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Cyclic fluctuation of daily acetylcholine esterase activity in *Spodoptera littoralis* (Boisd.) moth

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ABSTRACT

The present study was executed to assess the fluctuations of esterases and acetylcholine esterase (AChE) rhythmic activity in adult central nervous system (CNS) of the cotton leafworm *Spodoptera littoralis* (Boisd.). Series of the experiments dealt with esterases rhythmic in CNS and electrophysiological experiments on the tarsus sensilla were conducted. The esterases activities in either male or female heads and thorax of *S. littoralis* were assayed 24 times (an hour interval), 8 times (3 hours intervals), 4 times (6 hours intervals) and 2 times (12 hr intervals) in the day. The obtained results showed that the cyclic fluctuation of AChE activity was significantly higher in the head and lower in the thorax during different day times. The highest AChE daily activity levels occurred in the female and male at 4 O'clock (scotophase), while the lowest activity levels occurred at 12 O'clock (photophase). Also, results showed that the averages of AChE phase activity levels were higher in the female of 3-4 days old after eclocion and male of 2-4 days after eclocion. In addition, contact chemoreceptors in tarsus, sensilla showed higher sensitivity to different chemicals, i.e., sodium chloride and sucrose in the scotophase than in photophase.

Key words: *Spodoptera littoralis*, acetylcholine esterase, electrophysiology, circadian, rhythm, contact chemoreceptors.

INTRODUCTION

Circadian clocks are endogenous timing mechanisms that generate daily rhythms in all organisms from unicellular to humans. Circadian clocks provide temporal synchronization of life processes and adaptation of organisms to environmental cycles (Giebultowicz, 1999). Several behavioral rhythms are known in insects, such as those of pheromone production and release mating, or oviposition (Saunders, 1982 and Gaaboub and Halawaa, 2003). Some of these physiological rhythms have been well established in insects (Brady, 1974 and Vijayalakshmi and Babu, 1978).

The cyclic fluctuations of AChE activity were studied in nocturnals, moths, cockroach and cricket as well as in diurnals, boll weevil, desert locust and grasshopper. In moths, highly significant differences were found in ACh and AChE activity among the different tested light conditions and with the different day times (El-Aw, 1997). The influence of photoperiodism and the day time on the K_m and V_{max} values for esterases hydrolysing α-naphthylacetate in the 4^{th} and 6^{th} instar larval heads of *S. littoralis* was studied by Hashem (1989). The responsiveness of peripheral sensilla of *S. littoralis* was tested by many investigators (Schoonhoven *et al.*, 1991 and Simmonds *et al.*, 1991). A physiological functions in insect are fluctuated with deal rhythmicity (Ry), some of these rhythms (Rms) have been found when it has been necessary to produce standard insect material for a particular physiological study, the initial trials have yield inconsistent results at different times of day (Brady, 1974).

Esterases are multifunctional enzymes that catalyze the hydrolysis of ester linkages irrespective of chain length or solubility and regulate a variety of tissue functions. The most significant esterase is the AChE as it regulates nervous impulses in insect CNS (El-Aw, 1997). ACh is the major neurotransmitter in insects and many insecticides, which degrades ACh, inhibits the AChE and prevents cholinerigic toxicity (Bose, 1990). The presence of true ChE (EC 3.1.1.7) hydrolysing ATCh in the nervous tissue (NT) of *Prodenia littura* may be important in the functioning of the nervous system (Mehrotra and Chandra, 1974). AChE was highest in the head of *Dacus cucurbita* and *S. littoralis* followed by thorax and was lowest in the abdomen (Shukla and Srivastava, 1980).

The present work aimed to investigate the rhythmicity of AChE enzyme and the response activity during the life span of *S. littoralis*. Also, the sensitivity of the contact chemoreceptors, sensilla chaticum was carried out under different conditions. The correlation between AChE activity and the contact chemoreceptors will be detected to find their role in the interpretation of the receptor mechanism of insect.

