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Esterase activity and detection of carboxylesterase and phosphotriesterase in female desert locust *Schistocerca gregaria* (Forskal) in relation to tissues and ages

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ABSTRACT

General esterases activity in the different body tissues of adult females of 2-day and 13-day-old *Schistocerca gregaria* (Forskal) were measured spectrophotometricaly and carboxylesterase and phosphotriesterase were characterized by electrophoresis method. Esterase activities were studied in the tissues of brain, fore-, mid-, hindgut and ovary of the two ages in the presence of α-naphthyl acetate as a substrate and ethylenediamine tetraacetic acid (EDTA) and profenofos as inhibitors. Spectrophotometric analysis indicated that the esterase activity in adult *S. gregaria* tissues was significantly higher in the tissues of mid-, foregut, ovary and brain of 2-day-old females than those of 13-day-old, respectively. Meanwhile, the activity was greater in the hindgut tissue of 13-day-old females than in 2-day-old ones. Non-denaturing polyacrylamide gel electrophoresis revealed differences in isozyme composition. Three to ten esterase bands were detected in the different body tissues of the two ages of adult females. These bands were characterized as phosphotriesterase and carboxylesterase by using the two inhibitors. Bands Est-1, Est-2, Est-3 and Est-6 were found to be carboxylesterase in the different tissues, while band Est-4 was only detected to be phosphotriesterase.

Key words: *Schistocerca gregaria*, carboxylesterase, phosphotriesterase, isozymes, tissues, ages, esterase activity.

INTRODUCTION

Esterases are classified as hydrolases, a large and diverse group of enzymes that catalyze the hydrolysis of a wide range of aliphatic and aromatic esters, choline esters and organophosphorous compounds (Dauterman, 1985).Esterases act on molecules that are completely dissolved in water, hydrolyzing carboxylesterases into alcohol and carboxylate. They may break down cholesterol and are important in the resistance to insecticides and plant secondary substances (Klowden, 2007).

Phosphotriesterase enzyme is a monomeric, spherical protein having a molecular weight of 39,000. A single zinc atom is bound to the enzyme and is required for catalytic activity. Incubation with metal chelating compounds, o-phenanthroline, EDTA, or 2, 6-pyridine dicarboxylate inactivate the enzyme (Dumas *et al*., 1989; Horne *et al*., 2002). Carboxylesterase is well investigated with regard to its importance in organophosphate insecticide resistance in various pest species. Recent studies suggest that elevated esterase activities (as measured with α -naphtyl acetate) are associated with resistance to organophosphorous insecticides (Zhao *et al*., 1996) and are correlated with frequencies of profenofos resistance (Harold and Ottea, 2000). The level of insect esterase highly variable depending on the life stage, sex, tissue, hormones, strain, food , environmental conditions and numerous other factors (Devorshak and Roe,1999). Generally, studies of phosphotriesterases in invertebrates such as insects are very limited, probably a consequence of low activity of

phosphotriesterases in these insects (Guedes *et al*., 1997). On the other hand, carboxylesterases also appeared to be the predominant enzyme in most tissues of many insects (Kapin and Ahmad, 1980; Park and Kamble, 1999).

Therefore, this study was under taken to determine the esterase activity in different body tissues at different ages of adult female *Schistocerca gregaria* and to detect carboxylesterases and phosphotriesterases in these tissues.

MATERIALS AND METHODS

Insects:

Colony of *Schistocerca gregaria* (Forskal) (*S.gregaria*) was reared on a diet of green leaves of clover (*Trifolium alexandarium*) at 28-34˚C, 40-50% R.H. and daily illumination of 12h as described by Tawfik *et al*. (1997). Newly enclosed adult females were removed for dissection of tissues at ages 2 days $\pm$ 6hrs and 13 $\pm$ 1 days.

Dissections

Ten adult of sexually immature (2-day-old) and mature (13-day-old) females *S.gregaria* were dissected in 1.15 M KCl to obtain the following tissues: brain, foregut, midgut, hindgut and ovary. Guts were cut, open and undigested food and fecal materials were expelled. All discrete organ systems were of high degree of purity. All tissues were stored overnight in 1.15MKCl at 2 $\pm$1˚C.

