Effects of Dimilin on Young *Spodoptera littoralis* (Boisd.) Larvae

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ABSTRACT

Experiments were conducted under laboratory conditions to determine the susceptibility of the first three larval instars of *Spodoptera littoralis* (Boisd.) to the antimoulting agent"dimilin". Obtained results indicated that the response of 1st, 2nd,and 3rd,instar larvae of *S.littoralis* were abnormal, because the low concentration of 12.5 ppm or 25 ppm proved sometimes either more effective than, or equally effective against *S.littoralis* as the higher concentration (e.g.50ppm), This result might be attributed to the mode of action of the subject synthesis inhibitor under investigation or to the amount of contaminated diet consumed by each of the tested larval instars. Moreover, this compound might exhibit phagostimulant or deterrent effects against young larval instars. Also, the contact activity of the compound might contribute to the fact that the1st, instar larvae with relatively more exposed surfaces succumbed to death in periods (LT_{50}=43-60 hrs) shorter than those needed for the 2nd, (LT_{50}= 68-72 hrs)or the 3rd, (LT_{50} =51 60 hrs) instar larvae. To investigate the effect of the exposure period to dimilin on the larval mortality rates, three groups of each of the first and the second instar larvae of *S.littoralis* were allowed to feed on castor bean -oil leaves that were dipped in certain concentration of dimilin. Feeding on contaminated leaves proceeded for different exposure periods of 24,48,72 hrs, after which the larvae were offered untreated leaves and mortality data were recorded. The lowest concentration of 12.5 ppm was more potent than the other tested concentrations. However statistical analysis of the data showed that the higher concentration accelerated mortality as the LT_{50} values were 60, 47& 43 hrs for 50,25&1 2.5 ppm treatments respectively. On the other hand, continuous exposure period for 72 hrs, to the high concentration of treatments being 25 &50 ppm accelerated death and the LT_{50} values were 75 & 72 hrs, respectively. In fact,while the LT_{50}value for 12.5 ppm was 68 hrs.

Key words: *Spodoptera littoralis*, dimilin, artificial diet, castor bean oil leaves.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisd.), is a polyphagous insect pest. The host range of *S. littoralis* covers over 40 families, containing at least 87 species of economic importance (Eppo, 1999); (Salama *et al.,* 1970). On most crops, damage arises from extensive feeding of larvae, leading to complete stripping of the plants. On cotton, the larvae feed on the leaves may also bore into the bud or young bolls and consume the whole contents, causing them to be shed or dry up (Bishara, 1934). The chemical control of *S. littoralis* has been extensively reported, especially in cotton fields in Egypt. Numerous organophosphorus, synthetic pyrethroids and other insecticide groups have been used, with appearance of resistance in many insects (Issa *et al*., 1984a; 1984b; Abo-El-Ghar *et al*., 1986). However, compulsory limitation of the application of synthetic pyrethroids to one per year on cotton in Egypt has reduced the resistance (Sawicki, 1986). An alternative to chemical control for truck crops is of major interest, not only in order to avoid upsetting the biological balance, but also to lift the threat of pollution. One of such possibility is insect growth regulators (IGRs).The chitin synthetic inhibitors are chemical insecticides that disturb insect-specific, sometimes mite-specific, physiological processes that do not exist in vertebrates, including humans. Current commercial IGRs include chitin synthesis inhibitors "CSIs", interfering with molt, juvenile hormone analogs that disturb metamorphosis and/or prevent reproduction, and ecdysone (ecdysteroid) agonists that induce an untimely lethal molt. Diflubenzuronis the oldest CSI with insecticidal activity, discovered in the early 1970's. It was also the first registered commercial CSI under the trade name Dimilin. It affects insects by ingestion. Diflubenzuron is used against a wide range of insects, especially against leaf-chewing species. Binyason (1979) as well as some other investigators studied the effect of dimilin either on the eggs, on the advanced larval instars e.g. the 5th and 6th Instars, on pupae or on adults of *S. littoralis*. The present study aimed to test dimilin on young *S.littorlis* larvae.

