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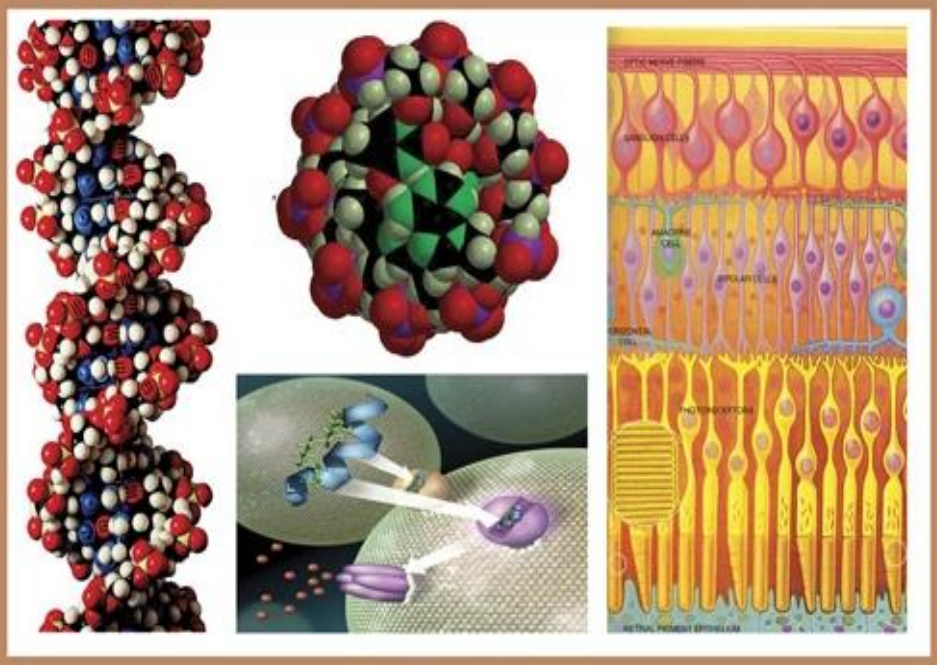

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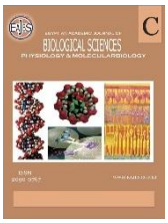

Biochemical and Physiological Reactions for Field Strain of Cotton Leafworm, *Spodoptera littoralis* (Boisd.) as An Exposure-Response to Temperature Under Climatic Change

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ABSTRACT

It is important to understand the effects of temperatures on Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) which damages a wide variety of crops in the Middle East and is one of the major economic pests of cotton in Egypt. Therefore, the investigating was designed to study the effect of fluctuation temperatures on the field strain in Abnob city, Assuit Governorate during the 2017 and 2018 cotton growing seasons. On the other hand, the results compared with the laboratory strain under constant temperature (25°C). The biochemical parameters were measured on the 4th larval instars for both strains. Biochemical analyses were performed to determine the effects of fluctuation and constant temperatures on field and laboratory strain on total proteins, lipids, carbohydrates and free amino acids as well as metabolic digestive, neural and detoxification enzymes. It's clear from the results that the GST, GOT, AchE, Alpha esterases, Beta esterases as well as, the activity levels were increased significantly by field strain. While the free amino acids, total proteins, proteases, lipases, amylase were decreased significantly by field strain and no significant changes were detected between field and laboratory strains in total carbohydrates, total lipids, trehalase and GPT.

INTRODUCTION

Climate changes are considered the most important factors that affected insects in terms of physical fitness and metabolism Rinehart *et al.*, 2000. Temperature is a profound biotic environmental factor that induces physiological changes in insects resulting in rapid metabolic variation, which can lead to disorders affecting their lives Parmesan and Yohe 2003. Many authors concluded that, as a result of global warming, the frequency and degree of appearance of high temperatures are predicted to increase substantially Easterling *et al.*, 2000 and Deffenbaugh, 2005. In addition, Parmesan, 2006 noted that the change of climate may cause insect species to spread at the mean rate of 6.1 km per decade. It can influence insect pests and the size of damage caused by them: directly through their development, reproduction and distribution and indirectly by altering host physiology and defining mechanisms. Moreover, temperatures tend to enhance insect survival because they accelerated metabolism which may lead to higher consumption, growth, and development rates.

