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EFFICIENCY OF CHITIN SYNTHESIS INHIBITORS, INSECTICIDES AND THEIR MIXTURES AGAINST THE FOURTH LARVAL INSTAR OF THE COTTON LEAFWORM

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Abstract

The effect of chitin synthesis inhibitors when applied singly or in binary mixtures with conventional insecticides against different ages of fourth instar larvae of the cotton leafworm *Spodoptera littoralis* was studied throughout the present investigation. The results obtained indicate that the most suitable age of fourth instar larvae was 24 hr to evaluate the efficiency of chitin synthesis inhibitors. Conventional insecticides and their binary mixtures with chitin synthesis inhibitors were more potent to early and middle age of such instar (8-24 hr). All the tested compounds induced lower effect when used against the older larvae (40-64 hr).

Regarding the effect of chitin synthesis inhibitors on the performance of insecticides, DC 902 mixture showed an obvious antagonistic effect, while Catabron mixture revealed an additive effect. On other hand, additive and potentiation effects occurred in case of Deenate mixture.

INTRODUCTION

Ingestion of chitin synthesis inhibitors by insect larvae disturbed endocuticular deposition during molting process because it blocks chitin synthesis (Mulder and Gijswijt, 1973). The blockade of chitin synthesis occurs due to disruption of the function of connecting N-acetylglucose amine moieties to the chitin chain in spite of that the coupling of uridine diphosphate-N-acetylglucose amine (UDPAG), the ultimate chitin precursor, to the chitin synthetase still proceeds (Post *et al.* 1974).

Results obtained from the bioassay techniques adopted by the Ministry of Agriculture differ too much according to the age of the fourth instar larvae of *S.littoralis* used (Watson *et al.*, 1984; Abdel-Megead *et al.*, 1986; El-Hamaky *et al.*, 1987; Khidr *et al.*, 1991; El-Hamaky *et al.*, 1993).

So, the aim of the present work is to find out the most susceptible age of fourth larval instar of *S.littoralis* to chitin synthesis inhibitors, conventional insecticides and their binary mixtures under laboratory conditions.

MATERIALS AND METHODS

1. Test insect: For the present study, 8, 16,24,32,40,48,56 and 64 hr old 4th instar larvae of cotton leafworm, *S.littoralis*, were taken from a colony reared in the laboratory according to the method adopted by El-Defrawi *et al*, 1964.

2. Insecticides used: The following insecticides and their rates in grams active ingredients (a.i) per feddan were used:

2.1. Conventional insecticides: Curacron (profenofos), 72%, EC at 473 g/fed; Dursban (chlorpyrifos), 48% EC at 480 g/fed. and Lannate (methomyl), 90% SP at 270 g/fed.

2.2. Chitin synthesis inhibitors (CSI): IKI (chlorfluazuron), 5% EC at 27 g/fed.; XRD, 5% EC at 30 g/fed. and Dimilin (diflubenzuron), 25% WP at 40 g/fed.

2.3. Binary mixtures : DC 902, (Dursban 480 g + XRD 30 g/fed.); Catabron, (Curacron 473 g + IKI 27 g/fed.) and Deenate, (Lannate 270 g + Dimilin 40 g/fed.).

The above mentioned insecticides were sprayed twice on June 26th and July 20th on cotton during 1996 cotton season at Sakha Agric. Res. Station, using knapsack sprayer Model CP3 with 200 litres water per feddan.

