydrate concentration was 6.81, 8.87 and 9.36 at 25, 30 and 35°C temperature respectively. The difference in carbohydrate concentration at 20 and 25°C was 6.66 mg/g body weight The concentration gradually decreases at 25 and 30°C and starts increasing at 35°C. The free amino acid concentration was maximum at 25 and 30°C temperature (6.84 & 8.53 mg/g body weight). The free amino acid concentration at **1**5, 20 and 35°C temperature were 3.36, 4.99 and 5.41 mg/g body weight respectively. The amino acid concentration in *S. frugiperda* was minimum at lowest and highest temperature whereas maximum amino acid concentration was recorded at optimum temperature (30°C) in the present experiments.

**Table 2: Effect of different temperatures on biochemical properties of *S. frugiperda***

| Temp.(°C) | Total proteins (mg/g.b. wt.) Mean ± SE | Total lipids (mg/g.b. wt.) Mean ± SE | Total carbohydrates (mg/g.b. wt.) Mean ± SE | Free amino acids (mg /g.b.wt.) Mean ± SE |
|---|---|---|---|---|
| 15°C | 6.36 ± 0.2 | 16.19 ± 0.11 | 10.88 ± 0.28 | 3.36 ± 0.25 |
| 20°C | 8.63 ± 0.10 | 12.89 ± 0.12 | 13.47 ± 0.24 | 4.99 ± 0.23 |
| 25°C | 4.55 ± 0.11 | 6.07 ± 0.53 | 6.81 ± 0.095 | 6.84 ± 0.20 |
| 30°C | 5.69 ± 0.29 | 7.20 ± 0.36 | 8.87 ± 0.35 | 8.53 ± 0.13 |
| 35°C | 7.57 ± 0.17 | 8.20 ± 0.08 | 9.36 ± 0.11 | 5.41 ± 0.15 |

**Fig 1. Effect of different temperatures on biochemical properties of 4th instar larvae of *S. frugiperda***




## 4. Discussion

The metabolism, behaviour and ecology of any organism will be influenced by the environmental temperature [15]. The energy gained through metabolic activities used for vital activities such as movement, growth, reproduction and nutrition. In general, female insects store more carbohydrates, proteins and lipids than males in both pre adult and post adult stages. Temperatures influence insect survival by speeding up their metabolism, to increase intake of food, growth, and development**.** The metabolic activities in insects increases with rise in temperature. The tolerance to variable temperatures provided through modifying metabolic activities [16] .

Protein plays potential role in growth, development, morphogenesis and metabolism of insects [17]. Malik and Malik (2009) reported that *Bombyx mori* L. larvae and pupa when exposed to high temperature, the protein levels were decreased in haemolymph [18]. In the present investigation, the protein concentration was more in lower temperature regime, minimum at optimal temperature regime and highest at maximum temperature. The protein structure and the biochemical rate and physiological reaction rate is affected by the exposure to high temperature [19]. The high temperature kills the insects' cells by denaturing proteins, altering membrane, enzyme structure and properties. Neven (2000) conclude that in high temperature due to denaturation of proteins death occurs. The higher protein concentration recorded at 35˚C indicate that *S. frugiperda* larvae capability to survive in higher temperature compare to other caterpillars [10].

Insects store food as lipids because it can be store without extensively increasing body weight. Lepidoptera and Orthoptera uses lipids as main energy source [20]. The lipids effect

the membrane fluidity and viability of stored energy resources. In *Philosamia ricini* lipid composition was important trait for their fitness response to temperature [21]. The lipid concentration was highest at lowest temperature 15 and 20°C and these findings correlate with results of Sonmez and Gulel, 2008 [22]. They found that at 25 and 30°C lipid concentration in bean beetle *Acanthoscelides obtectus* was equal. The storage efficacy of lipids in *Spodoptera exigua* larvae was lower at 18°C than at 26°C [23]. In the present investigation also the lipid concentration was lowest at 25°C.

The main energy producing molecules in caterpillars are carbohydrates and they mostly depend upon on leaves. The carbohydrates have a positive influence on life span of insects whereas proteins help in body mass. Sonmez and Gulel (2008) reported that the amount of total carbohydrates and proteins were decreased at lower temperatures in bean beetle *Acanthoscelides obtectus* [22]. In the present investigation carbohydrate levels were higher at lowest temperature regimes which is contradicting to the Sonmez and Gulel, 2008 [22] findings. The bean beetle is a storage pest whereas *S. frugiperda* is field pest and reared on artificial diets at different temperature regimes. This might have helped to increase the carbohydrate level in *S. frugiperda* at lower temperature. Kostal 2011 concluded that total free amino acid doubled during winter on *Pymhocoris apterus* [24]. They also revealed that the Pro and α- ala amino acids significantly contributed to the seasonal incidence. In the present investigation, lowest temperature regimes recorded lowest amino acid levels.

## 5. CONCLUSION

In the present study the level of carbohydrate, protein and lipids were higher at lower temperature regimes whereas the amino acids concentration was lowest. The higher temperature 35°C also recorded higher level of carbohydrate, protein, lipids and amino acid concentration. This shows the ability of fall armyworm to adapt to varied climatic conditions through their metabolic activities.

## DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

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