LABORATORY EVALUATION AND FIELD APPLICATION OF BACTERIAL AND FUNGAL INSECTICIDES ON THE CITRUS FLOWER MOTH, *PRAYS CITRI* MILLER (LEP.: HYPONOMEUTIDE) IN LIME ORCHARDS IN EGYPT

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Abstract

The efficacy of two commercial products of Bactospine and Bio-fly on *Prays citri* of lime trees was studied under laboratory and field conditions throughout the two seasons of 1996 and 1997. Laboratory results indicated that the pathogenic effect of Bactospein, *Bacillus thuringiensis* (berliner) was higher than that of Bio-fly, *Baeuveria bassiana* in spite of the later was applied with higher doses. Bactospine at the rate (2 ccl1 I.W.) affected the larvae more than adults, that treatment reduced no. of eggs/female by 81.70% and decreased the larval infestation by 90.08%. Bio-fly at the highest concentration (4 cc/1 I.W.) reduced the infestation with the eggs, the larvae and the pupae by 65.83%, 68.45% and 72.55%, respectively. Advanced effects of bacterial and fungal treatments on the final reduction rates of *P.citri* infestation were recorded. Field applications revealed that two sprays with Bactospeine or Bio-fly gave good control of *P.citri* more than one spray. Bactospeine at the rate (2 cc/1 I.W.) and Bio-fly at the rate (2 cc/1 I.W.) reduced the infestation by (89.82-93.43%) and (76.32-78.82%), respectively. In all cases, higher doses of bioinsecticides produced potentiation effects of infestation reduction. It is recommended that two sprays of Bactospeine at a rate (2 cc/1 I.W.) is adequate effective to reduce the population of *P.citri* in lime orchards during the main flowering period of trees without need of chemical insecticides.

INTRODUCTION

During the main flowering period of citrus, the citrus flower moth *Prays citri* Mill. (lep.: Hyponomeutiedae) attacks citrus specially lime and lemon trees. Eggs are deposited on the petals and the calyces of the flowers and/or flower-buds. Upon hatching, the larvae bore and feed through the organs within the flowers. Pupae occur in the flowers, leaves and forks of twigs. Previous information on *Prays citri* control measures using against this pest in Egypt (Shehata, 1982 and Attia *et al.*, 1992). Other control methods were: microbial control with bacterial pathogen *(Bacillus thuringiensis)* by Carles (1984) in France; or with fungal pathogen by Kamburov (1986) in South Africa, phermone traps were used by Benfatto (1990) in Italy and parasitoids were applied by Moreana *et al.* (1993) in Spain.

The new trend of the present study aims to replace the biological agents instead of the chemical control. Therefore, laboratory evaluations and field applications were tested on the different stages of *P.citri* using the microbial products of Bactospeine and Bio-fly.

MATERIALS AND METHODS

The present investigation on controlling of *P.citri* Mill. using bacterial and fungal treatments was evaluated under laboratory conditions at the Agricultural Experimental Station and that was applied in two lime infested orchards at Abies, Alexandria, Egypt, during the seasons of 1996 and 1997.

Two commercial products of bioinsecticides were tested:

1. Bactospeine F.C. [a.i., *Bacillus thuringiensis* (berliner), 8500 International Units AK/mg.] was supplied by Duphar B.V., Weesp, Holand.

2. Bio-fly F.C.[a.i., *Beauveria bassiana* $3X10^7$ spores/mg.] was supplied by El-Nasr Bio-insecticides and Fertilizers Company, El-sadaat, Egypt.

Three concentrations of each of microbial product were prepared, Bactospeine at the rates of 1.0,1.5 and 2.0 cc/l 1.w. and Bio-fly at the rates of 2.0,3.0 and 4.0cc/1 l.w.

Laboratory evaluation: During the main flowering season of lime trees in 1996, a stock culture of badly infested flowers with larvae and pupae of *P.citri* was collected and kept in 30 X 30 X 20 cm wooden rearing cages.

The stock culture was used to study the effect of bioinsecticides on the different stages of the insect, as well as, to determine the advanced effect of these materials on the final reduction rate of infestation.

