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Biochemical investigation of metabolic content and enzymes on diapaused and active larvae of pink bollworm *Pectinophora Gossypiella* (Saunders)

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Abstract
A comparative study between diapaused *Pectinophora gossypiella* (Saunders) male and female larvae from five cotton varieties (Giza 70, 86, 87, 88 and 92) cultivated in Kafr El-Sheik governorate and the active laboratory 4th instar larvae, in the biochemical contents (triglycerides, free fatty acids and total proteins) and the metabolic enzymes (alkaline phosphatase, L.D.H., GOT and GPT), showed high significance in Triglycerides, free fatty acids in male and female of some varieties of diapause larvae than with the active larvae. While, the content of total protein was high in diapause male larvae of Giza 88 followed by Giza 70 and Giza 92 and was low in all female diapause larvae. Alkaline phosphatase, L.D.H. and GPT were higher in the active larvae than the diapause.

Keywords: *Pectinophora gossypiella***,** diapause, biochemical enzymes

Introduction
Pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is a serious key pest of cotton, the larvae of pink bollworm feed on flowers, squares and bolls of cotton. Newly hatched larvae drill into cotton bolls and complete its larval development inside the boll feeding upon seeds. When the 4th instar larvae exit from cotton bolls, it leaves rounded exit hole which is characteristic symptom of pink bollworm damage.
Commonly, diapause is associated with the storage of metabolic reserves regardless of the stage of development in which it occurs (Denlinger, 2002) [16]. Diapause in insects represents a complex dynamic process characterized by several specific physiological and behavioral features (Tauber *et al*., 1986, Denlinger 1991) [15]. Many diapausing insects differ physiologically from their non-diapausing counterparts (Danks 1987) [13]. Some biochemical investigations have been conducted to describe these aspects of diapause induction and development Li *et al*. (2002).
Diapause is a hormonally controlled cessation of development that is a metabolically expensive life history strategy (Hahn and Denlinger, 2007, 2011; Emerson *et al*., 2009) [23, 24, 19]. Insects have to expend energy constantly, and if they are not feeding, they must live on reserves that have been accumulated in prior periods of food abundance. Energy reserves may be stored in animal cells as glycogen or triglycerides. Enzyme differences between diapause and no diapause pink bollworm, *Pectinophora gossypiella* (Saunders), larvae were investigated by Abd E1 Fattah *et al.* (1972) [1] and Slugs *et al.* (1975) [46].
The present work gives special significance in understanding the physiological strategy adopted by *P. gossypiella* to withstand winter harsh conditions; the trial may help in developing enhanced techniques in IPM programs. We try to spot on the bio chemical metabolic reserves content and enzymes activates in male and female of the diapause and no diapause pink bollworm larvae from five cotton varieties.

Materials and Methods
Insects
Mature field 4th instar larvae of the pink bollworm *P. gossypiella* were collected from five cotton varieties (Giza 70, 86, 87, 88 and 92) cultivated in Kafr El-Sheik Governorate, Egypt.
Collecting the cotton bolls from field then holding the bolls under the surrounding conditions for several days. Infested bolls with mature 4th larvae of pink bollworms begin leaving the bolls searching for pupation or still remain inside cotton seeds as diapause sites. These larvae are placed in Petri dishes under paper towels and left to incubate in room temperature conditions, those larvae which not pupating within 10 days were assumed to be in diapause.

Preparation of Insect
The diapause and active laboratory larvae were prepared as described by Amin (1998) [4]. They were homogenized in distilled water (50mg/1ml). Homogenates were centrifuged at 8000 r. p. m. for 15 min at 2°C in a refrigerated centrifuge. The deposits were discarded and the supernatant, which is referred as enzyme extract, can be stored at -20 °c in a deep freezer till use for biochemical assays.

Biochemical Measurements
Triglycerides, free fatty acids, total proteins, Alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined colorimetrically according to the methods of Sadasivam and Manickam (1991) [39], Powel and Sith (1954) [34], Bradford (1976) [8] Reitman and Frankle (1957) [36], respectively. Lactate dehydrogenase (LDH) derived from the formulation recommended by the German Society for Clinical Chemistry (DGKC, 1972).

Statistical Analysis
The available data values were statistically analyzed with one-way analysis of variance (ANOVA) ($P < 0.05\%$)

(Snedecor 1952) [41] And Duncan's multiple range test of means (Duncan 1955) [18] being used by COSTAT program.

