

LABORATORY EVALUATION OF THREE DIFFERENT BIOCIDES AGAINST THE FIELD COTTON LEAF WORM STRAINS *Spodoptera littoralis* (Boisd.)

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ABSTRACT

Laboratory tests were conducted to determine the efficiency of Abamectin 1.8% EC, Xentari 10.3% Granule and MVP II 20% FL against the 2nd instar larvae of Monofia, Gharbya and Behera field strains of the cotton leafworm *Spodoptera littoralis*. Manshiat Ganzor, Ewasna and Etay El-Barod water were used for dilution of the biocides concentrations and were tested against for Gharbya, Monofia and Behera strains. The results indicate that Abamectin at the different concentrations caused high mortality after 14 days to the 2nd instar larvae of the three tested field strains. All larvae tested failed to reach successful pupation. Behera cotton leafworm strain was the most susceptible to MVP II while Monofia strain was the least. The LT₅₀'s for Monofia, Behera and Gharbya strains were 7.46, 2.70 and 4.69 days at the concentration of 10.00ml/L. The data also revealed that the 2nd instar larvae of Behera strain was more susceptible to Xentari than the larvae of Monofia and Gharbya. The LT₅₀'s were 2.44, 1.99 and 4.74 days at the concentration of 0.6ml/L for Monofia, Behera and Gharbya strains, respectively.

Data showed that pH, conductivity and salinity for Behera (Etay El-Barod) water were higher than that of Gharbya (Manshiat Ganzor) and Monofia (Ewasna) water. The usage of Behera water for dilution resulted in increasing the toxicity of the tested biocides for the larvae of the cotton leafworm. This phenomenon can be benefits since the great majority of waters used for dilution in Egypt are alkaline. Increasing in toxicity as a result of increasing conductivity may be due to augmenting penetration of the toxicant.

INTRODUCTION

Environmental contamination of air, water, soil and food as affected by means's agricultural and social activities has become a threat to the continued existence of many plant and animal communities of the ecosystem and many ultimately threaten the human race. Therefore, the increasing concern among the environmental groups, the farmers and the general public about adverse effects of the chemical pesticides have been cited. Because of these concerns, many farmers in several places of the world have shifted away from the conventional farming systems to sustainable (agroecological, organic) systems. Controlling insects, weeds and diseases without chemicals is a goal of sustainable strategies, and evidence for its feasibility is encouraging.

A central component of sustainable farming is the biological control techniques. The concept of biopesticides is being ecofriendly as well as environmentally safe. Successful bioinsecticides products are based on *Bacillus Thuringiensis* Berliner that produces a selectivity toxic protein in the

form of inclusion or crystal within the cell. This protein crystal is the active component in *B.t.* products and consists of the protoxin from one or more delta endotoxins.

About 50 strains of *B.thuringiensis* have been isolated from different insects and classified into 12 groups by esterase patterns and by serological and biochemical tests *B.thuringiensis* and its relative have been tested against many insect species, mainly lepidoptera, many of them were found highly susceptible to these bacteria (Burges, 1981 and Feeby, 1999)

Therefore, the present investigation was carried out to study the efficiency of Abamectin, Xentari and MVPΠ against the 2nd instar larvae of the different field cotton leafworm strains.

MATERIALS AND METHODS

1-Maintenance of the strains:

The field strains of the cotton leafworm *Spodoptera littoralis* (Boisd.) were obtained from Monofia , Gharbeya and Behera governorates. These strains were kept under normal laboratory conditions at 25±2 °C and 70 ±5% relative humidity. The egg-masses were kept separately until eggs hatched and then provided with castor-oil leaves. The colonies were maintained for three generations.

2- Biocides used:

- a) Abamectin (vertimec 1.8% EC): A mixture containing a minimum of 80% avermectin B1a(5-o-demethyl avermectin A1a) and a maximum of 20% avermectin B1b(5-o-demethyl-25-de(1-methyl propyl-25-(1-methylethyl) avermectin A1a). A nature product produced by the soil microorganisms *Streptomyces averemittilis*.
- b) Xentari 10.3% (water dispersible granule) based on *Bacillus thuringiensis* sub. sp.. aizawai lepidoptera active toxin. Produced by Abbott Laboratories Nourth Chicago, USA.
- c) MVPΠ 20% aqueous flowable, a genetically engineered bacterium that produces delta endotoxin derived from *B.t.* sub. sp.. Kurstaki. The active ingredient consists of endotoxin protein crystals which are encapsulated in dead *Pseudomonas fluorescens* cells, produced by Mycogen USA.

3-Preparation of concentrations:

Formulated biocides Abamectin 1.8% , MVPΠ 20% and Xentari 10.3% were used in this study. The concentrations were 0.15, 0.30 and 0.60 ml/L 2.50, 5.00 and 10.00 ml/L and 0.60 1.20 and 2.40 ml/L for Abamectin, MVPΠ and Xentari respectively. The concentrations were prepared using field water dilution.

