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EFFICIENCY OF THE BIOINSECTICIDE, AGERIN (*Bacillus thuringiensis*) ALONE AND ITS MIXTURE WITH THREE INSECTICIDES AGAINST COTTON LEAFWORM *Spodoptera littoralis* (BOISD.)

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ABSTRACT

The Efficiency of the bioinsecticide, Agerin (*Bacillus thuringiensis*) alone and its mixtures with three insecticides on cotton leafworm *Spodoptera littoralis* (Boisd.) was investigated . Data indicated that Lufenuron was the highest effective insecticides against both the 2nd and the 4th instar larvae of *S. littoralis* followed by Chlorfluazuron , Chlorpyrifos and B.t , respectively .

All tested mixtures decreased the toxicity according to Co-Toxicity factor . The tested mixture (B.t + Chlfluazuroorn) gave the high level of antagonism effect followed by the mixture (B.t + Lufenuron) . Concerning the joint action of combination (B.t + Chlorpyrifos) , it produced additive effect when tested on the 2nd instar larvae, while it gave antagonism effect when tested on the 4th instars larvae of *S. littoralis* .

The field efficiency of tested insecticides (when used singular) can be arranged according to the general mean of reduction percentage during two seasons in a descending order as follows : Chlorfluazuron , Chlorpyrifos , Lufenuron and B.t they were 89.26, 82.60, 77.83 and 67.15%, respectively . The addition of B.t to Chlorpyrifos gave raising the general mean of reduction percentage for two seasons, in contrast the other two tested insecticides gave a reduction but less when applied without added to Bt . The same trend were obtained when applied three tested insecticides at the half recommended rate alone or mixed with the same dose of Bt on *S. littoralis* but with less level of reduction .

INTRODUCTION

Bacillus thuringiensis subsp. *kurstaki* Berliner is the most common bioinsecticide used to cotton leafworm *Spodoptera littoralis* (Boisd.) in Egypt . depending on the dose ingested , larvae may die from starvation or septicemia , or thy may survive but suffer sublethal effects such as feeding inhibition , reduced pupal weight , prolonged development and increased incidence of developmental polymorphism (Ramachandran *et al.* 1993, Pedersen *et al.* 1997 and Moreau and Bauce 2001) . Resistance to pesticide is probably the biggest challenge facing pesticide researchers today . Consequently, insecticides from different chemical groups with different mode of actions and also some of their combinations should be tested against *S. littoralis* to help developing a sound control program in the future (Ghoneim, 2002) . The combination of such bioactive agent with insecticides was investigated as attempt to increase their efficiency on *S. littoralis* and reduce the amounts of insecticide release in the environment which is appreciable from the environmental safety point of view (Aly and El-Dahan, 1987) .

So, the objective of this study was to evaluate the susceptibility of the 2nd and 4th instars larvae of the cotton leafworm *S. littoralis* (Boisd.) to *Bacillus thuringiensis* (Agerin) as a bioinsecticide , Chlorpyrifos as organophosphorus insecticide as well as Chlorfluazuron and Lufenuron as

insect growth regulators. In addition assessment the joint effect of the combinations of B.t with each other tested insecticides. Also, evaluate the field efficiency of B.t when applied alone or mixed with the complete or half recommended rate of the same tested insecticides against *S. littoralis* .

MATERIALS AND METHODS

Tested Insecticides:

1- *Bacillus thuringiensis* subsp. *kurstaki* Berliner (Agerin® 6.5% W.P.) contain 32000 IU/mg as bioinsecticide.
2- Chlorpyrifos (Dursban® 48%E.C.) as organophosphorus insecticide.
3- Chlorfluazuron (Atabron® 5% E.C.) as insect growth regulator .
4- Lufenuron (Sorba® 5% E.C.) as insect growth regulator .

I. Laboratory Experiment :

A laboratory strain of the cotton leafworm *S. littoralis* (Boisd.) was reared in the laboratory under constant conditions of 25°C±1 and 70 ± 5% RH and kept of any contamination with chemicals till the time of study as described by El-Defrawi *et al.* (1964) .

