

TOXICOLOGICAL STUDIES OF SOME MICROBIAL COMPOUNDS AND THEIR MIXTURES AGAINST 2nd INSTAR LARVAE OF COTTON LEAFWORM *Spodoptera littoralis* (Boisd).

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ABSTRACT

The effect of various microbial pesticides and its binary mixtures against 2nd instar larvae of cotton leafworm *Spodoptera littoralis* (Boisd), were studied. Results of laboratory experiments revealed that LC_{50} and toxicity index of the tested compounds against 2nd instar larvae of cotton leafworm were (0.52, 4.81, 147.003, 180.30, 205.30, 205.35, 249.9 and 1065.90 PPM.) for Spinetoram, Spinosad, Abamectin, Diple DF, Agerin, Protecto and Viroset respectively. The toxicity index were (100, 10.81, 0.35, 0.29, 0.25, 0.21 and 0.05 %) for mentioned obvious tested compounds respectively.

Co-toxicity factors of the binary mixture of (Spinetoram+ Diple DF), (Spinetoram + Agerin), (Spinosad + Diple DF), (Spinosad + Agerin), (Abamectin +Diple DF) and (Abamectin + Agerin) and LC_{25} levels against 2nd instar larvae of cotton leaf worm *S.littoralis* were (-30 and - 49.46), (+ 528.5, + 30) and (+ 657.14 and + 390), respectively.

INTRODUCTION

The cotton leafworm *S.littoralis* is one of the main pests in Egypt with polyphagous habit feeding on many crops. This insect is found almost all the year round, causing great damage to the different parts of plants.

Pesticide research in the agricultural business generally continuous to emphasize studies on the development and use of synthetic broadly toxic compounds. Although synthetic organic pesticides appear to provide a solution to the problems of pest control, it has become apparent that repeated application and excessive reliance on synthetic pesticides can be an inadequate method of control. Health and environmental problems and increasing pest resistance to many of these synthetic pesticides, clearly indicated that the basic research must be directed to the discover a new safer types of pest control agents in order to insure high production and preservation of agricultural products.

Recently the discovery and characterization of the soil actinomycete *Saccharopolyspora spinosa* represented a novel opportunity to develop a profile of progressive insect management tools. Sparks *et al.* (1998).

The effect of *Bacillus thuringiensis* and Viricides against the lepidopterous pests were studied by Hunter *et al.*(1997), , Mabrouk and El-Abbas (2002), Murillo, *et al.* (2003), Gergis *et al.*(2005) and Anwar (2006) and Anwar and El-Gindy (2006) .

The present study aimed to investigate the activity of various microbial pesticides and their mixture against 2nd instar larvae of cotton leafworm *S.littoralis* under laboratory conditions.

MATERIALS AND METHODS

The present study was carried out at Plant Protection Institute, Dakahlia Branch in order to study the effect of different biopesticides belonging to various groups either alone or in combinations with each other using recommended doses against 2nd instar larvae of cotton leaf worm *S.littoralis*.

I – Tested compounds

- 1- Protecto 9.4 %, a commercial product of *Bacillus thuringiensis* var. Kurstaki. Rate : 300 gm / Feddan.
- 2- Diple DF 6.4 %, a commercial product of *Bacillus thuringiensis* SubSp. Kurstaki 32000 IU / mg.
Rate : 300 gm / Feddan.
- 3- Agerin 6.5 %, a commercial product of *Bacillus thuringiensis* 32000 IU / mg.
Rate : 300 gm / Feddan.
- 4- Viroset 4 % a commercial product of *S.littoralis* nuclear polyhedrosis virus. Rate : 300 gm / Feddan.
- 5- Abamactin (Vertimec 1.8 % E)
Amixture containing minimum of 80 % avermectin B_{4a} (5-0-dimethyle avermectin A_{1b}) and maximum of 20 % avermectin B_{1b} (5-0-dimethyl-25- de – (1-methyl propyl – 2.5 (1- methyl ethyl) avermectin A_{1a}.
Rate : 40 / cm³ feddan.
- 6- Spinosad : (Tracer 24 % SC)
is a naturally occurring mixture of two active (Spinosyn A and D) produced by the fermentation of the soil actinomycete, *Saccharopolyspora spinosa*.
Rate: 50 cm³ / feddan.
- 7- Spinetoram 12 % SC.
(C₄₂H₆₉NO₁₀ + C₄₃H₆₉NO₁₀)
Rate : 100 cm³ / feddan.

