Egyptian Journal of Biology, 2004, Vol. 6, pp 136 - 146

 . Egyptian British Biological Society (EBB Soc)

Effect of 6-methyl-5-hepten-2-one on acetylcholinesterase activity, growth and development of *Spodoptera littoralis*

Talaat E. Emara

Zoology Department, Faculty of Science, Menoufyia University, Shebin El-Kom

ABSTRACT

A kinetic analysis of the interaction of *Spodoptera littoralis* and electric eel acetylcholinesterase with acetylthiocholine iodide substrate and 6-methyl-5-hepten-2-one inhibitor revealed the differences between these enzymes. *S.littoralis* AChE had Michaelis constant (KM) of 3.5×10^{-4} M while the electric eel AChE had a KM of 2.8×10^{-4} M. The 6-methyl-5-hepten-2-one competitively inhibited AChE, where the determined inhibition constant (Ki) values were 2.16 and 0.40×10^{-3} M for AChE from *S.littoralis* and electric eel, respectively. The inhibitor 6-methyl-5-hepten-2-one, a ketone compound, was assessed to evaluate the effect on growth and development of *S.littoralis.* The ketone compound significantly decreased the percentage of egg hatching and increased the larval mortality. The present observations clearly indicated that the larval treatment induced a significant decrease on pupal weight, percentage pupation and adult emergence, while the larval mortality, larval duration and pupal duration were increased.

KEYWORDS: 6-methyl-5-hepten-2-one, acetylcholinesterase, *Spodoptera littoralis*

INTRODUCTION

S. littoralis Boisd is one of the major cotton pests which cause a considerable damage to many vegetables and crops. The rising consumption of the synthetic insecticides has led to a number of problems such as pollution, insect resistance and residual toxicity.

Intensive studies were carried out so as to evaluate control procedures which can replace the current use of synthetic insecticides. Chemical communication in insects can be significantly affected by synthetic biologically active compounds, related or not to their sex phermones (Renou & Guerrero 2000). Esterases, dehydrogenases and oxidases present in the male antennae inactivate the sex phermones in insects (Kasang *et al*. 1989). Inhibition of these enzymes may lead to a decreased capability by the insect to identify new phermone molecules, and therefore a new approach to insect control can be processed (Prestwich 1986).

Molecules that resemble the substrate in its transition state geometry may be specifically recognized by enzymes. By virtue of this resemblance, such molecules bind much tighter to the enzyme than do the parent substrates. These compounds offer an interesting experimental approach to the design of novel, potent enzyme inhibitor of high specificity (Brodbeck *et al*. 1979).

Many insect phermones are structurally related to the long chain alcohols, esters, ketones and aldehydes (Bestmann & Vostrowsky 1980). This diversity of functional groups, however, is invariably attached to an alkyl chain that is very hydrophobic. Hydrophobic binding is established as the mechanism by which many organic compounds inactivate proteins (Hansch *et al*. 1965). Acetylcholinesterase enzyme is very susceptible to inhibition by hydrophobic ligands, such as methyl carbamate and organophosphate insecticides (Hansch & Deutsch 1966). Potencies as anticholinesterases, increased gradually with increasing length of the alkyl chain. Phermone chain length has been predicted on theoretical grounds as within the range of 5-20 carbons (Wilson & Bossert 1963) indicating that phermone structure is consistent with possible inhibition of

acetylcholinesterase. Trifluromethyl ketones are known as potent inhibitors of a number of esterases and proteases, such as acetylcholinesterase, chymotrypsin or human liver carboxylesterases (Ashour & Hammock 1987). The aim of this work was to evaluate the insecticidal and inhibition effects of 6-methyl-5-hepten-2-one (an alarm phermone ketone) on acetylcholinesterase and development in *S. littoralis*.

MATERIALS AND METHODS

The cotton leafworm, *S. littoralis* was reared in the laboratory on castor bean leaves, *Ricinus communis*. Adults were provided with 10 % sucrose solution and supplied with strips of soft tissue paper as substrate for oviposition. Larvae were reared in glass jars (250 c.c) until pupation.

The pheromone 6-methyl-5-hepten-2-one phermone was purchased from Aldrish chemical company. Acetylcholinesterase (electric eel); 5,5`-dithiobis-2-nitrobenzoic acid (DTNB); Acetylthiocholine iodide; eserine were purchased form Sigma chemical company.