A. Toxicity tests :

A series of concentrations (in water) for each insecticide was prepared on the active ingredient (a.i) based on ppm by diluting the commercial formulation. Castor-bean leaves were dipped for 30 seconds in each concentration then left to dry for one hour. The 2^{nd} and 4^{th} instars larvae of each tested strain were confined with treated leaves in glass jars covered with muslin for 24 hrs. Test also included a non treated control in which leaves were dipped in water (as a check). Treated leaves were then removed and fresh untreated leaves provided for four days. Three replicates (each of 20 larvae) were tested for each concentration. The average of mortality percentage was corrected using Abbott's formula (1925). The corrected mortality percentage of each compound was statistically computed according to Finney (1971). From which the corresponding concentration probit lines (LC-p lines) were estimated in addition to determine 25, 50 and 90% mortalities, slope values of tested compounds were also estimated . In addition, the efficiency of different compounds was measured by comparing the tested compound with the most effective compound by using the following equation : Toxicity index = LC_{50} of the most effective compound / LC_{50} of the tested compound x 100 (Sun , 1950).

B. Determination of the joint action :

The tested insecticides were applied each at LC_{25} level when used singly . To test the joint action of the tested insecticides , equal volumes of the two insecticides were added together [Agerin and each insecticide 1:1 (w/w)] . Three replicates with twenty larvae each were used in each treatment . Mortality counts were recorded after five days of treatment . To determine the effect of applying pairs of insecticides , the expected LC_{25} concentrate of each insecticide in the paired combination was calculated from its

corresponding LC-p lines , there for , the expected mortality for the mixture of two insecticides was the sum of the expected mortalities of each of the concentrate used in the combination . To evaluate the joined effect of the different pairs of used insecticides , the equation of the co-toxicity factor of Mansour *et al.,* (1966) was applied .

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

This factor was employed to differentiate the results into three categories . A positive factor of (+20) or more meant potentiation , a negative factor of (-20) or more meant antagonism , and any intermediate value (i.e. between -20 and +20) was considered only additive effect .

II. Field Experiment :

Experiments were conducted at Aga district , Dakahlia Governorate during the two successive seasons 2004 and 2005 to evaluate the field efficiency of the bioinsecticide Agerin (*Bacillus thuringiensis* subsp. *kurstaki* Berliner) alone and mixed with the complete or half recommended rate of three insecticides (Chlorpyrifos , Chlorfluazuron and Lufenuron) on cotton leafworm *S. littoralis* (Boisd.). The fields were cultivated with Giza 86 cotton variety and the normal agricultural practices were applied . The experimental area was divided into plots of 42 m^2 each and the treatments were arranged in randomized complete blocks with four replicates each. Plots were isolated from each other by unplanted corridors (1 m width) that separated replicates . A motor sprayer was used to spray the chemical dilutions . The volume of spray solution was 40 litres /feddan . The number of larvae of the cotton leafworm *S. littoralis* (Boisd.) was recorded on 25 plants at random from the inside rows of each plot before the first spray and often 2,5,7,11 and 14 days and so the percent of reduction was calculated by using Henderson and Tilton (1955) equation .

RESULTS AND DISCUSSION

I. Laboratory Experiment :

A. Toxicity tests :

Data presented in Table (1) and Figure (1) showed that Lufenuron was the highest effective insecticides against the 2nd instar larvae of *S. littoralis* followed by Chlorfluazuron , Chlorpyrifos and B.t , respectively . The toxicity index being 55.645, 5.255 and 0.0003 % based on LC_{50} of Lufenuron 100% , respectively .

Concerning the efficiency of tested insecticides against the 4th instar larvae of *S. littoralis* (Table 2 & Figure 1), also Lufenuron was the most effective insecticides followed by Chlorfluazuron , Chlorpyrifos and B.t , respectively . The toxicity index being 52.838, 28.430 and 0.0016 % based on LC_{50} of Lufenuron 100% , respectively .