Faster development, in turn, may lead to population increases via reduced generation time and decreased warmer late winter and early-spring Bale *et al*., 2002. Metabolism, growth and reproduction increase exponentially with warming. Global warming is producing increases in both average temperatures and in the frequency and severity of heatwaves Stone *et al*., 2010. Moreover, because insects can be killed by short exposure to an extremely high temperature, heat treatments can be applied to control horticultural and stored-product pests, with few insecticidal applications, decreasing the environmental threat, Cui *et al.*, 2008 and Hansen *et al.,* 2011. Recent studies by many authors suggested that the conditions in the upper elevation and also in higher latitude would become more suitable for organisms, because global warming will exceed the threshold of tolerance, in almost all insect species, which, in turn, accelerates cellular energetic demands, Englund *et al*., 2011; Lemoine and Burkepile, 2012. Insect metabolic rates are highly sensitive to temperature, roughly doubling with an increase of 10°C across the full range of regularly experienced temperatures Berggren *et al.,* 2009. Temperature affects diffusion, membrane fluidity, nucleic acid stability, salt and gas solubility, and, significantly, the behaviour of enzymes Lee *et al.,* 2007. The Egyptian cotton leafworm, *Spodoptera littoralis* is distributed throughout the world. It is a serious or major pest of cultivated crops primarily in tropical and subtropical regions, in Africa, Southern Europe, the Middle East and Asia Pineda *et al.,* 2004. *S. littoralis* is one of the major economic pests of cotton in Egypt that causes considerable damage to many other vegetables and crops Dahi *et al.,* 2009. *S. littoralis*, attacks all major crops in Egypt, including cotton, clover, corn, cabbage, cowpea, castor bean, sweet potato, lettuce, tomato, pepper, okra, mulberry, soybeans, etc. El-Aswad *et al.,* 2003. Biochemical parameters such as total proteins, lipids, carbohydrates and free amino acids as well as the activity levels of enzyme groups (metabolic, digestive, nervous and detoxifying enzymes) are among the most important characteristics that may change with global warming (climate change) Aida *et al.,* 2018.The aim of the study was to observe the changes that resulted from climatic changes in the previous years on the cotton leafworm *S. littoralis* biochemical parameters

MATERIALS AND METHODS

Maintenance of *Spodoptera littoralis* Culture (laboratory strain):-

The original colony of the cotton leafworm *S. littoralis* was obtained from a well-established culture at the Department of Cotton Leafworm; Plant Protection Research Institute. The insects were maintained under constant conditions of 25 ± 2^0 C, 70 ± 5% R.H. and 12:12 (L: D) photoperiod, separately. Larvae were reared on fresh castor oil leaves**,** (*Ricinus communis* L.) supplied daily in sufficient amounts. Maintenance of the different developmental stages was conducted according to the method described by Gamil, 2004.

Biochemical Bioassay (Sample Preparation):

1-Apparatus:

Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer after homogenation, supernatants these larvae were then homogenized in phosphate buffer (PH.7) were kept in a deep freezer at -20ºC till used for biochemical assays. A double beam ultraviolet/visible spectrophotometer was used to measure the absorbance of coloured substances or metabolic compounds.

2-Preparation of Insects for Analysis:

The insects were prepared as described by

Amin 1998. They were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants, which are referred to as enzyme extract, can be stored at least one week without appreciable loss of activity when stored at less than zero ˚C.

-Total soluble protein as described by Bradford (1976)

-Total carbohydrates according to Dubois *et al.,* (1956).

-Total lipids according to Knight *et al.,* (1972).

-Free Amino acid assayed by ninhydrin reagent according to Lee and Takabashi (1966).

Enzymes Assay:

The Following Enzymes Activities Were Determined:

-Proteases activity was measured as described by Lee and Takabashi (1966) and Tatchell *et al.*, (1972).

-Carbohydrates hydrolysing enzymes; as described by Dubois *et al.,* (1956)., Crompton and Birt (1967).

-Amylase, Trehalase and Invertase were determined by the method of Ishaaya and Swiriski (1976) and Amin, (1998) using starch, trehalose and sucrose as substrates.

-Lipase activity was measured as described by Knight *et al., (*1972).

-Acetyl choline-esterase activity was determined using acetylcholine bromide (AChBr) as substrate according to the method described by Simpson *et al.,* (1964).

-Non-specific α and β esterase activities were measured as described by Asperen, (1962) using α naphthol acetate and β naphthol acetate, respectively, as substrates.

-Glutathione S-transferase activity (GST) was determined spectophotometrically at 340 nm according to the method of Habig *et al.* (1974).

-Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase-Reitman and Frankel 1957.

- (GOT) were determined calorimetrically according to the method of Reitman and Frankle (1957)

Statistical Analysis:

Data from all experiments were subjected to analysis of variance (ANOVA) Using the computer program SAS (Statistical Analysis Systems).

RESULTS AND DISCUSSION

Field stress (climate change) is considered one of the most important factors affecting the field strain of *S. littoralis* due to the different and fluctuating temperatures and nitrogen ratios that it is exposed to before affecting the type of food. Temperature rise leads to increased metabolic rate, thus decreasing the development period and causing an increase in stored foods Lale *et al*., 2003; Taveras *et al*., 2004; Khrüt *et al.*, 2006; Coracini *et al.*, 2007. In addition, a number of physiological stress responses occur in insects as a result of variations in temperature. One reaction to thermal stress is the generation of reactive oxygen species (ROS), which can be harmful by causing oxidative damage. as reported by Ali *et al*., 2017. Data in Fig.1 indicate the average monthly temperatures during 2017 and 2018 cotton growing seasons at Assuit Governorate and the field strain of *S. littoralis* exposure to these averages of temperatures during the two successive seasons.