Since the brain is rich in cholinesterases, the frozen heads of *S.littoralis* larvae were homogenized in ice-cold phosphate buffer (20 mM, pH 7.2, containing 10 g/L Triton X-100) using a glass homogenizer in an ice bath. The homogenate was centrifuged at 15,000 r.p.m for 30 minutes at 4°C in a cooling centrifuge. The supernatant was used for the AChE assay (Bryony *et al*. 1988). Electric-eel AChE was used as a purified preparation (i.e. as a reference enzyme). Electric-eel AChE was dissolved in 0.5 ml of 1 % gelatin and diluted 1:200 with distilled water, yielding a solution of 5 units/ml. Solutions of DTNB and of acetylthiocholine iodide were made up in 0.1 M phosphate buffer, pH 8.0. The stock solution of 6-methyl-5-hepten-2-one was made by dissolving in 1 ml acetone, then made up to 20 ml in phosphate buffer. Dilutions to obtain test concentrations were made by using phosphate buffer at pH 8.0.

The Ellman (1961) assay was used for AChE. 40 µl of enzyme (one unit) was added to Eppendorf assay tubes (1.5 ml), each containing 40 µl of 10 mM DTNB, 10 µl of different concentrations (9.46, 7.05, 4.73, 3.54, 2.36 and 1.18 $\times 10^{-4}$ M) of acetylthiocholine iodide and 1 ml of different concentrations (5.67, 11.35, 17.03 and 22.71 $\times 10^{-4}$ M) of pheromone solution. Eppendorf tubes were incubated at 37 °C for 15 min and the reaction stopped by the addition of 10 µl of 0.1 M eserine. There were 3 replicates per assay; controls used phosphate buffer instead of pheromone. Test and control assays were corrected by blanks for non-enzymatic hydrolysis of the substrate. Absorbance was measured at 412 nm using a UV/VIS spectrophotometer. Data were analysed by a double-reciprocal plot (Lineweaver & Burk 1934) to determine the affinity of AChE for its substrate (K_M values) and by a Dixon (1953) plot to determine the nature of the inhibition as well as the inhibition constants (K_i).

Evaluation of insecticidal activity on the various life stages was done as follows: by dipping eggs (0-24 hrs old) in different concentrations of the pheromone; and by allowing second-instar larvae to feed for three days on castor bean leaves treated with different concentrations of pheromone. Hatched larvae from treated eggs were reared on castor bean leaves. Biological parameters such as larval mortality, larval duration, pupal weight, pupal duration, pupation percentage, adult emergence and adult malformation were determined. Data were statistically analyzed based on one-way ANOVA analysis using SPSS 11.0.

RESULTS

The kinetic study, determining the K_M values, looked for *in vitro* differences in affinity for the substrate acetylthiocholine iodide of two different AChEs, from *S. littoralis* heads and from electric eel. AChE from *S. littoralis* shows slight less affinity (K_M = 3.5 $\times 10^{-4}$ M) for acetylthiocholine iodide than the same enzyme from electric eel (2.8 $\times 10^{-4}$ M) (Fig. 1 A and B). Fig. 1 also shows that K_M values increased with the presence of the pheromone inhibitor, rising to 5.0 $\times 10^{-4}$ M for *S. littoralis*, and 3.5 $\times 10^{-4}$ M electric eel.

Dixon plots (Fig. 2 A and B) confirmed that inhibition by the pheromone was competitive, as indicated by increasing inhibition associated with decreasing substrate concentration and by the intersections in the Dixon plots. The inhibition constants (K_i) were 0.40 and 2.16 $\times 10^{-3}$ M for AChE from electric eel and *S. littoralis*, respectively. K_i for AChE from *S. littoralis* was significatly higher than K_i for electric-eel AChE. Thus AChE from *S. littoralis* was less sensitive to the inhibitor 6-methy-5-hepten-2-one than AChE from electric eel.

The effects of 6-methyl-5-hepten-2-one on the growth and development of *S. littoralis* are given in Tables 1 and 2. The percentage of eggs hatched was significantly affected, and this effect was dose-dependent (Table 1), as was the mortality of larvae treated as eggs. The percentage larval mortality when treated as early 2nd instars also significantly increased with increasing dose (Table 2, Fig 3); larval and pupal durations were significantly extended with dose. There was a significant decrease in pupal weight (Table 2). The percentage of individuals pupating, emerging as adults significantly decreased, or emerging as malformed adults significantly increased with increasing dose, irrespective of when the treatments were applied (Tables 1 and 2; Fig. 3). Typical malformations induced were shrinkage and folding of wings, wings appearing as rudiments and antennae shorter than controls (Tables 1 and 2).

