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EFFECT OF CERTAIN INSECTICIDES IN ENHANCING THE POTENCY OF *BACILLUS THURINGIENSIS* AGAINST THE COTTON LEAFWORM *SPODOPTERA LITTORALIS*

[42]

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ABSTRACT

The joint action of deltamethrin, chlorpyrifos-ethyl, methomyl and hexaflumuron with the biocides Xentari, Protecto and Dipel2x against the 2nd instar larvae of the cotton leafworm *Spodoptera littoralis*_was studied. The results indicate that the toxicity of the tested biocides was greatly enhanced by the LC_{50} and LC_{25} of deltamethrin in admixture with each of them. The LC_{25} of deltamethrin increased the toxicity of all experimental biocides by co-toxicity factors ranging between 46.67 and 92.94. Results also indicate that all tested biocides were potentiated when used in admixture with chlorpyrifos-ethyl particulary when used at its LC_{25} concentration. On the contrary, methomyl when used at the two sublethal concentrations caused antagonistic or additive effects for Xentari, Protecto and Dipel 2x. Most combinations of tested biocides with hexaflumuron resulted in additive effect. However, when hexaflumuron was used at its LC_{25} in admixture with the (recommended rate) of the tested biocides a reasonable synergism level was produced.

It may be concluded that the use of low rates of conventional insecticides such as pyrethroids and some organophosphorus compounds in combination with B.t. proved to be suitable to control the cotton leafworm Spodoptera littoralis.

INTRODUCTION

The bacterial biocide *Bacillus thuringiensis* has been used for insect control because of its environmental safety and specificity to most of the lepidopterous target insect pests. It is difficult, however, to effectively manage populations of the cotton leafworm with microbial insecticides alone because of their short half life, low insecticidal activity, and photo-decomposition. Attempts have been made to combine microbial and chemical insecticides to increase control efficacy and reduce the use of chemicals. **Dabi (1988)** repoted that the efficacy of insecticides could be increased when lower doses of *Bacillus thuringiensis* were applied to cotton bollworm. Pyrethroid insecticides historically have provided excellent control of many agricultural pests. However, widespread use of these insecticides has resulted in increase tolerance. **Wang *et al* (1994)** and **Tan *et al* (1998)** found that sublethal exposure of the boll worm *Helicoverpa armigera* to a *Bacillus thuringiensis* insecticide resulted in a decrease in Pyrethroid resistance. **Brickle *et al* (2001)** cited that lower rates of lambada-cyhalothrin, spinosad, and thiodicarb could be used for control of *Helicoverpa*

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zea populations in dry land transgenic *B.t.* cotton,system.

The purpose of the present study is to investigate the joint action of various insecticides, representing different groups, with the experimental *B.t.* biocides against the cotton leaf worm *Spodoptera littoralis*.

MATERIALS AND METHODS

Chemicals used

Three bio-insecticide formulations and four insecticides belonging to different functional groups were used throughout this investigation .

A. Bio-insecticides

1. Dipel 2X: It is a wettable powder formulation, based on *Bacillus thuringiensis* subsp. *kurstaki*. It contains lepidopteran active toxin 6.4% and 93.6% inert material, produced by Abbot laboratories, North Chicago IL-USA.

2. Xentari: It is a water dispersible granular formulation, based on *Bacillus thuringiensis* subsp.*aizawia*. It contains lepidopteran active toxin 10.3% and 89.7% inert material, produced by Abbot laboratories, North Chicago, USA.

3. Protecto: It is a wettable powder formulation, based on *Bacillus thuringiensis* subsp. *kurstaki*.. It contains lepidopteran active toxin 9.4% and 90.6% inert material, produced by the Plant Protection Research Institute, Agricultural Research Center, Dokki, Cairo, Egypt.

B. Insecticides

1. Chlorpyrifos – Ethyl: (Dursban 48% E.C), provided by : Dow chemical Co.

2. Methomyl: (Lannate 90% SP), provided by : Du pont de Nemours, USA.

3. Deltamethrin: (Decis 2.5 % E.C), provided by : Bayer Crop Science .

4. Hexaflumuron: (Growth Regulator) (IGR) (Consult 10% E.C), provided by : Dow Agro-Sciences .

Biological activity

The second-instar larvae of lab-strain of *Spodoptera littoralis* (Boisd), normally fed on castor–bean leaves, was used. Mortality percentages for insecticides and IGR were recorded after 24 and 72h, respectively, by leaf dipping method. The corrected percent mortalities were statistically computed according to **Finney (1971)**.

