TOXICOLOGICAL AND BIOCHEMICAL EFFECTS OF PRECOCENE II AGAINST COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.)

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(Manuscript received 31 March 2019)

Abstract

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is very polyphagous insect against the most economic plants all over the world. The traditional synthetic chemical insecticides had dramatic effect on the living organisms including man. Anti-juvenile hormones; precocene II effect on the cotton leafworm, *Spodoptera littoralis* were assayed under laboratory conditions. (PrecoceneII (6,7-dimethoxy-2,2-dimethylchromene) was the main constituent isolated from *Ageratum houstonianum* plant. The compound caused toxicity against the larvae with (LC_{10} was 5.59 mg/l), (LC_{25} was 24.69 mg/l) and (LC_{50} was 128.53 mg/l). There was some morphogenic effect obtained due to effect of precoceneII on larvae, pupae and adult stages. On the other hands the biochemical analysis of the cotton leafworm revealed that precocene II caused significant increase in chitinase, protease and total carbohydrates with (17.32, 50.17 and 36.85 %), respectively comparison to control.

Keywords: Biochemical analysis, toxicology, Precocene II, IGRs, Anti-juvenile hormone, *Spodoptera littoralis*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is a most important polyphagous pest, while it is widely distributed all over the world. Larvae of this pest can feed on many of the economically important plant species belonging to 40 families and the rate of development has a strong nutritional component (Brown and Dewhurst, 1975).

Commonly, the control of this pest has largely been depending on the synthetic insecticides; chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids (Baldwin and Graves, 1991). Among the problems associated mainly with the excessive application of synthetic insecticides is rising the resistant populations of pathogens and pests and environmental hazards.

For the production of foodstuffs of such quality to be possible, it is necessary among other things, to reduce the risks associated with excessive application of synthetic insecticides.

The corpora allata is endocrine gland of insect which secrete juvenile hormones that affect the regulation of metamorphosis and the development of gonads. Juvenile

hormone (JH) is necessary for insect development throughout the immature stages (Staal, 1986). It works alone or in combination with ecdysone and its metabolite 20E (20-hydroxyecdysone) in regulating various functions (Nijhout, 1994). JHs play important roles in several physiological processes, such as reproduction, diapause, behavior, polymorphism, migration, metabolism and innate immunity (Truman and Riddiford, 2007; Flatt *et al.,* 2008).

Most compounds that belong to the IGR class are not stomach or neurotoxic poisons, but have a unique mode of action that disrupts the molting process or cuticle formation in insects (Smagghe and Degheele, 1994) or interferes with the hormonal balance of insects (Dhadialla *et al.,* 1998), they are characteristically slow acting against a narrow range of sensitive stages of the insects' life cycle with harmful effect against target pest.

Anti-juvenile hormone compounds (Precocene) are effective insect growth regulators of some insect species (Bede *et al.,* 2001), where inhibiting the biosynthesis of juvenile hormones of the corpora allata and the insufficiency of these substances exert some abnormalities in certain biological phenomena, which are controlled by the juvenile hormones (Bowers, 1985).

Plants defend themselves by producing natural products that are effective against herbivores. Precocene II is a chromene derivative from 2,2-Dimethylchromenes which are characteristic natural products found in many species of the Asteraceae (Proksch and Rodriguez, 1983). Bowers et al., (1976) showed that the Precocene II (6,7-dimethoxy-2,2-dimethylchromene) is a compound isolated from *Ageratum houstonianum* plant which reveal the growth-disrupting properties of certain species of insects by causing a deficiency of juvenile hormone in insects.
Precocene II is known as an active anti-juvenile hormone in susceptible insect species because of its lethal action in a broader range of insects
(Randriaminahy, 1992).

Thus, the aim of the current study was to investigate the influence of precocene II on the toxicological and biochemical parameters on the 4th instar larvae of *S. littoralis*.