DISCUSSION

The main finding is that AChE from *S. littoralis* showed a lower affinity for acetylthiocholine iodide than AChE from electric eel. Booth & Lee (1971) reported that cricket-head AChE showed slight less affinity for acetylthiocholine than bovine erythrocyte AChE. More relevantly to the current results, the K_M values of the antennal esterase of *S. littoralis* and *Sesamia nonagrioides* differ, with *S. littoralis* esterase having a lower affinity for the substrate (Quero *et al*. 2003). In contrast, bovine erythrocyte AChE showed lower affinity for acetylthiocholine than housefly-head AChE (Hellenbrand & Krupka 1970).

In competitive inhibition, both inhibitor and substrate compete for the same active site on the enzyme. The compound 6-methyl-5-hepten-2-one was a competitive inhibitor of AChE from *S. littoralis* and from electric eel. The affinity of the enzyme decreased with the presence of the inhibitor. Similarly, the inhibitor 3-hydroxy phenyltrimethyl ammonium increased KM values of fly-head AChE (Hellenbrand & Krupka 1970). AChE from *S. littoralis* was less sensitive to the inhibitor 6-methyl-5-hepten-2-one than AChE from electric eel. Similar results were obtained by Ryan *et al*. (1992), who reported that the K_i value was 0.38 mM for 3-methyl-2-cyclohexen-1-one (a sex pheromone) against electric eel AChE. The decreased potency of inhibition of 6-methyl-5-hepten-2-one against *S. littoralis* AChE might be assigned to the non-purified state of *S. littoralis* AChE in comparison with electric-eel AChE. *In vitro*, K_i values of Curacron and RH 218

(organophosphorus insecticides) of *Heliothis armigera* AChE were 0.399 and 0.276 mM respectively (El-Sebae *et al*. 1980).

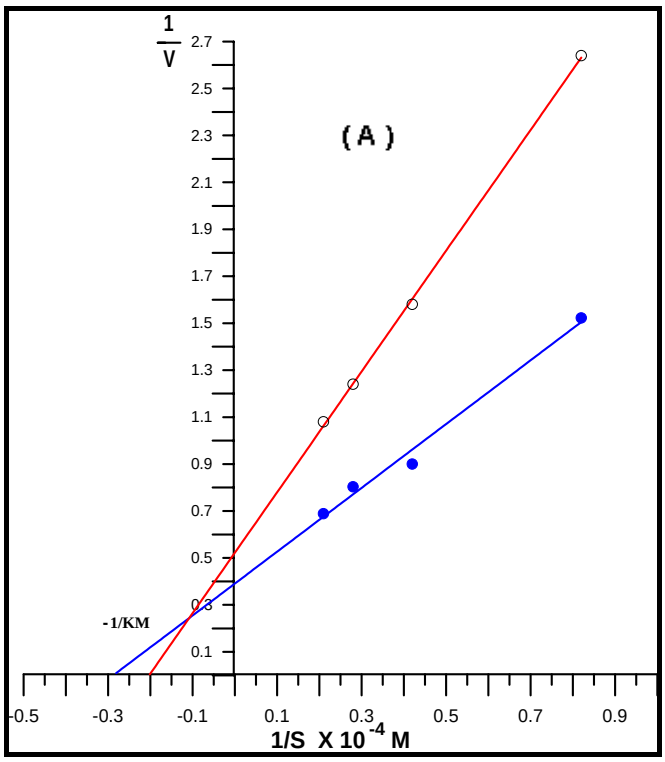
Compounds that inhibit AChE cause acetylcholine to accumulate at the cholinergic site, producing continous stimulation of cholinergic nerve fibres throughout the central and peripheral nervous systems. This effect can lead to paralysis and death: Corbett *et al*. (1984) reported that knockdown of the honey bee and house fly was associated with 60 % and 27 % AChE inhibition, respectively.

In vitro, 3-octylthio-1,1,1-trifluropropan-2-one was a very potent inhibitor of the antennal esterases (enzymes responsible for the catabolism of the pheromone) of the Egyptian armyworm, *S. littoralis* (Rosell *et al*. 1996). Trifluromethyl ketones are known as potent inhibitors of a number of esterases and proteases, such as acetylcholinesterases and human liver carboxylesterases (Ashour & Hammock 1987; Brodbeck *et al*. 1979). In general, the trifluromethyl ketones exhibit higher inhibitory potency than the corresponding difluromethy ketones (Quero *et al*. 2003). *In vitro*, the hydroxamic acid 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one inhibited the activities of carboxypeptidases, aminopeptidases, glutathion s-transferase and esterase (Castanera *et al*. 1998). *In vivo*, hydroxamic acid in the diet of the aphid *Rhopalosiphum padi* inhibited the activities of esterases by 50-75 % and inhibited glutathione transferase by 30 % (Mukanganyama *et al*. 2003).