Results

Triglycerides, free fatty acids, total proteins content and the activities of alkaline phosphatase (ALP), Lactate dehydrogenase (LDH),glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in diapause and active male and female larvae of *P. gossypiella* from five cotton varieties (Giza 70, 86, 87, 88 and 92) cultivated in Kafr El-Sheik Governorate, Egypt are presented in Table (1, 2).

Table 1: Changes in some biochemical contents and enzymes activity of diapause and active *P. gossypiella*4th instar male larvae between five cotton varieties (Giza 70, 86, 87, 88 and 92) cultivated in Kafr El-Sheik Governorate.

Sample	Triglycerides (mg/g.b.wt)	Free Fatty Acids (µgtriolein/g.b.wt)	Total Proteins (mg/g.b.wt)	Alkaline Phosphatase (Ux103 /g.b.wt)	LDH (U/g.b.wt)	GOT (U/g.b.wt)	GPT (U/g.b.wt)
Giza70	2.86^{c}±0.15	4099.67^{a}155.63	47.51^{b}±2.92	49.33^{b}±2.08	1.94^{c}±0.19	34.03^{b}±2.76	7.55^{c}±0.31
Giza 86	3.28^{b}±0.076	1883.33^{e}±125.83	22.04 ^{d}±0.95	68.67^{b}±4.16	7.43^{b}±0.74	17.16^{e}±1.95	7.70^{c}±0.20
Giza 87	3.66^{a}±0.22	2443.67^{cd}±59.75	20.96 ^{d}±2.16	49.00^{b}±5.29	2.56^{c}±0.29	21.07^{de} ±3.16	10.04^{b}±0.47
Giza 88	3.82^{a}±0.079	2507.33^{c}±91.22	58.23^{a}±4.12	44.00^{b}±4.36	1.73^{c}±0.21	52.30^{a}±2.31	8.06^{c}±0.11
Giza92	3.89^{a}±0.96	3096.67^{b}±99.62	43.00^{c}±2.07	79.67^{b}±4.73	2.27^{c}±0.23	28.10^{c}±1.22	8.30^{c}±0.36
Active	1.51^{d}±0.225	2273.33^{d}±200.33	38.58^{c}±1.58	3038.33^{a}±151.180	13.82^{a}±2.68	24.20^{cd}±1.81	12.17^{a}±0.77
F	101.02	108.22	101.77	1116.17	52.92	90.76	53.97
P-value	.0000***	.0000***	.0000***	.0000***	.0000***	.0000***	.0000***
LSD	0.27	231.95	4.47	110.02	2.05	4.07	0.76

Table 2: Changes in some biochemical contents and enzymes activity of diapause and active *P. gossypiella*4th instar female larvae between five cotton varieties (Giza 70, 86, 87, 88 and 92) cultivated in Kafr El-Sheik Governorate.

Sample	Triglycerides (mg/g.b.wt)	Free Fatty Acids (µgtriolein/g.b.wt)	Total Proteins (mg/g.b.wt)	Alkaline Phosphatase ($Ux10^3$ /g.b.wt)	LDH (U/g.b.wt)	GOT (U/g.b.wt)	GPT (U/g.b.wt)
Giza 70	3.68^{d}±0.202	4680.33^{a}±159.32	51.15^{cd}±2.06	82.00^{b}±3.00	1.80^{d}±0.26	13.26^{d}±1.31	8.18^{c}±0.23
Giza 86	4.81^{b}±0.11	3123.00^{d}±124.53	62.83 ^{b}±2.11	105.33^{b}±8.39	3.14^{c}±0.17	23.27^{b}±1.15	9.40^{b}±0.3
Giza 87	4.48^{c}±0.101	4007.33^{b}±94.21	48.20^{d}±2.62	188.33^{b}±10.12	5.06^{b}±0.35	22.30^{b}±0.82	7.60^{d}±0.13
Giza 88	6.56^{a}±0.09	3630.00^{c}±112.69	54.07^{c}±2.97	112.00^{b}±7.55	1.37^{d}±0.16	18.07^{c}±0.27	8.06^{cd}±0.21
Giza 92	6.69^{a}±0.17	3360.67^{d}±126.78	55.07^{c}±1.53	154.67^{b}±17.62	2.11^{cd}±0.26	18.80^{c}±1.22	8.34^{c}±0.32
Active	1.93^{e}±0.166	3787.33^{bc}±186.77	71.63^{a}±3.66	5503.33^{a}±431.55	8.02^{a}±1.48	26.29^{a}±2.31	10.60^{a}±0.54
F	452.70	47.32	33.40	464.14	46.64	35.85	37.97
P-value	.0000***	.0000***	.0000***	.0000***	.0000***	.0000***	.0000***
LSD	0.26	244.59	4.60	313.88	1.15	2.36	0.55