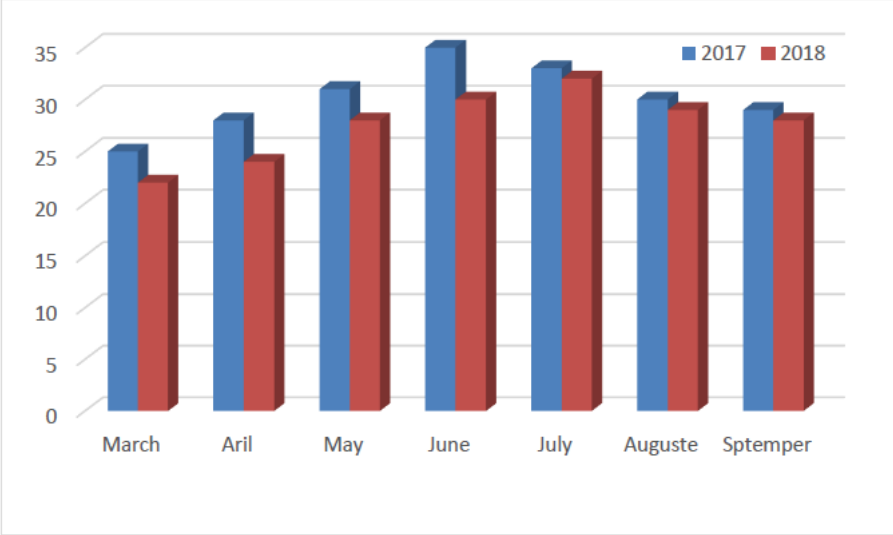

Fig. 1: The monthly average of temperature during 2017 and 2018 cotton growing seasons at Assuit Governorate

Biochemical Analysis:

Climate change, especially temperature, is one of the most important factors profound effects on chemical and biochemical reactions Hochachka and Somero, 2002. The higher kinetic energy of biochemical reactions speeds up the rate of metabolic processes, scaling up to affect the physiology and behavior of individual organisms Angilletta, 2009. Protein has always been an interesting biochemical tool for insect biochemists because of its potential role in growth, development, morphogenesis and many intermediaries of the metabolic pathway of insects, Kar *et al*., 1994.

Results in Table.1 & Fig. 2 (A) indicated that the levels of total proteins recorded in haemolymph of 4th larval instars in field strain represented by (23.34 ± 1.78) mg/g.b.wt, was less than the laboratory strain recorded (31.34 ± 0.89). These results explained by Aida *et al.*, 2018 revealed that *S. littoralis* growth rates increase with Field stress and the larvae consume more protein-rich diets. The results obtained here agree with the finding of Kingsolver, 2006 and Lee and Roh2010.

Also, similar were recorded by, Nagata and Kobayashi 1990 had reported an increase in protein synthesis during the feeding stage in *bombyx mori* in haemolymph and increased the soluble protein level during pupal development could be attributed to the compensatory replacement of protein that utilized for the formation of pupation. Moreover, Martin *et al.*, 1969 reported the increase of protein levels in haemolymph during oncogenic development of 5th larval instars of silkworm *bombyx mori*. The increase of protein levels of haemolymph is due to the synthesis of new proteins by tissue and release into haemolymph. Hachiya *et al*., 2007 reported that proteins denature more rapidly at higher temperatures, which, in turn, requires greater rates of protein synthesis and repair to maintain basic cellular function. In contrast, Malik and Malik 2009 were found when the larvae and pupae were exposed to selected higher temperatures a significant decrease in the protein levels of haemolymph. A relatively higher increase in the free amino acid levels in the haemolymph presumably provides protective cover to tissues against high temperature by an increase in osmolality and reduction in evaporative water loss. On the other hand, Kiran *et al.*, 1998 reported that the fat body synthesizes a number of proteins and releases them into the haemolymph during the active larval

period. This clearance was confirmed by Willmer *et al*., 2004 they reported that high temperature affected all biological processes including the structure of proteins and biological members and rates of biochemical and physiological reactions. Moreover, Gullan and Cranston, 2005 concluded the high temperature tended to kill insects cells by denaturing proteins, altering membrane, enzyme structures and properties, by the loss of water (dehydration) and they offer a rich potential for pest management strategies. While the previous study carried by Neven 2000 on the codling moth. The moth subjected to acute heat treatments had a maximal carbon dioxide evolution rate, respiration increase in response to increasing temperatures to a critical upper limit. After this point, respiration decreased. So, death occurs soon after the respiration rate drop even if the insect returned to a normal thermal condition indicating that death cells, death is occurring and denatured protein. Lepidopteran larvae may be particularly vulnerable to environmental warming because their rapid growth rates demand high protein foods Lee *et al*., 2004 Ehsan *et al.*, 2011 were revealed the fourth larval instars of *Pistachio* white leaf borer reared at 25ºC had a lower level of glycogen and a higher level of protein in comparison with those larvae reared at 35ºC. Glycogen is a storage form of energy; therefore, temperature rise leads to increase metabolic rate, decrease development period and cause an increase in stored foods. Protein content in larvae reared at 25ºC was significantly higher than those reared at 35ºC, but glycogen content in the larvae reared at 35ºC was more than larvae reared at 25ºC.