MATERIALS AND METHODES

Insect rearing:

The susceptible strain of the cotton leafworm, *Spodoptera littoralis* (Boisd.) was obtained from the Cotton Pesticides Evaluation Research Department in plant protection Research institute, Dokki, Giza, Egypt, and was maintained in climatic chamber under optimum conditions of 25°C± 2 and 65± 5% RH and (16L:8D) light: dark photoperiod. The culture was kept and reared on castor bean leaves as described by El-Defrawi *et al.*, (1964).

Precocene II:

Precocene II was obtained from Sigma-Aldrich Chemical Company. It was dissolved in acetone solvent, 1gm: 1ml, and serial concentrations was prepared.

Toxicological test:

By using leaf dipping technique, Castor bean leaves were dipped for 30 seconds in each concentration (50, 100, 200, 400 and 800 mg/l) then left to dry. The treated leaves were offered to newly molted 4th instar larvae of *S. littoralis* for 48 hrs. Parallel with control larvae without any treatment. Mortality percentages were recorded after 24 hrs., then corrected according Abbott's formula (1925). From the corrected mortality percentages the corresponding toxicity lines (LC-P lines) were estimated in addition to determine $LC_{10,}$ LC_{25} and LC_{50} values and their confidence limits, slope values of tested extract were also estimated.

Biochemical assay:

The survived larvae after feeding on the leaves treated with the different concentrations of precocene II were taken to determine the biochemical activity of total carbohydrate, chitinase and protease enzymes.

Preparation of homogenate samples for biochemical analysis:

By using a Teflon homogenizer (Mechianika Precyzyjna Warszawa type MPN-309-Poland) at 500 rpm, the collected larvae were homogenized in distilled water for 3 minutes. Homogenates were collected in cold tubes (on ice) previously coated with crystals of phenylthiourea to prevent melanization. by using (BECKMAN GS-6R Centrifuge), the cold tubes were Centrifuged for 10 min. at 6000 rpm at 5°C, the formed supernatant fluid during the centrifugation was divided to aliquots each of (0.5 ml) and it was kept at -20 °C until analysis.

Biochemical impacts:

1- **Chitinase enzyme activity**

   According to Ishaaya & Casida (1974) method, the free aldehydic groups of hexoaminase liberated on chitin digestion is determining using 3.5-dinitrosalicylic acid as a reagent to assay chitinase.

2- **Protease enzyme activity:**

   Protease was assayed by using Tachell et al., (1972) method which evaluate the increase of the free-amino acids that are separated from albumin in the period of incubation at30 ºC for an hour.

3- **Total carbohydrate assay:**

   Total carbohydrates were estimated in acid of sample by the phenol-sulphuric acid reaction of Dubois *et al*., (1956). Total carbohydrates were extracted and prepared according to Crompton and Birt (1967).

Morphogenic effect:

The treated 4th instar larvae of *S. littoralis* with precocene II produced many abnormalities for the different developmental stages. This was including abnormal larvae, pupal-larval intermediate and abnormal adults.

RESULTS AND DISCUSSIONS

Toxicological impacts:

The experiments were carried out to evaluate the toxicological activity of Precocene II against the 4th instar larvae of *S. littoralis* under laboratory conditions.

From data in Table (1) Precocene II concentrations caused lethality and morphogenic effects against the 4th instar of *S. littoralis*.

Table 1. Accumulative mortality percentages of *S. littoralis* larvae after treatment with precocene II under laboratory conditions

Concentrations (mg/l)	Accumulative mortality percentages %		
	3 days post treatment	7 days post treatment	14 days post treatment
50	3.33	16.67	33.33
100	5	20	46.67
200	8.33	33.33	60
400	15	45	66.67
800	16.67	53.33	76.67

Table 2. Toxicity data of Precocene II against the 4th instar larvae of *S. littoralis* under laboratory conditions.

