Egypt. Acad. J. biolog. Sci., 2 (1): 133-141 (2009)
E.mail. egyptianacademic@yahoo.com ISSN: 1687 – 8809
Received: 11/3/2009 www.eajbs.eg.net

# Embryonic and post-emergence changes of acid and alkaline phosphatases in the cotton leaf worm, *Spodoptera littoralis* (Boisd.)

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## ABSTRACT

The acid and alkaline phosphatase activities were studied in the developing egg of the cotton leaf worm, *Spodoptera littoralis* at different time intervals (ages, 0 - 72 h). The activities of these enzymes were also studied in aging ovary and testis of post-emerged adult at the same intervals (ages, 0 – 72 h). The post-emerged adults were grouped into mated and non-mated females and males.

Acid phosphatase activity was noticed to be cyclic with the embryonic development and was higher than that of alkaline phosphatase. Alkaline phosphatase activity was slightly increased in the freshly laid eggs but decreased more sharply than acid phosphatase during embryonic development. In all groups of post-emerged adults, acid phosphatase activities were much greater than those of the alkaline phosphatase at any given stage of progressively increasing age. Acid and alkaline phosphatase activities were also noticed to be cyclic in the post-emerged mated and non-mated adults. A conspicuous difference was noted between the activities of these enzymes in ovaries and testes of mated and non-mated adults. This phenomenon could be due to the periods of spermatozoa production in the testes and yolk accumulation in oocytes and ovulation. Mating could be also a factor that affected the phosphatases in both males and females.

**Key words:** *Spodoptera littoralis*, acid phosphatase, alkaline phosphatase, spectrophotometer, eggs, ovaries, testes.

## INTRODUCTION

Eggs of most insect species have special organelles, called yolk spheres, filled with protein (vitellin) that is the most important reserve of amino acids for the embryo development during embryogenesis. The vitellogenins that are vitellin precursors are present in the hemolymph and are internalized by the oocyte via receptor-mediated endocytosis (Raikhel and Dhadialla, 1992). After oocyte fertilization and during embryogenesis an intensive protein metabolism take place which involves mainly break down of pre-existing yolk reserves (vitellin) and the conversion of these into tissue and organ specific proteins. Several enzymes involved in this process have been characterized in the past years (Fagotto, 1990; Yamamoto and Takahashi, 1993; Izumi *et al*., 1994; Liu *et al*., 1996; Cho *et al*., 1999).

Acid phosphatases (EC. 3.1.3.2) are widely distributed enzymes and occur as multiple forms and isozymes. The role of acid phosphatase in processing of yolk during development was first recognized by Lemanski and Aldoroty (1974). In addition acidification of yolk platelets during *Xenopus laevis* (Fagotto and Maxfield, 1994), and tick development (Fagotto, 1991) was demonstrated to correlate with yolk

utilization. Alkaline phosphatase (EC. 3.1.3.1) is also known to cause the breakdown of cytoplasmic inclusion during embryogenesis and at the same time the products of this process transform in some way the morphogenetic property of the cleavage energids which later become included in the primordial cells (Seidel, 1936; 1960).

Therefore, the present study is an attempt to examine the pattern of variations in the activity of acid and alkaline phosphatase during embryonic development of *Spodoptera littoralis* at different ages. Attempts have also been made to correlate the changes in phosphatase activity to ovaries and testes development in mated and nonmated post-emerged adult of *Spodoptera littoralis* at different ages.

## MATERIALS AND METHODS

**Insect colony:**

*Spodoptera littoralis* were provided from the Department of Plant Protection Research Institute, Dokki-Giza. Successive generations of *Spodoptera littoralis* were maintained at 25±1˚C and 70±3%R.H. Larvae were fed on castor oil leaves, *Ricinus communis*, while the adults were fed on 20% sucrose solution.

Preparation of samples for phosphatases determination:

Males and females of *S. littoralis* were collected immediately after emergence and maintained at approximately 25±1˚C for a number of hours designated in each experiment. Males and females were grouped in mated and non-mated groups. At intervals of 0, 6, 12, 24, 36, 48, 60 and 72- hour (h) after emergence, 0.2 gm of testes and ovaries of mated and non-mated adults were removed carefully and collected in distilled water then frozen at -20 ˚C. 0.2 gm of fertilized eggs was collected at the same intervals after laying. All the samples were homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for ice for 3 minutes. Homogenates were centrifuged at 6000 r.p.m. for 10 minutes at 5˚ C. the supernatants were stored at -20˚ C. until analysis.