3. Laboratory tests : The initial and bio-residual activity of the tested insecticides against the selected ages of 4th larval instar of cotton leafworm were studied in the laboratory. In this respect 8, 16, 24, 32, 40, 48 56 and 64 hr old larval stage of 4th instar were subjected to the feeding technique according to the method adopted by the Ministry of Agriculture of Egypt for evaluating the efficiency of chitin synthesis inhibitors (CSI) and their binary mixtures with conventional insecticides. In such technique, the feeding period was started immediately after insecticidal application and extended for 15 days. The feeding period was divided into three successive intervals of 5 days each (i.e. 0-4, 5-9 and 10-14 days). In each time interval, the larvae were fed on treated cotton leaves for 2 days and the alive larvae were fed on untreated leaves for 3 days. The larvae of each selected age were divided into batches of 50 larvae each (5 replicates with 10 larvae). One batch was fed on treated cotton leaves collected during the first two days of the first interval (0-4 days) and then on untreated leaves for the next three days. Likewise, another batch was fed on the leaves collected during the second period (5-9 days). Also, the same number of larvae was used for the last period (10-14 days).

Mortality count in each treatment was recorded daily for 5 successive days and the total number of dead larvae was calculated at the end of each period.

The co-toxicity factor equation of Mansour *et al.* (1966) was used to evaluate the joint effect of different pairs of insecticides/CSIs. The factor was used to differentiate the results into three categories. A positive factor of 20 or more means potentiation, a negative factor of 20 or more means antagonism and any intermediate value (-20 to + 20) was considered additive effect.

RESULTS AND DISCUSSION

Data presented in Tables 1, 2 & 3 show the bio-activity of some chitin synthesis inhibitors and conventional insecticides applied either singly or in binary mixtures against different ages of 4th larval instar of *S.littoralis*.

Table 1 shows that the younger 4th instar larvae was more susceptible to CSIs than the older ones. The efficiency of the three tested compounds was highly noticed against the 8,16 and 24 hr old of the 4th larval instar. In this respect, the average cumulative percent mortalities for the three tested periods of chlorfluazuron, XRD and diflubenzuron induced 58.7, 64.7 & 68.7; 42, 46.7 & 54 and 44, 48.7 & 50.7% mortality, respectively. Meanwhile, the ages of 48, 56 and 64 hr old were more tolerant to the aforementioned insecticides as they induced 49.3, 43.3& 36.7; 38, 33.3 & 29.3 and 30, 22.7 & 14% mortality, respectively. The data also revealed that chlorfluazuron induced highly initial effect and long bio-residual activity followed by XRD and diflubenzuron.

The results obtained in Table 2 show that the efficiency of the tested conventional insecticides was more pronounced with the younger ages of 4th larval instar of cotton leafworm. This may be correlated with the rate of penetration of such toxicants within the cuticle. The effect of profenofos, chlorpyrifos and methomyl decreased with the increase of the larval age. Profenofos induced 82.7, 82, 78, 71.3, 67.3, 66, 65.3 and 62.7% mortality during 15 days after treatment to 8, 16, 24, 32, 40, 48, 56 and 64 hr larval age of the 4th intar, respectively. The same trend was observed by chlorpyrifos and methomyl, but profenofos was more effective followed closely by chlorpyrifos then methomyl against the tested ages. This may be due to the short residual effect of methomyl.

Table 3 indicates that the binary mixtures of CSIs and conventional insecticides induced high intial effect and long residual activity with the younger ages of

Table 1. Effect of different ages of 4th instar larvae of *S.littoralis* on the efficacy of chitin synthesis inhibitors (CSIs).

Chitin synthesis inhibitors	Age of tested larvae (hr)	% mortality at indicated periods			Average mean of the 3 periods
		0-4 days	5-6 days	10-14 days	
Chlorfluazuron	8	70	62	44	58.67
	16	72	70	52	64.67
	24	78	74	54	68.67
	32	70	58	46	58
	40	68	54	42	54.67
	48	60	48	40	49.33
	56	56	40	34	43.33
	64	50	34	26	36.67
XRD	8	50	40	36	42
	16	54	48	38	46.67
	24	60	54	48	54
	32	54	46	42	47.33
	40	52	40	32	41.33
	48	48	38	28	38
	56	40	36	24	33.33
	64	38	30	20	29.33
Diflubenzuron	8	56	38	38	44
	16	60	46	40	48.67
	24	62	48	42	50.67
	32	50	40	32	40.67
	40	44	36	28	36
	48	40	28	22	30
	56	30	20	18	22.67
	64	20	14	8	14

Table 2. Effect of different ages of 4th instar larvae of *S.littoralis* on the efficacy of certain conventional insecticides.