Toxicity parameters	LC_{10} (Confidence limits) mg/l	LC_{25} (Confidence limits) mg/l	LC_{50} (Confidence limits) mg/l	Slope ± SE
	5.59 (1.27-12.82)	24.69 (10.07- 41.57)	128.53 (90.39-170.07)	0.941± 0.139

By using LC-P program; LC_{10}, LC_{25}, LC_{50} and slope values were used as parameters in evaluation the insecticidal activity of this compound. The LC_{10}, LC_{25}, LC_{50} values were recorded in Table (2), where they were; 5.59, 24.69 and 128.53 mg/l, respectively.The lower and upper confidence limits were ranged between 1.27-12.82, 10.07-41.57 and 90.39-170.07 mg/l for LC_{10}, LC_{25}, LC_{50} respectively, as shown in Table (2).

As mentioned before according to Bowers *et al.,* (1976) noted that precocene II induces premature metamorphosis and/or cessation of other juvenile hormone-mediated functions.

The Chemicals of Plants produce a resistance against herbivorous insects under natural conditions. Due to the antihormonal activity of precocene II, a metabolic fate in the insect's diet has received considerable attention (Bergot *et al.,* 1980; Ohta *et al.,* 1977; Soderlund *et al.,* 1980).

The obtained results are compatible with Srivastava and Proksch (1991); which found that precocene II has shown an exhibit venomousness and feeding deterrence to the Noctuid species which are a type of herbivorous insects.

Morphogenic abnormality:

After treatment 4th larval instars with precocene II produced morphogenic effect to larvae, pupae and adult stages of *S. littoralis*. These abnormalities appeared that larva was wrinkled with black colour and pupa was appeared as larval-pupal intermediate form, while adult was emerged without wing and shortened of wing or immobilized legs.

Biochemical impacts:

The biochemical parameters of the survived larvae of *S. littoralis* after treatment with the sub-lethal concentration were evaluated; the effect of precocene II on some enzyme activities related to metamorphosis; chitinase, protease and total carbohydrates.

Theses biochemical impacts were undertaken as an attempt to interpret the primary mechanism of action of this concentration.

Table 3. Biochemical parameters in haemolymph after treatment the 4th instar larvae of *S. littoralis* with the LC_{50} of Precocene II.

Parameters	Control	Precocence treatment	Change %
Chitinase µg N-acetyl glucosamine /min./g b. wt	1472 ± 54.79	1727 ± 48.22*	17.32
Protease µgalanin /min./g b. wt	45.13 ± 1.02	67.77 ± 1.45*	50.17
Total Carbohydrates	6.16 ± 0.01	8.34 ± 0.13*	36.85

Results in Table (3) and Fig. (1) Showed that treatment of the 4th instar larvae with the sub-lethal concentration of precocene II caused a significant at P< 0.5 increase in chitinase activity with (17.32 %) more than in control. While protease enzyme activity with 50.17 % more than control, and total carbohydrates with 36.85 % more than control.

Insect juvenile hormones (JH) might antagonize JH-action (Bowers, 1976). It was found that that activity of chromenes (precocene II) isolated from *Ageratum houstonianum* plant. In sensitive insects treated with these compounds (anti-JHs). JH-deprivation is signaled by precocious metamorphosis in immature stages and sterility in adult female's insects (Anthonsen & Chantarasakul, 1970; Bowers *et al.,* 1976; Pratt, 1983; and Staal, 1986).