Homogenization and centrifugation

Tissues were drained of KCl solution and rinsed with 0.04 M phosphate buffer, pH 7.0. Rinsed tissues were then homogenized in 5ml of phosphate buffer for 3min in an iced-chilled glass homogenizer. The homogenates were transferred to glass centrifuge tubes (10.2 cm) and spun at 1200 *g* for 20 min at 4˚C. The 1200 *g* supernatants were used directly as enzyme source.

Protein determination:

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

Enzyme assays:

Total esterase activity was measured according to Mostafa *et al*. (2003) with some modifications. α -naphtyl acetate (C-2) was used to measure the substrate specificity.

The interaction of esterase enzyme with an insecticidal substrate (profenofos and EDTA 1x10 M each) was studied.

Electrophoresis:

Nondenaturing native polyacrylamide gel electrophoresis (PAGE) was done according to Mostafa *et al*. (2003).

Detection of carboxylesterase and phosphotriesterase:

This was accomplished by the use of two inhibitors: profenofos, inhibitor of carboxylesterase and EDTA (ethylenediamine tetraacetic acid) inhibitor of phosphotriesterase. Each gel was incubated in the same buffer containing 100 µl of each inhibitor solution for 30 min at room temperature before starting the reaction with substrate. Gels and cellophane membrane were placed in the drying solution containing 0.3 % glycerol and 20 % methanol, and the fixed gels were dried between 2 sheets of filter paper in a gel dryer. An image of the dried gel was carried out using Epson GT-9500 scanner then transferred into an image analyzer Phoretix 1D Quantifier to integrate the gel data.

RESULTS

Enzyme activity:

Figure (1) showed that esterase activity in all of the five tissues of the two ages of females examined. Analysis of the specific activity data showed the presence of significant variations in the different tissues. Most noticeable was the high specific activity of the hindgut tissue enzyme followed by the tissues of mid-, foregut, the ovary and the brain of the 2-day and 13-day- old of the adult females.

The tissue distribution given in Figure (1) was computed from total protein content of tissue supernatants and their specific activities. Spectrophotometric analysis indicated that esterase activity was significantly higher in tissues of mid-, foregut, ovary and brain of 2-day-old females (21.24, 14.5, 13.85, and 7.39 µmol/min/mg protein, respectively) than those of 13-day-old ones (16.1, 7.53. 5.62 and 5.10 µmol/min/mg protein, respectively). Meanwhile, it was greater in the hindgut tissue of 13-day-old females (41.3 µmol) than that in 2-day-old ones (38.71 µmol).

Figure (1) indicated that α- naphthyl acetate esterase activity in the brain, fore-, mid-, hindgut and ovary tissues of the 2-day-old females were strongly inhibited by EDTA (inhibition percentage: 73.75, 74.5, 67.66, 67.66 and 76.9%, respectively) and profenofos (inhibition percentage: 77.54, 68.3, 70.34, 67 and 72.78%, respectively). While, in the 13-day-old females the esterase activity in the brain, fore- and midgut tissues was partially inhibited by EDTA (59.61, 41.57, and 51.56%, respectively) and profenofos (57.25, 40.24, and 49.5%, respectively).

In contrast, the α- naphthyl acetate esterase activity in the ovary and hindgut tissues of 13-day-old females was strongly inhibited by the two inhibitors (74% and 88.43% for EDTA respectively; 63.88% and 91.43% for profenofos, respectively).