Protein determination:

The protein concentrations of eggs and tissue supernatants were determined by method of Bradford (1976) using bovine serum albumin as a standard.

**Enzyme assays:**

Acid and alkaline phosphatase activities were determined according to El-Sheikh (2006). The reaction medium contained 100 µl of enzyme solution, 100 µl of p-nitro-phenol (0.69 mg / 10ml distilled water) and 300 µl of appropriate buffer. The reaction mixture was incubated for 1 hour at room temperature, and then stopped by adding 1 ml of 0.1N NaOH in case of acid phosphatase and by 1ml of 0.02N NaOH in case of alkaline phosphatase. The change in absorbance was measured at 410nm against control.

## RESULTS

**I- Embryonic changes of acid and alkaline phosphatases:**

The activity of both acid and alkaline phosphatase was studied in *S. littoralis* eggs at different ages during the course of embryonic development. As shown in figure (1), the activity of both acid and alkaline phosphatase was very low at the onset of egg deposition. The activity of acid phosphatase appeared to be cyclic during the embryonic development. During this period, the enzyme exhibited peak activity at the age of 6, 36 and 60 h, and low activity at the age of 12, 48 and 72 h; another picture was found for the alkaline phosphatase in the homogenates of *Spodoptera* eggs. The

activity of this enzyme rose until the age 12h, and thereafter decreased gradually throughout the different ages. At the age of 72h, the alkaline phosphatase activity was slightly increased. It was indicative from the present observations that the activity of acid phosphatase was higher than of alkaline phosphatase during the course of embryonic development.

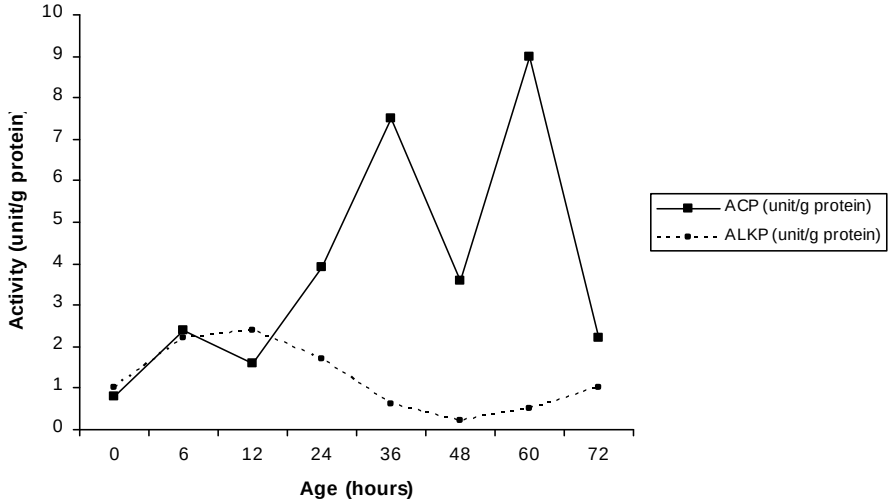


Fig. (1): Activity of acid and alkaline phosphatases (unit/g protein) in developing eggs at different ages of *S. littoralis*

**II- Post-emergence changes of acid and alkaline phosphatases:**

The activity patterns of acid and alkaline phosphatases in gonads of post-emerged females and males, mated and non-mated *S. littoralis* were illustrated in figures 2 and 3, respectively. In both ovaries and testes, acid phosphatase activities were much greater than those of alkaline phosphatase at any given stage of progressively increasing age.

Acid phosphatase activity proved to be cyclic through 72h post-emergence. It could be observed from figure (2) that initially the activities of acid phosphatase increased steadily in mated and non-mated males and reached a maximum at the age of 6h in the case of mated males and at 24h in the case of non-mated males. After attaining the peaks, the activities dropped progressively in the mated males until reach 36h of age but in the case of non-mated males the activity dropped sharply at the age of 36h.

The dropped level of this enzyme in mated males remained unchanged with minor fluctuations during the remainder ages, until reached 72h of age where the activity was increased. But the dropped level of this enzyme activity in non-mated males continued until 48h age. From this age an interesting increased in acid phosphatase activities were noticed reaching a second maximum at the age 60h. This activity, with a slight drop remained quite high until 72h of age of the non-mated males.

