ACUTE AND LATENT BIOINSECTICIDAL ACTIVITIES OF PRECOCENES (I AND II) ON DIFFERENT DEVELOPMENTAL STAGES OF THE BERSEEM GRASSHOPPER, *EUPREPOCNEMIS PLORANS PLORANS* CHARP. AND THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

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Abstract

The aim of the this investigation was to study the bioinsecticidal activities of precocenes (I and II) on the berseem grasshopper, *Euprepocnemis plorans plorans* Charp. and the cotton leafworm, *Spodoptera littoralis* (Boisd.). Scored percentages for mortalities and abnormalities, as well as, the shortened nymphal duration had occurred due to ecdysitropic effect. The complete sterility resulted from grasshopper treatment proved that the insects were as if chemically allatoectomized by precocene II. Biochemical assays on the latent stages for total protein, total cholesterol, integument chitin and whole body lipids content, in addition to some biochemical parameters indicated that precocenes can play a role as insecticides and/or chemosterilants.

INTRODUCTION

The environmental hazards of insecticides used are still controversial (Everts, 1990), and necessitates the urgent search for new and equally effective, but safer alternatives.

The unique feature of insect endocrine system could provide some insecticides with a relatively new mode of action, such as precocenes which are naturally occurring cheromones, found in plants in the genus *Ageratum* and interfere with the juvenile hormone (J.H.) and possess anti-allatropic properties in insects from several orders (Unnithan *et al.*, 1980).

The berseem grasshopper, *Euprepocnemis plorans plorans* Charp. is one of the most economic species for grasshoppers. It caused a severe damage to planted crops of the Nile Delta and harmful grasshoppers were also reported (Showler, 1995). Also, the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) causes serious damage to most of vegetables and field crops. Thus, the present work was directed to study the resultant effects of hormone deficiency due to treatment by precocenes (I and II) on *E. plorans plorans* and *S. littoralis*. The effects of these compounds, whether acute or latent on metamorphosis, development rate, metabolic rate, reproductive potential and some other biological parameters of the tested insects were also investigated.

MATERIAL AND METHODS

Rearing: The berseem grasshopper was reared according to the method described by Hunter-Jones (1966), while the cotton leafworm was reared according to El-Defrawi *et al.* (1964).

Chemicals: The substances assayed were:

Precocene (PI): 7-methoxy-2,2-dimethyl-3-chromene).
Precocene (PII): 6,7-methoxy-2,2-dimethyl-3-chromene).

Treatment: Each compound (PI or PII) was dissolved in acetone (Critchley and Almeida, 1973) and topically applied by means of microapplicator onto the thoracic dorsa of *S. littoralis* newly moulted larvae. Fourth larval instar received 25 µg/g b.wt. Control larvae received acetone only. After treatment, the tested larvae were supplied with fresh castor bean leaves and reared for daily biological observation and selecting specimens for biochemical analysis. PII was applied topically to the pronotum of *E. plorans plorans* at the rate of 50µg/g b.wt. of newly moulted fourth and fifth nymphal instars.Duration and development of the tested insects were obtained by using Dempester's equation (1957). The rate of development was calculated according to the following equation: Rate of development = 100/mean duration in days. Mortality and abnormality data were corrected using Abbott's formula (1925). The reproductive potential of the resulting adults were evaluated using the formula of El-Ibrashy and Abou-Zeid (1972).Each experiment was replicated three times and every replicate contained 10-15 individuals.Haemolymph was collected into a small vials containing traces of phenylthiourea to prevent melanization by puncturing the pleural membrane of the grasshopper third thoracic segment, while that of the cotton leafworm was obtained by cutting off the first abdominal proleg. The collected haemolymph was centrifuged using refrigerated centrifuge at 2500 rpm for ten minutes and stored at a deep freeze till use for biochemical analysis.

Total proteins were determined by the method of Gornal *et al.* (1949), total cholesterol was estimated according to Richmond (1973), whole body lipids content was determined by the gravimetric method of Loveridge (1973), while chitin content was estimated according to Karl and Koga (1986).

The data were introduced to computer to be analyzed using Studentds t- test.