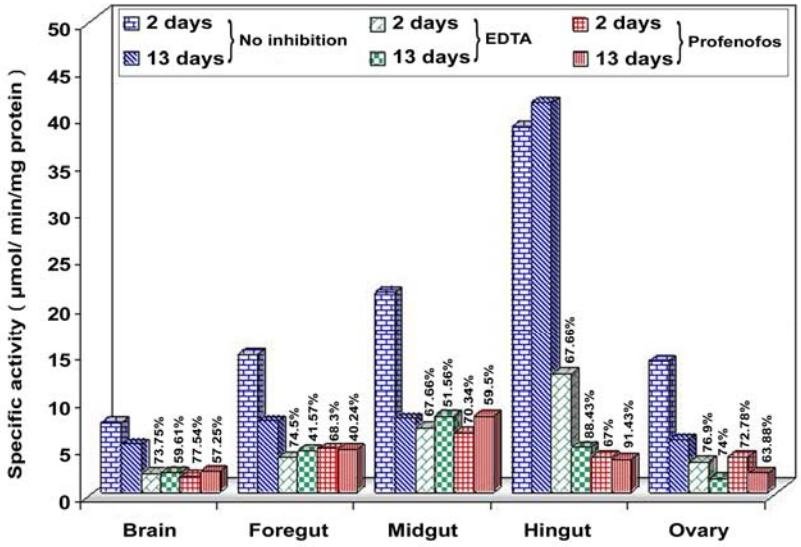

Fig. 1: Specific activity and inhibition percentage of esterases in the different body tissues of 2-days and 13-days old adult females *Schistocerca gregaria* using □–naphthyl acetate as a substrate and EDTA and profenofos as inhibitors by spectrophotometric assay.

Gel electrophoresis:

Esterase isozyme analysis with native polyacrylamide gel electrophoresis revealed 10 bands in the brain of the two ages. These bands were designated as 1-10 according

to their mobility. Est-1 was the slowest migrating band and Est-10 the fastest. Tissues of the 2-day-old females have Est- 1,6,7,8 and 9 in the foregut tissue, Est- 5,6,7,8 and 9 in the mid- and hindgut tissues, while ovary tissue exhibited Est-5, 6, 7, 8,9,10 and 3 specific isozymes designated as a, b and c . In the 13-day-old females Est- 7, 8 and 9 were dominant in the foregut tissue, Est-7, 8, 9 and 10 were dominant in the midgut tissue and Est-4, 6, 7 and 9 were dominant in the hindgut tissue. On the other hand, the 13-day-old females characterized by the appearance of two newly isozymes in the ovary tissues, Est-1 and Est-4 in addition to the dominant isozymes Est-5,6,7,8,9 and 10 with the disappearance of the specific isozyme esterase c (Fig.2).

Detection of carboxylesterase and phosphotriesterase:

The sensitivity of esterase bands to different insecticidal substrates was evaluated visually according to their stain density. Bands inhibited by organophosphorous compounds (profenofos) were classified as carboxylesterase, those inhibited by EDTA were grouped as phosphotriesterase.

Incubation of the tissues homogenates polyacrylamide gel of the two ages in EDTA resulted in complete inhibition of band Est-4 in the brain of the two ages; tissues of hindgut and ovary of 13-day-old females. Incubation of the gel with profenofos caused complete inhibition for bands Est-1, Est-2, Est-3 and Est-6 in the two ages of the brain; Est-1 and Est-6 in the foregut tissue; Est-6 in the midgut tissue of 2-day-old females and Est-6 in the hindgut tissue and ovary of the two ages of the females (Fig.2). According to the previous classification, band Est-4 showed properties of phosphotriesterase. Bands Est-1, Est-2, Est-3 and Est-6 were found to be carboxylesterase.

DISCUSSION

In the present study, spectrophotometric analysis indicated that the esterase activity in adult *S.gregaria* tissues was significantly higher in the mid-, foregut tissues, ovary and brain of 2-day-old females than those of 13-day-old, respectively. Meanwhile, the activity was greater in the hindgut tissue of 13-day-old females than in 2-day-old ones. This result was in good agreement with those of *Lymantria dispar* L. by Kapin and Ahmad (1980). They stated that elevation of the esterase activity in the early days of development is correlated with the increasing rate of food consumption and growth and maturation of adult females. Contradictory results by Pruett *et al*. (2000, 2001) on the horn flies, presented that the general esterase activity increases with aging and that newly emerged flies have a much lower level of general esterase activity than do more mature flies.