- **Comparison of biochemical content of triglycerides, free fatty acids and total proteins of diapause and active *P. gossypiella* larvae**

Triglycerides were significantly increased in *P. gossypiella* diapause male larvae of Giza 70 and Giza 86varieties by (2.86 and 3.28 mg/g.b.wt), respectively compared with (1.51 mg/g.b.wt) in control. While, non-significant increase in triglycerides content of *P. gossypiella* diapauses male larvae of Giza 87, Giza 88 and Giza 92 varieties by (3.66, 3.82 and 3.89 mg/g.b.wt), respectively, Table (1).On the other hand, triglycerides significantly increase in *P. gossypiella* diapause female larvae of Giza 70, Giza 86 and Giza 87 varieties by (3.68, 4.81 and 4.48mg/g.b.wt), respectively, relative to (1.93mg/g.b.wt) in the active larvae. And, non-significant increase in diapause female larvae of Giza88 and Giza92 varieties by (6.56 and 6.69mg/g.b.wt), respectively, Table (2).

Free fatty acids content were significantly increased in *P. gossypiella* diapause male larvae of Giza 70 variety followed by Giza 92, Giza 88 then Giza 87 varieties by (4099.67, 3096.67, 2507.33 and 2443.67µg triolein/g.b.wt) compared with 2273.33µg triolein/g.b.wt in the active one. Whereas, there was significant decrease in diapause male larvae of Giza 86 variety by (1883.33 µg triolein/g.b.wt)Table (1).While, there was significant increase in *P. gossypiella* diapause female larvae of Giza 70 and Giza 87 varieties by (4680.33 and4007.33µg triolein/g.b.wt), respectively, whereas, a significant decrease in diapause female larvae by (3123.00, 3630.00 and 3360.67µg triolein/g.b.wt), respectively, of Giza 86, Giza 88 and Giza 92 varieties compared with (3787.33 µg triolein/g.b.wt)in the active female larvae Table (2).

It is clear that total protein content was significantly increased in diapause male larvae of Giza 88 followed by Giza 70 and Giza 92 varieties by (58.23, 47.51 and 43.00 mg/g.b.wt), respectively, compared with (38.58 mg/g. b. wt.) in the active male larvae, while, there was decrease in diapause male larvae of Giza 86 and Giza 87 varieties by (22.04 and 20.96 mg/g.b.wt), respectively, Table (1). Whereas, there was significant decrease in the diapause female larvae of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties by (51.15, 62.83, 48.20, 54.07 and 55.07 mg/g.b.wt) compared with (71.63 mg/g.b.wt) in active female larvae, Table (2).

Triglycerides, free fatty acid and total protein contents in all or active female larvae of the five varieties were more than all diapaused or active male larvae.

- **Comparison of biochemical activity of Alkaline Phosphatase, LDH, GOT and GPT of diapause and active *P. gossypiella* larvae**

Metabolic activity of alkaline phosphatase was higher in *P. gossypiella* active male and female larvae compared with the diapause larvae of all varieties. Whereas, metabolic rates of alkaline phosphatase were highly decreased (49.33, 68.67, 49.00, 44.00 and 79.67 $Ux10^3$/g.b.wt) in the diapaused male larvae of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties, respectively compared with (3038.33 $Ux10^3$/g.b.wt) in the active male larvae Table (1). Also, alkaline phosphatase metabolic rates were highly decreased

(82.00, 105.33, 188.33, 112.00 and 154.67 Ux10^3/g.b.wt) in the diapause female larvae of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties, respectively compared with (5503.33 Ux10^3/g.b.wt) in the active female larvae Table (2).

As, L.D.H. activity was higher in both *P. gossypiella* active male and female larvae than in diapaused larvae. LDH activity was decreased significantly in diapause male larvae of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties by (1.94, 7.43, 2.56, 1.73 and 2.27U/g.b.wt), respectively, than active male larvae by (13.82U/g.b.wt), showed in Table (1). Also, LDH activity was decreased significantly in diapause female larvae of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties by (1.80, 3.14, 5.06, 1.37 and 2.11U/g.b.wt), respectively, than active female larvae by (8.02U/g.b.wt), showed in Table (2).