On contrary, in both mated and non-mated females, the activity of acid phosphatase decreased at the age of 6h and remained unchanged in the mated females until reached the age of 48h where the level of activities increased at 60h age and dropped again at 72h. In the ovaries of non-mated females there were two maxima in the acid phosphatase activity, one at the age of 12h and the other at the age of 48h. At the other ages the acid phosphatase exhibited lower activity.

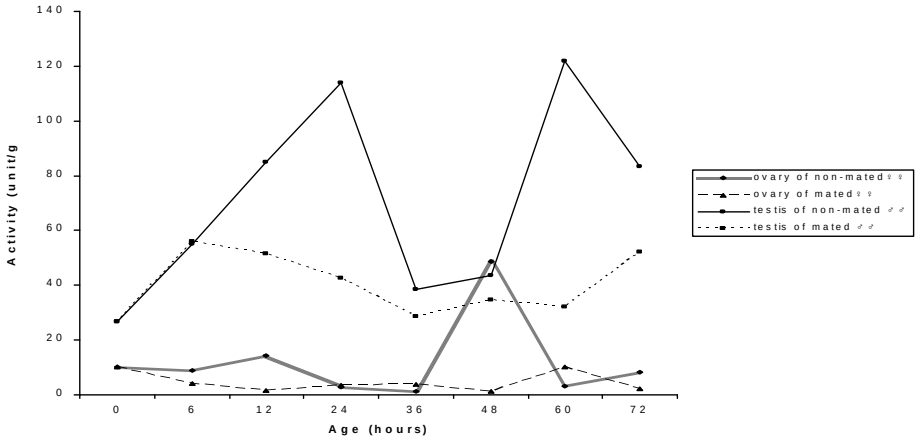


Fig. (2): Activity of acid phosphatase (unit/g protein) at different ages of gonads of non-mated and mated females and males of *S.littoralis*.

The alkaline phosphatase activities were always at a low level in both sexes (Figure 3). However in mated males and in non-mated females and males, the alkaline phosphatase activities appeared to be cyclic at the different ages of the post-emerged adult. In non-mated males, the alkaline phosphatase exhibited low level of activities in the intervals of ages from 0- 48h then the activity increased and reached a peak at the age of 60h. On the other hand, maximal activities of this enzyme were observed at two ages of ovaries of non-mated females. One of this peaks at the age of 12h, and the second at the age of 48h. On the contrary, the activities of the alkaline phosphatase in ovaries of mated females were at low level at all ages of post-emerged females except at the age of 60h which exhibited smaller and minor peak.

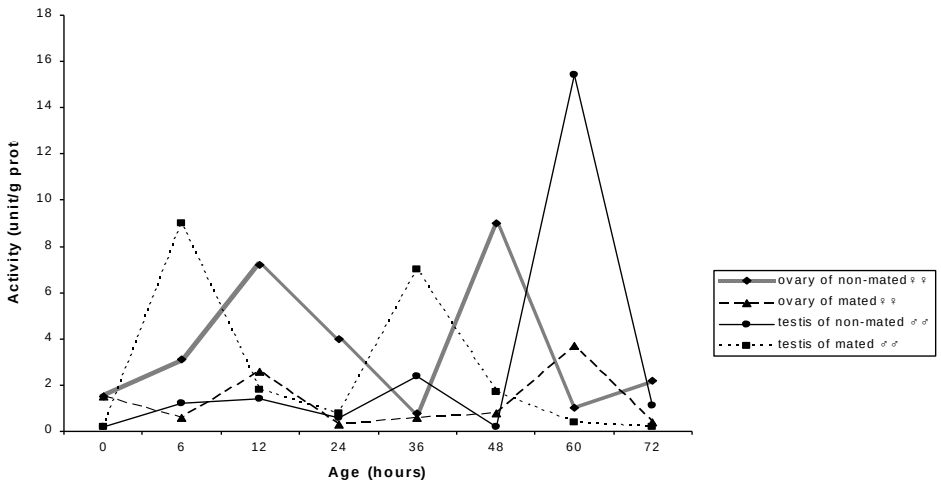


Fig. (3): Activity of alkaline phosphatase (unit/g protein) at different ages of gonads of non-mated and mated females and males of *S. littoralis*.