RESULTS AND DISCUSSION

Biological effects of precocenes: The data in Table 1 revealed that PII when applied to the 4th nymphal instar of the berseem grasshopper, it prolonged the nymphal period, decreased the rate of development, increased the percentage of deformation or mortality and decreased the weight gain (except females). But the treatment of the 5th nymphal instar elicited quicker significant impact of ecdysitropic effect, i.e. greatly imbalanced the normal level of J.H. and ecdysone that ensuring the successful moult, consequently, led to higher percentage of deformation and delayed the rate of development.

In spite of the treatment with precocene caused rapid irreversible breakdown of corpora allata (CA), it had a juvenilization eeffect. This effect was probably the result of synthesis or release of J.H. during the breakdown of the glands (Miall and Mordue, 1980) and probably was responsible for showing such abnormalities after treatment by this compound.

The results in Table 2, more or less, go on line with the previous ones, except that PI was more effective than PII on the fourth larval instar and gave good indications of allatectomization iin the sixth instar. Soderlund *et al.* (1980) concluded that the apparent insensitivity was not due to the lack of intrinsic sensitivity of their corpora allata to this allatotoxin, but may be due to the detoxification of this compound. Keely and Fuchus (1978) stressed that precocene was toxic compound, causing mortality in the mosquito *Aedes aegypti* due to inhibition of trypsin, synthesis. This inhibition acts as "allatocidal agent" causing atrophy and necrosis of corpora allata. It is known that J.H. are vital ones that affect many biological processes such as vitellogenesis, but the reduction of body weight due to the treatment with precocene might be due to reduction of digestive enzymes as a result of J.H. suppression. Mandal *et al.* (1983) found that allatectomy resulted in a significant reduction in the activity of protease, lipase and trypsin. Our results agree with those of Unnithan *et al.* (1980), Lange *et al.* (1983), Mathai and Nair (1984) and Tanaka and Pener (1995).

On the other hand, juvenile hormones which are produced by CA are isoprenoids that play many roles in the regulation of insect physiology, including reproductive potential (Wyatt and Davey, 1996) and since precocene causes degeneration of CA, the studying of the delayed effect on reproduction becomes of valuable interest. Table 4 shows that treatment of one day old last nymphal instar of *E.plorans plorans* led to great reduction in the number of offspring which reached to 98.6%.

Biochemical effects of precocenes : Table 5 shows that precocenes significantly increased the haemolymph total proteins of the berseem grasshopper females at all experimental days, but in males at only days 10 and 12. Concerning *S. littoralis,* Table 3, this increase was very high in pupae treated during the fourth larval instar with PI, but in haemolymph of the larval stage, they tend to decrease. In locusts and grasshoppers, the first detection of vitellogenesis in haemolymph was at the sixth day after adult emergence, the oocyte maturation and the high release of J.H. by corpora allata was at the tenth day, while vitellogenesis and release by the fat body and uptake in oocytes is at day 12 (Tobe and Pratt, 1975). J.H. had been implicated in controlling the synthesis of proteins. Since precocene had an anti-juvenile hormone effect, it was expected that the total proteins to be decreased. The results however were contradictory and were difficult to interprete.

Haemolymph cholesterol level was sharply reduced in treated grasshoppers at all days of analysis. But precocene had a dual effect on *S. littoralis* which was dose dependent. In this respect, Cassier *et al.* (1988) stated that cholesterol in insects served as a precursor of ecdyson.

Whole body lipids content of treated grasshopper showed a significant reduction at days 6 and 10, but at day 12, lipids increased in both sexes. Also, the adults of the cotton leafworm showed increased level of lipids, while larvae and pupae showed a dramatic reduction in these metabolites. This might cause developmental arrest, thus leading to abnormalities or the formation of larval-pupal intermediates. Detailed studies were carried out by Schneider *et al.* (1995), who reported that in *Locusta*, J.H. stimulated both vitellogenesis production by the fat body and uptake by the developing oocytes and the adipokinetic reaction. The reduction of lipids including cholesterol, specially at the larval stage might influence the hormonal synthesis leading to the failure in moulting.

