

TOXICOLOGICAL AND LETENT EFFECTS OF CERTAIN COMPOUNDS ON THE BLACK CUTWORM *AGROTIS IPSILON* (HUFN) IN THE LABORATORY.

BY

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ABSTRACT

This study carried out in the laboratory to evaluate the toxicity of chloropyrifos 48% (chlorozan) one of the organophorus insecticides, neemix (azadirachtin 4.5%) and Dipel-2x (*Bacillus thuringiensis*) against *Agrotis ipsilon*. Data obtained showed that, chlorozan had the greatest lethal effect on 4th instar larvae of *A.ipsilon*. The highest mortalities (98%) had been occurred after the treatment with 0.125ppm concentration at 72h. Data also revealed that the concentration of Neemix 4.5% lead to lower mortality percentage for 4th instar larvae of *A. ipsilon* treatments with *Bacillus thuringiensis* (Dipel-2x) on the 4th instar larvae of *A. ipsilon* indicated that, there was no larval mortality during the first day after treatment. The mortality increased by time dapsing after treatment with Bt. Pathogen and reached 60% after 7 days post treatment with 2g/100ml Dipel- 2X.

INTRODUCTION

The lepidopterous insects are considered the most polyphagous insect pest in Egypt. They attack more than 70 different field crops including vegetables, fruit trees, ornamental plants, medicinal and aromatic plants, besides weeds and wild plants. One of this pests, is the black cutworm, *Agrotis ipsilon* causes serious damage to different parts of plants beside cutting of the stems under ground causing death of seedlings. In

Egypt, changing of climatic and environmental conditions are not drastic enough to play an important role in the size of infestation. So our work in this part carried out in laboratory to evaluate the toxicity of one organophorus insecticides (chloropyrifos 48%), one plant extract (Neemix 4.5%)and also one bacterial insecticide (Diple-2x) against 4th instar larvae of *Agrotis ipsilon*.

MATERIAL AND METHODS

The culture of *A. ipsilon* used in this study originated from eggs obtained from susceptible laboratory strain established in the Black cutworm Department, of Plant Protection Research Institute, Dokki, Giza. The culture was maintained and built up under 27 + °C 4th instar larvae were chosen randomly and individually left without feeding for 24 hours before treatment. All experiments were also conducted under the same temperature.

1) Insecticides and concentrations:

- a- Chemical insecticide chloropyrifos 48% (chlorozan)
(0.125 – 0.063 – 0.032 – 0.02 – 0.01) ppm
- b- Plant extract Neemix (azadirachtin 4.5%)
(100 – 50 – 25 – 12.5 – 6.3)ppm
- c- *Bacillus thuringiensis* var. Rurstaki Dipel-2x (32000 international Units)
(2 – 1 – 0.5 – 0.25 – 0.125 gm/100ml)

2) Treatment of the larvae:

Hundred of 4th instar larvae of *Agrotis ipsilon*, for each concentration in 4 replicates were used: Each Replicate was of 25 larvae and each larva transferred into a small plastic jar. Larvae were starved for 24 hours and then fed for 24 hours on (caster bean) leaves discs dipped for 10 seconds in the various concen-

trations then air dried. After 24 hours of treatment the survived larvae were transferred into another clean jars, and supplied daily with untreated leaves until pupation. Mortality was recorded 1,2,3 day after the treatment with Chlorozan, 1, 2, 3, 4, 5 day Neemix and 1, 2, 3, 4, 5, 6, 7 days to Dipel-2x.

RESULTS AND DISCUSSION

Effect of various compounds on the 4th instar larvae of *A. ipsilon* under laboratory condition.

The effect of Chlorozan concentrations as tested on the fourth larvae instar of *A. ipsilon* is given in Table (1). Generally results showed moderate mortality after 24h at the three higher concentration and increased gradually within the second and third day to reach very high mortality (98%) by the treatment of 0.125ppm moderate (66%) at 0.063ppm. Data demonstrated in Table (2) revealed that the neemix 4.5% lead to lower mortality percentages against 4th instar larvae of *A. ipsilon* at the higher two concentrations. The highest mortality (83%) was recorded after 6 day with 100ppm. The lower tested concentrations (6.25 - 12.5 & 25ppm) had the lowest effect on 4th instar larvae of *A. ipsilon* even after 5 days causing 19, 29 & 39% mortality, respectively.