Conventional insecticides	Age of tested larvae (hr)	% mortality at indicated periods			Average mean of the 3 periods
		0-4 days	5-9 days	10-14 days	
Profenofos	8	94	82	72	82.67
	16	92	82	72	82.00
	24	92	78	64	78.00
	32	90	68	56	71.33
	40	88	60	54	67.33
	48	86	60	52	66.00
	56	88	58	50	65.33
	64	86	54	48	62.67
Chlorpy	8	90	80	70	80.00
	16	92	78	68	79.33
	24	88	76	70	78.00
	32	82	64	60	68.67
	40	80	58	50	62.67
	48	78	56	44	59.33
	56	78	54	42	58.00
	64	76	54	40	56.67
Methomyl	8	90	48	30	56.00
	16	90	42	26	52.67
	24	88	38	24	50.00
	32	82	32	20	44.67
	40	80	30	18	42.67
	48	76	30	16	40.67
	56	70	24	14	36.00
	64	64	20	10	31.33

Table 3. Effect of different ages of 4th instar larvae of *S.littoralis* on the efficacy of three binary mixtures of CSIs and conventional insecticides.

Binary mixtures	Age of tested larvae (hr)	% mortality at indicated periods			Average mean of the periods
		0-4 days	5-6 days	10-14 days	
DC 902	8	100	92	80	90.67
	16	100	92	78	90.00
	24	100	90	78	89.33
	32	98	88	70	85.33
	40	96	82	66	81.33
	48	90	78	60	76.00
	56	88	70	54	70.67
	64	88	66	42	65.33
Catabron	8	100	94	88	94.00
	16	100	94	84	92.67
	24	100	96	84	93.33
	32	100	90	78	89.33
	40	98	82	70	83.33
	48	96	78	62	78.67
	56	94	74	58	75.33
	64	90	70	50	70.00
Deenate	8	100	90	70	86.67
	16	100	90	70	86.67
	24	98	90	70	86.00
	32	96	88	64	82.67
	40	96	78	58	77.33
	48	88	70	48	68.67
	56	88	66	40	64.67
	64	84	56	36	58.67

Table 4. Toxicity of pairs combinations of CSIs/insecticide to different ages of 4th instar larvae of *S.littoralis* .

Mixtures	Age of tested larvae (hr)	Co-toxicity factor at the indicated periods		
		0-4 days	5-9 days	10-14 days
DC 902 (Dursban 480 g+XRD 30 g/ fed.)	8	-39.02	-36.11	-31.03
	16	-39.02	-39.47	-37.10
	24	-41.18	-40.79	-33.90
	32	-38.75	-30.16	-31.37
	40	-38.46	-28.07	-31.25
	48	-38.36	-27.78	-34.78
	56	-38.89	-28.57	-35.71
	64	-35.29	-25.00	-43.24
Catabron (Curacron 473 + IKI 27 g/fed.)	8	-28.58	-21.67	-16.98
	16	-31.51	-25.40	-20.75
	24	32.43	-26.15	-28.81
	32	-26.47	-18.18	23.53
	40	-25.76	-16.33	-14.63
	48	-23.81	17.02	-13.89
	56	-20.34	-17.78	-12.12
	64	-21.05	-16.67	-16.67
Deenate (Lannate 270 g + Dimilin 40 g/ fed.)	8	-31.51	+4.65	+2.94
	16	-33.33	+2.27	+6.06
	24	-34.67	+4.65	+6.06
	32	-27.27	+22.22	+23.08
	40	-22.58	+18.18	+26.09
	48	-24.14	+20.69	+26.32
	56	-12.00	+50.00	+25.00
	64	0	+64.71	+100.00

the 4th larval instar than older one. In this respect Catabron proved to be the superior compound against 8, 16, 24 and 32hr larval age, recording the highest initial effect and the longest residual activity. The general mean percent mortality reached 94, 92.7, 93.3 and 89.3 followed by DC 902 as it induced 90.7, 90, 93.3 and 85.3% mortality. Meanwhile, Deenate showed inferior performance recording 86.7, 86.7, 86 and 82.7%.