In the present study, the number of total lipids as seen inTable.1 & Fig. 2(B) were recorded in haemolymph and are no significant changes in the total lipid levels recorded between field strain and laboratory strain (5.64 ± 0.21) and (5.84 ± 0.25) respectively. These results may be due to an increase in food consumption at lower degrees of temperatures to provide energy (laboratory strain). These results agree with the findings. Hochachka and Somero, 1973., revealed the increase in the contents of total lipids, free fatty acids and phospholipids to increase in food consumption The increase in free fatty acids may provide the lipoprotein enzymes, with an environment to modulate the latter's activity at low temperature. On the other hand, many authors Ellis *et al*., 2002; Costamagna and Landis, 2004 reported that the orders Lepidoptera and orthoptera use lipids and stored carbohydrates as the main energy source. Lee and Roh 2010 concluded that lipids storage efficiency was lower in larvae of *Spodoptera exigua* at 18ºC than at 26 ºC, and was similar to those at 34 ºC. Dooremalen *et al*., 2011 found that in *Philosamia ricini*, lipid composition may be an important trait underlying fitness response to temperature, because it affects membrane fluidity as well as the viability of stored energy reserves. With the observed increase in the phospholipid content, larvae showed greatly increased activity of those mitochondrial enzymes which were membrane-associated, and thus the former retained their reticular structures intact during cold exposure. Therefore, *Philosamia ricini* larvae were exposed to a higher temperature of 35°C, monovalent cations like Na+ and K+ increased whereas divalent cations like Ca2+ and Mg2+ decreased, the percent changes were observed being more at 36°C than at highest at 31°C. The haemolymph monovalent captions were hyper-feeding larvae and divalent captions were hypo-regulated at the quantity of silk synthesized by their silk glands.

The results in Table.1 & Fig. 2(C), revealed that the amount of total carbohydrates recorded in haemolymph and is no significant changes in the total carbohydrates levels recorded that field

and laboratory strain were (19.27 ± 0.22) and (19.34 ± 0.4) mg/g.b.wt respectively. These results disagree with Sonmez and Gulel 2008, they concluded that a low temperature decreases the total carbohydrates and protein amounts of the pest *Acanthoscelides obtectus*. They recommended that Storage should be kept at 10-15°C so that, *A. obtectus* and other possible pests give minimal damage to crops.

In the current study as seen in Table.1 & Fig. 2 (D), it is clear that the levels of free amino acids had significant changes less in the haemolymph of fourth larval instars in field strain they were (372 ± 5.69) mg/g.b.wt. then the laboratory strain was (432.34 ± 12.47) mg/g.b.wt These results agree with Michiyo *et al.*, 1997, they concluded that a low temperature decreases the effects of temperature, diapause and aerobic conditions on the levels of amino acids in overwintering larvae were analyzed. Amino acids levels rose at low temperature on the larval of *E. leucotaeniella*.

Table 1: The activity levels of biochemical in 4th S. *littoralis* larval instars for field and laboratory strains**.**

Strains	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 Ug alanine/ml Mean ± SE
Field strain	23.34 ± 1.78 [b]	5.64 ± 0.21 [a]	19.27 ± 0.22 [a]	372 ± 5.69 [b]
laboratory strain	31.34 ± 0.89 [a]	5.84 ± 0.25 [a]	19.34 ± 0 . 4 5 [a]	432.34 ± 12.47 [a]
Note: Means with the same letter in the same column are not significantly different				

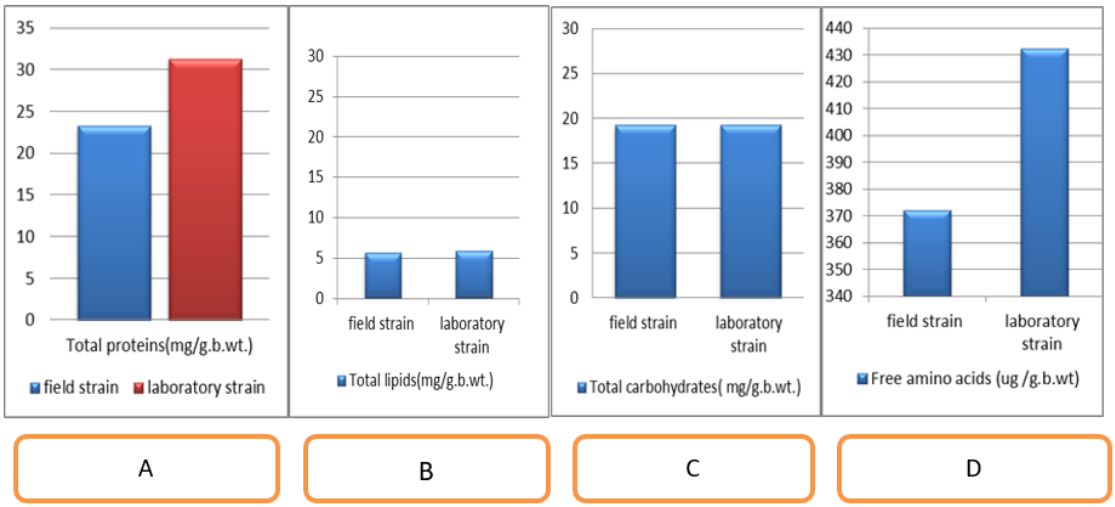

Fig. 2: The activity levels of biochemical of *S. littoralis* 4th larval instar for field and laboratory strains.