Data of the effect of *Bacillus thuringiensis* on the 4th larval instar of *A. ipsilon* tabulated in Table (3) indicate that there was no larval mortality during the first day after treatment with different tested concentrations of Dipel-2x. The mortality among the 4th larval instar slightly increased to reach 60, 49, 29, 21 and 13% for concentrations of 2, 1,

0.5, 0.25 & 0.125 g/100ml, respectively after 7 day. The corresponding percentage after 4 days of treatment increased to reach (22, 22, 11, 10 & 4%) at the same concentration of dipel-2x respectively while after 7 days the mortality reached (60, 49, 29, 21 & 13%) at the same concentration. The mortalities among untreated 4th larval instar were zero% within the same testing intervals .

These results agree with those obtained by Elham and El-Sayed (1991) and Khurana and Kamshik (1991) who studied and evaluated the efficacy of organo-phosphates (chlorpyrifos) for control of *Agrotis ipsilon*.

The result of neemix agree with those obtained by El-Sayed (1983a) who reported that the neem oil caused 100% mortality larval instar of *S. littoralis* when fed on treated leaves with 0.2 to 0.5 % concentration. In this scale Senh *et al.* (1981); Rizk *et al.* (1977) and Rizk *et al.* (1981) found that the susceptibility of *S. littoralis* & *Agrotis ipsilon* were decreased with the increase of the larval age post treatment with *B. thuringiensis* and the first three instars were more susceptible than the older once.

Table (1): Effect of Chlorozan (Chlorpyrifos) on 4th larval instar of *Agrotis ipsilon* at different time

Conc. ppm	Mortality (%) Period after treatment		
	24 h.	48 h.	72 h.
0.125	44	90	98
0.063	26	46	66
0.032	20	32	43
0.02	6	14	20
0.01	0	0	7

Table (2): Effect of Neemix (azadirachtin) on 4th of larval instar of *Agrotis ipsilon* at different time.

Conc. ppm	Mortality (%)				
	Period after treatment				
	24 h.	48 h.	72 h.	96 h.	120 h.
100	18	47	61	75	83
50	10	27	39	47	52
25	0	1	11	24	32
12.5	0	0	5	18	29
6.25	0	0	5	11	19

Table (3): Effect of Dipel-2x on 4th larval instar of *Agrotis ipsilon* at different time.

Conc. g/100ml	Days after treatment						
	1 day	2 day	3 day	4day	5 day	6day	7day
2	0	4	12	22	31	44	60
1	0	4	12	22	31	47	49
0.5	0	0	2	11	17	23	29
0.25	0	2	7	10	15	17	21
0.125	0	0	2	4	5	9	13

Latent effects:

Larval duration:

Data in Tables (4, 5 and 6) showed that chlorozan, Neemix and Dipel-2x compounds caused prolonged larval duration for the survival larvae after treatment, but the influence of the three compound proved to be non-significant. Mean larval duration increased from 24.3 days in untreated check to about 27.3, 27.4 and 27.02 days for chlorozan, Neemix and Dipel-2x, respectively.

Pupal duration

Results obtained in Tables (4, 5 and 6) showed that pupal duration of *Agrotis ipsilon* increased, significantly, as influenced by chlorozan, Neemix, Dipel-2x compared with untreated larvae. Mean pupal duration increased from 9.5 day in control check to about ranged between 9.5 – 11.3 days for the three compounds under study.

Table (4): Latent effects of chlorozan on 4th larval instar of *A. ipsilon*

Conc. ppm	% Mortality	Larval Duration (days)	Pupal Duration (days)	% Pupation	No. of adults Emergence	% Adults emergence	% malformation		No. of Eggs/ female	No. of Hatching	%H
							Pupae	adults			
0.125	98 ^a	27.8	10	2	1 ^c	50	0 ^d	0 ^c	0	0 ^a	0
0.063	89 ^b	27.8	9.5	11	6 ^d	54.5	9.1 ^c	0 ^c	0	0 ^d	0
0.032	74 ^c	27	10	24	16 ^c	66.7	12.5 ^b	6.3 ^a	253	114.3 ^c	45.3
0.02	52 ^d	27	10	48	38 ^b	79.2	8.3 ^c	5.3 ^{ab}	299.3	158.7 ^b	53
0.01	25 ^e	27	10.3	75	61 ^a	81.3	16 ^a	4.9 ^b	543.7	370 ^a	68.1
Mean	67.6	27.32	9.96	32	24.4	66.34	9.18	3.3	219.2	128.6	33.28
Control	0	24.3	9.5	96	86	89.6	0	0	923.7	808.7	87.5
F	2604.58	2.286 _{NS}	0.369 _{NS}		2779.38		681.15	207.4		175557.68	
L.S.D	2.701	0.945	1.54		2.209		1.086	1.007		1.722	

Table (5): Latent effects of Neemix extract on 4th larval instar of *A. ipsilon*.