## DISCUSSION

### I- Embryonic changes of acid and alkaline phosphatases:

The wide occurrence of phosphatases in animal tissues is thought to be associated with (a) transport of metabolites, (b) metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrates, and (c) synthesis of protein. The importance of these enzymes in embryonic tissues has also been repeatedly pointed out by Moog (1946) and Boell (1955). In the present study, the activities of acid and alkaline phosphatases were determined in relation to the various morphogenetic events occurring during the embryogenesis of *S. littoralis*. Acid and alkaline phosphatases were present from the onset of egg deposition. A similar trend has also been observed in *Drosophila* by Yao (1950 a, b) and Prakash and Reddy (1979). Sidhu *et al*. (1984) reported that the presence of acid and alkaline phosphatase from the beginning of the egg development of *Chilomenes sexmaculata* indicates that transcription of these enzymes take place right during the period of egg maturation.

The activity of acid phosphatase in the present work was observed to increase during the first 6 hours of embryonic development while that of alkaline phosphatase was increased during the first 12 hours. Mukesh *et al*. (1980) stated that these period of enzymes activities in *Chilomenes sexmaculata* correspond to a period of active yolk protein lysis as was evident from an increase level of free amino acids. They also assumed that this time of enzymes activities is the time for active movement of cleavage energids. Chapman (2002) reported that vitellophages formed in the beginning of embryonic development have a variety of functions. One of these functions is the breakdown of yolk. Breakdown or lysis of yolk protein facilitates the cleavage in the egg as mentioned by Klowden (2007).

As embryogenesis proceeds, the acid phosphatase activity was increased and reached the highest level at 60 followed by 36 h of age. The alkaline phosphatase activity was decreased to reach the lowest level in the eggs at 48 h of age. But at 60 and 72 h of age the level of activity increased slightly. The rise in the activity of acid phosphatase with advancement of embryogenesis was in conformity with the observations of Fitzgerald (1949) in the eggs of grasshoppers; Chino (1961) and Sridhara and Bhat (1963) in the eggs of silk worm; Sidhu *et al.* (1984) in the eggs of *Chilomenes sexmaculata* and Fialho *et al.* (2002) in the eggs of *Rhodnius prolixus.* Such an enhancement in the activity of acid phosphatase may be considered as one of the many factors responsible for rapid breakdown of yolk protein in the early stages and also in keeping a relatively low level of protein content in general, during later stages of embryonic development of *Corcyra cephalonica* as mentioned by Chaubey and Bhatt (1988). Naqvi and Ashrafi (1968) stated that the higher acid phosphatase activity in eggs of *Schistocerca gregaria* indicates that this enzyme is related to developmental processes which include tissue growth, tissue transformation, and excessive transport of materials. This view was further strengthened by the fact that the highest activity of the acid phosphatase was present in the late embryonic stage, where most of the organs and systems are in the state of formation. Mukesh *et al*. (1980) also reported that further higher activity of acid phosphatases during mid and late stages of embryogenesis may be related with the process of cellular multiplication and differentiation during phase of development.

### II- Post-emergence changes of acid and alkaline phosphatases:

In the present work, the activities of acid and alkaline phosphatase were studied in four groups of post-emerged adult, ovaries of mated females, ovaries of non-mated females, testes of mated males and testes of non-mated males. Generally

the activities of acid phosphatase in the four studied groups were higher than that of alkaline phosphatase. Similar results had also been observed by Barker and Alexander (1958); Pant and Dyna Morris (1972) and Houk and Hardy (1984).

On the other hand, the acid phosphatase activities in mated and non-mated males were higher than that in mated and non-mated females. Barker and Alexander (1958) studied the acid and alkaline phosphatases in house flies of different ages. They observed that the activities of phosphatases in females were greater than in males. Houk and Hardy (1984) also studied the activity of alkaline phosphatase in male and female of *Culex tarsalis.* They concluded that no significant differences were noted between the specific activities of the enzymes of males and females.