MATERIALS AND METHODS

Tested insect:

Moths of *S. littoralis* (Boisd.) were obtained from a stock culture maintained under constant conditions of 26.0 ± 2.0 °C and 70.0 ± 5.0 % relative humidity in the Department of Entomology, Faculty of Agriculture, Moshtohor. The culture was reared on castor leaves.

Preparation of homogenates:

Heads or thoraxes of the adults of both male and female insects were collected to determine the specific activity of esterases. The head and thorax were homogenate with phosphate buffer (pH 7.4) under ice conditions. Homogenates were centrifuged for 20 min at 6000 xg and the supernatants were obtained.

Determination of protein and AChE activity:

Protein was determined according to the protein-dye binding method of Bradford (1976), which was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 ml 95 % ethanol and 100 ml 85 % (w / v) phosphoric acid made the dye solution. To 5 ml of this solution, 0.1 ml homogenate preparation was added, and the contents were mixed by vortexing. Absorbance was measured at 595 nm against a reagent blank using a Spectronic 21D Spectrophotometer. The concentration was estimated from a standard curve of concentrations of bovine serum albumin (BSA).

Acetylcholine esterase activity was determined by measuring the rate of hydrolysis of acetylcholine iodide ($3X10^{-3}$ M) in 0.1M sodium phosphate buffer (pH 8) according to Ellman *et. al.*, (1961).

The specific activities of esterase (AChE) were determined in the head and thorax homogenates of both sexes of *S. littoralis* adults. The investigation of the diel rhythmic changes in the AChE activities under life span after eloucion were carried out 24 times (an hour interval), 8 times (3 hours intervals), 4 times (6 hours intervals) and 2 times (12 hr intervals, at 12 O'clock and 24 O'clock) in the day.

The statistical analysis adapted in such randomized complete block design (RCBD) was the analysis of variance (ANOVA) of the factorial experiment (Steel and Torrie, 1981).

Electrophysiology experiments:

Responses from individual sensilla (sensilla chaeticum) to chemical stimuli on the ventral side of the tarsus and in the antennae were recorded using the tip recording technique (Hodgson *et al.*, 1955). The potentials were amplified and filtered using AC amplifiers. A blunt glass microelectrode filled with salt solution (1M NaCl) was placed over the shaft of the sensillain to stimulate the chemosensory afferents. Controlled movements of this electrode were used to deflect the sensillum so as to elicit spikes in the mechanosensory afferents. The same electrode was therefore used simultaneously to evoke and record the spike of the afferents. The displacement of a sensillum did not deform its short and stout shaft.

RESULTS AND DISCUSSION

The AChE activity of *S. littoralis* moth through life span was estimated from eclousion to 72 hr old. This activity started after 18, and 30 hr for male and female, respectively and rised up gradually to a maximum activity after 36 and 48 hours from eclousion in male and female, respectively (Fig. 1). This result was similar to that of Bebas *et al.*, (2002) in which the maximum activity of AChE in the head of *S. littoralis* was achieved after 36 and 48 hours in male and female, respectively.