Table 1. Susceptibility of 2nd instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested insecticides.

Tested insecticides	LC_{25}(ppm) its limits at 95%	LC_{50}(ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	Slope	Toxicity index(%)
B.t	3726.51 2261.47 8664.85	22432.62 9323.71 42334E+1	679420 86946.20 10841E+5	0.865 ± 0.251	0.0003
Chlorpyrifos	1.08 1.004 1.167	1.313 1.221 1.412	1.904 1.77 2.048	7.941 ± 0.923	5.225
Chlorfluazuron	0.012 0.002 0.031	0.124 0.056 0.212	10.117 3.821 64.754	0.670 ± 0.116	55.645
Lufenuron	0.014 0.007 0.026	0.069 0.038 0.126	1.481 0.812 2.702	0.963 ± 0.092	100.00

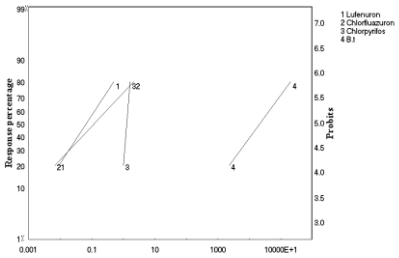
Toxicity index = LC_{50} of the most effective insecticide / LC_{50} of the tested insecticide x 100

Table 2. Susceptibility of 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.) to tested insecticides.

Tested insecticides	LC_{25}(ppm) its limits at 95%	LC_{50}(ppm) its limits at 95%	LC_{90}(ppm) its limits at 95%	Slope	Toxicity index(%)
B.t	8768.53 5010.13 37448.67	38206.90 14278.64 76672E+1	626180 92741.13 26771E+4	1.055 ± 0.283	0.0016
Chlorpyrifos	1.643 1.492 1.775	2.128 1.975 2.313	3.478 3.065 4.203	6.004 ± 0.697	28.430
Chlorfluazuron	0.307 0.198 0.432	1.145 0.823 1.703	13.976 7.227 39.219	1.179 ± 0.144	52.838
Lufenuron	0.032 0.012 0.065	0.605 0.296 1.544	164.99 32.880 2481.06	0.526 ± 0.072	100

Toxicity index = LC_{50} of the most effective insecticide / LC_{50} of the tested insecticide x 100

2nd instar larvae



4th instar larvae

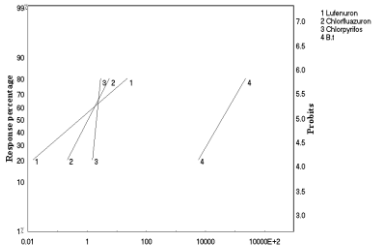


Fig.(l): Log concentration probit lines of susceptibility of 2nd and 4th instars larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) to tested insecticides.

B. The joint effect of the tested mixture of insecticides :

The joint action data of the combinations of B.t with each other tested insecticides against 2nd and 4th instars larvae of *S. littoralis* at LC_{25} level are shown in Table (3) . Data clearly indicated that all tested mixtures decreased the toxicity according to Co-Toxicity factor . The tested mixture (B.t + Chlorfluazuron) gave the high level of antagonism effect – 51.86 and – 79.31

followed by the mixture (B.t + Lufenuron) which gave – 43.76 and – 32.43 against 2nd and 4th instars larvae of *S. littoralis* , respectively .

Concerning the joint action of combination (B.t + Chlorpyrifos) , it produced additive effect when tested on the 2nd instar larvae (- 19.98) , while it gave antagonism effect (- 30.29) when tested on the 4th instars larvae of *S. littoralis* .

It could be concluded that all tested combinations didn't gave any positive effect but in contrast it gave negative effects under laboratory condition . So, unfavorable the bioinsecticide B.t (Agerin®) mixed with tested insecticides Chlorpyrifos , Chlorfluazuron or Lufenuron .