The tested compound decreased the hatching of treated eggs and this decrease was dose-dependent. The developmental success of larvae treated as eggs or as larvae was reduced and this reduction was also dependent on the dose and on the treated stage. Such reported effects of 6-methyl-5-hepten-2-one are in consistent with other researchers who concluded that: pure terpenes and terpene – related compounds which are AChE inhibitors (Sebae *et al*.1981, Meisner *et al*.1982, Ryan and Byrne 1988); and various ketone compounds which are inhibitors of AChE and esterase enzymes (Ryan *et al*.1992, Castanera *et al*.1998, Reddy *et al*.2002) deleteriously affect growth, development and behaviour of insects in various ways.

The anti-acetylcholinesterase activity of 6-methyl-5-hepten-2-one, as measured from *in vitro* studies, might be due to deleterious effects on the hatching of treated eggs and development of larvae. Habibulla & Newburgh (1973) detected AChE activity in the fertilized eggs of *Galleria mellonella* and such activity was found to be greater at advanced stages of development. They mentioned that inhibition of AChE activity by eserine significantly decreased normal metamorphosis, suggesting that normal metamorphosis might be under neuronal control and altered by the increased amount of acetylcholine resulting from AChE inhibition.

The observed inhibition of AChE activity and the deleterious effects of the tested ketone compound on the development of eggs and larvae might trigger studies on the use of ketone compounds in future pest control strategies.



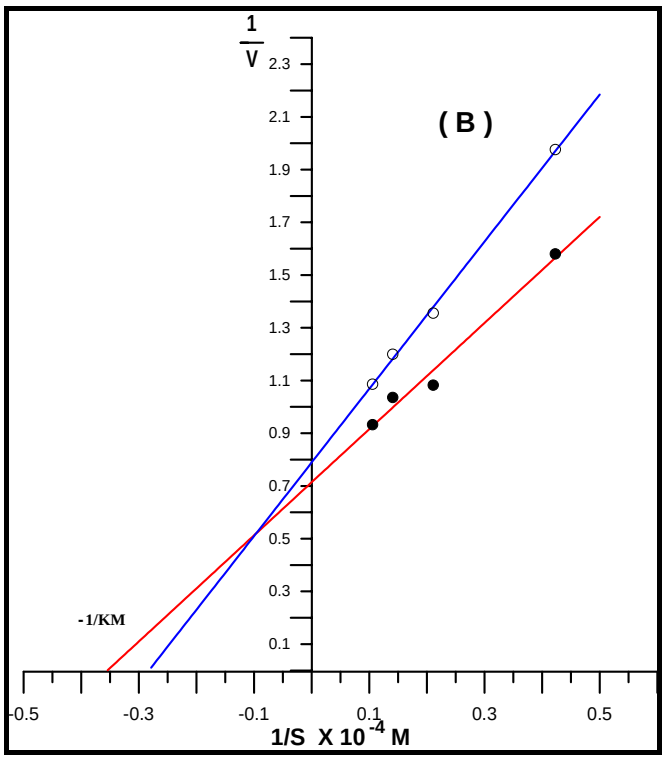


Fig.1: **Double reciprocal plot to ascertin the affinity (KM values) of *S. littoralis* AChE (A) and electric eel AChE (B) for acetylthiocholine iodide in the absence (●—●) and presence (○—○) of inhibitor.**

KM, calculated from the intersection of the line on the horizontal axis
KM=3.5 and 2.8×10^{-4} M for ●—● line from *S. littoralis* and electric eel AchE respectively.
KM=5.0 and 3.5×10^{-4} M for ○—○ line from *S. littoralis* and electric eel AchE, respectively.

Table 1: Effects of 6-methyl-5-hepten-2-one on the development of *S. littoralis* treated as eggs (0-24 hrs old)