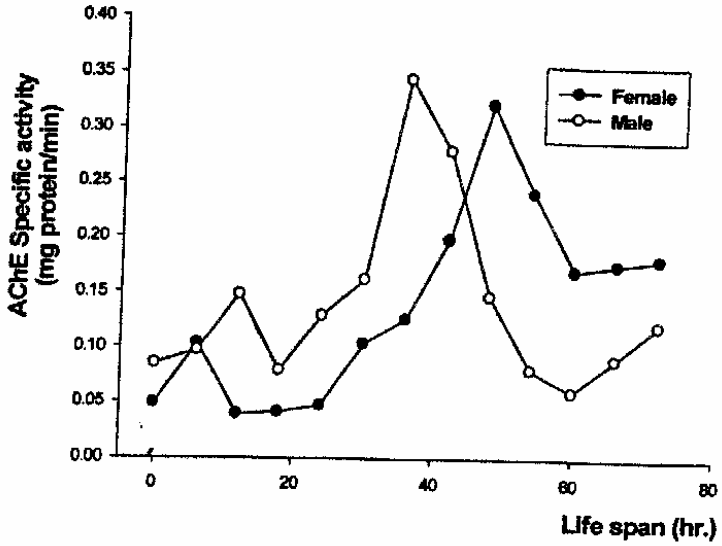
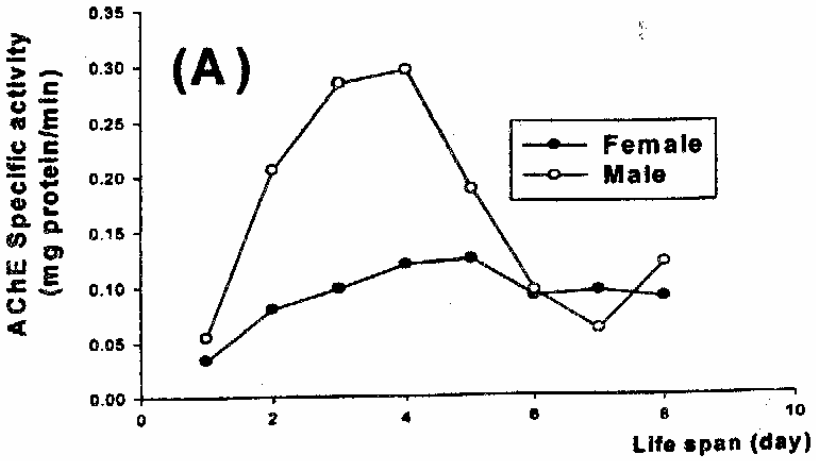


Fig. (1): Specific activity of AChE for *S. littoralis* moths every 6 hours after eclusion.

The AChE activity was determined in the heads of both male and female moths of *S. littoralis* at two times 12 O'clock in photophase and 24 O'clock in scotophase through eight days. The AChE activity was lower in the photophase than in scotophase (Fig. 2). In addition, the patterns of the activity of AChE at 24 O'clock in the scotophase showed a fluctuated rhythm correlated with age after eclousion. The maximum activities of AChE were detected in the second day in male and third day in female moths. The AChE activity levels of *S. littoralis* head homogenates were generally higher in the female than in the male in all tested moths The results indicated highly significant differences in AChE activity during the life span, daytime as well as between male and female (Table 1).



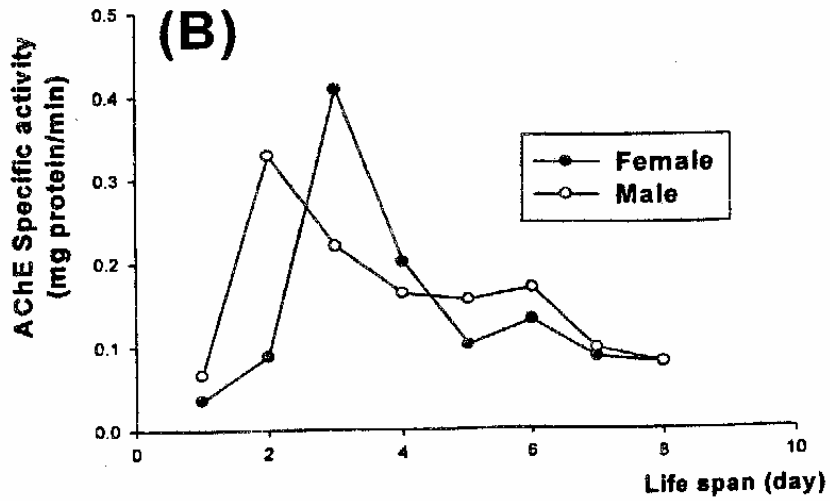


Fig. (2): Specific activity of AChE for *S. littoralis* moths during life span of 1st to 8th day
(A) At 12 O'clock (photophase)
(B) At 24 O'clock (scotophase)

Table (1) Mean squares of specific activity of AChE in head capsules and thorax of *S. littoralis* male and female affected by life span intervals, sex and their interactions.