MATERIALS AND METHODS

Rearing the cotton leafworm

The cotton leafworm *S.littoralis*was reared in a mass culture on semi-artificial diet used by Hegazi *et al*. (1977).This diet is made of: kidney beans 155 grams, Medical dried yeast 30 grams, Methyl-p-hydroxybenzoate 3.5 grams, Ascorbic acid 3.5 grams, Agar 14 grams, Formaldehyde 1.5 ml, Water (total) 650ml.

Test insecticide Dimilin:

(48% SC) was provided by Philips Daphar B.V. company. This compound belongs to the benzoyl-phenyl-urea group. It is commonly known Diflubenzuron (DFBZ) with the chemical name of 1-(4-

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Chlorophenyl)-3-(2,6-difluorobenxoyl) urea. The Molecular weight is 310.7.While the Empirical formula is C_{14} Hg cl F_2N_2 O_2.

Susceptibility of young *S.littoralis* larvae to dimilin toxicity

Tests were achieved using the semi-artificial medium or castor-oil leaves as larval diets under laboratory conditions.

A-Use of semi-artificial medium

Preliminary tests were carried out to determine the suitable series of concentrations of the tested material. Each concentration was dissolved in 10 ml water and then mixed with the diet to obtain an end volume of 100 ml diet. The latter mixture was stirred for about 5 minutes by means of an electric stirrer while the diet was still soft at 50-55˚C and left for about 2hrs to solidify to be suitable as larval diet (Kares,1978). The solidified diet was cut to small cubical pieces, ca. cm^3, and distributed into small plastic cups. The larvae were distributed inside the previous cups at the rate of 10 larvae/cup and three replicates were made to investigate the effect of each tested concentration. The larvae were firstly starved for ca. 6hrs in order to obtain rapid simultaneous ingestion of the diet and then were allowed to feed continuously on these bioassay feeding diets. Tested larvae were provided daily with fresh diet until death or successful development to the adult stage.

Three concentrations of "dimilin" 12.5, 25 were used and 50ppm|100 ml diet. The above concentration tests were evaluated against the 1st, 2nd and 3rd larval instars.

b- Use of castor been-oil leaves

The above mentioned concentrations of dimilin, used with the semi-artificial diet, were also carried out in case of castor-oil leaves. Each concentration was prepared in 100 ml water and about 6 clean leaves of castor been-oil leaves were dipped in the insecticide suspension for about 5 seconds and then left to dry for one hr. The dipped leaves were distributed into clean plastic cups. The same larval instars of *S.littoralis* and the same replicates, carried out in the case of treated artificial diet were also used. Larvae treated with dimilin were 1stand the 2ndinstars. Larvae pertaining to either of the aforementioned instars were offered to dimilin-treated castor been-oil leaves reared with dimilin for 24,48 or 72 hrs and were then left to feed on untreated leaves until death or successful development to the following stages. All dimilin-treated larvae were held at laboratory conditions at 25± 2˚C and 60±4% R.H with 14 hrs. light photophase.

Statistical analysis

Percentages of corrected mortality were assessed according to Abbot's formula (1925):

$$\frac{\text{\% test mortality- \% control mortality}}{\text{100- \% control mortality}} \times 100$$

LC_{50} values at 5% confidence limits, and slopes of regression lines were determined by using the probit analysis statistical method of Litchfield and Wilcoxon (1949) Analysis of variance and Duncan's multiple range test (Duncan, 1955) were applied to estimate significance of differences among the treatments of tested larvae .