Table 3. Joint action of the tested mixture of insecticides against 2nd and 4th instars larvae of *Spodoptera littoralis* (Boisd.) at LC_{25} level .

Tested mixture	2nd instar larvae			4th instar larvae		
	Expected(%) mortality	Observed(%) mortality	Co-Toxicity factor	Expected(%) mortality	Observed(%) mortality	Co-Toxicity factor
B.t+Chlorpyrifos	46.29	37.04	- 19.98	68.75	47.92	- 30.29
B.t+Chlorfluazuron	50.00	24.07	- 51.86	60.42	12.50	- 79.31
B.t+Lufenuron	59.26	33.33	- 43.76	77.08	52.08	- 32.43

II. Field Experiment :

Data in Table (4) showed that the field efficiency of the bioinsecticide , Agerin (*Bacillus thuringiensis*) alone and other complete dose of three insecticides on cotton leafworm *S. littoralis* (Boisd.) during 2004 and 2005 seasons .The initial effect (after two days from spraying), Chlorpyrifos was the superior in activity gave 99.13 and 83.09% reduction in infestation followed by Chlorfluazuron and Lufenuron gave (75.16 and 44.24%) and (48.81 and 53.75%) whereas B.t gave the least effect (24.54 and 36.40 %) reduction during 2004 and 2005 seasons, respectively ..

Regarding the mean of residual effect percentage for tested insecticides, Chorfluazuron showed the longest residual effect gave 94.97 and 98.33% followed by Lufenuron , Chlorpyrifos and B.t with values (80.80 and 88.13%) , (78.32 and 82.61%) and (76.23 and 76.42 %) during two seasons , respectively .The efficiency of tested insecticides (when used singular) can be arranged according to the general mean of reduction percentage of two seasons in a descending order as follows : Chlorfluazuron , Chlorpyrifos , Lufenuron and B.t they were 89.26, 82.60, 77.83 and 67.15%, respectively .

Concerning the field efficiency of B.t (Agerin) when mixed with other complete dose of previous three insecticides on cotton leafworm *S. littoralis* (Boisd.) , data indicate the addition of B.t to Chlorpyrifos gave raising the general mean of reduction percentage during two seasons with value 95.08% reduction , in contrast the other two tested insecticides gave a reduction but less than when applied without added to Bt (82.77 and 64.49%) .

The same trend of effects were obtained when applied three tested insecticides at the half recommended rate alone or mixed with the same dose of Bt on *S. littoralis* but with less level of reduction (Table 2) . In respect of the efficiency of tested insecticides when used singular in a descending order as

follows : Chlorfluazuron, Chlorpyrifos, B.t and Lufenuron , they gave 87.72, 75.26, 67.15 and 60.08% according to the general mean of reduction percentage of two seasons respectively .

Table 4. Field efficiency of the bioinsecticide, Agerin (*Bacillus thuringiensis*) alone and mixed with the complete dose of three insecticides on cotton leafworm during 2004 and 2005 seasons .

Treatments	Rate of application / feddan	Season	% Reduction after spraying						General mean of % Reduction	General mean of two seasons
			Initial effect after 2 days	Residual effect (days)				Mean of % Residual effect		
				5	7	11	14			
Alone										
B.t	500 gm	2004	24.54	75.80	100.00	100.00	29.11	76.23	65.89	67.15
		2005	36.40	100.00	100.00	87.92	17.75	76.42	68.41	
Chlorpyrifos	1000 ml	2004	99.13	86.54	100.00	69.25	57.49	78.32	82.48	82.60
		2005	83.09	100.00	100.00	83.06	47.39	82.61	82.71	
Chlorfluazuron	400 ml	2004	75.16	79.88	100.00	100.00	100.00	94.97	91.01	89.26
		2005	44.24	100.00	100.00	100.00	93.32	98.33	87.51	
Lufenuron	160 ml	2004	48.81	63.19	100.00	100.00	60.00	80.80	74.40	77.83
		2005	53.75	100.00	100.00	100.00	52.53	88.13	81.26	
Mixed										
B.t + Chlorpyrifos	500 gm + 1000 ml	2004	96.24	88.09	100.00	100.00	91.22	94.83	95.11	95.08
		2005	85.77	100.00	100.00	100.00	89.43	97.36	95.04	
B.t + Chlorfluazuron	500 gm + 400 ml	2004	70.05	80.90	100.00	100.00	69.37	87.57	84.06	82.77
		2005	35.19	100.00	100.00	100.00	72.21	93.05	81.48	
B.t + Lufenuron	500 gm + 160 ml	2004	67.25	42.05	95.65	90.54	27.74	63.99	64.65	64.49
		2005	11.52	100.00	100.00	100.00	10.15	77.54	64.33	