The joint action of the binary combinations of candidate tested insecticides (at their LC_{25} and LC_{50}) and biocides (at their full, ¾ and ½ recommended rates) was determined according to the equation of the co-toxicity factor given by **Mansour *et al* (1966)**. as followes :

$$CF. = \frac{Observed\ .mortality\ .(\%) - expected\ .mortality\ .(\%)}{expected\ .mortality\ .(\%)} X100$$

A positive factor of 20 or more is considered potentiation, a factor of – 20 or less means antagonism, and a value between -20 and + 20 is an additive effect expected .

RESULTS AND DISCUSSION

The data shown in **Table (1)** indicate that deltamethrin was the most toxic insecticide against the 2[nd] instar larvae of *Spodoptera littoralis* while methomyl was the least effective.

Data presented in **Table (2)** indicate that the experimental biocides (Xentari, Protecto and Dipel 2x) at their different tested rates were generally potentiated when used in admixture with LC_{25} and LC_{50} of deltamethrin. The highest potency was observed at LC_{25} of deltamethrin with the recommended rate of the tested biocides. The LC_{25} of deltamethrin increased the toxicity of all experimental biocides by co-toxicity factors ranging between 46.67 and 92.94.

The combined effect of deltamethrin with *B.t.* is probably related to the mode of action of synthetic pyrethoid. Pyrethrum affects the peripheral and central nervous systems resulting in rapid paralysis. Deltamethrin may also affect sodium and potassium permeability of insect cells and nitrogen metabolism. The parasporal crystal endotoxin of *B.t.* also causes rapid paralysis of the midgut and damages the selective permeability of the midgut epithelium of susceptible insects **(Fast and Angus, 1965)**.

Mortality decreased with increased deltamethrin rate at the highest *Bacillus thuringiensis* concentration. A possible explanation for this observation is that the higher concentrations of both the bacterial endotoxin and the neurotoxic pyrethroid would cause rapid knockdown and inhibit the test larvae from consuming enough bacterial spores to cause high mortality.

Table 1. Toxicity of some insecticides against 2nd instar larvae of the cotton leafworm, *Spodoptera littoralis*

Insecticides used	LC_{25} (ppm)	LC_{50} (ppm)	Slope
Chlorpyrifos – Ethyl	2.237	2.69	2.83 ± 0.57
Deltamethrin	0.879	1.39	1.44 ± 0.37
Methomyl	21.50	26.7	2.25 ± 055
Hexaflumuron	2.930	4.40	1.05 ± 0.37

Table 2. The joint action between tested *B.t.* biocides of deltamethrin against the 2nd instar larvae of *Spodoptera littoralis* after 24 h.

Treatment		Expected Mortality %	Observed Mortality %	Co-Toxicity factor
Xentari (103 pp m) (a)	Alone	9.0		
	+ Delta. LC_{25}	25.0 + 9.0 = 34.00	65.6	+ 92.94
	+ Delta. LC_{50}	50.0 + 9.0 = 59.00	71.0	+ 20.34
Xentari (77.25 pp m) (b)	Alone	4.0		
	+ Delta. LC_{25}	25.0 + 4.0 = 29.00	37.5	+ 29.31
	+ Delta. LC_{50}	50.0 + 4.0 = 54.00	67.2	+ 24.44
Xentari (51.5 pp m) (c)	Alone	2.0		
	+ Delta. LC_{25}	25.0 + 2.0 = 27.00	34.8	+ 28.89
	+ Delta. LC_{50}	50.0 + 2.0 = 52.00	65.3	+ 25.58
Protecto (141 pp m) (a)	Alone	5.0		
	+ Delta. LC_{25}	25.0 + 5.0 = 30.00	44.0	+ 46.67
	+ Delta. LC_{50}	50.0 + 5.0 = 55.00	75.35	+ 37.00
Protecto (105.75 pp m) (b)	Alone	3.0		
	+ Delta. LC_{25}	25.0 + 3.0 = 28.00	39.6	+ 41.43
	+ Delta. LC_{50}	50.0 + 3.0 = 53.00	67.5	+ 27.36
Protecto (70.5 pp m) (c)	Alone	0.0		
	+ Delta. LC_{25}	25.0 + 0.0 = 25.00	39.0	+ 56.00
	+ Delta. LC_{50}	50.0 + 0.0 = 50.00	61.0	+ 22.00
Dipel2x (64 pp m) (a)	Alone	7.0		
	+ Delta. LC_{25}	25.0 +7.0 = 32.00	61.2	+ 91.25
	+ Delta. LC_{50}	50.0 + 7.0 = 57.00	79.6	+ 39.65
Dipel2x (48 pp m) (b)	Alone	4.0		
	+ Delta. LC_{25}	25.0 +4.0 = 29.00	36.25	+ 25.0
	+ Delta. LC_{50}	50.0 + 4.0 = 54.00	64.8	+ 20.00
Dipel2x (32 pp m) (c)	Alone	0.0		
	+ Delta. LC_{25}	25.0 + 0.0 = 25.00	32.8	+ 31.25
	+ Delta. LC_{50}	50.0 + 0.0 = 50.00	53.4	+ 6.80