4- Method of application:

Castor-oil leaves were dipped for 15 seconds in the biocides solutions, then left to dry. Second instar larvae of *Spodoptera littoralis* of the experimental strains was used about 200±20 larvae were put in glass jars (1liter) and provided with treated castor-oil leaves. After 48 hours the larvae

were transferred to another jar and provided with untreated leaves until pupation. Moreover 200±20 larvae of tested field strains were used as control and provided with untreated castor-oil leaves until pupation.

Insects resulted from the treatments were maintained to determine the different biological criteria. These were percentage of mortality in larval and pupal span, percentage of emergency, percentage of deformation in pupa and in the adult, as well as egg hatchability and percentage sterility. For mating experiments, the resulting morphologically normal adults were grouped in pairs (male and female) and each pair was placed in small cage provided with tafla leaves *Nearium oleander* served as an oviposition site. The percentage of sterility was calculated according to the equation of Topozada and El-Defrawi (1966). The LT_{50} values of candidate biocides were assessed (Finney, 1971).

5- The following Physico-Chemical properties of water used for dilution were determined:

- a- pH at 20°C using orien pH meter.
- b- Conductivity expressed as millsiemens meter (mS/m)
- c- Percentage of salinity employing the conductimeter (YSI).

Table (1): Some Physico-chemical properties of Gharbya (Mashiat Ganzor), Monofia (Ewasna) and Behera (Etay El-Barod) water used for dilution.

Water sample	pH at 20°C	Conductivity mS/m	% salinity
Gharbya Manshiat Ganzor	7.5	883	0.03
Monofia Ewasna	7.3	511	0.02
Behera Etay El- Barod	7.9	1386	0.20

RESULTS AND DISCUSSIONS

1. At Monofia Governorate :

1.1 Effect of Abamectin 1.8% EC:

Results in table 2 show that after 14 days of treatment, all the concentrations tested caused high mortality; 95.50%, 97.50% and 99.45%, occurred in the case of 0.15 ml/L., 0.30 ml/L. and 0.60 ml/L. respectively. The initial mortality were 70.00%, 75.00% and 80.90%. All the treated larvae failed to reach successful pupation.

1.2 Effect of MVP11 20% aqueous flowable:

The results in table 2 gave a very low percentage of mortality varied from 19.23 and 32.50%, and increased by the lapse of time .the percentages of pupation were 23.75, 20.63 and 21.26% and adult formation ranged between 15.00 and 10.00. The results show deformation of pupal by 1.88 and 3.88% in case of concentrations of 2.50 and 5.00 ml/L.

1.3 Effect of Xentari 10.3% granule:

As shown in table 2 during 14 days after the treatment, the mortality percentages increased by the lapse of time. the mortality percentages

were 79.00, 82.00 and 93.00%. after 14 days of treatment for the concentrations of 0.60, 1.20 and 2.40gm/L. The percentages of pupation were 7.33, 4.00 and 3.13% and adult formation ranged from 2.67 and 0.88% for the same concentrations, respectively.

2- At Behera Governorate:

2.1 Effect of Abamectin 1.8% EC:

Results in table 3 showed that 14 days after treatment, all the tested concentrations caused high mortality percentages among the 2nd instar larvae. The mortality percentages ranged between 95.00, 97.50 and 100.00% for 0.15, 0.30 and 0.60ml/L, respectively. All treated larvae failed to reach pupation.

2.2 Effect of MVP II 20% aqueous folwable:

Results obtained in table 3 revealed very few initial mortality larvae varied from zero, 12.50 and 45.00%, while it increased by the lapse of time reaching 70.00, 75.00 and 86.00% after two weeks of treatment with 2.50, 5.00 and 10.00ml/L. The data show that all treatments of larvae with the different concentrations drastically reduced percentage of pupation, percentage of emergency and caused 100% sterility.

2.3 Effect of Xentari 10.3% granule:

Results in table 3 indicate that the mortality percentages after 14 days from treatment were 83.50, 89.50 and 97.00% and the data also indicate that the sterility was 96.02, 98.37 and 100% for the concentrations of 0.60, 1.20 and 2.40gm/L respectively.

3- At Gharbya Governorate:

3.1 Effect of Abamectin 1.8%:

Data in table 4 showed that, Abamectin at the rate of 0.60 ml/L caused 45.50% initial kill. The highest mortality percentage obtained after 14 days from treatment were 94.00, 94.50 and 96.00% for 0.15, 0.30 and 0.60 ml/L. All the treated larvae with these concentrations failed to reach successful pupation.