Gilbert and Huddleston (1965) studied the testicular acid phosphatase in giant silk moth. They found that homogenates of testes extirpated from giant silk moth adults contain relatively active and non-specific acid phosphatases. The testes enzymes are much more proficient than those found in the ovaries. Our results revealed that phosphatase found in testes of *S. littoralis* may be lysosomal phosphatase. This hypothesis confirmed by Stay (1959) who determined histochemically that both the cytoplasm and nucleus of testicular cells in *Phormia regina* contain phosphatase. Gilbert and Huddleston (1965) also found that small percentage of total acid phosphatase in giant silk moth can be of lysosomal origin. Moreover, Ribolla *et al.* (1993) showed that this enzyme is likely to be lysosomal phosphatase.

Our results also showed that the activities of acid and alkaline phosphatases appeared to be cyclic in the four groups at the different ages of the post-emerged adults. These results agreed with that observed by Houk and Hardy (1984). This phenomenon may be due to the periods of spermatozoa production in the testes (Stay, 1959 and Klowden, 2007) and yolk accumulation in oocytes and ovulation (Chapman, 2002).

Chapman (2002) stated that the function of follicle cells change during oocyte development and the follicle cells produce some minor yolk proteins and perhaps some of the enzymes that will later be involved in processing the yolk and producing egg shell or chorion. Funk (2001) supported this statement since he found the alkaline phosphatase in the portion of the ovariole surrounding the terminal oocyte which is the location where the egg chorion is formed and begins to harden. Fialho *et al.* (2002) reported that acid phosphatase has long been recognized in insects as one of the main yolk associated enzymes activities.

The present study revealed that acid phosphatase activity was higher in non-mated males than in mated ones. Alkaline phosphatase activity was higher in non-mated males than in mated ones except at the age of 6, 36 and 48 h. The role of these enzymes in the biochemistry of the insect testes or in spermatogenesis is not understood till now. It is possible that the phosphatases in the insect testes may be contributed to inorganic phosphate reactions leading to the biosynthesis of DNA during spermatogenesis or to the vast number of ubiquitous reactions requiring phosphorylation (Gilbert & Huddleston, 1965).

The present work showed that acid and alkaline phosphatase activities were higher in non-mated female than mated ones except at 36 and 60 h and 60 h of age, respectively. Peaks of phosphatases in our results may be related to the intervals between successive ovulation. Mating causes females to oviposit while virgin females usually do not lay eggs (Chapman, 2002). The oviposition is induced by peptides or other substances transferred to the female by the male during sperm transfer (Gillott, 2003). Generally yolk accumulation is largely restricted to the oocyte nearest the

oviduct in each ovariole (terminal oocyte). The succeeding oocytes remain relatively small until the first discharge from the ovariole into the oviduct, ovulation, hence there is an interval between successive ovulations from any single ovariole. However, in many Lepidoptera, yolk accumulation occurs simultaneously in a number of oocytes in each ovariole and fully developed oocytes are stored before oviposition. After mating, the mature oocytes are moved into the oviduct by the process of ovulation. But in non-mated females, oocytes may be destroyed and their contents resorbed by the insect as a result of non-mating (Chapman, 2002). This increase in phosphatases could be related to yolk accumulation in the terminal oocytes in the ovarioles. However, the decrease could be related either to ovulation in mated females or destruction and resorption of the mature oocytes in non-mated females. This hypothesis needs further investigations.

## REFERENCES

Barker, R. J. and Alexander, B. H. (1958). Acid and alkaline phosphatases in house flies of different ages. Ann. ent. Soc. Amer. 51: 255-257.

Boell, E. J. (1955). Energy exchange and enzyme development during embryogenesis. In Analysis of Development (Edited by Willier B. H., Weiss P. A. and Hamburger V.), pp. 520-555. Saunders, Philadelphia.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analyt. Biochem. 72: 248-254.

Chapman, R. F. (2002). The insects: structure and function, 4th ed. Cambridge, U.K. Cambridge University Press.

Chaubey, S. N. and Bhatt, R. S. (1988). Changes in the levels of nucleic acid, protein, total free amino acid and glycogen and activity of acid phosphatase in the eggs, during normal embryogenic development of rice moth, *Corcyra cephalonica*, Stainton (Lepidoptera: Pyralidae). Insect Biochem. 18: 443-447.

Chino, H. (1961). Enzymatic pathways in the formation of sorbitol and glycerol in diapausing egg of the silkworm, *Bombyx mori* (L.) II. On the phosphatases. J. Insect Physiol. 6: 231-240.