II – Tested insects

Cotton leafworm *Spodoptera littoralis*.

Laboratory strain of the cotton leafworm *S.littoralis* was maintained under conditions of 25 ±1°C and 70 ±5 % R.H which reared on castor bean leaves according to the methods described by El-Defrawy *et al.* (1964).

III – Assessment of biocidal activity:

Serial concentrations of the tested biocides in water were prepared. Castor bean leaves were dipped for 15 second in each solution of the biocides under investigation, then left to dry before being offered to the tested insects which starved for 4-6 hours. The treated leaves were replenished with untreated leaves. Each concentration included 3 replicates. Leaves in untreated check was only treated with water (Merdan, 1968). Mortality were recorded for two days post-treatment, mortality percentages were estimated and corrected for natural mortality according to Abbott's formula (1925) then

subjected to probit analysis by Finney's method (1971). To evaluate the joint effect for the pairs of the tested toxicants, the equation of Mansour *et al.* (1966) was used as the following :

$$\text{Co-toxicity factor} = \frac{\text{Observed \% mortality} - \text{Expected \% M}}{\text{Expected \% mortality}} \times 100$$

RESULTS AND DISCUSSION

The biocidal activity of various tested compounds against 2nd instar larvae of cotton leafworm *S.littoralis* are summarized in Table (1). In this table data showed that Spinetoram was the most effective followed by Spinosad, Abamectin, Diple DF, Agerin, Protecto and Viroset. The LC₅₀ values were (0.52, 4.81, 147.00, 180.30, 205.35, 249.90 and 1065.90 ppm) respectively. The toxicity index values were (100, 10.81, 0.35, 0.29, 0.25, 0.21 and 0.05 %). Based on (LC₅₀ Spinetoram 100 %) respectively. It is clear that spinetoram was the most effective followed by Spinosad while Abamectin and Bt compounds gave a moderate effects and Viroset seem to be the lowest one.

The obtained results agree with Jansens *et al.* (1984) who tested 4 preparation of *B.thuringiensis* against 1st – 5th instar larvae of the noctuid *S.littoralis*. the formulation known as Thuricide WP, Diple WP and ABG 6105 contained strains of *B.thuringiensis* subsp. Kurstaki (Serotype 3a3b). ABG 6105 caused the highest larval mortality followed by Diple WP and Bactospeine I. Salama *et al.* (1991) reported that Diple 2x (B.t) at 200-250 gm / feddan was effective against *S.littoralis* on Trifolium.

Hunter *et al.* (1997) reported that the effects of infecting larvae of *S.littoralis* with the mixture of granulosis virus (GV) and nuclear polyhedrosis virus (NPV) gave hybrid infection. Survival of *S.littoralis* larvae followed mixed infection was longer than that following infection with NPV alone and shorter than that following infection with GV alone.

Table (1): Toxicity effect of certain microbial pesticides against 2nd instar larvae of cotton leafworm *S.littoralis*.

Tested compounds	LC ₂₅ at 95 % confidence limit (PPM)	LC ₅₀ at 95 % confidence limit (PPM)	LC ₉₀ at 95 % confidence limit (PPM)	Slope value	Toxicity index at LC50
Spinetoram	0.26	0.52	1.19	2.41	100
Spinosad	1.004	4.81	94.53	0.99±0.27	10.81
Abamectin	8.11	147.003	36170.34	0.74± 0.25	0.35
Diple DF	31.20	180.30	5098.10	0.88±0.97	0.29
Agerin	93.59	205.35	913.95	1.98± 4.4	0.25
Protecto	67.10	249.9	3046.10	1.18±0.93	0.21
Viroset	44.90	1065.90	43646	0.491± 0.33	0.05

Raslan (2002) found that initial kill as mortality % were 100, 97, 91 and 81 three days after spraying Spinosad 24 % SC at 48, 36, 24 and 12 ml / feddan respectively. Only 0, 3, 7 and 11 larvae were remaining alive after

rearing the tagged egg masses that have alive larvae and or alive / dead. Girgis *et al.* (2005) reported that *Bacillus thuringiensis* (xentari-10.3 % granul) Sub species. Kurtski (MVPII 20 % FL) and microbial toxin (Abamectin 1.8 % EC) were tested against *S.littoralis*. the results showed that the Abamectin was the most potent bio-compound in reducing the population of *S.littoralis* followed by Xantari and the lowest results was obtained with MPVII.