The specific activity of esterase in hindgut tissue of the two ages of *S. gregaria* was high followed by that in midgut tissue. The esterase activity may be primarily due to carboxylesterase as mentioned by Hendry *et al*. (1975). They stated that the role of esterase in the hindgut tissue is unclear, but since the hindgut tissue plays a role in excretion, it is conceivable that the carboxylesterase is involved in the hydrolysis of metabolically inert and undesirable esters. The elevation of the esterase activity in the hindgut tissue might be also attributed to the presence of bacteria in the gut locust. Dillon *et al*. (2000) proved that guaiacol, a key component of a pheromone derived from locust faecal pellets that promotes the aggregation of locusts, is produced by bacteria in the locust gut. Moreover, this elevation might be due to the absorptive function of the hind gut, where Nation (2002) stated that the fate of enzymes is not known, they are rarely found in the hindgut lumen, although no inhibitors are secreted

by any region of the gut or Malpighian tubules probably they are absorbed in the hindgut tissue.

The high concentration and specific activity of the esterase in the midgut raised the question of the functional role of this enzyme. It was believed by some investigators that in insects, the midgut esterases were involved in the absorption / digestion of dietary lipids (Kapin and Ahmad, 1980). Park and Kamble (1999) assumed that the main fraction of the total esterase activity in the German cockroach exists in the midgut because of its digestive function. Nevertheless, midgut esterases play a role in insect resistance to insecticide (Whyard *et al*., 1994) and to allelochemicals (Lindroth, 1989).

The specific activity of esterase in ovary of *S. gregaria* was high at 2-day-old females than at 13-day-old ones. The esterase activity appeared to be primarily due to carboxylesterase.

Native polyacrylamide gel electrophoresis is a valuable technique in the study of insect esterases and insecticide resistance. Esterase isozyme patterns after staining for enzymatic activity can be compared with each other to detect the activity of esterase in the different tissues. Moreover, the characteristics of each esterase isozyme can be determined by the addition of specific inhibitors in the process of enzymatic staining of gels. In the present study, comparison of esterase isozyme patterns between the different tissues and the two ages revealed that, 10 different esterase bands stained with α-naphthyl acetate were detected in the brain of 2-day- and 13-day-old adult female *S. gregaria*. Five different bands were detected in fore-, mid- and hindgut tissues and 9 bands in ovary of 2-day-old adult females *S. gregaria*. In 13-day-old adult female, the number of bands decreased to 3 bands in foregut tissue, 4 bands in mid- and hindgut tissues; while it increased to 10 bands in ovary. These results are in agreement with Ruvolo- Takasusuki and Collet (2000). They observed differences in number of zones of esterase activity depending on the tissue, age, sex or phase of the development in African honey bees, *Apis mellifera.* The present work indicated also that isozymes 7 and 9 are common to all tissues while, a and b are specific to ovary and isozyme c is specific to the age of ovary. Shaurab *et al*. (1999) found that 6 esterase bands were capable of hydrolyzing α- naphthyl acetate in three ages of *Spodoptera littoralis*. Two of them were detected in the ovaries of the three physiological ages studied.

In the present study the specific inhibitors (profenofos and EDTA) were used to detect the carboxylesterases and phosphotriesterases at the different chosen tissues. Caroxylesterases were observed to be the dominant in the brain, fore-, hindgut tissues and ovary of the two ages and midgut tissue in 2-day-old females, while phosphotriesterases were detected only in the brain of 2-day-old adult females and hindgut tissue of 13-day-old adult ones. Both the carboxylesterases and phosphotriesterase were detected in the brain tissues of 2-day-old and 13-day-old adult females suggested the possibility that the esterases in the brain might play an important role in insecticide resistance of *S.gregaria* as mentioned by Park and Kamble(1999) on *Blattella germanica*. Rooveldt and Tawfik (2005) also demonstrated that phosphotriesterase from *Pseudomonas diminuta* is an extremely efficient metalloenzyme that hydrolases a variety of compounds including organophosphorous nerve agents. On the other hand, the occurrence of both carboxylesterases and phosphotriesterases in the ovary tissue of 13-day-old and the presence of carboxylesterases in 2-day-old females might explain the correlation between ovarian maturation and occurrence of esterases as mentioned by Krishnamurthy and Umakanth (1997) and Shaurub *et al*. (1999). They may be also