RESULTS AND DISCUSSION

Susceptibility of *S.littoralis* larvae to diet incorporated with location

Results in Table (1) show the response of the 1st, 2ndand 3rdinstar larvae of *S.littoralis* fed from the beginning of each instar on semi-artificial diet containing different concentrations (ppm) of dimilin used as ppm. As tabulated in Table (1) the low concentration of 12.5 ppm or 25 ppm caused either to be more effective than , or equally effective against *S.littoralis* larvae as the higher concentration (50 ppm) ($F = 3791.31$, $df = 3,8$ $P< 0.05$). This result might be attributed to mode of action of the chitin synthetic inhibitor under investigation or to the amount of contaminated diet consumed by each of the tested larval instars. First instar was significantly susceptible than older ones ($F = 144.3$;$df = 3,8$;$P< 0.05$). On the other hand, Binyason (1979) reported that dimilin has neither deterrent nor phagostimulant effects on the 5th& 6thinstar of larvae *S.littoralis*. He also reported that the checking effect of this compound on the tested larvae was enhanced with the dose increase as 25 ppm, of the compound caused 50% prohibition of the adult formation, while 250 ppm caused 100% effect. The deviation of the differences was in the recovery and exposure periods. Moreover, this compound might exhibit phagostimulant or deterrent effects on younger instars. To clarify this point, tests should be done on the basis of the delivery of dosages calculated as µg compound/g larval body weight. The contact activity of the compound under investigation, reported before by Mulder &Gijsnijt (1973), Mulder&Swennen, 1973 and Binyason (1979) might contributes to the fact that 1st instar larvae with relatively more exposed surface succumbed to death in few hours (LT_{50} =43-60 hrs)shorter than those needed for the 2nd (LT_{50} =68-75hrs) or the 3rd (LT_{50} =51-60 hrs.) instar larvae (Table 2).

Inhibition of reproduction in *S.littoralis* with the antimoulting compound, dimilin was obtained by larval treatment (Radwan *et al*., 2009). Promising results were obtained when using the antimoulting agent, dimilin,

against phytophagous insects *e.g.Agrotis ipsiolon*, *Pectinophor agossypiella* and *S.littoralis* Binyamen *et al*. 2014; Abd El-Salam *et al*., 1979.

Young instars liable to repeated molting to develop to adult stages are expected to suffer from dimilin as a molting inhibitor (Tamaki& Turner,1974) or prohibiting chitin synthesis (Post &Vincent,1973) than those more grown up larvae or pupae which are supposed to undergo less number of molts during their development. The present results further indicate that after relatively long exposure periods of 120 and 144 hrs covering the period needed by each of the tested instars to molt, the tested concentrations, were equally toxic (F = 1566;df = 3,8; *P*< 0. 05). (Table1). All treated larvae succumbed to death before reaching the prepupal stage as they suffered mortality during molts between larval instars, a result which is in accordance with the findings of Ascher&Nemny (1976) they found that *S.littoralis* larvae weighing 100-150 mg each, when fed continuously for 2 days on dimilin-baited alfalfa or wheat bran, dead during molting between larval instars interference, while larvae weighing more than 150 mg died in the prepupal stage. Concerning the present range of concentrations of dimilin (12.5-50 ppm), it was found to be far less than those investigated by Abd-el-Salam *et al* (1979) against *A.ipsilon* (3750 ppm), but relatively higher than the concentrations used against *P.gossypiella* (1-10 ppm) (Flint *et al*., 1978).

Table 1. Corrected mortality percentage of *S.littoralis* larvae, fed continuously on semi-artificial diet incorporated with different concentrations of dimilin

			Exposure time	(hrs).		
Concentration of Dimilin (ppm)	**24**	**48**	**72**	**96**	**120**	**144**
			1st. instar	**larvae**		
0.00	0.00	0.00	0.00	0.00	0.00	3.33
12.5	20.00	46.66	80.00	90.00	100.00	100.00
25	16.66	46.66	80.00	86.66	96.66	100.00
50	3.33	30.00	70.00	86.66	93.33	96.66
			2nd. Instar	**larvae**		
0.00	0.00	0.00	0.00	0.00	0.00	0.00
12.5	3.33	20.00	53.33	86.66	93.33	96.66
25	10.00	13.33	50.00	73.33	80.00	90.00
50	13.33	16.66	50.00	73.33	83.33	93.33
			3rd. instar	**larvae**		
0.00	3.33	3.33	3.33	3.33	3.33	3.33
12.5	23.00	50.00	56.66	70.00	83.33	93.33
25	20.00	43.33	53.33	60.00	80.00	83.33
50	20.00	46.66	63.33	86.66	93.33	100.00

ANOVA among instars: F = 144.3; df = 3,8; *P*< 0. 05
ANOVA among hours :F = 1566;df = 3,8; *P*< 0. 05.
ANOVA for concentrations: F = 3791.31; df = 3,8; *P*< 0. 05.