Data in Table (2) revealed that binary mixtures of (Spinetoram + Agerin) showed the most effective antagonism followed by Spinetoram + Diple DF). Values of co-toxicity factor were (- 49.46 and – 42.86) respectively. Whereas the mixtures of (Spinosad + Diple DF), (Spinosad + Agerin), (Abamectin +Diple DF) and (Abamectin + Agerin) gave a potentiation. Co-toxicity factors of the obvious mixtures were (+528.57, +30 + 657.14 and + 390) respectively. It can be concluded that the tested compounds Spinetoram, Spinosad and Abamectin gave a good results alone but Spinotram mixtures with Bt compounds gave antagonism due to highest effects against Bt compounds, while the mixture of Spinosad and Abamectin with Diple DF and Agerin gave potentiation effects, so it can be used mixing with the Bt compounds if it necessary to use mixtures.

Table (2):Joint action effect of binary mixtures of some tested compounds against 2nd instar larvae of *S.littoralis* at LC₂₅ level.

Tested compounds	% Expected Mortality	% Observed Mortality	Co-toxicity	Joint action category
Spinetoram				
+ Diple DF	90	63	-30	Antagonism
+ Agerin	93	47	-49.46	
Spinosad				
+Diple DF	7	44	+ 528.57	Potentiation
+Agerin	10	13	+30	
Abamectin				
+Diple DF	7	53	+ 657.14	Potentiation
+Agerin	10	49	+ 390	

The obtained results are in agreement with those of Anwar (2006) who found that the binary mixtures of Chloropyrifos + Abamectin gave a good results. Co-toxicity factors were + 83.02 and + 54.9 for 2nd and 4th instar larvae of cotton leafworm respectively. Also the auther reported that the joint action effect of binary mixtures of Beta-cyflthrin + Abamectin and Beta-cyflthrin + Spinosad against 2nd instar larvae of cotton leafworm gave antagonism effects and the co-toxicity factor were (-28.57) and (-100) respectively. LC₅₀' values of Spinosad and Abamectin were (21.4 and 150.88 ppm) against 2nd instar larvae of cotton leafworm *S.littoralis*.

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دراسات السمية لبعض المركبات الميكروبية ومخاليطها ضد يرقات العمر اليرقى الثانى لدودة ورق القطن.

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أجريت هذه الدراسة معمليا لاختبار سمية بعض المركبات الميكروبية وبعض مخاليطها المختلفة على يرقات العمر الثانى لدودة ورق القطن . وقد أظهرت النتائج مايلى :

1- قيم الجرعة النصفية ضد يرقات العمر الثانى لدودة ورق القطن كانت (٠,٥٢ ، ٤,٨١ ، ١٤٧,٠٠٣ ، ١٨٠,٣٠ ، ٢٠٥,٣٥ ، ٢٤٩,٩٠ و ١٠٥٦,٩٠ جزء فى المليون) للمركبات اسبينوترام ، سبينوساد ، أبامكتين ، دايبيل دف ، أجرين ، بروتكتو و فيروست على الترتيب. بينما كان دليل السمية للمركبات المختبرة (١٠٠ ، ١٠,٨١ ، ١,٣٥ ، ٠,٢٩ ، ٠,٢٥ ، ٠,٢١ . ٠,٠٥ ، %) (على اعتبار القيم الجرعة النصفية القاتلة لمركب اسبينوترام ١٠٠ %) على الترتيب.

2- أظهرت الدراسة التأثير المشترك لمركب اسبينوترام عند خلطه مع دايبيل دف والأجرين على يرقات العمر اليرقى الثانى وكانت كما يلى (- ٣٠ ، - ٤٩,٤٦) تأثيرا تضاديا. بينما كان التأثير المشترك لمركب اسبينوساد والأبامكتين عند خلطهما مع الدايبيل دف والأجرين على يرقات العمر اليرقى الثانى كما يلى : (+ ٥٢٨٥٧ ، + ٣٠) و (+ ٦٥٧,١٤ ، + ٣٩٠) على الترتيب وكانت ذات تأثيرا تنشطيا .

لذلك لاينصح بخلط سبينوترام مع الدايبيل دف والأجرين ولكن يمكن خلط اسبينوساد والأبامكتين مع الدايبيل دف والأجرين اذا دعت الضرورة لذلك.

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