Concerning chitin content, the treatment caused it to be slightly increased in adult grasshoppers, while in *S.littoralis* it sharply decreased for both pupal stage and unhatched pupae .This might influence the speed of penetration of insecticides.

In conclusion, the obtained results indicate that there was difference in the sensitivity of a given pest to the different types of precocenes, as well as, in the susceptibility of the treated instars. Emphasizing by their latent effects and their bioactivity, precocenes proved their effectiveness as environmentally benign control agents which might lead to a new approach in an integrated pest control programmes.

Table 1. Initial and latent bioinsecticidal activities of antihormone precocene II on the berseem grasshopper, *E. plorans plorans.*

Treated	Nymphal duration (days)			Rate of development		% weight gain change		% mortality	
instar	4th instar	5th instar	4th+ 5th instars	4th instar	5th instar	Male	Female	4th instar	5th instar
Fourth	12.4 (11-14)	14.5 (14-15)	26.9	8.06	5.90	-54.85	177.95	11.55	29.15
Fifth	6.25 (5-7)	4.1 (4-6)	10.36	-	24.39	-26.04	-33.95	-	78.20
Check	6.25 (5-7)	7.1 (7-9)	13.35	16.00	14.08	-	-	-	-

Table 2. Bioinsecticidal activities of precocenes against the cotton leafworm, *S. littoralis* (Boisd.).

Treated instar	Insecticide used	Dose (µg/g b.wt.)	% weight gain change			% larval mortality		% prepupal and pupal abnormalities	% pupation	% emergence	% duration change	
			Larva	Pupa	Adult	Acute	Latent				Larva	Pupa
4th	PI	40	-75.66	0.00	0.00	38.00	100.00	0.00	0.00	0.00	-46.15	-
		25	-57.66	-44.4	0.00	36.00	84.38	40.00	15.63	0.00	-30.77	-
	PII	40	-32.00	1.35	-26.94	2.00	56.67	16.66	43.33	66.66	-23.08	27.27
		25	-2.66	3.93	0.31	0.00	25.00	6.66	75.00	80.00	-15.38	9.09
6th	PI	40	-29.80	-51.88	0.00	50.00	20.00	50.00	0.00	0.00	15.66	-
		25	-26.46	-21.93	0.00	5.00	15.79	33.33	0.00	0.00	21.88	-
Control		0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	100.00	0.00	-

Table 3. Effect of precocenes on chitin content and biochemical components of the cotton leafworm *S. littoralis* (Boisd.)

Treated instar	Insecticide used	Chitin content in cuticle (mg)		Whole body lipid content (mg/g b.wt.)			Total cholesterol				Total proteins			
		Pupae (7 days old)	Unhatched pupae				Haemolymph (mg%)	Whole body (mg/g b.wt.)			Haemolymph (mg%)	Whole body (mg/g b.wt.)		
				Larvae	Pupae	Adults		Larva	Pupa	Adult		Larva	Pupa	Adult
4th	PI	-	-	23.08	-	-	8.99	8.99	-	-	0.80	18.77	-	-
		6.30	0.00	13.12	18.75	-	2.51	1.71	7.05	-	0.97	24.13	54.90	-
	PII	19.65	0.60	314.18	152.24	107.14	7.91	17.08	12.01	40.03	0.93	16.74	8.90	10.17
		16.13	7.20	381.57	68.08	105.26	4.81	70.84	20.43	16.63	0.57	12.83	8.00	15.23
6th	PI	2.50	0.00	140.35	23.26	-	26.7	18.14	11.41	-	1.52	15.50	17.00	-
		5.00	3.75	39.22	44.44	-	6.24	23.05	10.48	-	0.84	13.95	9.90	-
Control		21.75	21.75	359.85	65.10	68.97	7.56	24.05	24.99	5.15	0.83	12.00	8.71	13.50

Table 4. Effect of precocene II on reproductive potential of the berseem grasshopper, *E. plorans plorans* treated as one day old last nymphal instar and paired with normal ones.