Whereas : (a) = full rate, (b) = ¾ recommended, (c) ½ recommended rates of the tested biocides, respectively

Table 3. The joint action between tested *B.t.* biocides of cloropyrifos- –ethyl 48 % E.C. against the 2nd instar larvae of *Spodoptera littoralis* after 24 h.

Treatment		Expected Mortality %	Observed Mortality %	Co-Toxicity factor
	Alone	9.0		
Xentari (103 pp m) (a)	+ Cloro. LC_{25}	25.0 + 9.0 = 34.00	60.1	+76.76
	+ Cloro. LC_{50}	50.0 + 9.0 = 59.00	87.4	+ 48.14
	Alone	4.0		
Xentari (77.25 pp m) (b)	+ Cloro. LC_{25}	25.0 + 4.0 = 29.00	42.8	+47.59
	+ Cloro. LC_{50}	50.0 + 4.0 = 54.00	83.3	+54.26
	Alone	2.0		
Xentari (51.5 pp m) (c)	+ Cloro. LC_{25}	25.0 + 2.0 = 27.00	35.6	+31.85
	+ Cloro. LC_{50}	50.0 + 2.0 = 52.00	70.9	+36.37
	Alone	5.0		
Protecto (141 pp m) (a)	+ Cloro. LC_{25}	25.0 + 5.0 = 30.00	51.8	+ 72.67
	+ Cloro. LC_{50}	50.0 + 5.0 = 55.00	61.5	+11.82
	Alone	3.0		
Protecto (105.75 pp m) (b)	+ Cloro. LC_{25}	25.0 + 3.0 = 28.00	47.6	+70.00
	+ Cloro. LC_{50}	50.0 + 3.0 = 53.00	57.2	+ 7.92
	Alone	0.0		
Protecto (70.5 pp m) (c)	+ Cloro. LC_{25}	25.0 + 0.0 = 25.00	45.6	+82.4
	+ Cloro. LC_{50}	50.0 + 0.0 = 50.00	53.0	+6.00
	Alone	7.0		
Dipel2x (64 pp m) (a)	+ Cloro. LC_{25}	25.0 +7.0 = 32.00	48.0	+50.00
	+ Cloro. LC_{50}	50.0 + 7.0 = 57.00	68.6	+20.35
	Alone	4.0		
Dipel2x (48 pp m) (b)	+ Cloro. LC_{25}	25.0 +4.0 = 29.00	41.4	+ 42.76
	+ Cloro. LC_{50}	50.0 + 4.0 = 54.00	64.9	+20.19
	Alone	0.0		
Dipel2x (32 pp m) (c)	+ Cloro. LC_{25}	25.0 + 00 = 25.00	41.2	+ 64.80
	+ Cloro. LC_{50}	50.0 + 00 = 50.00	60.0	+20.00

Whereas : (a) = full rate ,(b) = ¾ recommended, (c) ½ recommended rates of the tested biocides , respectively

Table 4. The joint action between tested *B.t.* biocides of methomyl 90 % SP against the 2nd instar larvae of *Spodoptera littoralis* after 24 h.