Table 2. The median lethal time of 1st., 2nd. &3rd. instar *S.littoralis* larvae fed continuously on semi-artificial diet contaminated with known concentrations of Dimilin

Concentration (ppm)	**Instar tested**	**LT_{50} in hours**	**LT_{50}**	**S**			
12.5	1st.	43	1.86	51.60	35.83	2.36	1.47
25		47	1.93	57.34	38.52	2.47	1.51
50		60	1.56	70.20	51.28	1.92	1.27
12.5	2nd.	68	1.47	76.16	60.71	1.68	1.00
25		75	1.58	87.75	64.10	1.83	1.36
50		72	1.6	82.08	63.16	1.92	1.33
12.5	3rd.	55	2.28	66.0	54.83	3.00	1.73
25		60	2.63	72.0	50.00	3.73	1.85
50		51	1.81	62.22	41.80	2.26	1.45

S: The slope function.. Confidence limits S (5% probability) of

Table 3. Corrected mortality percentages of the 1st instar larvae of *S. littoralis* fed continuously on castor bean-oil leaves contaminated with certain concentrations of dimilin

Concentration (ppm)	Exposure time (days).							
	2	5	8	11	14	17	20	23
				24 hrs				
0.00	0.00	3.33	3.33	3.33	6.66	6.66	6.66	10.00
12.5	16.66	26.66	43.33	63.33	70.00	73.33	80.00	83.33
25	23.33	36.66	46.66	63.33	66.66	70.00	70.00	70.00
50	20.00	46.66	56.66	66.66	70.00	80.00	83.33	86.66
				48 hrs				
0.00	0.00		3.33	6.66	6.66	6.66	6.66	6.66
12.5	20.00		66.66	70.00	70.00	73.33	76.66	76.66
25	30.00		56.66	6.00	66.66	70.00	70.00	70.00
50	20.00		60.00	60.00	63.33	63.33	66.66	70.00
				72 hrs				
0.00	3.33	6.66	6.66	6.66	10.00	10.00	10.00	
12.5	40.00	6.66	70.00	73.33	76.00	86.66	86.66	
25	30.00	56.66	70.00	80.00	80.00	80.00	83.33	
50	26.66	63.33	90.00	90.00	90.00	90.00	100.00	

ANOVA among feeding periods:F =1036.5; df = 2,6; *P*< 0. 05
ANOVA among days: F =1856.1; df = 2,6; *P*< 0. 05
ANOVA among concentrations: F =12714.9; df = 2,6; *P*< 0. 05

Table 4. Corrected mortality percentage of the 2nd instar larvae of *S. littoralis* fed fed continuously on castor bean-oil leaves contaminated with certain concentrations of dimilin

Concentration in (ppm)	Exposure time (days)						
	2	5	8	11	14	17	20
			24 hrs				
0.00	0.00	0.00	3.33	3.33	6.66	6.66	10.00
12.5	6.66	33.33	43.33	56.66	63.33	70.00	70.00
25	6.66	23.33	30.00	43.33	46.66	56.66	56.66
50	16.66	43.33	50.00	53.33	60.00	66.66	70.00
			48 hrs				
0.00	0.00		6.66	6.66	10.00	10.00	
12.5	16.66		60.00	66.66	70.00	80.00	
25	26.66		76.66	76.66	80.00	80.00	
50	13.33		70.00	73.33	76.66	80.00	
			72 hrs				
0.00	0.00	3.33	3.00	6.66			
12.5	23.33	63.33	73.33	80.00			
25	26.66	86.66	96.66	96.66			
50	30.00	73.33	90.00	90.00			

ANOVA among feeding periods : F =2968.30; df = 2,6; *P*< 0. 05
ANOVA among days : F == 2264.4; df = 6,4; *P*< 0. 05
ANOVA among concentration : F =10646.1; df = 3,8; *P*< 0. 05

Although all the aforementioned concentrations tested by oral administration on larval food, the exaggerations was noticed by using very high concentrations (Abd-El-Salam *et al.,*1979). The exposure period to the treated food might be the sole reason to compensate for such conflict.