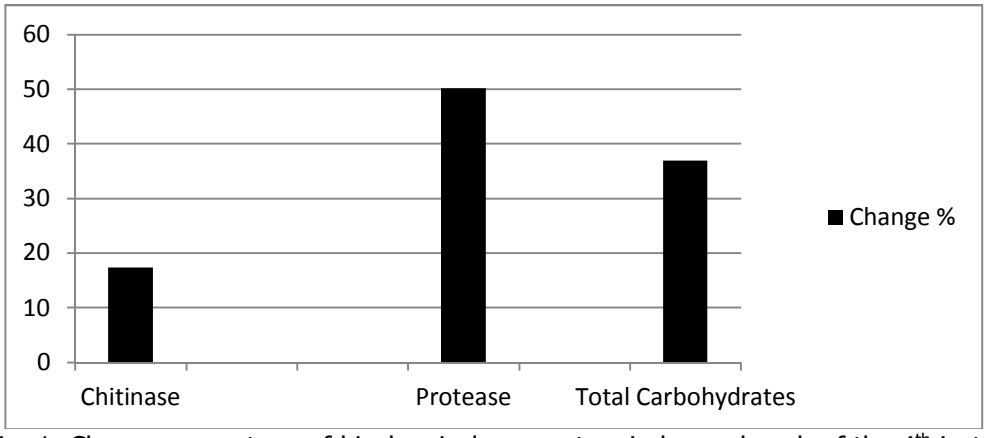

Fig. 1. Change percentage of biochemical parameters in haemolymph of the 4th instar larvae of *S. littoralis* after treatment with LC_{50} of Precocence II.

REFERENCES

1. Abbott, W.S. 1925. A method ofcomputing the effectiveness of aninsecticide.*J. Econ. Entomol.*, 18:265-267.
2. Anthonsen, T. and S. Chantarasakul 1970. Ageratone and dihydroageratone, new benzofuran derivatives from *Ageratum houstonianum* Mill. *Acta Chem. Scand*. 24: 721-722.
3. Baldwin, J.L. and J.B. Graves 1991. Cotton insect pest management. *LA Coop. Ext. Serv. Bull.,*1829.
4. Bede, J.C.; Teal, P.E.; Goodman, W.G. and Tobe, S.S. 2001. Biosynthetic pathway of insect juvenile hormone III in cell suspension cultures of the sedge *Cyperusiria. Plant Physiol.*, 127(2): 584–593.
5. Bergot, B.J.; K.J. Judy; D.A. Schooley and L.W. Tsai 1980. Precocene II metabolism: comparative *in vivo* studies among several species of insects and structure elucidation of 2 major metabolites. *Pestic. Biochem, Physiol.*, 13: 95-104.
6. Bowers, W.S. 1982. Toxicology of the precocenes. In Insecticide Mode of Action (Coats, J. R., ed.), pp. 403-427. Academic Press, New York.
7. Bowers, W.S. 1985. Comprehensive Insect Physiology Biochemistry and Pharmacology, G.A. Kerkut and L.I. Gilbert., Vol. 8, Pergamon Press, 551-564.
8. Bowers, W.S.; T. Ohta; J.S. Cleere and P.A. Marsella 1976. Discovery of insect anti-juvenile hormones in plants. *Sci.* 193: 542-547.
9. Brown, E.S. and C.F. Dewhurst 1975. The genus *Spodoptera* (Lepidoptera: Noctuidae) in Africa and the Near East. *Bull. Entomol. Res.,* 65: 221–261.
10. Crompton, M. and Birt. 1967. Change in the amount of carbohydrates, phosphagen, and related compounds during the metamorphosis of the blowfly *Luciliacuprina. J. Insec. Physiol.,* 13: 1575-1595.
11. Dhadialla, T.S.; G.R. Carlson; D.P. Le. 1998. New insecticides with ecdysteroidal and juvenile hormone activity.*Ann. Rev. Entomol.*, 43: 545–569.