Conc. ppm	% Mortality	Larval Duration (days)	Pupal Duration (days)	% pupation	No. of adults emergence	% adults emergence	% malformation		No. of Eggs/ female	No. of Hatching	%H
							Pupae	adults			
100	98 ^a	28	10.5 ^a	9	5 ^a	55.6	22.2 ^a	0 ^c	0	0 ^d	0
50	82 ^b	28	10.5 ^a	15	10 ^d	66.7	20 ^b	0 ^c	241.3	119 ^d	49.3
25	70 ^c	27.5	10.5 ^a	28	20 ^c	71.4	10.7 ^{bc}	7.1 ^a	429.7	260.3 ^c	60.6
12.5	60 ^d	27.3	10.3 ^a	40	30 ^b	75	12.5 ^b	4 ^b	388	263 ^b	67.8
6.25	44 ^e	26	9.3 ^b	54	45 ^a	83.3	9.3 ^c	4.7 ^b	446.3	336.3 ^a	75.4
Mean	70.8	27.36	10.32	29.2	22	70.4	14.94	3.16	301.06	195.92	50.62
Control	0	24.3	9.5	96	86	89.6	0	0	923.7	808.7	87.5
F	1594.5	1.30 _{ns}	8.625		772.5		165.35	135.82		103394.95	
L.S.D	2.450	2.346	0.490		2.508		2.113	1.264		1099	

Table (6): Latent effects of Dipel-2x on 4th larval instar of *A. ipsilon*.

Conc. g/100ml	% Mortality	Larval Duration (days)	Pupal Duration (days)	% Pupation	No. of adults emergence	% Adults emergence	% Malformation		No. of Eggs/ female	No. of Hatching	%H
							Pupae	adults			
2	65 ^a	28.8 ^a	11.3 ^a	36	28 ^c	77.8	13.9 ^a	0 ^d	203	125.3 ^c	61.7
1	53 ^b	28.5 ^a	10.8 ^b	47	37 ^d	78.7	10.6 ^b	14.7 ^a	251	159 ^a	63.3
0.5	37 ^c	27 ^b	9.8 ^{bc}	63	50 ^c	79.4	11.1 ^b	10.4 ^c	223	162.3 ^c	72.8
0.25	27 ^d	26 ^c	10 ^{bc}	73	58 ^b	79.5	10.9 ^b	12.1 ^b	472	332 ^b	74.6
0.125	17 ^e	24.8 ^d	9.5 ^b	86	72 ^a	83.7	8.1 ^c	12.5 ^b	538	452.6 ^a	84.1
Mean	39.8	27.02	10.28	61	49	79.82	10.92	9.94	337.4	244.24	71.3
Control	0	24.3	9.5	96	86	89.6	0	0	923.7	808.7	87.5
F	213.125	202.29	9.396		1161.61		78.55	1200.8		115686.8	
L.S.D	6.002299	0.561	1.216		2.339		1.103	0.789		1.943	

Pupation percentage:

Analysis of variance of the data given in Tables (4, 5 and 6) indicates highly significance of Chlorozan and Neemix on percentage of pupation, but Dipel-2x showed non-significant effect. Mean percent of pupation decreased from 96% in untreated check to 75,54, and 86% at 0.01, 6.25ppm and 0.125g/100ml. concentration for Chlorozan, Neemix and Dipel-2x respectively. The results agree with that recorded by Rizk *et al.* (1977) who found that the pupation rate of *S. littorata* was decreased when larvae treated with Dipel-2x and thuricide HP. The reduction in pupation increased with the increase in bacterial concentration, the percentage of pupation of *S. littoralis* was decreased with increasing of Egyptian neem (Zanzalakht) (Emara, *et al.*, 2002).

Malformation percentage of pupal and adults:

The presented data in Tables (4, 5 & 6) indicate that the effect of Chlorozan and Neemix on percentage of malformation of emerged moths was significant whereas the influence of dipel-2x proved to be highly significant.

The impact of different concentrations of each compound on this biological aspects was highly significant for Neemix, Chlorozan and dipel-2x, respectively. Increasing the concentration of Neemix induced a gradual increase in percentage of malformation of emerged adults to show the highest of emerged adults of (22.2 & 20%) with the highest concentration (100 & 50 ppm). On the other hand in the case of dipel-2x & Chlorozan show percent malformation of emerged adults of 13.9%, 16% & 0.01% and 2g/100ml concentration, respectively.

These results agree with those obtained by El-Sayed (1983 b) who found that Neem extract caused malformation in adults of *S. littoralis*. Zanzalakt extracts caused malformation in the different stages of *A. ipsilon* (Abo-El Ghar *et al.*, 1994) and *S. littoralis* (Emara *et al.*, 2002).