Cho, W-L.; Tsao, S-M.; Hays, A.R.; Walter, R.; Chen, J-S.; Snigirevskaya, E.S. and Raikhel, A.S. (1999). Mosquito cathepsin B-like protease involved in embryonic degradation of vitellin is produced as a latent extraovarian precursor. J. Biol. Chem. 274: 13311-13321.

El-Sheikh, T. A. (2006). Biological and biochemical effects of *Serratia marcescens* (Eubacteriales: Enterobacteria) as microbial agent and the chitin synthesis inhibitor Lufenuron on the cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Bull.ent. Soc. Egypt, Econ. Ser. 32: 113-125.

Fagotto, F. (1990). Yolk degradation in tick eggs: I. Occurrence of Cathepsin L-like acid proteinase in yolk spheres. Archs. Insect Biochem. Physiol. 14: 217-235.

Fagotto, F. (1991). Yolk degradation in tick eggs: III. Developmentally regulated acidification of the yolk spheres. Develop. Growth& Differ. 33: 57-66.

Fagotto, F.; Maxfield, F. R. (1994). Changes in yolk platelet pH during *Xenopus laevis* development correlate with yolk utilization. A quantitative confocal microscopy study. J. Cell Sci. 107: 3325-3337.

Fialho, E.; Silveira, A. B.; Masuda, H. and Silva-Neto, M. A. (2002). Oocyte fertilization triggers acid phosphatase activity during *Rhodnius prolixus* embryogenesis. Insect Biochem. Mol. Biol. 32: 871-880.

Fitzgerald, L.R. (1949). The alkaline phosphatase of the developing grasshopper egg. J. Exp. Zool. 110: 461-487.

Funk, C.J. (2001). Alkaline phosphatase activity in whitefly salivary glands and saliva. Arch. Insect Biochem. Physiol. 46: 165-174.

Gilbert, L. I. and Huddleston, C. J. (1965). Testicular acid phosphatase in the giant silkmoth. J. Insect Physiol. 11: 177-194.

Gillott, C. (2003). Male accessory gland secretions: Modulators of female reproductive physiology and behavior. Annu. Rev. Entomol.48: 163-184.

Houk, E.J. and Hardy, J. L. (1984). Alkaline phosphatase of the mosquito, *Culex tarsalis* Coquillett. Comp. Biochem. Physiol. (B) 78: 303-310.

Izumi, S.; Yano, K.; Yamamoto, Y. and Takahashi, S.Y. (1994). Yolk proteins from insect eggs: structure biosynthesis and programmed degradation during embryogenesis. Review. J. Insect Physiol. 40: 735-746.

Klowden, M. J. (2007). Physiological systems in insects. Oxford, Elsevier Inc. pp: 147-149.

Lemanski, L. F. and Aldoroty, R. (1974). Role of acid phosphatase in the breakdown of yolk platelets in developing amphibian embryos. J. Morph. 153: 419-426.

Liu, X.; McCarron, R.C. and Nordin, J. H. (1996). A cysteine protease that process insect vitellin. Purification and partial characterization of the enzyme and the proenzyme. J. Biol. Chem. 271: 33344-33351.

Moog, F. (1946). The physiological significance of the phosphomonoesterases. Biol. Rev. 21:41-59.

Mukesh, D.; Sidhu, D.S. and Kumar, N. (1980). Total concentration of free amino acids and corresponding morphogenetic changes during the embryogenesis of *Chilomenes sexmaculata* (Coleoptera: Coccindellidae). J. N. Y. Entomol. Soc. 88: 115-119.

Naqvi, S. N. and Ashrafi, S. H. (1968). Phosphomonoesterase activity in the life cycle stages of *Schistocerca gregaria*. Can. Ent. 100: 649-655.

Pant, R. and Dyna Morris, I. (1972). Variation in glycogen, total free sugars, protein, alkaline and acid phosphatases, citrate and inorganic phosphorus level in fat body of *Philosamia ricini* (Eri-silkworm) during development. J. Biochem. 71: 1-8.

Prakash, H. S. and Reddy, G. S. (1979). Development variability in the acid and alkaline phosphatase activity in *Drosophila rajasekri.* Indian. J. Exp. Biol. 17: 801-803.

Raikhel, A. S. and Dhadialla, T. S. (1992). Accumulation of yolk proteins in insect oocytes. Annu. Rev. Entomol.37: 217-251.