Treatment		Expected Mortality %	Observed Mortality %	Co-Toxicity factor
	Alone	9.0		
Xentari (103 pp m) (a)	+ Metho. LC_{25}	25.0 + 9.0 = 34.00	32.8	- 3.53
	+ Metho. LC_{50}	50.0 + 9.0 = 59.00	37.6	- 36.27
	Alone	4.0		
Xentari (77.25 pp m) (b)	+ Metho. LC_{25}	25.0 + 4.0 = 29.00	25.2	- 13.10
	+ Metho. LC_{50}	50.0 + 4.0 = 54.00	32.2	- 40.37
	Alone	2.0		
Xentari (51.5 pp m) (c)	+ Metho. LC_{25}	25.0 + 2.0 = 27.00	10.3	- 61.85
	+ Metho. LC_{50}	50.0 + 2.0 = 52.00	31.2	- 40.00
	Alone	5.0		
Protecto (141 pp m) (a)	+ Metho. LC_{25}	25.0 + 5.0 = 30.00	26.7	- 11.00
	+ Metho. LC_{50}	50.0 + 5.0 = 55.00	37.1	- 32.55
	Alone	3.0		
Protecto (105.75 pp m) (b)	+ Metho. LC_{25}	25.0 + 3.0 = 28.00	17.6	- 37.14
	+ Metho. LC_{50}	50.0 + 3.0 = 53.00	27.1	- 48.87
	Alone	0.0		
Protecto (70.5 pp m) (c)	+ Metho. LC_{25}	25.0 + 0.0 = 25.00	6.8	- 72.80
	+ Metho. LC_{50}	50.0 + 0.0 = 50.00	25.0	- 50.00
	Alone	7.0		
Dipel2x (64 pp m) (a)	+ Metho. LC_{25}	25.0 +7.0 = 32.00	24.0	- 25.00
	+ Metho. LC_{50}	50.0 + 7.0 = 57.00	38.0	- 33.33
	Alone	4.0		
Dipel2x (48 pp m) (b)	+ Metho. LC_{25}	25.0 +4.0 = 29.00	22.7	- 21.72
	+ Metho. LC_{50}	50.0 + 4.0 = 54.00	32.1	-40.5
	Alone	0.0		
Dipel2x (32 pp m) (c)	+ Metho. LC_{25}	25.0 + 00 = 25.00	17.4	- 30.40
	+ Metho. LC_{50}	50.0 + 00 = 50.00	30.3	- 39.40

Whereas : (a) = full rate ,(b) = ¾ recommended, (c) ½ recommended rates of the tested biocides , respectively.

Table 5. The joint action between tested *B.t.* biocides of hexaflumuron 10 % E.C. against the 2[nd] instar larvae of *Spodoptera littoralis* after 72 h.

Treatment		Expected Mortality %	Observed Mortality %	Co-Toxicity factor
Xentari (103 pp m) (a)	Alone	22.4		
	+ Hexa. LC_{25}	25.0 + 22.4= 47.4	57.4	+ 21.09
	+ Hexa.. LC_{50}	50.0 + 22.4= 72.4	84.3	+ 16.44
Xentari (77.25 pp m) (b)	Alone	18.4		
	+ Hexa. LC_{25}	25.0 + 18.4= 43.4	44.4	+ 2.30
	+ Hexa.. LC_{50}	50.0 + 18.4= 68.4	73.1	+ 6.87
Xentari (51.5 pp m) (c)	Alone	9.2		
	+ Hexa. LC_{25}	25.0 + 9.2= 34.2	34.2	0.0
	+ Hexa.. LC_{50}	50.0 + 9.2 = 59.2	67.8	+14.50
Protecto (141 pp m) (a)	Alone	13.3		
	+ Hexa. LC_{25}	25.0 + 13.3 = 38.30	51.6	+34.73
	+ Hexa.. LC_{50}	50.0 + 13.3= 63.3	77.9	+ 23.06
Protecto (105.75 pp m) (b)	Alone	10.2		
	+ Hexa. LC_{25}	25.0 + 10.2= 35.2	39.1	+11.1
	+ Hexa.. LC_{50}	50.0 + 10.2 = 60.2	63.6	+ 5.65
Protecto (70.5 pp m) (c)	Alone	4.0		
	+ Hexa. LC_{25}	25.0 + 4.0= 29.00	37.2	+ 28.27
	+ Hexa.. LC_{50}	50.0 + 4.0= 54.00	61.4	+13.70
Dipel2x (64 pp m) (a)	Alone	18.4		
	+ Hexa. LC_{25}	25.0 +18.4= 43.4	67.7	+ 55.99
	+ Hexa.. LC_{50}	50.0 + 18.4= 68.4	77.6	+ 13.45
Dipel2x (48 pp m) (b)	Alone	15.3		
	+ Hexa. LC_{25}	25.0 +15.3= 40.3	56.6	+ 40.45
	+ Hexa.. LC_{50}	50.0 + 15.3= 65.3	67.7	+ 3.67
Dipel2x (32 pp m) (c)	Alone	7.1		
	+ Hexa. LC_{25}	25.0 + 7.1 = 32.1	40.0	+ 24.61
	+ Hexa.. LC_{50}	50.0 + 7.1 = 57.1	63.3	+ 10.86

Whereas : (a) = full rate ,(b) = ¾ recommended, (c) ½ recommended rates of the tested biocides , respectively.

The joint action between LC_{25} and LC_{50} of chloropyrifos – ethyl and tested *Bacillus thuringiensis* biocides against the 2nd instar larvae of *Spodoptera littoralis* is tabulated in **Table (3)**. All experimental biocides were almost potentiated when used in admixture with chloropyrifos– ethyl specially at its LC_{25}. The toxicity was increased by a co - toxicity factor ranging between 31.85 (Xentari) and 82.4 (Protecto).