Accordingly, a significant increase in GOT activity of diapause male larvae of Giza 88 followed by Giza 70 and Giza 92varieties by (52.30, 34.03and 28.10 U/g.b.wt), respectively compared with (24.20 U/g.b.wt) in the active male larvae. While, a significant decrease in GOT activity of diapause male larvae of Giza 86 and Giza 87 by (17.16 and 21.07U/g.b.wt), respectively, than active male larvae, showed in Table (1).

While, the metabolic rate of GOT decreased significantly in diapause female larvae of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties by (13.26, 23.27, 22.30, 18.07 and 18.80U/g.b.wt), respectively compared with (26.29 U/g.b.wt) in the active female larvae of *P. gossypiella*, showed in Table (2).

Nevertheless, GPT activity was significantly greater in the active male and female larvae than the diapause larvae of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties by (7.55, 7.70, 10.04, 8.06 and 8.30U/g.b.wt) compared with (12.17 U/g.b.wt) in the active male larvae Table (1). And, were (8.18, 9.40, 7.60, 8.06 and 8.34U/g.b.wt) of Giza 70, Giza 86, Giza 87, Giza 88 and Giza 92 varieties compared with (10.60 U/g.b.wt) in the active female larvae Table (2).

Discussion

Diapause is the primary mechanism for survival in temperate environments. Diapause is a genetically determined response by which an insect enters a dormant state in response to environmental cues which indicate the onset of unfavorable conditions (Mansingh, 1971) [29].

The fat bodies are present all over the tissue (Jensen and Borgesen, 2000) [27]. The basic cell of the fat body is the adipocyte, characterized by the presence of numerous lipid droplets. Triglycerides are the major component of the lipid droplets, and at the end of the feeding period lipid droplets occupy most of the intracellular space along with glycogen and protein granules (Dean *et al.,* 1985) [14]. Lipid is the main fat body component, and more than 90% of the lipid stored is triglyceride (Canavoso *et al.*, 2001) [10].

Fatty acids are rapidly taken up by the fat body and readily incorporated into triglyceride and, in smaller amounts, into other glycerides and phospholipids (Soulagesand Wells, 1994) [44]. The amount of fatty acid or acetate incorporated by the fat body is dependent on the developmental stage and feeding status of the insect (Pontes *et al*., 2008) [33].

Insects have to expend energy constantly, and if they are not feeding, they must live on reserves accumulated in periods of food abundance. Glycogen and triglyceride are the energy reserves in animal cells (Steele, 1982) [47].

As our results many others indicated that triglyceride and fatty acids to be most important compound accumulated in diapause than non-diapaused larvae Hahn and Denlinger, (2007) [23]. They estimated that an increase in the accumulation of lipid stores is common in (most) diapause-destined or overwintering stages of insects. The amount of reserves accumulated in the fat body differs among insect species. However, lipid is always the major component of the fat body, representing more than 50% of the dry weight (Ziegler, 1991) [50]. Most overwintering insects end winter with substantially fewer lipid stores than they began with, which suggests that lipids are a primary source of overwintering fuel (Sinclair, 2015) [43].

Vukašinović*et al.,* (2013) [49] this study compares the composition and biophysical properties of lipids in non-diapason and diapason fifth instar larvae of *Ostrinia nubilalis*. The majority of fat body lipids in both of these physiological states were comprised of 90% triacylglycerol's. Adjustments of fatty acid compositions are likely to be an important component of winter diapause mechanisms, possibly maintaining the fluidity of cell membranes and the functionality of the organism during lower winter temperatures. Han *et al*., (2008) [25] found that the higher level of free fatty acids in non-diapause larvae is consistent with higher metabolic rates in larvae of the pine caterpillar. The total content of protein and free fatty acids in female of all the tested varieties were higher than in male larvae. Similar profiles were identified in closely related to Shairra *et al.* (2016) [42] noted that 4th instar larvae of *P. gossypiella* female showed a slight significant higher total protein contents than male. Also the contents of the free fatty acids were higher in female than male. In general, the problem of protein synthesis is intimately related to metabolism of nucleic acids (Abdel- Mageed 2018) [2]. Also, Salama and Miller, (1992) [40] found that a drop in the concentration of fat body proteins of *P. gossypiella* coincided with a corresponding increase in hemolymph proteins, which suggests an active release of protein from the fat body into the hemolymph during the development of diapause. This explain the decrease of total protein in all female diapause larvae of the five varieties and in the male larvae of the varieties Giza 86 and Giza 87 that we had tested compared to the active female and male larvae. In contrary, Amiri and Bandani (2013) [5] found no significant difference in protein content among different treatments diapause laboratory reared and natural habitat adults of pre-diapause male and female adults had less available energy than diapause male and female adults in all samples including laboratory reared, cold treated, and natural habitat. Rostom *et al.,* (1992) [38] reported that the concentration of total protein and fat body of *P. gossypiella* fourth instar larva increased in diapause and in early ages of termination of diapause relative to that in the active phase. While in our result it increased only in diapause male larvae of G70, G88 and G92. As, Raina (1980) [35] estimated comparison of the total body protein of male and female larvae, pupae, and adults of a diapause and 2 non-diapause strains of the *P. gossypiella* indicated that the protein content of diapause larvae strains was significantly higher than of the non-diapause strain.