regulating the juvenile hormone titer as mentioned by Chapman (2002) who stated that the hemolymph usually contains a number of esterases. One of their functions is to regulate juvenile hormone titer. Forever, further studies are needed to classify the esterase of ovary and to detect the principle role of the esterase in the ovary maturation.

REFERENCES

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

Chapman, R. F. (2002). The Insects: Structure and function.4th Edition, Cambridge University Press.

Dauterman, W. C. (1985). Insect metabolism: Extramicrosomal, pp: 713-730. In Kerkut, G. A. and Gilbert, L. I. (eds.), Comprehensive insect physiology, biochemistry and pharmacology, vol.12, Pergamon, Oxford.

Devorshak, C. and Roe, RM. (1999). The role of esterases in insecticide resistance. Reviews in Toxicology, 2 (7-8):501-537.

Dillon, R. J.; Vennard, C. T. and Charnley, K.A. (2000). Pheromones: Exploitation of gut bacteria in the locust. Nature, 403 (851):1000-1038.

Dumas, D. P.; Caldwell, S. R.; Wild, J. R. and Raushel, F. M. (1989). Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*.J. Biol. Chem.264 (33):19659-19665.

Guedes, R. N.; Zhu, K. Y.; Dover, B. A. and Kambhampati, S. (1997). Partial characterization of phosphotriesterases from organophosphate-susceptible and resistant populations of *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Pestic. Biochem. Physiol., 57:156-164.

Harold, J. A. and Ottea, J. A. (2000). Characterization of esterases associated with profenofos resistance in the Tobacco Budworm, *Heliothis virescens* (F.). Arch.Insect Biochem. Physiol., 45: 47-59.

Hendry, L. B.; Kostele, J. G.; Hindenlang, D. M.; Wichmann,J. K.; Fix, C.J. and Korzenoiski, S. H. (1975). Chemical messengers in insects and plants. In Recent Advances in Phytochemistry. Biochemical Interaction Between plants and Insects (Ed by Wallace J. W. and Mansell R. L.) vol. 10, pp. 351-348. Plenum Press, New York.

Horne, I.; Harcourt, R. L.; Sutherland, T. D.; Russel, R. J. and Oakeshott, J. G. (2002). Isolation of a *Pseudomonas monteilli* strain with a novel phosphotiesterase. Appl. Environ. Microbiol.68 (7): 3371-3376.

Kapin, M. A. and Ahmad, S. (1980). Esterase in larval tissues of Gypsy moth, *Lymantria dispar* (L.): Optimum assay conditions, quantification and characterization. Insect Biochem. 10:331-337.

Klowden, M. J. (2007). Physiological systems in insects.Oxford, Elsevier Inc.

Krishnamurthy, N. B. and Umakanth, R. S. (1997). Contribution of esterase isozymes during mating in silkworm, *Bombyx mori* L. Ind. J. Sericult. 36: 11-16.

Lindroth, R.L. (1989). Biochemical detoxication: Mechanism of differential tiger swallowtail tolerance to phenolic glycosides. Oeclogia, 81: 219-224.

Mostafa, Z. K.; Ramadan, K. S. and Rashad, E. M. (2003). General characterization of esterases from the peach fruit fly, adult *Bactrocera zonata* Saund. (Diptera: Tephritidae). Egypt. J. Biochem. Mol.Biol. 21:329-340.

Nation, J. L. (2002). Insect physiology and biochemistry. New York, CRC Press.

Park, N. J. and Kamble, S. T. (1999). Distribution and inhibition of esterases in body tissues of susceptible and resistant German cockroaches (Dictyoptera: Blattellidae). Ann. Entomol. Soc. Am. 92 (4):556-562.