Fecundity:

Statistical analysis in Tables (4, 5, & 6) indicate that the effects of both compound and their concentrations were highly significant. Mean number of laid eggs/female decreased from 923.7 egg/female in untreated check to (543.7, 446.3 & 538) egg /female at the lowest concentration increasing the concentration of either neemix and dipel-2x induced gradual reduction in number of laid eggs/female while in case of chlorozan there is

no number of eggs laid in the high concentration these result are similar to obtained by Abo El-Ghar *et al.* (1994) who found that acetone and ethanol extracts of zanzalkht caused decrease in the number of egg / female and percentage of hatching of *A. ipsilon* eggs .

Fertility of eggs:

Statistical analysis of variance of the data given in Tables (4, 5, & 6) shows that the percent of egg hatching was highly significantly affected by Chlorozan but the influence of Neemix and dipel-2x proved to be non - significant at the lowest concentration. Mean percentage of egg hatching decreased from 87.5- in untreated check to (68.7, 75.4 & 84.7%) for Chlorozan Neemix and dipel-2x respectively at the lower concentration.

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تأثيرات سمية وحيوية لبعض المركبات علي الدودة القارضة تحت الظروف المعملية

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** معهد بحوث وقاية النباتات - الدقى - القاهرة

أجرى هذا البحث بهدف تقدير السمية لكل من المركبات الكيميائية والطبيعية والحيوية والكلوروزان والنيمكس والدايبل - 2x بالترتيب على العمر الرابع ليرقة الدودة القارضة . وأسفرت النتائج المتحصل عليها أن الكلوروزان وهو المبيد الكيميائي له قدرة كبيرة في قتل يرقات تلك الحشرة والذي أعطى 98% موت بعد 72 ساعة من التعريض للمعاملة بتركيز 0,125 (جزء في المليون) . وأوضحت النتائج أيضا أن تأثير النيمكس على العمر اليرقي الرابع للدودة القارضة كان ضعيفا جدا. وأنه لم يحدث موت إطلاقا في اليوم الأول بعد استخدام تركيزات المبيد الحيوي (دايبل - 2x). وقد لوحظ من الدراسة أن نسب الموت المتسببة من المركب الحيوي قد زادت من 2، 7، 2، 12، 12، 12% موت وذلك عند التركيز 0,125، 0,25، 0,5، 1، 2 جرام لكل 100 مل ماء على الترتيب بعد ثلاثة أيام بينما في اليوم السابع وصلت نسب الموت إلى 13، 21، 29، 49، 60% لنفس التركيزات السابقة . وبالنسبة لقياس التأثيرات البيولوجية لهذه المركبات (الكلوروزان والنيمكس والدايبل - 2x) علي نمو وتطور الحشرة بعد معاملة يرقات العمر الرابع فقد أسفرت النتائج أنه بالنسبة لكل من:

- 1- فترة الطور اليرقي: اتضح من النتائج أن التركيزات العالية للمبيدات الثلاثة لها تأثيراتها الغير معنوية علي تلك الفترة مقارنة بالكنترول حيث كانت هناك زيادة ملحوظة في فترة الطور اليرقي فكانت 27,3، 27,4، 27، 27 يوم للمبيدات (الكلوروزان ، النيمكس ، والدايبل - 2x) علي التوالي مقارنة بالكنترول (24,3 يوم) .
- 2- فترة طور العذراء: زادت هذه الفترة زيادة معنوية ملحوظة عند تعريضها للمبيدات الثلاثة (الكلوروزان والنيمكس والدايبل - 2x) مقارنة بالكنترول .
- 3- نسبة التعذر: أثبتت التحليلات أنه يوجد تأثير معنوي للمركبات (الكلوروزان - النيمكس) علي نسبة التعذر ولكن الدايبل - 2x كان ضعيف التأثير .
- 4- نسبة التشوه في العذراء والحشرة الكاملة: أشارت البيانات المتحصل عليها أن تأثير الكلوروزان والنيمكس علي نسبة التشوه كان معنويا ولكن تأثير الدايبل - 2x كان عالي المعنوية .
- 5- الخصوبة: أشارت التحليلات الإحصائية أن تأثير المركبات المستخدمة كانت عالية المعنوية علي خصوبة الحشرة لذلك انخفض عدد البيض الموضوع لكل أنثى عنه في الكنترول .
- 6- نسبة الفقس: أظهرت النتائج المتحصل عليها انخفاض نسبة الفقس للبيض للمركبات الثلاثة (الكلوروزان والنيمكس والدايبل - 2x) عنه في الكنترول.