On the other hand, the efficacy of the tested binary mixtures was decreased when offered to older larvae, in such case Catabron, DC 902 and Deenate caused 78.7, 75.3 & 70,76, 70.7 & 65.3 and 69.7, 64.7 & 58.7% mortality to 48, 56 & 64 hr age of 4th instar larvae, respectively.

All insecticides tested showed lower effect with the larvae aged from 40 to 64 hr, because at that time, larvae reached the end of such instar and prepared itselves for new molt. Larvae refused to eat, moved slowly and the new cuticle of 5th instar was almost formed under older one (cuticle of 4th instar). So, the effect of CSIs to interfere with the formation of the new cuticle was nearly absent. Moreover, the slight efficiency of conventional insecticides may be attributed due to drop of food consumption and stopped motion at the time of ecdysis.

Table 4 demonstrated the co-toxicity factor as a criterion to evaluate the role of CSIs on the potentiality of the used insecticides against *S.littoralis* larvae. Data clearly indicate the misleading understanding of the co-toxicity factor in the case of initial effect. All treatments showed antagonistic effects when CSIs were mixed with insecticides, which could be due to the high potency of these insecticides immediately after application.

Also, data show the significant role of the age of 4th instar larvae in determining the behaviour of CSIs in relation to the bio-residual activity of insecticides in their mixtures. XRD caused antagonistic effects on the performance of chlorpyrifos within the period of experiment to all ages of 4th instar larvae. Chlorfluazuron caused an antagonistic effect on profenofos for the ages of 8 to 32 hr within the 2nd and 3rd period of experiment, while it caused additive effects to the other ages. On other hand, diflubenzuron caused additive effect on methomyl to ages (8 to 32 hr) and potentiation effect against the older ages (40-64 hr) of 4th instar larvae.

In conclusion, the most suitable age to judge on the efficiency of chitin synthesis inhibitors applied singly or in binary mixtures with conventional insecticides was 8 to 24 hr old of the 4th larval instar.

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دراسة تأثير مثبطات تخليق الكيتين والمبيدات التقليدية ومخاليطهما علي يرقات العمر الرابع لدودة ورق القطن

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درس تأثير استخدام ثلاث مركبات من مثبطات تخليق الكيتين منفردة وكذلك ثلاث مركبات من المبيدات التقليدية علاوة علي ثلاث أخري من مخاليط المجموعتين معا علي يرقات العمر الرابع لدودة ورقة القطن في مراحل سنية مختلفة ويمكن تلخيص النتائج المتحصل عليها في النقاط التالية :

١ – اليرقات في سن ٢٤ ساعة من العمر الرابع كانت أكثر ملائمة لتقييم فعالية مثبطات تخليق الكيتين منفردة.

٢ – اليرقات في سن ٨ – ٢٤ ساعة كانت الأفضل في حالة تقييم فعالية المبيدات التقليدية منفردة أو مخاليطها مع مثبطات تخليق الكيتين.

٣ – كانت جميع المركبات المستخدمه أقل تأثيراً عندما عوملت بها اليرقات المتقدمة في السن من العمر الرابع (٤٠ –٦٤ ساعة).

٤ – استخدام المخاليط اظهر تباينا واضحا في النتائج، فبينما اعطي مركب DC 902 تضاد في التأثير نجد ان مركب كاتبرون كان له تأثير اضافة في حين تراوح تأثير مركب دينيت بين الاضافة والتنشيط .