Concentration ppm	Number of eggs treated	Number of eggs hatched Mean ± S.E	Percentage of eggs hatched Mean ± S.E	Larval mortality % Mean ± S.E	Corrected larval mortality Abbott (1925)	Pupation % Mean ± S.E	Adult emergence % Mean ± S.E	Adult malformation % Mean ± S.E
0	684	564.00 ± 66.72	82.26 ± 1.42	10.18 ± 1.28	-	89.82 ± 1.28	83.83 ± 2.24	6.90 ± 1.76
400	488	372.66 ± 19.53	76.26 ± 2.02	14.93 ± 1.80	5.28	84.94 ± 1.88	74.01 ± 1.51	9.90 ± 0.44
800	501	373.33 ± 29.62	74.77 ± 0.28	20.52 ± 0.93	11.51	79.47 ± 0.93	70.30 ± 0.54	11.03 ± 0.71
1600	495	306.65 ± 58.11	60.41 ± 0.97	33.48 ± 1.14	25.94	66.51 ± 1.14	63.54 ± 2.80	17.21 ± 1.41
3200	436	170.00 ± 15.27	38.96 ± 1.71	62.01±2.33	57.70	37.99 ± 2.33	49.21 ± 0.81	17.39 ± 0.51
F value		10.84	149.15	173.02	-	168.47	51.24	17.66
P value (P< 0.05)		0.0013	0.0004	0.0001		0.0003	0.0002	0.0001

d.f. values = degree of freedom = (4 , 10) *P < 0.05 = Significant*

Table 2: Effect of 6-methyl-5-hepten-2-one on the development of S. littoralis treated as larvae (early 2nd instar)

Concentration ppm	Percentage Larval mortality Mean± S.E	Corrected Larval mortality Abbott (1925)	Larval duration (days) Mean ± S.E	Pupation % Mean ± S.E	Pupal weight (mg) Mean ± S.E	Pupal duration (days) Mean ± S.E	Adult emergence % Mean ± S.E	Adult malformation % Mean ± S.E
0	11.45 ± 0.99	-	17.72 ± 0.68	88.88 ± 0.68	328.73 ± 4.54	7.61 ± 1.91	86.50 ± 0.79	4.53 ± 0.64
400	19.99 ± 1.95	9.64	18.96 ± 0.54	77.00 ± 3.88	288.83 ± 1.56	10.35 ± 0.27	78.63 ± 0.90	6.32 ± 0.61
800	22.51 ± 0.44	12.49	20.36 ± 0.51	77.82 ± 0.59	269.13 ± 1.97	11.44 ± 0.23	74.90 ± 0.68	11.20 ± 0.72
1600	35.14 ± 3.31	26.75	22.51 ± 0.54	64.85 ± 3.31	238.40 ± 1.66	12.73 ± 0.12	65.60 ± 0.40	14.43 ± 0.38
3200	52.06 ± 4.18	45.86	23.43 ± 0.90	47.93 ± 4.18	193.43 ± 6.53	13.40 ± 0.70	50.23 ± 1.39	17.06 ± 0.52
F value	37.21		13.23	27.37	180.67	6.03	240.73	80.80
P value (P< 0.05)	0.0003		0.0012	0.0004	0.0001	0.0103	0.0004	0.0001