In addition, whether lipid content increased in larvae reserves prior to diapause or not may be related to the naturally diapause preparatory period. For example, bolls of cotton plants have greater lipid content while diapause

destined larvae only accumulate greater lipid reserves than non-diapause larvae when fed high-fat bolls, and not when fed lower-fat in squares (Clark and Chadbourne, 1962) [11]. This observation highlights the importance of ecological conditions for diapause physiology and consideration of experimental conditions when performing research on diapause physiology. Foster & Crowder (1976) [22] recently reported the variation of fatty acid content in geographical strains of diapause pink bollworms.

May be the variation in the free fatty acid and protein content in pink bollworms larva is related to the variation in the composition of the cotton seed varieties.

Mohamed *et al.* (2009) [3] reported significant differences between varieties when conducted a combined analysis for seven seasons to study seed chemical quality, oil content %, protein content % and Gossypol content (mg/g). Giza 45 and Giza 85 gave the highest values for oil % while Giza 45 had less protein percentage beside Giza 88 and Giza 89. Giza 90 is the best for protein content %. Giza 86 minimizes gossypol content (mg/g).

Our results referred to the correlation between the activity of the enzymes and the total protein content. Where GPT and GOT play an important role in protein metabolism. A sharp decrease or increase in the level of above enzymes effect oxygen consumption in insects (Pant and Morris, 1972). However, inhibition of phosphatase and lactic dehydrogenase level shows tissue necrosis in insects (Ishaaya and Casida, 1980) [26].

Alkaline phosphatase (ALP, E.C.3.1.3.1) are the hydrolytic enzymes, which hydrolyze phosphate monoesters under alkaline milieus (Bai *et al*., 1993) [6]. Also, this enzyme may act as hydrolase during the final stages of digestion, gonad maturation and metamorphic molts (Rhadha and Priti, 1969) [37]. Their activities are low during the larval molting stage and increased gradually after molting (Miao, 2002) [30]. ALP is active in tissues with active membrane transport in insect, such as intestinal epithelial cells (Caglayan, 1990) [9]. It is responsible for cytolysis of tissues during the insect development (Dadd, 1970) [12].

Bradfield, (1946) [7] concluded that the enzyme is located in cells most active in the synthesis of fibrous proteins. They occur in so many locations that their functions must be many and varied. Also, Moog (1946) [31] has considered phosphatases in relation to calcification, in transport mechanisms, and in relation to growth and differentiation.

Lactate dehydrogenase (also called lactic acid dehydrogenase or LDH) is an enzyme found in almost all body tissues. It plays an important role in cellular respiration, the process by which glucose (sugar) from food is converted into usable energy for cells. Lactate dehydrogenase is an enzyme found in nearly all living cells. LDH catalyzes the conversion of lactate to pyruvate and back, as it converts NAD^+ to NADH and back. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another. In this respect, Abd-El Fattah *et al.*, (1972) [1] found that the activities of lactic dehydrogenase (L.D.H.), alkaline phosphatase and GPT declined in the diapause larva at various degrees. While GOT increased in activity in diapause larva and decreased during diapause termination in 4th instar larvae of pink bollworm.

Conclusion

Our results showed that there were apparent varieties linkage associations of the enzyme activities and biochemical metabolic reserves in diapause and non-diapause of male and female of pink bollworm larvae with the different mentioned cotton varieties.

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