		Life span from 1st to 8th day			
		At 12 O'clock in head capsules		At 24 O'clock in head capsules	
S.O.V.	d.f	Mean Square	Fc.	Mean Square	Fc.
Rep.	2	0.0025	0.454	0.0122	1.0517
Sex (S)	1	0.069	12.54	0.0008	0.0689
Time(T)	7	0.0081	1.454	0.0468	4.034*
(S) x (T)	7	0.0131	2.381*	0.0196	1.689
Error	30	0.0055		0.0116	

		Life span interval for 3 days old insect					
		1 hr interval in head capsules from 24 to 7 O'clock		3 hr interval in head capsules		3 hr interval in thorax	
S.O.V.	d.f	Mean Square	Fc.	Mean Square	Fc.	Mean Square	Fc.
Rep.	2	0.405	3.139	.27	2.5	0.0339	3.2
Sex (S)	1	0.206	15.96**	1.15	10.45*	0.638	5.54
Time(T)	7	0.152	11.78**	0.196	1.78	0.016	1.41
(S) x (T)	7	0.0688	5.33**	0.053	0.48	0.0734	0.649
Error	30	0.0129		0.11		0.0113	

		Life span interval for 3 days old insect	
		6 hr interval in head capsules	
S.O.V.	d.f	Mean Square	Fc.
Rep.	2	0.00676	0.6035
Sex (S)	1	0.0786	7.017
Time(T)	12	0.0785	7.008**
(S) x (T)	12	0.0088	0.785
Error	50	0.0112	

* Significant
** Highly significant

The maturity of reproductive system was complete in the second and third day for male and female, respectively (Giebultowicz, 1999 and Bebas *et al.*, 2002). The averages of AChE activity levels, in both sexes were higher in the scotophase than in the photophase, after overnight retention in the vas deferens lumen. The exit of sperm bundles from the testis to vas deferens is restricted to a few hours in the evening, and subsequent transfer of sperm from the vas deference to seminal vesicles occurs only within a few hours in the morning in gypsy moth, *Lymantria dispar* (Riemann *et al.*, 1974, Riemann and Giebultowicz, 1991, Giebultowicz *et. al.*, 1996 and Bebas, *et al.*, 2002). In *Periplanta americana* reared under LD (light dark) 12 hr: 12 hr conditions the CNS AChE and electrical activities were maximum at 24 O'clock (midnight) and minimum at 12 O'clock in which ACh content showed an opposite rhythm (Vijayalakshmi *et al.*, 1977).

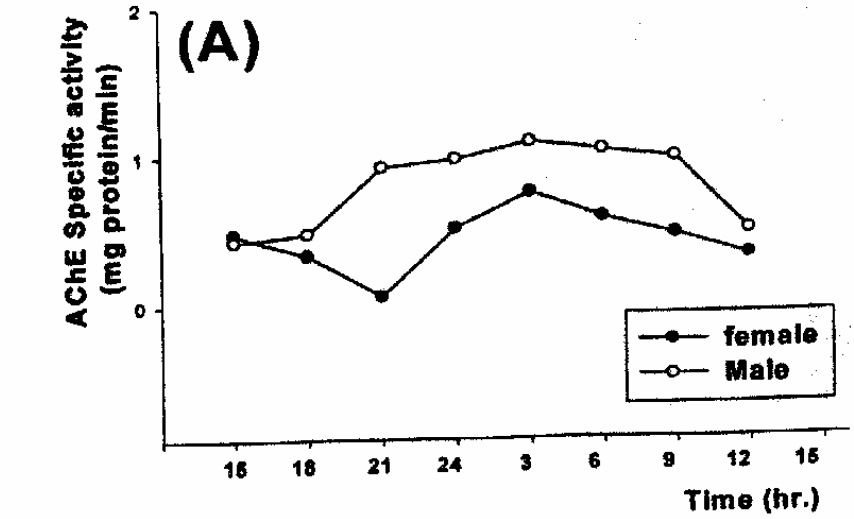
Figures (3 A & B) represent the AChE activity levels at each 3 hours during 24 hours, starting at 15 O'clock. The obtained results revealed that, the maximum activity in head occurred at 4 O'clock (in the scotophase) for male and female, while the minimum activity lied between 12 O'clock and 18 O'clock (in the photophase). The specific activity values of AChE in the head homogenates of both sexes were ranged from 0.337 to 0.743 and from 0.435 to 1.028 (mg protein / min) for male and female, respectively. The fluctuation in AChE activity levels were more pronounced in the head capsule than thorax.