d.f. values = degree of freedom = (4 , 10) **P < 0.05 = Significant**

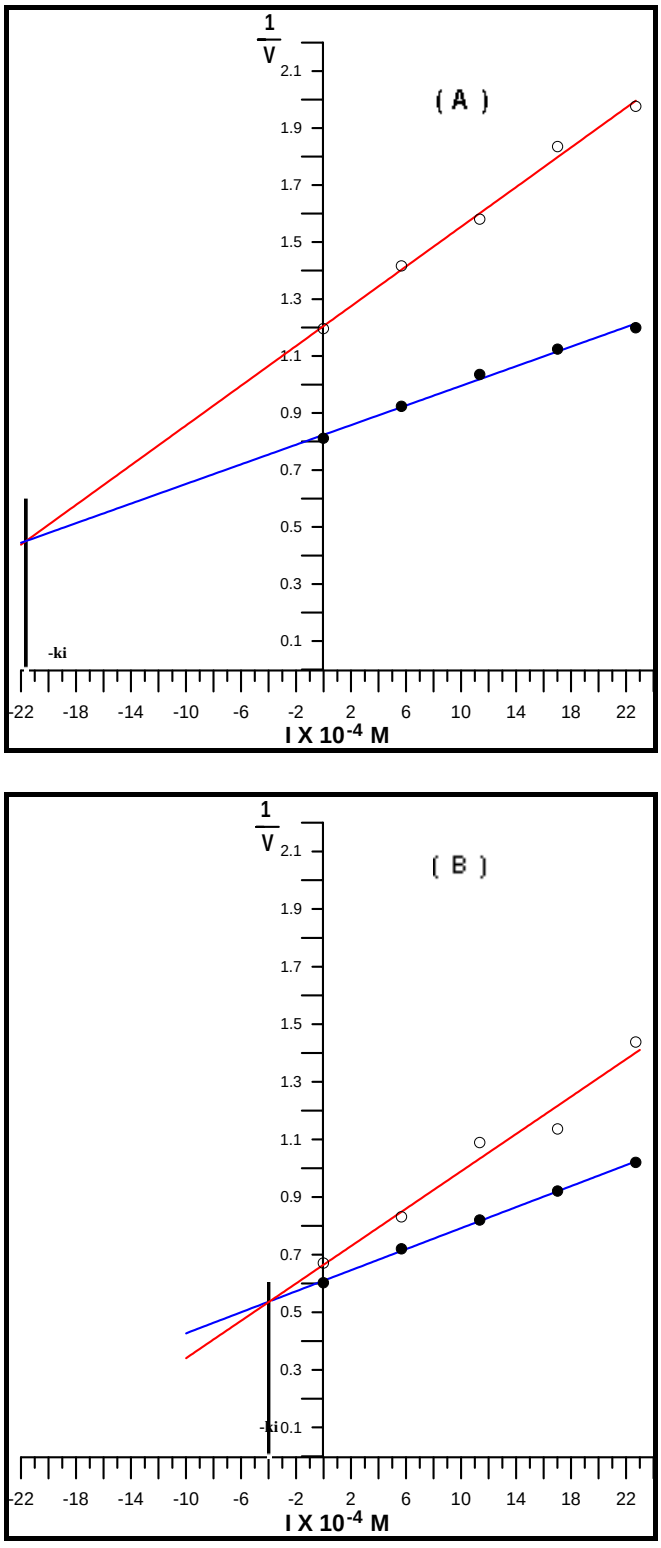


Fig.2: **Dixon plot derived from the inhibition of *S. littoralis* AChE (A) and electric eel AChE (B) by 6-methyl–5-hepten-2-one.**

Substrate concentrations: ●—● 9.46×10^{-4} M and o—o 4.73×10^{-4} M

Ki, calculated from the point obtained from the extension of the intersection of lines on horizontal axis

Ki = 2.16 and 0.40×10^{-3} M for *S. littoralis* and electric eel AChE, respectively.