Pruett, J. H.; Oehler, D. D.; Kammlah, D. M. and Guerrero, F. D. (2000). Evaluation of horn flies (Diptera: Muscidae) from a pyrethoid susceptible colony for general and permethrin esterase activities. J. Econ. Entomol. 93:920-924.

Pruett, J. H.; Kammlah, D.M. and Guerrero, F. D. (2001). Variation in general esterase activity within a population of *Haematobia irritans* (Diptera: Muscidae). J. Econ. Entomol. 94 (3): 714-718.

Roodveldt, C. and Tawfik, D. S. (2005). Directed evolution of phosphotriesterase from *Pseudomonas diminuta* for heterologous expression in *Escherichia coli* results in stabilization of the metal-free state. Protein Engineering, Design& Selection.18 (1): 51-58.

Ruvolo-Takasusuki, M.C. and Collet, T. (2000). Characterization of *Nasutitermes globiceps* (Isoptera: Termitidae) Esterases. Biochem. Genet., 38(11/12):367-375.

Shaurub, E. H.; Ali, G. M.and Megahed, F. M. (1999). Esterase isozymes in the ovaries of gamma-irradiated cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Bull. Ent. Soc. Egypt, Econ. Ser. 26:109-122.

Tawfik, A.I.; Osir, E.O.; Hassanali, A. and Ismail, S.H. (1997). Effects of juvenile hormone treatment on phase changes and pheromone production in the desert locust, *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae). J. insect Physiol.43:1177-1182.

Whyard, S.; Downe, A. E. and Walker, V.K. (1994). Isolation of an esterase conferring insecticide resistance in the mosquito *Culex tarsalis.* Insect Biochem. Mol. Biol., 24: 819-827.

Zhao, G.; Rose, RL.; Hodgson, E. and Roe, RM. (1996). Biochemical mechanisms and diagnostic microassays for pyrethroid, carbamate and organophosphate insecticide resistance in the Tobacco Budworm, *Heliothis virescens*. Pestic. Bochem. Physiol., 51: 57-67.