Enzyme Assay*:*

The optimum temperature for enzyme reactions is generally defined as the temperature at which the maximum reaction rate is achieved Takaya, 2005. The fact that each enzyme behaves differently under various temperature regimes highlights the fact that each enzyme has an optimum temperature activity. Carbohydrate hydrolyzing enzymes (amylase, trehalase and invertase), proteases, and lipases are the

three main enzymes involved in the digestion of food by insects Callaghan *et al.,* 2002.

The results obtained here that recorded in Table 2 & Fig. 3 (A and B) the activities of the carbohydrate hydrolysing enzymes (amylase, Trehalase) levels that recorded in haemolymph were no significant changes in field strain for laboratory strain (133.67 ± 1.21), (135 ± 3.47) mg/g.b.wt for amylase and trehalase (164.67 ± 7.76), (165 ± 2.31) mg/g.b.wt. As for invertase in Table 2 & Fig. 3 (C) the activity levels of were increased significantly in field strain (912.0 ± 6.62) mg/g.b.wt than laboratory strain (852.7 ± 7.58) mg/g.b.wt.

Proteases, which are also known as endopeptidases, enrol an important function in protein digestion. These enzymes begin the protein digestion process by breaking internal bonds in proteins. The amino acid residues vary along the peptide chain, therefore, different kinds of proteinases are necessary to hydrolyze them. Based on the active site group and their corresponding mechanism, digestive proteinases can be classified as serine, cysteine, and aspartic proteases Terra and ferreira, 2012. In the current study in Table 2 & Fig. 3 (D) appeared that the activity levels of protease enzymes were decreased significant in field strain (19.7 ± 0.93) mg/g.b.wt than laboratory strain (23.04 ± 0.8) mg/g.b.wt. Pant and Gupta 1979 noted that a significant reduction in enzyme level at field strain that had high proteolytic activity in the second larval instar of *Philosamia Ricini* declined steadily till late 5^{th} instar development. The decrease was attributed to the composition of food ingested. The low proteolytic activity was due to the host plants which are rich sources of amino acids and which are consumed in large quantities by this insect. The two major proteases classes in the digestive systems of phytophagous insects are the serine and cysteine proteases Haq *et al*., 2004. Moreover, Srinivasan *et al*., 2008 had reported on the midgut enzymes of various pests belonging to Lepidoptera. Serine proteases are known to dominate the larval gut environment and contribute to about 95 % of the total digestive activity in Lepidoptera.

Lipases (triacylglycerol–acyl-hydrolase EC 3.1.1.3), which catalyzes the hydrolysis of fatty acid ester bonds, are widely distributed among animals, plants and microorganisms Naumff, 2001. In the present study as seen in Table 2 & Fig. 3 (E), the activity levels of lipase in the midgut of larvae increased significantly in laboratory strain than field strain (40.3±0.46) and (36.4 ±1.4) Ug oleic acid/g.b.wt, respectively. These results disagree with the finding of some previous authors Aida *et al.,* 2018 recorded the activity levels decreased significantly when the temperature raised at 25, 30 and 35ºC.They were 820.33, 810.0 and 789.0 Ug oleic acid/g.b.wt respectively .There were significant changes between them. Ishaaya *et al.,* 1971 concluded that enzymatic activity increased when the temperature rose from 10 to 32°C. At 10°C (i.e. below the threshold of larval development), both proteolytic and amyl lytic activities in the midgut wall were less than 10 percent of that obtained at 32°C.

Detoxification enzymes in insects are generally demonstrated as the enzymatic defense against foreign compounds and play a significant role in maintaining their normal physiological functions Mukanganyama *et al.,* 2010. Several defensive mechanisms and biochemical reactions are involved in the detoxification processes against any temperature Stress. These mechanisms predominantly involve either metabolic detoxification of the temperature stress before it reaches their damage or the sensitivity changes of the larvae. The most common metabolic resistance mechanisms involve esterases,

glutathione S-transferases (GSTs). Generally speaking, the increase of activity of detoxification enzymes is the most universal resistant mechanism in insects. An elevation in the activity of such enzymes but surprisingly, this assumption couldn't be achieved as GST relatively decreased exposed to a different constant temperature. GSTs also play an important role in stress physiology and have been implicated in intracellular transport and various biosynthetic pathways Wilce and Parker, 1994.