Effect of exposure periods on larval mortality rates

The effect of feeding the first and the second *S. littoralis* larval instars on castor bean-oil leaves dipped in certain concentration of 12.5, 25 & 50 ppm . As shown in Tables 3and 4. The lowest concentration of 12.5 ppm proved more potent than the other concentration tested (for the 1st instar: F =12714.9; df

= 2,6; $P< 0.05$;for the 2nd instar: F =10646.1; df = 3,8; $P< 0.05$). However statistical analysis of the data showed that the higher concentration accelerated mortality as the LT_{50} values were 60,47 & 43 hrs for 50,25&12.5 ppm treatments in respect. On the other hand, increasing the exposure period to 72 hrs, the high concentration treatments of 25 &50 ppm accelerated death as the LT_{50}values were 75 & 72 hrs in respect, while the LT_{50} for 12.5 ppm was 68 hrs. To be highly potent the residues of Dimilin under field conditions should be stable enough to cover a period not less than 72 hrs which is needed to let the larvae ingest sufficient quantities of the compound to produce 100% failure of adult formation.

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الملخص العربي

تأثير الديميلين Dimilin على يرقات دودة ورق القطن الصغيرة سبودبترا ليتولارز

رضا عبد السميع هندى، حسين موسى، عصمت حجازى

أجريت عدة تجارب تحت ظروف المعمل لتحديد قابلية الأطوار الأولى من يرقات دودة ورق القطن- سبودبترا ليتولارز إلى مادة مضادة الانسلاخ. وأشارت النتائج المتحصل عليها الى ان استجابة العمر الأول والثاني والثالث لدودة ورق القطن المغذاه باستمرار من بداية كل عمر، على بيئة شبه صناعية محتوية على تركيزات الديميلين المختلفه والمحسوبة كجزء في المليون. كانت النتائج غير متوقعة وذلك لان التركزيات المنخفضه (١٢,٥ أو٢٥ جز فى المليون) اثبتت احيانا اما أنها كانت أكثر تأثيرا على دودة ورق القطن أو مساويه للتركيزات المرتفعه ٥٠ جزء فى المليون. ربما تعزى هذه النتائج إلى سلوك المبيد المنظم للنمو المختبر أوالى كمية البيئة المستهلكه الملوثه لكل من العمر اليرقى المختبر. علاوة على ذلك فانه ربما يظهر هذا المركب تأثيرا مشجعا للتغذية، أو تأثيرا مانعا للتغذية ضد الأعمار الصغيرة ايضا ربما يعزى نشاط المركب كمبيد بالملامسة الى أن يرقات العمر الاول ذات السطوح الاكثر عرضه للمبيد، استسلمت للموت فى فترات(٤٣ -٦٠ ساعه لقتل ٥٠ % من الافراد) وهى أقصر من تلك التي يحتاجها العمر الثاني (٦٨ -٧٢ ساعه لقتل ٥٠ % من الافراد). ولكى نعرف تأثير فترة التعريض للديملين على معدلات موت اليرقات، فـأنه تم عمل ثلاث مجموعات ليرقات دودة ورق القطن لكل من العمر الأول والثانى ووسمح لها بالتغذية على ورق خروع مغمور في تركيزات معينه من الديميلين، وكانت التغذية على الأوراق الملوثه مستمرة لفترات تعريض مختلفة وهى ٢٤و٤٨ و٧٢ ساعه ثم قدم لليرقات بعد ذلك أوراق غير معاملة حتى سجلت نتائج الموت. وقد أفاد التحليل الاحصائى للنتائج ان التركيزيات المرتفعه اسرعت من الموت حيث كانت قيم الزمن المتوسط القاتل ل٥٠ % من الافراد هو ٤٣ و٤٧ و٦٠ ساعة لمعاملات التركيزات (٥٠ و٢٥ و١٢,٥ جزء في المليون) على التوالي. وعندما كانت فترة التعريض ٧٢ ساعه، وجد أن معاملات التركيزات ٢٥و٥٠ جزء في المليون اسرعت من الموت حيث كان الوقت اللازم لموت ٥٠% من الافراد هي ٧٠ و٧٢ ساعه على التوالي، بينما كانت ٦٨ ساعه للتركيز ١٢,٥ جزء في المليون.

ولكى تكون متبقيات الديميلين ذات سمية فعالة تحت الظروف الحقلية فانه يجب ان تبقى هذه المتبقيات ثابته لفترة كافيه لا تقل عن ٧٢ ساعة حتى تستطيع اليرقات تناول كميات كافية من المبيد لتكون نسبة فشل تكوين الحشرات الكاملة ١٠٠%.