Data shown in **Table (4)** indicate the joint action between all the tested *B.t.*biocides, *and* LC_{25} and LC_{50} of methomyl against the 2nd instar larvae of *Spodoptera littoralis* The different tested rates of *B.t.* biocides exhibited antagonistic or additive effects when applied in admixture with methomyl at LC_{25} and LC50 .

Most combinations of biocides with the IGR (hexaflumuron) resulted in additive effect **Table (5)**. However, when the LC_{25} of hexaflumuron was used in admixture with the high concentration of all the tested biocides, a level of synergism was produced. The LC_{25} of hexaflumuron increased the toxicity by co- toxicity factors of 21.09, 34.73 and 55.99 for Xentari, Protecto and Dipel 2x, respectively.

Salama *et al* (1984) conducted some studies on the effects of chemical insecticides of different functional groups on sporulation yields of *B.t.* var *entomocidus*. Among the carbamates tested, carbaryl exhibited a more deleterious effect on the sporulation process of *B.t.* than methomyl. The pyrethroids and most organophosphorus compounds tested potentiated the activity of *B.t.*applied against *Spodoptera littoralis*. The carbamates, IGR diflubenzuron and a combination of methomyl and diflubenzuron showed an additive effect when jointly applied with *B.t.* varieties. The mild effect of pyrethroids on sporulation processes of *B.t.*compared to effect of other classes of chemical insecticides suggested little or no interference with the ecolgy and perpetuation of this useful bacterium at the site of application. Synergistic interactions suggest that application of pyrethroids with *B.t.* may be a safe and effective means for controlling *Spodoptera littoralis*

The use of low dosages of chemical insecticides such as pyrethroid and organophosphorous compounds combined with low dosages of B.t. formulations proved suitable as a means to control of *Spodoptera littoralis*. With these chemical insecticides - pathogen combinations, the insecticides rates can be reduced to a level which would minimize the environmental pollution and spore parasites, predators and biotic control agents. The reduced quantity of *B.t.*required would also reduce the cost of control.

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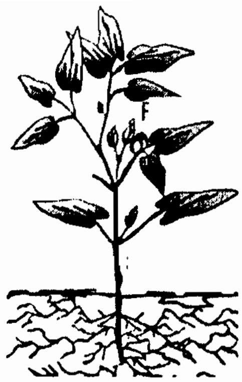

تأثير بعض المبيدات الحشرية فى زيادة فعالية المركبات البكتيرية باسيلس ثورنجنسيس ضد دودة ورق القطن

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تمت دراسة التأثير المشترك بين كل من مركبات الدلتامثرين والكلوربيريفوس - ايثيل والميثوميل ومنظم النمو الحشرى هيكسافليوميرون مع المركبات الحيوية البكتيرية زنتارى وبروتيكتو ودايبل ٢ أكس ضد العمر اليرقى الثانى لدودة ورق القطن .

اظهرت النتائج حدوث تنشيط ملحوظ للتركيزات المختلفة للمركبات الحيوية المختبره وذلك عند خلطها بكل من التركيز النصفى المميت والتركيز الذى يعطى ٢٥٪ موت للمبيد البيروثرودى دلتامثرين وكان ذلك أكثر وضوحا مع تركيز الدلتامثرين الذى يعطى ٢٥٪ موت حيث سبب زيادة فى سمية المركبات الحيوية بمعامل سمية تراوح بين ٤٦,٧٦ ، ٩٢,٩٤ .

على العكس من ذلك فعند استخدام مبيد الميثوميل بالتركيز النصفى المميت أوالتركيز الذى يعطى ٢٥٪ نسبة موت مع التركيزات المختلفة للمركبات الحيوية المختبرة قد ثبط كفاءتها أو أنة لم يؤدى الى زيادة الفاعلية ، كذلك اتضح أن منظم النمو الحشرى هيكسافليوميرون لم يؤثر على كفاءة المركبات الحيوية المختبرة الا عندما استخدم بالتركيز الذى يعطى ٢٥٪ نسبة موت مخلوطا مع التركيز الموصى بة لكل مركب حيوى مختبر حيث أعطى مستوى تنشيط لفاعلية هذه المركبات.

من هذا يتضح أن أستخدام المبيدات البيروثرودية أو بعض المبيدات الفوسفورية مخلوطا مع المركبات الحيوية البكتيرية قد يكون مناسبا فى مكافحة دودة ورق القطن علي الاقتصاديات المناسبة .

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