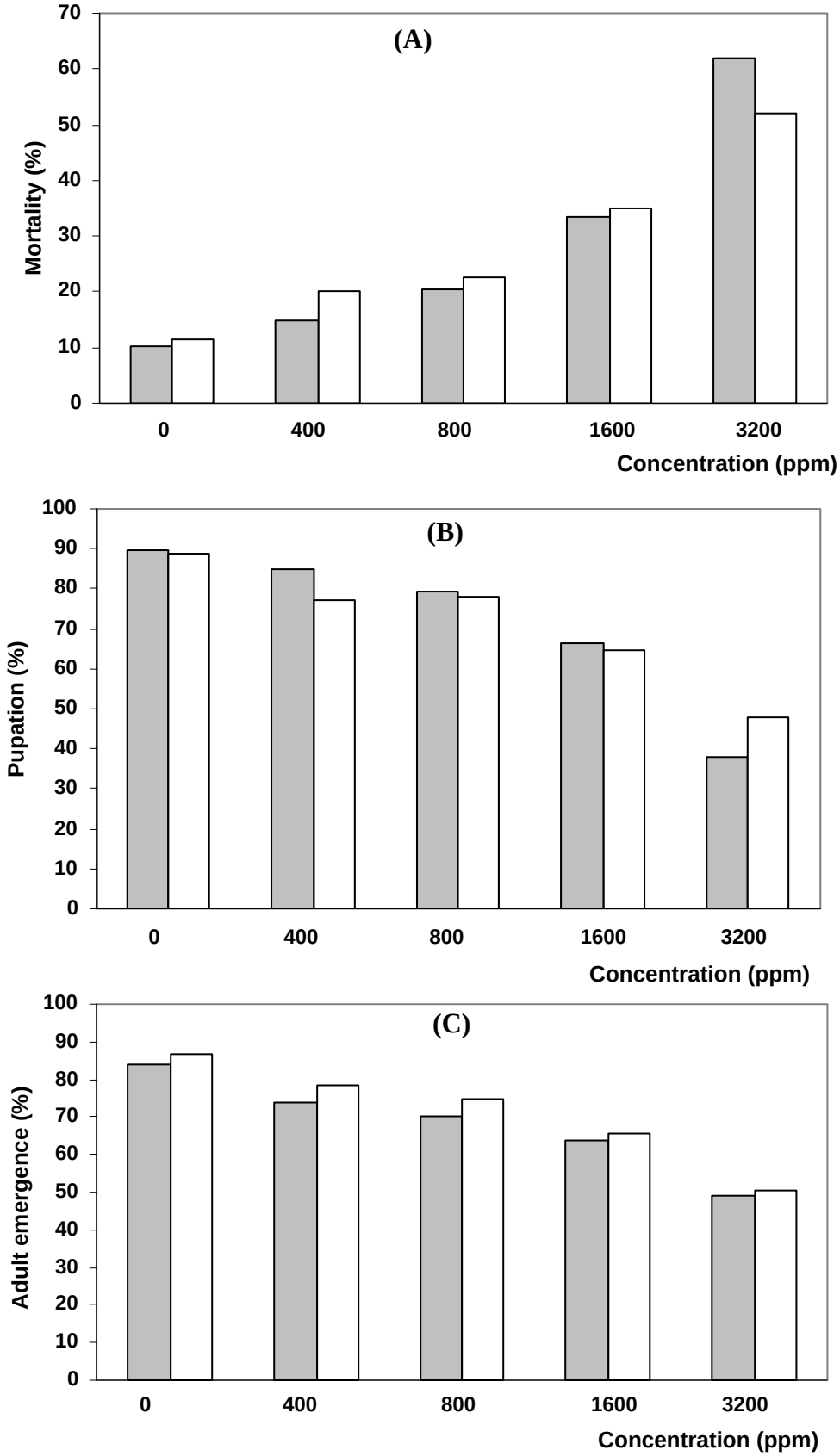


Fig. 3: Effect of 6-methyl-5-hepten-2-one on: (A) Mortality of larvae; (B) Pupation and (C) Adult emergence of *S.littoralis* treated as eggs (▩) or as early 2nd instar (□).

REFERENCES

Abbott WS (1925) A method of computing the effectiveness of an insecticide. J. Econ. Ent., 18: 265-267.

Ashour MBA & Hammock BD (1987) Substituted trifluoroketones as potent selective inhibitors of mammalian carboxylesterases. Biochem. Pharm., 36: 1869-1879.

Bestmann HJ & Vostrowsky O (1980) Chemistry of insect pheromones. In: Handbuch der Schaelings, R. Wegler, ed., Springer Verlag, Berlin, pp. 29-164.

Booth GM & Lee AH (1971) Distribution of Cholinesterases in insects. Bull. Wld. Hlth. Org., 44: 91-98.

Brodbeck U; Schweikert K; Gentinetta R & Rottenberg M (1979) Fluorinated aldehydes and ketones acting as Quasi-substrate inhibitors of acetylcholinesterase. Biochimica et Biophysica Acta, 567: 357-369.

Bryony CB, Colin AM & Janet H (1988) Purification and characterization of acetylcholinesterase from *Culex pipiens pipiens* (Say.). Pest. Science. 24: 278-280.

Castanera P, Ruiz M & Ortego F (1998) Effect of DIMBOA on growth and digestive physiology of *Sesamia nonagrioides* (Lepidoptera : Noctuidae) larvae. J. Insect physiol., 44: 95-101

Corbett JR; Wright K & Baillie AC (1984) The biochemical mode of action of pesticides, Academic Press, London.

Dixon M (1953) The determination of enzyme inhibitor constants. Biochemical J., 55: 170-171.

Ellman GL; Courtney KD; Andres V, Jr & Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95

El-Sebae AH; Soliman SA; El-Feel EAA; Galal I & Zeid MI (1980) Inhibition of AChE in relation to toxicity of some organophosphorus insecticides to *Spodoptera littoralis* and *Heliothis armigera*. Med. Fac. Landbouww. Rijksuniv. Gent., 45: 935-941

El-Sebae A; Sherby SI & Mansour NA (1981) Gossypol as an inducer or inhibitor in *Spodoptera littoralis* larvae. J. Environ. Sci. Health B., 16: 167-178.

Habibulla M & Newburgh RW (1973) Studies of acetyl-cholinesterase of the central nervous system of *Galleria mellonella*. Insect Biochem. 3: 231-242.

Hansch C & Deutsch EW (1966) The use of substituent constants in the study of structure-activity relationships in cholinesterase inhibitors. Biochim. Biophys. Acta, 126: 117-128

Hansch C, Kiehs K & Lawrence GI (1965) The role of substituents in the hydrophobic bonding of phenols by serum and mitochondrial proteins. J. Am. Chem. Soc., 87: 5770-5773

Hellenbrand K & Krupka RM (1970) Kinetic studies on the mechanism of insect acetylcholinesterase. Biochemistry, 9: 4665-4672.