(Values are means of 10 females)

Percentage of viable eggs per female in indicated eggs batch						Mean of egg pods/female	Mean of total eggs laid/female	Mean of total eggs hatched/ clutch	Fertile eggs %	Relative number of offsprings	% decrease in reproductive potential
1st	2nd	3rd	4th	5th	6th						
Precocene-treated females											
3.70	0.00	87.50	0.00	0.00	0.00	2.30	34.00	4.00	11.74	1.40	99.85
Precocene-treated males											
0.00	0.00	0.00	75.00	64.29	0.00	5.10	76.00	16.50	21.70	5.60	98.58
Precocene-treated males x females											
0.00	0.00	0.00	0.00	0.00	0.00	1.00	3.50	0.00	0.00	0.00	100.00
Untreated											
100.00	100.00	96.00	96.00	92.00	92.00	7.13	300.00	294.00	98.00	100.00	0.00

Table 5. Biochemical components of the berseem grasshopper, *E. plorans plorans* adults treated in the 5th nymphal instar by precocene II.

Age (days)	Sex		Haemolymph total Proteins (mg%)	Haemolymph total cholesterol (mg%)	Whole body total Lipids content (mg)	Integument chitin content (mg)
6	Males	Treated	12.00±0.064***	27.10±0.84***	2.28±0.30*	55.90±1.17***
		Control	17.70±1.30	53.30±1.12	4.44±0.60	43.20±1.76
	Females	Treated	16.20±1.30***	60.70±1.40***	5.34±0.80***	51.80±1.16**
		Control	5.70±0.60	98.90±1.46	9.75±0.90	42.50±1.75
10	Males	Treated	11.60±0.65***	40.90±1.60***	2.59±0.40***	56.30±1.18***
		Control	3.10±0.30	62.90±1.25	11.63±0.65	42.10±1.72
	Females	Treated	16.00±1.30*	111.10±6.40***	2.75±0.30***	49.30±1.10 ns
		Control	13.20±1.20	154.00±7.00	7.97±0.90	50.40±1.15
12	Males	Treated	14.40±1.23***	36.60±0.95***	13.37±1.30*	38.80±1.26***
		Control	62.20±1.48	49.30±1.66	10.98±0.62	49.30±1.13
	Females	Treated	62.20±1.48***	51.00±1.78***	9.46±0.90***	50.00±1.14***
		Control	3.30±0.50	67.70±1.15	1.93±0.03	38.20±0.90

Data are presented as means ± standard deviation.

.ns : non-significant

* : significant at the level 5%

** : significant at the level 1%

*** : significant at the level 0.1%

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التأثير الحيوى الفورى والمتأخر لمركبات البريكوسين (١ ، ٢) على أطوار النمو المختلفة لنطاط البرسيم ودودة ورق القطن

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من اجل تقييم الدور الحيوى الابادى لمادة البريكوسين ١، ٢ والتى تعيق عمل هورمون الحداثة (الشباب) فى تطوير برنامج المكافحة المتكاملة ضد آفة نطاط البرسيم وآفة دودة القطن ، تم تطبيق مركبات البريكوسين بالمعاملة السطحية بجرعات ٢٥ ، ٤٠ ميكروجرام لكل جرام من وزن الجسم على العمر الرابع والسادس لدودة ورق القطن ، وأوضحت النتائج أن البريكوسين الأول كان أكثر تأثيرا من البريكوسين الثانى وأن العمر السادس كان أكثر تأثرا وحساسية من العمر الرابع.

عند التطبيق على آفة نطاط البرسيم بجرعة ٥٠ ميكروجرام لكل جرام من وزن الجسم على العمر الرابع والخامس كان أهم النتائج المتحصل عليها هى أن العمر الخامس كان أكثر حساسية من العمر الرابع لحدوث انخفاض فى الوزن فى كل من الذكور والإناث مع تشوهات جسدية وانخفاض فى عمليات التمثيل الغذائى وخاصة الكوليسترول مما أدى إلى ارتفاع فى التأثير السمى، بينما التأثير المتأخر كان أكثر فعالية حيث أحدث تثبيط شديد لعمليات التمثيل الحيوى مما أدى إلى حدوث عقم للإناث والذكور وصل إلى ١٠٠٪ مما يؤكد دوره فى المكافحة الفسيولوجية.