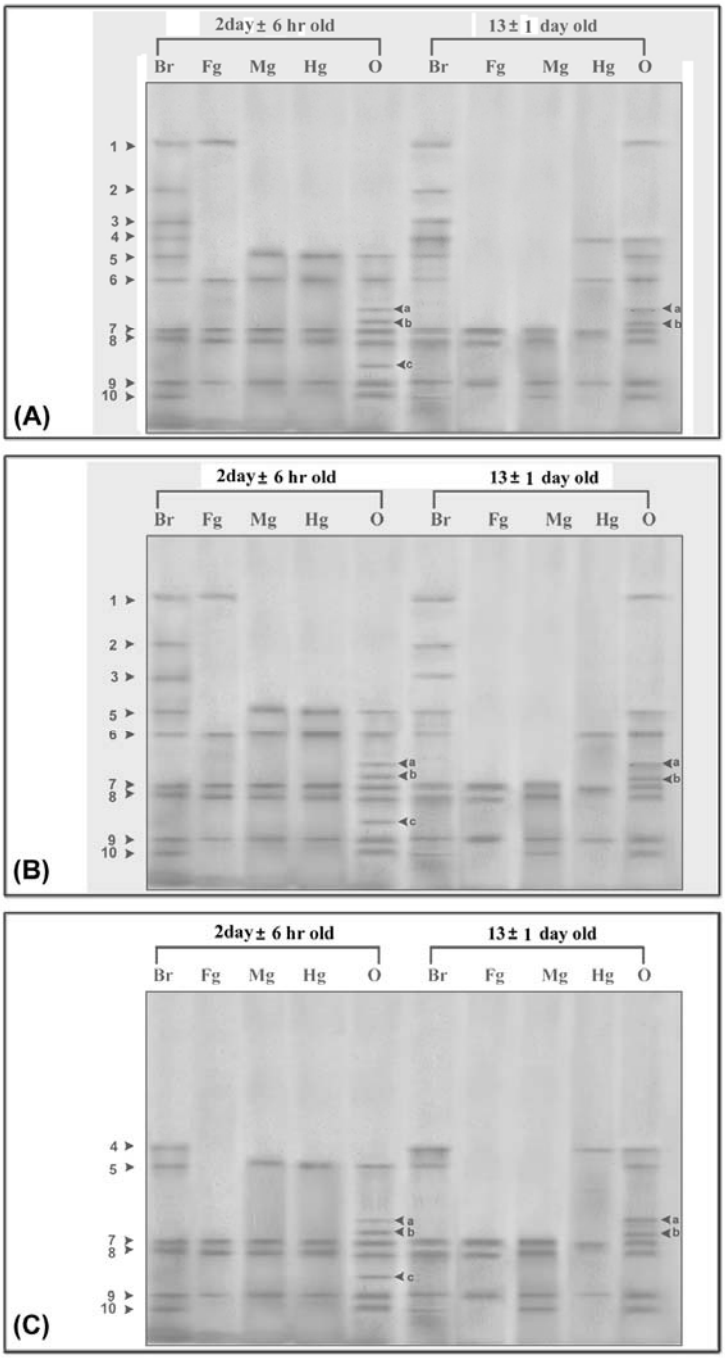

Fig. 2:(**A**)Esterase isozymes from the body tissues of 2-day and 13-day old adult females *Schistocerca gregaria*, separated by native polyacrylamide gel electrophoresis and visualized with α- naphthyl acetate as substrate. (**B**) Inhibition of esterase isozymes by EDTA. (**C**) Inhibition of esterase isozymes by profenofos.
Br, brain;Fg, foregut; Mg,midgut; Hg, hindgut; O,ovary.

ARABIC SUMMERY

النشاط الأنزيمى للأستيريز و الكشف عن الكربوكسيل أستيريز والفوسفوترای أستيريز فى إناث الجراد الصحراوى *شيستوسيركا جرجاريا* مرتبطاً بأختلاف الأنسجة و الأعمار

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تم دراسة النشاط الأنزيمى للأستيريز فى أنسجة الجسم المختلفة لأناث *الشيستوسيركا جرجاريا* عند عمرى يومان و ثلاثة عشر يوماً بأستخدام جهاز المطياف الضوئى وتم الكشف عن إنزيمى الكربوكسيل أستيريز و الفوسفوترای أستيريز المرتبطة بهذه الأنسجة و الأعمار بطريقة التفريد الكهربى. و قد تم قياس نشاط أنزيم الأستيريز فى أنسجة المخ و المعى الأمامى والمعى المتوسط والمعى الخلفى و المبيض فى العمرين السابقين فى وجود ألفا نفثيل أستيت كمادة للتفاعل و الأيثيلين داى أمين تيترا أسيتيك أسيد و البروفينوفوس كمثبطات لنشاط الأنزيمات. و قد وجد أن نشاط الأستيريز فى أنسجة المعى المتوسط و الأمامى و المبيض و المخ فى الأناث ذات عمر يومان أعلى من الأناث ذات ثلاثة عشر يوماً على التوالى. كما لوحظ زيادة نشاط الأستيريز فى أنسجة المعى الخلفى للأناث ذات عمر ثلاثة عشر يوماً عنها فى الأناث ذات عمر يومان.

و بأستخدام طريقة الفصل بالهجرة الكهربية على جل من نوع الأكريلاميد، فقد أظهر أنزيم الأستيريز من ثلاث الى عشر صور جزيئية فى أنسجة الجسم المختلفة للعمرين السابقين. و قد ميزت هذه الصور الجزيئية كلاً من الفوسفو ترای أستيريز والكربوكسيل أستيريز بأستخدام المثبطين. وقد كان أنزيم الكربوكسيل أستيريز ممثل فى الصور الجزيئية 1,2,3,6 بينما الفوسفوترای أستيريز ممثلاً فقط فى الصورة 4 و ذلك فى الأنسجة المختلفة للعمرين.