3.2 Effect of MVP II 20%:

Results obtained in table 4 revealed low percentage of mortality varied from 19.50, 21.50 and 38.50% increased after 14 days from treatment to 43.00, 71.00 and 83.00% with 2.50, 5.00 and 10.00 ml/L. The pupation were 42.50, 34.50 and 3.50 for the tested concentrations. Also the treatments reduced egg production and percentage of sterility ranged between 98.20, and 100%.

3.3 Effect of Xentari 10.3%:

Results in table 4 indicate that the mortality percentages after 14 days from treatment were 80.00, 86.00 and 93.50% with 0.60, 1.20 and 2.4gm/L respectively and the data also indicate that the sterility were 99.16, 100 and 100%.

Xentari treatment causes certain pathological effects in the mid-gut of larvae. Cross sections of mid-gut showed separation of the epithelial cells from the basement membrane as well as elongation, vaculization and break down of larval epithelium mid gut. Moreover Xentari causes disorganization and disintegration of pretrophic membrane (El-Lakwah *et al.* 1999). However,

El-Gemeiy (1992) reported that although the bacterial formulations (Dipel-2x, Florbac, Delfin and Bactospeino) showed high virulent action against the spiny bollworm larvae, only slight effect was reported on pupation and adult emergence from surviving larvae. It is also noted that the delayed effects of *Bt.* extended to the adults resulting from treated larvae, but some concentrations of former biocides had no effect on percent of pupation and adult emergence.

The discovery and development of avermectin endectocides, of which ivermectin is perhaps the foremost example (Hoston 1982), has provided the opportunity for evaluation of these compounds for the control and management of livestock ectoparasites. The avermectines offer the double advantage of having broad spectrum activity and efficiency at extremely low concentrations (Putter *et al.* 1981). There is little doubt that ivermectin levels in the blood serum of the animals acted to produce a very strong selective pressure on the ticks that survived (Ronald *et al.* 2001). Abamectin is an effective compound for controlling the cotton leafworm *S. littoralis*. Under standard laboratory conditions, the compound resulted in a total suppression of pupa and adult formation at the tested concentrations. In this respect, the effect of Emamectin against the cotton pests were studied by Isaac Ishaaya *et al.* (2002). They found that castor bean leaves treated with various concentrations of Emamectin and offered to 3rd instar *S. littoralis* resulted in 97% and 73% mortality at the concentrations of 0.4 and 0.08 mg Al/litre after 6 days of treatment.

The data in table 5 show that Behera cotton leafworm strain was the most susceptible to MVP11 while Monofia strain was the least. The LT_{50} 's were 7.46, 2.70 and 4.69 days at the concentration of 10.0 ml/l for Monofia, Behera and Gharbya strains, respectively. The susceptibility of the 2nd instar larvae of *S. littoralis* to Xentari in Monofia, Behera and Gharbya strains are shown in table 6 indicating that the larvae of Behera strain was more susceptible to Xentari than the larvae of Monofia and Gharbya. The LT_{50} 's were 2.44, 1.99 and 4.74 days at the concentration of 0.6 ml/l.

Data in table 1 show that pH, conductivity and salinity for Behera (Etay El-Barod) water were higher than those of Gharbya (Manshiat Ganzor) and Monofia (Ewasna). pH, conductivity and salinity were 7.9, 1386 mS/m and 0.20%, 7.5, 883 mS/m and 0.03% and 7.3, 511 mS/m and 0.02% for Behera, Gharbya and Monofia water respectively.

Data in tables 5 and 6 indicate that using Behera water for dilution resulted in increasing the toxicity of the biocides. Salama *et al.* (1989) reported that the combination of Dipel 2X with potassium carbonate significantly increased the efficiency of Dipel 2X. The delta-endotoxine effect was increased as increased from the pH 8 to 10 (Gringorten *et al.*, 1992). High activity of *B.t.* at pH 10 corresponds to the alkaline conditions in larval gut Tanada and Kaya, (1993). Furthermore Behle *et al.* (1997) represented that the activity of *B.t.* against the neonate European corn borer, *Ostrinia nubilalis* was decreased rapidly when exposed to field conditions. The degradation can be minimized by improving the formulation by adding alkaline gluten to spray formulations.

Table (5): The susceptibility of the 2nd instar larvae of *S. littoralis* to MVPII in Monofia, Behara and Gharbya strains.

Strain	Concentration ml/L	LT ₅₀ (days) 5%fiducail limits	Slope
Monofia	2.50	16.04(12.29-24.57)	1.01±0.15
	5.00	12.48(-----)	1.00±0.27
	10.00	7.46(5.88-9.76)	0.86±0.14
Behera	2.50	10.01(9.28-10.85)	3.21± 0.36
	5.00	8.12(4.41-19.53)	2.03 ±0.35
	10.00	2.70(2.01-3.34)	1.28 ±0.15
Gharbya	2.50	29.41(18.31-38.39)	0.76 ±0.15
	5.00	7.12(6.21-8.20)	1.53 ±0.15
	10.00	4.69(-----)	1.20 ±0.55

Table (6): The susceptibility of the 2nd instar larvae of *S. littoralis* to Xentari in Monofia, Behara and Gharbya strains.