Also, highly significant differences in AChE activities were found among day times. AChE activity was significantly higher in the head than in the thorax at all time intervals (Table 1). In addition, in the adult male and female, the average AChE phase activity was slightly higher between 2 to 4 O'clock in the scotophase. The ratio of AChE activity was higher in heads than in thorax in either sex. Exogenous factors like photoperiod and temperature have a great influence on the activity of the enzyme (Bauer, 1976). It was suggested that elevation in the head AChE activity levels of several lepidopteran species are in coincidence in the rising in the locomotor activity during the scototophase for the 4^{th} larval instar and during scotophase for the moths (Omino *et al.*, 1973). In contrast, in the case of the grasshopper *Poecilocerus pictus*, the average level of AChE during light hours was higher than during dark hours and vice versa for the ACh levels in the CNS (Vijayalakshmi and Babu, 1978). The activity of AChE in the whole body extract of boll weevil *Anthonomus grandis* was about 25 % greater during the photophase (insect is active) than during the night (Bull

and Lindquist, 1968). Rhythms controlled by circadian clock are self-sustained, persist in constant darkness and temperature at daytime period (close to a 24 O'clock). The endogenous periods of many rhythms were not close to 24 O'clock, and they are synchronized, or entrained, to the 24 hour day cycle of light and darkness and daily temperature fluctuation (Giebultowicz, *et al.*, 1997).

For further comparison of AChE activities in the scotophase, records were performed every an hour (Fig. 4). The highest activity occurred at 3 & 4 O'clock in male and female of *S. littoralis* moths, respectively. In general esterases in insect have been implicated in reproductive behaviour (Richmond *et. al.*, 1990, c. f. Parker *et al.*, 1991), phoromone and hormone metabolism (Hammock *et al.*, 1985). AChE is an important regulatory enzyme responsible for controlling the transmission of nerve impulses across cholinergic synapses where it acts to hydrolyze the excitatory neurotransmitter, acetylcholine (Huston and Roberts, 1985, c. f. Parker *et al.*, 1991). In *Manduca sexta*, experiments on preparatory behaviour indicate that a brain-independent circadian clock may control the phase of the ecdysteroid decline (Truman, 1984 and 1992). The physiology of insect circadian rhythm implicit the concept of biological clock is the assumption that it runs on some sort of biochemical mechanism. it has proved very elusive and attempts to measure its immediate products have generally been unrewarding, since it is impossible to know whether the substance examined is an important link in the clockwork itself, or some quite incidental secondary byproduct.

In many moth species sex pheromone-mediated mating behavior occurs during the scotophase period. Numerous examples from studies on female release and male response to pheromone indicate that the timing of this behavior, despite the influence of temperature, is governed by a circadian mechanism (Kanno, 1981, Kamimura and Tatsuki, 1994 and Linn *et al.*, 1996). The adaptive significance of a circadian-based regulation of mating behavior relates to added efficiency in the coordinated exchange between sender and receiver, and the occurrence of mating during optimal environmental condition (Linn, *et. al.*, 1996).



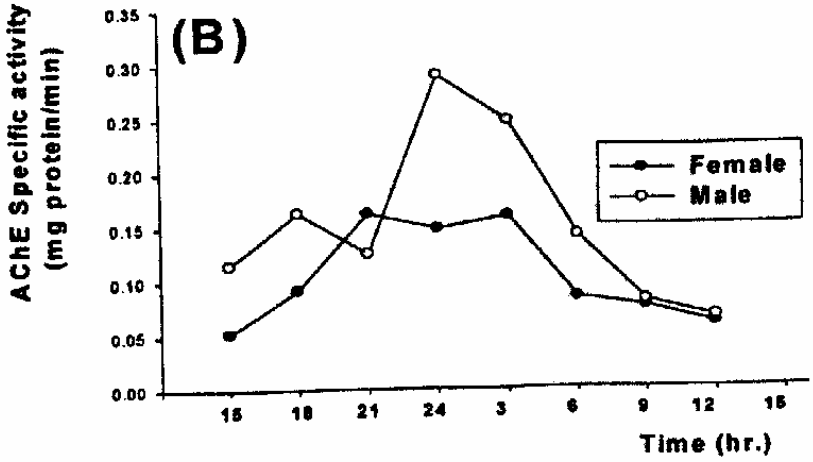


Fig. (3): Specific activity of AChE in for *S. littoralis* moths during the period of 15 to 12 O'clock for insect of 3 days old.
(A) Head capsuls
(B) Thorax