Table 2: The activity levels of digestive enzymes of 4th larval instars *S. littoralis* in field and laboratory strains**.**

Strains	**Protease Ug alanine/min/g.b.wt** Mean ± S.E	**Lipase Ug oleic acid/g.b.wt** Mean ± S.E	**Invertase Ug glucose/min/g .b.wt Mean ± S.E**	**Trehalase Ug glucose/min/g. b.wt** Mean ± S. E	**Amylase Ug glucose/min/g. b.wt** Mean ± S.E
Field strain	19.7 ± 0.93[b]	36.4 ±1.4 [a]	912.0 ± 6.62 [a]	164.67 ± 7.76 [a]	133.67 ± 1.21 [a]
Laboratory strain	23.04 ± *0.8* [a]	40.3±0.46 [b]	852.7 ± 7.58 [b]	165 ± 2.31[a]	135 ± 3.47 [a]
Note: Means with the same letter in the same column are not significantly different					

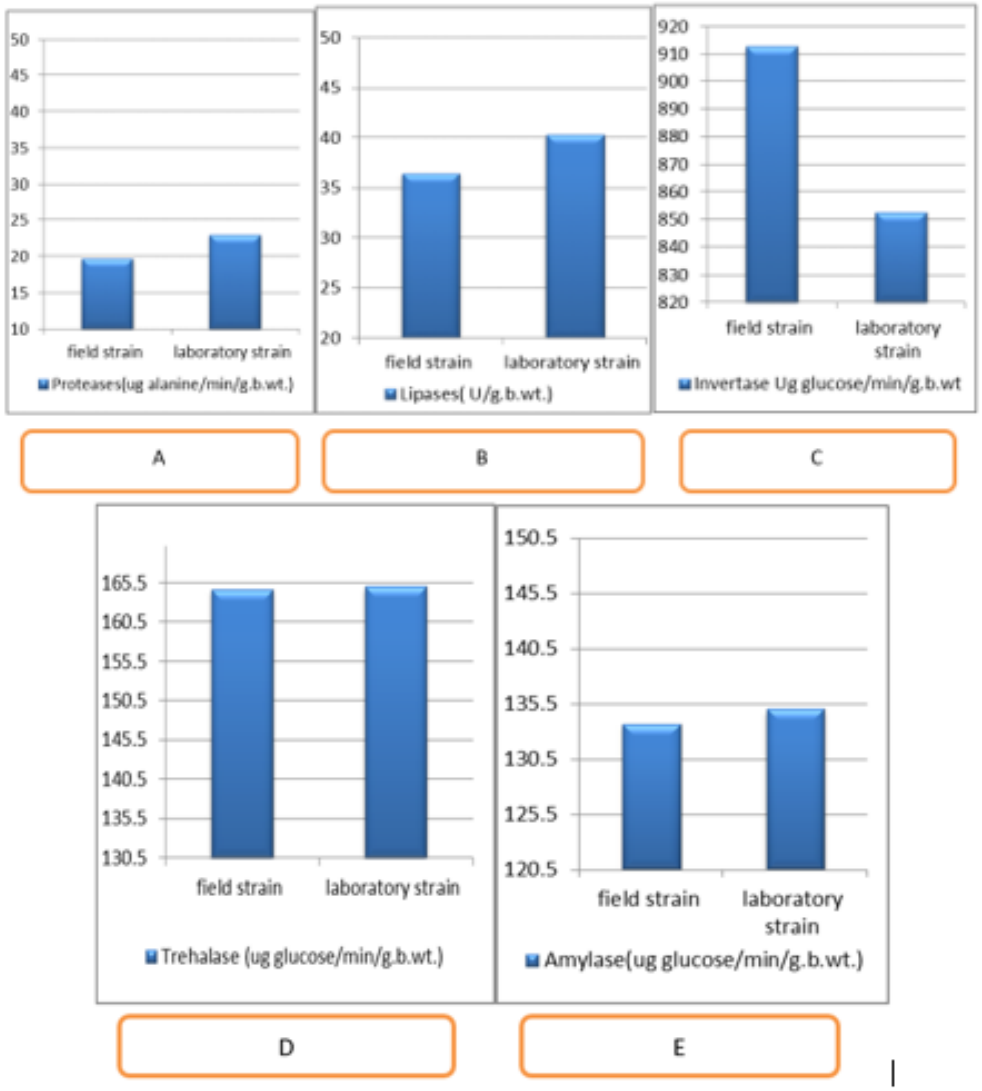

Fig. 3: The activity levels of digestive enzymes of *S. littoralis* 4th larval instar for field and laboratory strains

In the present study, it is clear in Table.3. & Fig. 4 (A) that level of glutathione S- transferases (GSTs) enzyme increased significantly in field strain (47± 1.53) conjugated/min/g.b.wt, while its activity level decreased at laboratory strain (38± 1.53) conjugated/min /g.b.wt. respectively. There was a significant difference between them. Whereas, there were significant differences between field strain and laboratory strain. In contrast, Zhang *et al.*, 2016 investigated that the activity levels of four antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione ne-S-transferase (GSTs), and peroxidase (POD) under heat stress (0, 4, 12, 16, 20, 24 and 28°C) for 4 and 12 hours, respectively they found that the activity of GSTs decreased obviously when the treatment temperature exceeded 24°C. This result disagrees with our results seen in Table.3.

Activity levels of glutamic oxaloacetic transaminase (GOT) enzymes as seen in Table.3& Fig. 4 (B) increased significantly at laboratory strain (320.67± 7.76) were as decreased in field strain (283±8.89) (Ux103/g. b. wt.), respectively. Activity levels of glutamic pyruvic transaminase (GPT) enzymes as seen in Table.3& Fig. 4 (C) gave no significant change between field and laboratory strain (86± 3.52) Ux103/g. b. wt. (85.34± 2.19) Ux103/g. b. wt. respectively. While activity levels of (GPT) at field strain and laboratory strain were not significantly different.