12. El-Defrawi, M.E.; A. Toppozada; N. Mansour and M. Zeid 1964.Toxicological studies on the Egyptian cotton leafworm, *Prodenialitura* (F.) I. Susceptibility of different larval instars of *prodenia* to insecticides. *J. Econ. Entomol.,* 57: 591-593.
13. Dubios, M.; K.A. Gilles; P.A. Rebersand F. Smith. 1956. Colorimetric method for determination of sugars and related substance. *J. Analy. Chem.,* 28:350-356.
14. Flatt, T.; A. Heyland; F. Rus; E. Porpiglia; C. Sherlock; R. Yamamoto; A. Garbuzov; S.R. Palli; M. Tatar and N. Silverman. 2008. Hormonal regulation of the humoral innate immune response in *Drosophila melanogaster*., *J. Exp. Biol.,* 211: 2712 – 2724.
15. Ishaaya, I. and J.E. Casida. 1974. Dietary TH-6040 alters composition and enzyme activity of housefly larval cuticle*. Pestic. Biochem. Physiol*., 4: 484-490.
16. Nijhout, H.F. 1994. Insect hormones. Princeton: Princeton Univ. Press, NewJersey, pp. 280.
17. Ohta, T.; R. J. Kuhr and W. S. Bowers. 1977. Radiosynthesis and metabolism of the anti-juvenile hormone, Precocene II. *J. Agric. Food. Chem*., 25, 478-481.
18. Pratt, G. E. 1983. The mode of action of pro-allactocidins. In Natural Products for *Innovative* Pest Management (Whitehead, D. L. and Bowers, W. S., eds), pp. 323-355. Pergamon Press, Oxford.
19. Proksch, P. and E. Rodriguez. 1983. Chromenes and benzofurans from the *Asteraceae*, their chemistry and biological significance. *Phytochemistry,* 22: 2335-2348.
20. Randriaminahy, M. 1992. Detoxification of naturally occurring chromenes in larvae of the generalist herbivore Spodoptera littoralis (Noctuidae), *Biochem. Syst. and Ecol*., 8: 711-722.
21. Smagghe, G. and D. Degheele (1994). Action of a novel nonsteroidal ecdysteroid mimics, tebufenozide (RH-5992) on insects of different orders. *Pest. Sci.,* 42: 85–92.
22. Soderlund, D.M.; A. Messeguerandand W.S. Bowers. 1980. Precocene II metabolism in insects: Synthesis of potential metabolites and identification of initial in vitro biotransformation products. *J. Agric. Food. Chem*., 28: 724-731.
23. Srivastava, R. E and P. Proksch. 1991. Contact toxicity and feeding inhibitory activity of chromenes from *Asteraceae* against *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Entomol. Gen.,*15: 265-274.
24. Staal, G.B. 1986. Anti-juvenile hormone agents. *Annu. Rev. Entomol.,* 31: 391-429.
25. Tachell, R.J.; S.F. Arman and F.N. Boctor. 1972. Biochemical and physiological studies of certain Ticks. (Ixodoidea). Z. Parsitenk ,9: 345-350.
26. Truman, J.W. and L.M. Riddiford. 2007. The morphostatic actions of juvenile hormone. *Insect Biochem. Mol. Biol*., 37: 761–770.

التأثيرات السمية والبيوكيميائية لمركب البريكوسين II ضد دودة ورق القطن، ***Spodoptera littoralis*** **(Boisd.,)**

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تعتبر دودة ورق القطن من أهم و أخطر الافات التى تصيب معظم المحاصيل الاقتصادية. استخدام المبيدات الحشرية التقليدية فى مكافحة هذه الافه لها اضرار عديدة علي البيئة وصحة الانسان. تم دراسة تأثير مستحضرمركب البريكوسين II على يرقات دودة ورق القطن . حيث ان مركب البريكوسين II هو المركب الرئيسى الذى تم فصله من نبات الاجرتيم. أظهرت نتائج السمية ان لمركب له تأثير سام ضد يرقات العمر الرابع لدودة ورق القطن وأن السمية تزداد بمرور الوقت بعد المعاملة، حيث بلغت قيم التركيز اللازم لقتل 10، 25، 50 % من الأفراد المعاملة هى (5.59 &mg/l 24.69 & 128.53) على التوالى. كما أظهر التحليل اليوكيميائى زيادة فى نشاط انزيمات الكيتينيز والبروتييز و الكربوهيدرات بنسبة (17.32& 50.17& 36.85 على التوالى مقارنة باليرقات الغير معاملة.