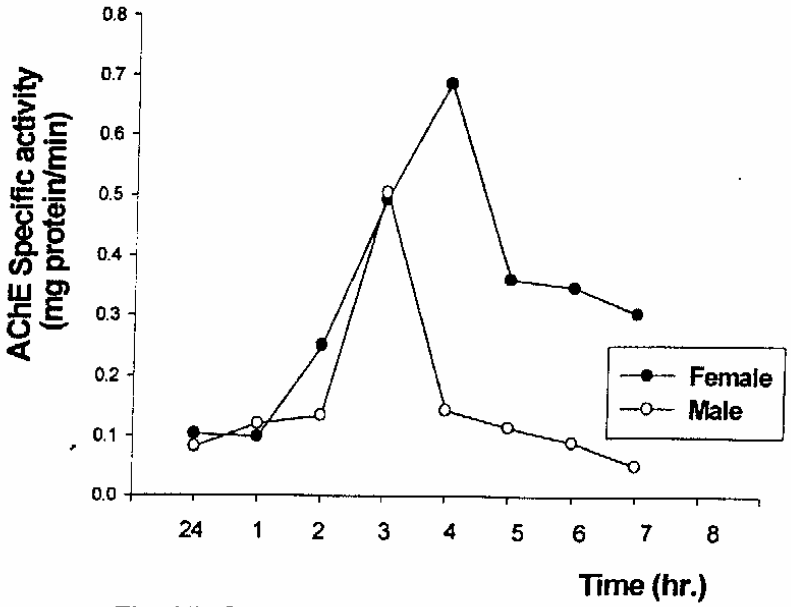


Fig. (4): Specific activity of AChE in head capsules of *S. littoralis* moths during 24 to 7 O'clock for insect of 3 days old.

The rhythmicity was observed in the electrophysiological response from sensilla in the scotophase and photophase, it was clear that response and sensitivity in sensilla to different chemicals were higher in the scotophase especially between 2 and 4 O'clock. The highest response was detected from 3:30 O'clock to 4 O'clock (Fig. 5). The result showed higher activity of moth in the scotophase as nocturnal insect when compared with diurnal insect.

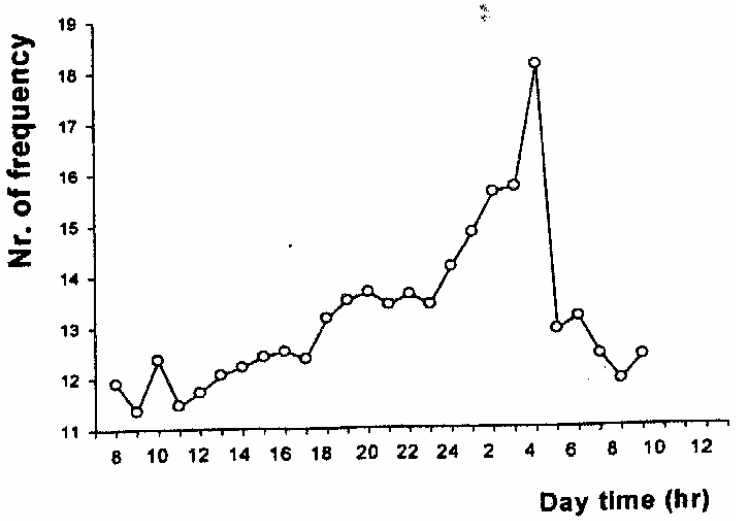


Fig. (5): Mean of the frequency number in female of 3 days old.