Table 3: The activity levels of metabolic enzymes of 4th larval instars *S. littoralis* in field and laboratory strains.

Strains	**GST (M mole sub conjugated/min/g.b.wt)** Mean ± S.E	**GOT -ALT (Ux10³/g.b.wt)** Mean ± S.E	**GPT-AST (Ux10³/g.b.wt)** Mean ± S.E
Field strain	47± 1.53[a]	283±8.89[b]	86± 3.52[a]
Laboratory strain	38± 1.53[b]	320.67± 7.76[a]	85.34± 2.19[a]
Note: Means with the same letter in the same row are not significantly different			

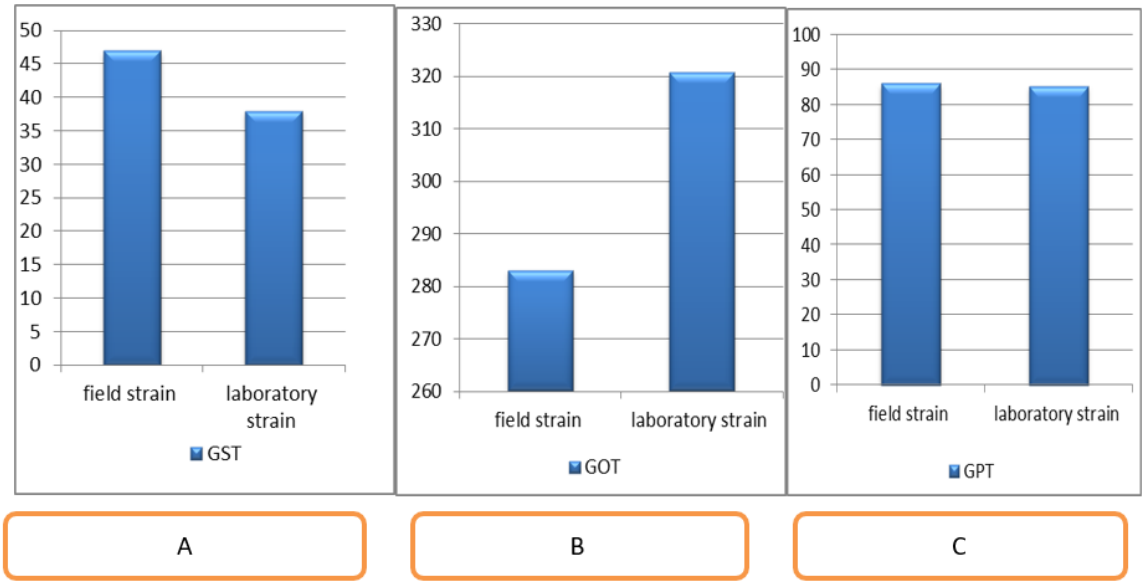

Fig.4: The activity levels of metabolic enzymes of *S. littoralis* 4th larval instar for field and laboratory strains.

Changes in the physiology of the nervous system and metabolism can be detected through the activity of acetylcholinesterase (AChE), alpha esterase (EST-α) and beta esterase (EST-β) Débora *et al.*, 2016.

The results whereas, found in Table.4 & Fig. 5 (A and B) appeared that the Alpha (EST-α) and beta esterase (EST-β) enzyme increased significantly at field strain (471.67 ± 0.41) Ugα-naphthol/min/g.b.wt. (264.67 ± 7.43) Ugβ-naphthol/min/g.b.wt respectively .Enzymes activity levels of alpha and beta decreased significantly at laboratory strain, they were (327 ± 7.58) Ugα-naphthol/min/g.b.wt and (236.34 ± 3.72) Ugβ-naphthol/min/g.b.wt. The results indicated that the activity levels of these enzymes are more significant. In the present study, as cleared in Table.4 & Fig. 5 (C). AchE enzyme activity appeared non-significantly different between field and laboratory strain (257.34 ± 6.75) Ug Ach Br/min/g.b.wt. and (235.34 ± 6.36) Ug Ach Br/min/g.b.wt respectively.

Table 4: The activity levels of acetylcholinesterase (AChE), alpha esterase (EST-α), and beta esterase (EST-β) of *4th larval instars S. littoralis* in the field and laboratory strains

Strains	Alpha esterase (EST-α) Ugα-naphthol/min/g.b.wt Mean ± S.E	Beta esterase (EST-β) Ugβ-naphthol/min/g.b.wt Mean ± S.E	Ache Ug Ach Br/min/g.b.wt Mean ± S.E
Field strain	471.67 ± 0.41 [a]	264.67 ± 7.43 [a]	257.34 ± 6.75 [a]
Laboratory strain	327 ± 7.58 [b]	236.34 ± 3.72 [b]	235.34 ± 6.36 [a]
Note: Means with the same letter in the same column are not significantly different			