Also, Chlorpyrifos was enhanced when mixed with B.t, they gave 93.93% reduction, while the addition of B.t to Chlorfluazuron, or Lufenuron gave less level of general mean of reduction percentage of two seasons compared to when applied singular (66.88 and 37.62%) .

Generally the joint action of thuringiensin (*Bacillus thuringiensis*) applied in binary mixtures with selected organophosphorus and pyrethroid insecticides against *S. littoralis* was investigated . B.t-insecticides combination were applied at the half recommended dose of seven insecticides in the field , the co-toxicity factor values indicated that B.t in its mixture with chlorpyrifos gave the highst potentiation effect (C.F. = +79.04 and +31.34 on the second and fourth larval instars , respectively after 5 days of exposure , while B.t when mixed with thiodicarb , methomyl , phospholan or alphamethrin gave an additive effect (Aly and El-Dahan, 1987) . Based on the LC_{50} values, the highest larvicidal activity of the selected insecticides alone was exhibited by chlorpyrifos, fenpropathrin and thuringiensin (Radwan *et al*. 1993) . Zidan *et al.* (1996) determined the insecticidal and biological activities of pyriproxyfen [juvenile hormone analogue], Dipel 2x (*B. thuringiensis*), and KZ mineral oil on the 4th instar larvae of *S. littoralis* . *B. thuringiensis* had the best LC_{50} value in the leaf dipping experiment and all 3 insecticides prolonged larval duration and adult longevity, and decreased pupal weights . The soil which mixed with 10 ml of insecticides, showed the great potential of all 3 insecticides for killing adult cotton leafworm larvae in soil . Mohamed and Ahmed (1990) reported that, in leaf-dip bioassays using castor leaves, sethoxydim (at 1 and 0.1%) the activity of *B. thuringiensis*

against 2nd instar larvae of the noctuid *S. littoralis*, whereas fluazifop at the same conc. was antagonistic .

Table 5. Field efficiency of the bioinsecticide, Agerin (*Bacillus thuringiensis*) alone and mixed with the half dose of three insecticides on cotton leafworm during 2004 and 2005 seasons .

Treatments	Rate of application / feddan	Season	% Reduction after spraying						General mean of % Reduction	General mean of two seasons
			Initial effect after 2 days	Residual effect (days)				Mean of % Residual effect		
				5	7	11	14			
Alone										
B.t	500 gm	2004	24.54	75.80	100.00	100.00	29.11	76.23	65.89	67.15
		2005	36.40	100.00	100.00	87.92	17.75	76.42	68.41	
Chlorpyrifos	500 ml	2004	96.05	75.99	77.80	62.99	58.40	68.80	74.25	75.26
		2005	67.18	100.00	100.00	75.05	39.08	78.53	76.26	
Chlorfluazuron	200 ml	2004	58.35	90.59	100.00	100.00	93.00	95.90	88.39	87.72
		2005	53.86	93.41	100.00	100.00	87.96	95.34	87.05	
Lufenuron	80 ml	2004	34.05	60.85	83.65	85.62	70.02	75.04	66.84	60.08
		2005	3.85	65.68	100.00	50.87	46.18	65.68	53.32	
Mixed										
B.t + Chlorpyrifos	500 gm + 500 ml	2004	95.11	99.16	100.00	100.00	87.22	96.60	96.30	93.93
		2005	74.63	100.00	93.49	100.00	89.62	95.78	91.55	
B.t + Chlorfluazuron	500 gm + 200 ml	2004	65.24	74.54	100.00	78.35	53.20	76.52	74.27	66.88
		2005	39.00	66.45	100.00	54.54	37.44	64.60	59.49	
B.t + Lufenuron	500 gm + 80 ml	2004	59.35	55.98	63.87	40.60	12.47	43.23	46.45	37.62
		2005	0.54	55.00	70.20	17.09	1.06	35.84	28.78	