Strain	Concentration gm/L	LT ₅₀ (days) 5%fiducail limits	Slope
Monofia	0.60	2.44(1.77-2.98)	1.28±0.20
Ewasna	1.20	1.89(1.07-2.52)	1.03±0.20
	2.40	0.75(0.23-1.17)	1.59±0.32
Behera	0.60	1.99(1.39-2.47)	1.39± 0.21
Etay El-Barod	1.20	1.13(0.62-1.58)	1.39 ±0.22
	2.40	1.15(0.70-1.51)	1.99 ±0.32
Gharbya	0.60	4.74(2.53-8.59)	2.35 ±0.41
Manshiat	1.20	1.19(0.65-1.66)	1.53 ±0.22
Ganzor	2.40	1.22(0.76-1.63)	1.59 ±0.23

The increase in efficiency in case of Behera water may be attributed to presence of certain nutrients which enhanced the penetration of candidate biocides through biological tissues and consequently increased their effectiveness. Abdel-Hai N.S. (2001) indicated that using alkaline water of artesian well for dilution the bio insecticides was correlated with increasing the toxicity of Dipel 2X, Ecotech and MVPII for the larval span and pupal span. Percentage mortalities to larval span of *S. littoralis* were 93.00, 92.50 and 89.50% increased to 98.00, 96.50 and 92.50% when tap and artesian well waters were used for dilution, respectively. The results revealed that tested biocides drastically reduced egg production and hatchability of eggs especially when the artesian well water was used for dilution the tested biocides.

The selectivity of action is a key advantage offered by *B.t.* products, particularly when they are used in IPM programs. This specificity means that *B.t.* products can be used to regulate pest populations without direct toxicity to the natural enemies of the target and secondary pests.

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التقييم المعملی لثلاثة مركبات حيوية ضد السلالات الحقلية لدودة ورق القطن
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تم تقويم كفاءة المبيدات الحيوية إمامكتين ١٨ القابل للأستحلاب والزنتاري ٣٠١% المحبب وكذلك أم في بي تو القابل للتدفق المهندس وراثيا ضد العمر اليرقي الثاني لثلاثة سلالات حقلية لدودة ورق القطن وهي سلالة المنوفيه والغريبه والبحيره معمليا.
تم تحضير محاليل المركبات الحيوية المختبرة باستعمال الماء الحقلی من منشية الجنزور محافظة الغربية وقويسنا محافظة المنوفيه و ايتای البارود محافظة البحيره.

ولقد وجد أن المركب الميكروبي إمامكتين أكثر المركبات الحيوية فعالیه ضد دودة ورق القطن حيث أعطى أعلى نسبة موت للعمر اليرقي الثاني للثلاثة سلالات الحقلية المستخدمه. ولقد وجد ان اليرقات المختبرة لهذه السلالات فشلت أن تكمل دورة حياتها وأن تصل الى طور العذراء. ولقد وجد أن يرقات العمر الثاني لسلالة البحيره أكثر سلالات حساسيه للمركب الحيوي أم في بي تو في حين كانت سلالة المنوفيه أقلهم حساسيه . وكان التركيز الوقتي النصفی الممیت LT₅₀ لسلالات المنوفيه والبحيره والغريبه ٧٦٤ ، ٢٧٤ ، ٩٦٤ يوم عند استخدام التركيز ١٠ سم^٢/لتر.

وكذلك وجد أن العمر اليرقي الثاني لسلالة البحيره أكثر حساسيه لمركب زنتاري عن يرقات المنوفيه والغريبه حيث كان التركيز الوقتي النصفی الممیت LT₅₀ ٢٧٤ ، ١٦٩ ، ٤٧٤ يوم عند استخدام التركيز ٦ سم^٢/لتر لكل من سلالات المنوفيه والبحيره والغريبه. ولقد أظهرت النتائج أن الأس الهيدروجيني pH والتوصيل الكهربی والملوحه لمياه التخفيف المستخدمه من محافظة البحيره (ايتای البارود) أنها أعلى من مياه الغريبه (منشية الجنزور) و المنوفيه (قويسنا).

ومن هذا يتضح أن استخدام مياه التخفيف ذات الأس الهيدروجيني pH يميل الى القلويه يزيد من فعالية المركبات الحيوية ضد دودة ورق القطن. وهذه الظاهره يمكن اعتبارها مفيده لأن أغلب المياه التي تستخدم في التخفيف في مصر تميل الى القلوية.