Kasang G, Nicholls M & Von proff L (1989) Sex pheromone conversion and degradation in antennae of the silkworm moth *Bombyx mori* L. Experientia, 45: 81-87

Lineweaver H & Burk D (1934) J. Am. Chem. Soc., 56: 658-666.

Meisner J; Fleischer A & Eizick C (1982) Phagodeterrency induced by (-)–carvone in the larvae of *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. Econ. Entomol., 75: 462-466

Mukanganyama S; Figueroa CC; Hasler JA & Niemeyer HM (2003) Effects of DIMBOA on detoxification enzymes of the aphid *Rhopalosiphum padi* (Homoptera: Aphididae). J. Insect Physiol., 49: 223-229

Prestwich GD (1986) Fluorinated sterols, hormones and pheromones: Enzyme-targeted disruptants in insects. Pest. Sci. 37: 430-440.

Reddy GV; Quero C & Guerrero A (2002) Activity of octylthiotrifluropropan-2-one, a potent esterase inhibitor, on growth, development, and intraspecific communication in *Spodoptera littoralis* and *Sesamia nonagrioides*. J. Agric. Food Chem., 50: 7062-7068.

Renou M & Guerrero A (2000) Insect parapheromones in olfaction research and semiochemical-based pest control strategies. Annu. Rev. Entomol., 48: 605-630.

Rosell G; Herrero S & Guerrero A (1996) New trifluromethyl ketones as potent inhibitors of esterases: 19 F NMR Spectroscopy of transition state analog complexes and structure. activity relationships. Biochem. Biophys. Res. Commun., 226: 287-292.

Ryan MF & Byrne O (1988) Plant-insect coevolution and inhibition of acetylcholinesterase. J. Chem. Ecol., 14: 1965-1975.

Ryan MF; Awde J & Moran S (1992) Insect pheromones as reversible competitive inhibitors of acetylcholinesterase. Invertebrate Reproduction and Development, 22: 31-38.

Quero C; Rosell G; Jimenez O; Rodriquez S; Bosch MP & Guerrero A (2003) New fluorinated derivatives as esterase inhibitors. Synthesis, hydration and crossed specificity studies. Bioorg. Med. Chem., 11: 1047-1055.

Wilson EO & Bossert WH (1963) Chemical communication among animals. Rec. Prog. Hormone Res., 19: 673-716.

الملخص العربى

نشاط مركب 6-methyl-5-hepten-2-one على النمو والتطور وإنزيم الآستيل كولين إستريز فـى دودة ورق القطن الكبرى "اسبودوبترا ليتورالس"

طلعـت السيـد عمـارة
قسم علم الحيوان- كلية العلوم - جامعة المنوفية- شبين الكوم

- تناول هذا البحث دراسة التحليل الحركى Kinetic analysis لتفاعلات إنزيم الأستيل كولين إستريز المـستخرج مـن رأس دودة ورق القطن الكبرى وأيضاً الخاص بسمكة الأنكليس (ثعبان الماء) electric eel مع المادة الخاضعة لتأثير الإنـزيم acetylthiocholine iodide substrate وأيضاً مع المركب المثبط للإنزيم 6-methyl-5-hepten-2-one وقد أظهرت الدراسـة إختلافـات بـين هـذه الإنزيمات.
- أظهرت النتائج أن قيم KM كانت $3.5 \text{ and } 2.8 \times 10^{-4}$ M لإنزيم دودة ورق القطن وإنزيم سمكة الإنكليس على الترتيب.
- لقد وجد أن المركب المستخدم أحدث تثبيطاً بشكل منافس للإنزيم حيث قيم K i كانت $2.16 \text{ and } 0.40 \times 10^{-3}$ M لإنـزيم دودة ورق القطن وإنزيم سمكة الإنكليس على الترتيب.
- أوضحت النتائج أن المركب المستخدم أدى إلى نقص معنوى فى النسبة المئوية لفقس البيض المعامل وأيضاً لوحظ زيادة فى نسبة موت اليرقات الناتجة من الفقس. كذلك لوحظ نقص معنوى فى عدد العذارى وعدد الفراشات الناتجة من البيض المعامل.
- الدراسة أوضحت تأثير معنوى على أطوار النمو التالية للعمر اليرقى الثانى المعامل بالمركب حيث لوحظ تنـاقص معنـوى فـى وزن العذارى، عدد العذارى الناتجة وأيضاً عدد الفراشات الناتجة.
- أوضحت النتائج زيادة معنوية فى موت اليرقات، فترة نمو اليرقات، فترة نمو العذارى وأيضاً التشوهات التى ظهرت فى طور الحـشرة الكاملة بسبب معاملة يرقات العمر الثانى بالمركب.