Al-Zubadi et al., (1988) investigated the compatibility of Bactospeine (a commercial preparation of *B. thuringiensis* subsp. thuringiensis) with several chemical insecticides in Iraq . Effective control of *S. littoralis* was achieved with mixtures of Bactospeine (at half the recommended dosage) with Decis 2.5% EC [deltamethrin], Sevin 85% WP [carbaryl] or Sumithion 50% EC [fenitrothion] . El-Hamaky *et al*. (1990) applied Cyfluthrin, triazophos and thiodicarb alone or as mixtures with *B. thuringiensis* (as Bactospeine) to cotton fields in Egypt, and the initial and residual mortality to 2nd and 4th instar larvae of the noctuid *S. littoralis* on treated leaves were determined in the laboratory. All treatments caused 95% initial mortality, but the microbial pesticide did not increase knockdown performance . Positive correlations were found between residual mortality and insecticide concentrations, as well as for feeding periods on treated leaves. Negative correlations were found for time after treatment. *B. thuringiensis* did not potentiate residual performance of the chemical insecticides.

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ليلى رجب على الجوهرى
قسم المبيدات- كلية الزراعة – جامعة المنصورة

في هذا البحث تمت دراسة فعالية المبيد الحيوي أجرين (باسيلس ثيرونجينسيز) منفرداً أو خلطا مع ثلاث مبيدات حشرية على دودة ورق القطن . أشارت النتائج إلى أن مركب ليفينيورون كان أكثر المركبات فعالية ضد يرقات العمر اليرقى الثاني لدودة ورق القطن يليه كلورفلوزيرون ثم كلوربيريفوس ثم أجرين على الترتيب ، أيضا أعطى ليفينيورون أعلى تأثير يليه كلورفلوزيرون ثم كلوربيريفوس ثم أجرين وذلك على العمر اليرقى الرابع لدودة ورق القطن على الترتيب . وجد أن كل المخاليط المختبرة قد خفضت من السمية وفقا لدليل السمية وقد أعطى الخليط (أجرين + كلورفلوزيرون) أعلى تأثير تضادي يليه الخليط (أجرين + ليفينيورون) بينما أعطى الخليط (أجرين + كلوربيريفوس) تأثير إضافة عندما اختبر على العمر اليرقى الثاني بينما كان له تأثير تضادي على العمر اليرقى الرابع لدودة ورق القطن .أوضحت النتائج انه يمكن ترتيب كفاءة المبيدات المختبرة عندما طبقت حقلياً وفقا لمتوسط الخفض خلال عامي الدراسة كالتالي كلورفلوزيرون ، كلوربيريفوس ، ليفينيورون ثم أجرين وكانت ٨٩,٢٦ ، ٨٢,٦٠، ٧٧,٨٣ ثم ٦٧,١٥% على الترتيب . و قد أدت إضافة أجرين إلى كلوربيريفوس إلى زيادة المتوسط العام للنسبة المئوية للخفض خلال سنتي الدراسة وعلى العكس أعطى كلا المبيدين الآخرين انخفاضاً ولكن بنسبة اقل من تطبيقيهما بدون إضافة أجرين .كذلك تم الحصول على نفس التأثير عندما طبقت الثلاث مبيدات الحشرية بنصف المعدل الموصى به سواء كانوا منفردين أو خلطا مع أجرين على دودة ورق القطن ولكن بنسبة كفاءة اقل.