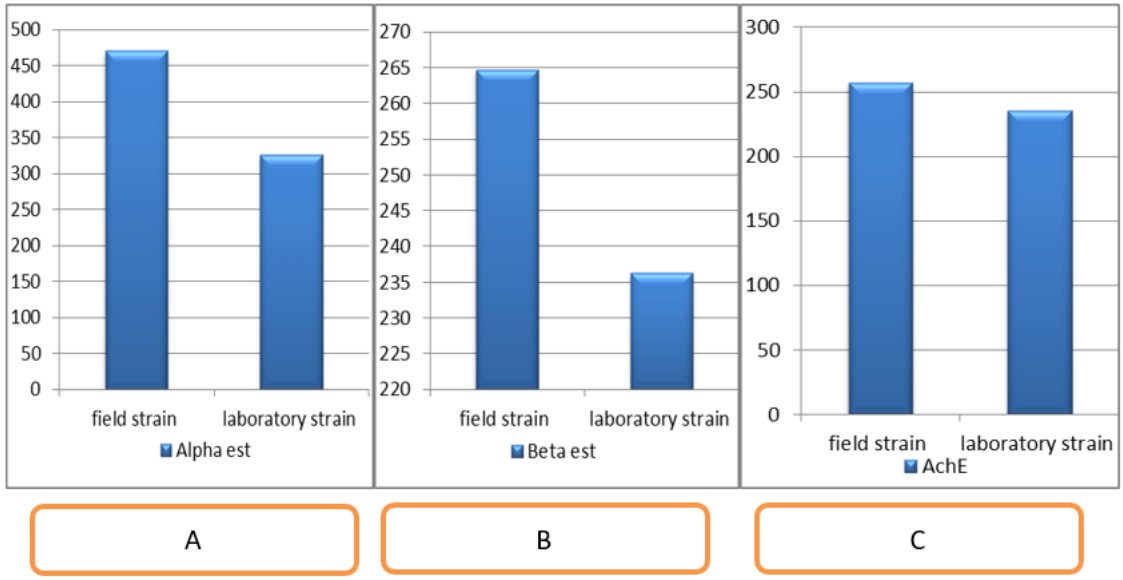

Fig. 5: The activity levels of acetylcholinesterase (AChE), alpha esterase (EST-α), and beta esterase (EST-β) of *S. littoralis* 4th larval instar for field and laboratory strains

Our results disagree with Debora *et al.*, 2016 who concluded that AChE activity decreased at higher temperatures and corroborated the results of Domingues *et al.*, 2007 who observed the activity of the AChE of *Chironomus. riparius Meigen*, is higher at 6 °C and 16 °C than at 26 °C. These results also

disagree with the finding of Débora *et al.*, 2016 who studied the effects of different temperatures on *Chironomus sancticaroli* where he found AChE activity decreased with increasing temperatures: at 20 and 25 °C, it was 69% and 59% lower than at 30 °C, respectively. No significant changes in enzyme activity were detected between field strain and laboratory strain in the activity of EST-α were observed enzyme activity increased by 44% and 45% respectively. The enzyme activity of EST-β was high at the field strain. At this strain, EST-β activity was 24% higher than at the laboratory strain. Singh *et al.*, 2013 concluded that *Pricini* when exposed to low-temperature causes alterations in the activity and kinetics of the tissue. Thus, AChE activity appears to be a potential biomarker towards the evaluation of the impact of cold stress on silkworms.

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ARABIC SUMMARY

ردود الافعال البيوكيميائية والفسيولوجية للسلالة الحقلية لدودة أوراق القطن كاستجابات للتعرض لدرجات الحرارة في ظل التغير المناخي الحالي

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من المهم فهم آثار درجات الحرارة على دودة أوراق القطن المصرية والتي تهاجم مجموعة كبيرة من المحاصيل في الشرق الأوسط، وهي واحدة من الافات الرئيسية للقطن في مصر. لذلك، تم تصميم هذه الدراسة لمعرفة تأثير درجات الحرارة اليومية على السلالة الحقلية في مدينة ابنوب بمحافظة أسيوط اثناء موسمي زراعة القطن 2017 و 2018 . ومن ناحية أخرى ، تم مقارنة النتائج مع السلالة الحقلية والمرباه تحت تاثير درجة حرارة ثابتة (25 درجة مؤية) وتم دراسة القياسات البيوكيميائية للعمر الرابع لكلا السلالتين. حيث تم اجراء التحاليل البيوكيميائية لتحديد اثر درجات الحرارة المتغيرة حقليا ودرجة الحرارة الثابتة معمليا وذلك علي مجموع البروتينات والدهون والكربوهيدرات والأحماض الأمينية الحرة وكذلك الإنزيمات الأيضية الهضمية والعصبية وإزالة السموم. وشملت الدراسة كل من GST ، GOT، AchE، ألفا وبيتااستريزس ، حيث زادت مستويات النشاط بسبب الاجهاد الحقلي .بينما انخفضت الأحماض الأمينية الحرة ، والبروتينات الكلية ، والبروتييز ، والليباز ، والأميلاز بشكل ملحوظ بسبب الإجهاد الحقلي ولم يتم الكشف عن تغيرات معنوية بين السلالات الحقلية والمخبرية في إجمالي الكربوهيدرات والدهون الكلية ، والترياليز، و GPT.