The rhythmicity of the mean AChE activities in the heads of adult male and female reached their peak of activities at 4 O'clock, at this time the sensitivity response of sensilla to stimulation by sucrose solution (50 mM mixed with 25 mM NaCl) was in a maximum value and fluctuation for response in daily stimulation was found (Fig. 6). Simmonds *et al.*, (1991) found that the responsiveness of peripheral sensilla on *S. littoralis* to sucrose was influenced by the age of the larvae and that, within a day, neural activity could differ between morning and afternoon. Schoonhoven *et al.*, (1991) showed that the responsiveness by using sinigrin and inositol on *S. littoralis* under laboratory conditions was affected by the timing of stimulation. In *Periplanta americana* reared under LD 12:12 conditions the CNS AChE and electrical activities were maximum at midnight and minimum at 12 O'clock as ACh content showed an opposite rhythm (Vijayalakshmi *et al.*, 1977).

It could be concluded that the AChE activity of *S. littoralis* moth was recorded to maximum level after 36 and 48 hr from eclousion in male and female, respectively. The activity was higher in the scotophase than in the photophase in particular at 3 and 4 O'clock. The correlation between AChE activity and the contact chemoreceptor of sensilla was significant. The

results indicated that knowing the time of the highest activity of AChE could be useful in Integrated Pest Management (IPM) program.

Fig. (6) Recording from tarsus sensilla (sensilla chaticum) to 50-mm sucrose mixed with 25 mM NaCl were used to stimulate the chemosensory afferent (A) at 15 O' clock and (B) at 3 O' clock.

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الإيقاع الدّوريّ اليومى لنشاط الأسيتايل كولين استيريز فى فراشة دودة ورق القطن

إبراهيم عبدالله جعبوب
قسم وقاية النبات، كلّيّة الزّراعة بمشتهر ،جامعة الزقازيق

الدّراسة الحاليّة نُفّذتْ لتقييم أثر فترة الحياة بعد الخروج من العذراء على معدل ومستوى نشاط الأسيتايل كولين استيريز في الجهاز العصبيّ المركزيّ فى الحشرات البالغة لدودة ورق القطن . تعاملت سلسلة التجارب مع معدل ومستوى نشاط الأسيتايل كولين استيريز في الجهاز العصبيّ المركزيّ و استخدام تكنيك الألكتروفسيولوجى لقياس معدل نشاط واستجابة الشعرات الحسية على رسغ الحشرة . وتم تقدير نشاط وكمية إنزيم الأسيتايل كولين استيريز فى الرأس والصدر لذكور وإناث فراشة دودة ورق القطن بمعدل ٢٤ مرة يوميا أى قياس معدل الأنزيم كل ساعة و ٨ مرّات فى اليوم أى قياس معدل الأنزيم كل ٣ ساعات و٤ مرّات فى اليوم أى قياس معدل الإنزيم كل ٦ ساعات و مرّتان فى اليوم أى قياس معدل الإنزيم كل ١٢ ساعة وأظهرت النتائج أن التذبذب الدّوريّ لنشاط الأسيتايل كولين استيريز كان عاليًا بدرجة كبيرة في الرّأس عن الصدر في أثناء أوقات اليوم المختلفة نهارا أو ليلا . مستويات نشاط إنزيم الأسيتايل كولين استيريز اليوميّة الأعلى حدثت في الأنثى و الذكر السّاعة ٤ فجرا بينما حدثت مستويات النشاط الأكثر انخفاضًا السّاعة ١٢ ظهرا . أيضًا أظهرت النتائج أن متوسّطات مستويات نشاط إنزيم الأسيتايل كولين استيريز كانت أعلى في الأنثى فى اليوم الثالث والرابع بعد الخروج من العذراء و فى الذكر فى اليوم الثانى و الثالث بعد الخروج من العذراء. بالإضافة لذلك أظهرت الدراسة الالكتروفسيولجية أن الشعرات الحسية وخاصة المستقبلات الكيماوية منها حساسية أعلى للموادّ الكيميائيّة المختلفة (ملح كلوريد الصوديوم أو السكروز) في فترة الظلام ليلا عن فترة الضوء نهارا .