Ribolla, P. E.; Daffre, S. and Bianchi, A.G. (1993). Cathepsin B and acid phosphatase activities during *Musca domestica* embryogenesis. Insect Biochem. Mol. Biol. 23: 217-223.

Seidel, F. (1936). Entwicklungs-physiologie des Insektenkeimes. Verh. Dtsch. Zool. Ges. pp. 291-336.

Seidel, F. (1960). Korpergrundgestalt und Keimkuthur. Zool. Ann. 164: 245-305.

Sidhu, D. S.; Dhillon, S. S. and Sandhu, S. K. (1984): Acid and alkaline phosphatase activity during embryonic development of *Chilomenes sexmaculata* Fabr. (Coleoptera: Coccinellidae). Acta entomol. Jugoslav. 20: 89-94.

Sridhara, S. and Bhat, J. V. (1963). Alkaline and acid phosphatase of silkworm, *Bombyx mori* (L.). J. Insect Physiol. 9: 693-701.

Stay, B. A. (1959). Histochemical studies on the blowfly, *Phormia regina* (Meigen). II. Distribution of phosphatases, dehydrogenases and cytochrome oxidase during larval and pupal stages. J. Morph. 105: 457- 493.

Yamamoto, Y. and Takahashi, S.Y. (1993). Cysteine proteinase from *Bombyx* eggs: role in programmed degradation of yolk proteins during embryogenesis. Mini review. Comp. Biochem. Physiol. 10B: 35-45.

Yao, T. (1950 a). Cytochemical studies on the embryonic development of *Drosophila melanogaster*. II. Alkaline and acid phosphatase. Quart. J. Micr. Sci. 91:79-88.

Yao, T. (1950 b). The localization of alkaline phosphatase during the post embryonic development of *Drosophila melanogaster*. Quart. J. Micr. Sci. 91: 79-88.

## ARABIC SUMMARY

تغير نشاط إنزيمىالفوسفات الحامضى و القلوى أثناء النمو الجنينى و ما بعد بزوغ الحشرة اليافعة لدودة ورق القطن سبودوبترا ليتوراليس (بولسد)

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تم دراسة نشاط إنزيمىً الفوسفات الحامضى و القلوى فى البيض أثناء النمو الجنينى لدودة ورق القطن *سبودوبترا ليتوراليس* فى فترات زمنية مختلفة ( أعمار من 0- 72 ساعة ). كما تم دراسة نشاط هذه الأنزيمات فى المبيض و الخصية للحشرة اليافعة بعد البزوغ فى نفس الفترات الزمنية ( 0- 72 ساعة). و قد تم تقسيم الحشرات اليافعة بعد البزوغ إلى إناث و ذكور متزاوجة و غير متزاوجة.

و قد لوحظ أثناء النمو الجنينى أن نشاط إنزيم الفوسفات الحامضى دوري و أعلى من نشاط إنزيم الفوسفات القلوى و ذلك خلال الفترات الزمنية من 0- 72ساعة. بينما أظهر نشاط إنزيم الفوسفات القلوى زيادة طفيفة فى البيض حديث الوضع ثم إنخفض بعد ذلك عن نشاط إنزيم الفوسفات الحامضى. و قد تم تفسير زيادة نشاط الأنزيمات فى الفترة الأولى من وضع البيض بأنه يصاحب تكسير المح إلى أحماض أمينية ، أما فى الفترات المتقدمة من العمر فالزيادة فى نشاط إنزيم الفوسفات الحامضى تصاحب تكون الجنين. و فى مجموعات الحشرة اليافعة بعد البزوغ فقد أظهر إنزيم الفوسفات الحامضى نشاطاً أعلى من نشاط إنزيم الفوسفات القلوى عند كل مراحل تقدم العمر. كما لوحظ أن نشاط إنزيم الفوسفات الحامضى دورياً أيضاً فى الحشرات اليافعة المتزاوجة و غير المتزاوجة. و قد أظهرت النتائج إختلافاً واضحاً بين نشاط الأنزيمات فى المبيض و الخصية للحشرات اليافعة المتزاوجة و غير المتزاوجة. و قد تم مناقشة هذه الأختلافات فى نشاط الأنزيمات التى قد ترجع إلى فترة تكون المنىً فى الذكور و فترة تكون البيض فى الأناث كما أن التزاوج قد يكون أحد العوامل المؤثرة فى نشاط الأنزيمات.