1. Effect of Bactospeine on the adult and larval stages

1.1. The adult stage: From stock culture, pupae were picked and then transfered separately into 1 X 4 cm specimen tubes covered with muslin. Upon moth emergence, pairs of moths were introducted into oviposition cages (chimney glass, about 8 cm in diameter and 16 cm high). Every cage was provided with a small plastic vial containing a piece of sponge-soaked in 10% sugar solution for feeding and 5 healthy flower-buds of lime to serve as oviposition sites. Cages were devided into 2 groups, each one consisted of 20 replicates. Bactospeine at the rate of 2 cc/1 l.w. was added to the feed-

ing solution of moths of the 1st cages group, while the 2 nd cages group left without treatment for check. Cages were examined daily to provide extra feeding solution and replace the buds with new ones.

Records of egg-laying capacity, oviposition period and adult longevity of male and femal moths were taken 10 days after treatment.

1.2. The larval stage: Random samples of small lime twigs containing 400 bud-flowers infested with larvae were distributed into 4 groups. Every group (20 twigs X 5 buds) devided into 5 replicates, each one (4 twigs X 5 buds) was kept in a small bottle with 2 cc water. Three groups were sprayed with one treatment of Bactospeine *i.e.* 1.0,1.5 & 2.0 cc/1 l.w., while the last group received only water and used for check. Spraying was made with atomizer (50 ml). Reduction percentage of larval infestation was calculated 7 days after treatment according to number of alive larvae in treated and untreated samples.

2. Effect of Bio-fly on the immature stages

2.1. The larval and the pupal stages: The above experimental design was repeated on another random samples of lime twigs infested with both larvae and pupae of *P.citri* were treated with Bio-fly at the rates of 2.0, 3.0 & 4.0 cc/1 l.w. Reduction percentages of each of larval and pupal infestation were recorded 10 days after treatment.

2.2. The egg stage: Newly *P. citri* eggs deposited on the petals of bud-flowers in the laboratory were kept in 20 Petri-dishes lined with filter papers, each dish contains petals with 40 eggs. Every 5 dishes (replicates) received one treatment of Bio-fly i.e. 2.0,3.0 &4.0 cc/1 l.w., the last 5 dishes only received water and used for comparison. Petales with eggs were treated by a syringe (3 ml).

Reduction percentage of egg hatchability was estimated 10 days after treatment.

Field application: Based on the results of the laboratory evaluation of *P. citri* control, 8 field applicantions were planned in order to control pest under field conditions during the main flowering period of lime trees in 1996 and 1997. In 1996, four experiments were carried out to determine the efficacy of one spray and two sprays with each of Bactospeine at the rate (2 cc/1 l.w.) and Bio-fly at the rate (4 cc/1 l.w.) on the reduction percentage of larval infestation. Field tests were conducted in two heavi-

ly infested lime orchards of similar age; one of them was selected for Bactospeine treatments and the other one was treated with Bio-fly. In each orchard, selected trees were devided into 3 sets (5 trees/set); the 1st and 2 nd sets received one spray and two sprays of bioinsecticides, respectively, the 3rd set was sprayed with water and used for control.

The 1st Spraying was applied on April 15; the 2nd spraying was done 7 days after the 1st application. Trees were inspected 2 weeks after the 1st treatment. Inspected samples consisted of 200 flowers/treatment (40 flowers/tree). Spraying was made by a compressed air knapsack sprayer (20 liter).

The similar previous field tests were repeated on another 30 infested trees during 1997 season for comparison.

Reduction percentages of *P.citri* infestation under laboratory and field conditions were based on the following formula:

% reduction of infestation = [(c-t/c] X 100

Where; c = the mean number of alive individuals in untreated

t= the mean number of individuals in treated.

The "F" test (Snedecor & Cochran, 1961) was calculated to check the significance between the concentrations used and reduction percentages of infestation. The "T" test (Dixon & Massey, 1969) was used in order to obtain information about the relationship between number of sprays and reduction percentages of insect infestation.

RESULTS AND DISCUSSION

The present data tabulated in Table 1,2,3,4 and 5 indicated that the bioinsecticides used under laboratory and field conditions gave variable efficiency on the reduction rates of *P. citri* infestation depended on the susceptible of insect stage, the effect of product applied and its concentration as well as the number of applications.

Laboratory Evaluation

1. Effect of Bactospeine on the adult and larval stages

1.1. The adult stage: Effect of Bactospeine at the rate of 2 cc/1 l.w. on adult longevity, oviposition period and no. of eggs/female of *P. citri* moths reared on lime fl-

owers under mean laboratory conditions of 25.6± 0.1°C and 56% R.H. are shown in Table 1.

1.1.1. Adult longevity: Data revealed that the longevities of untreated females and males, with respective lasted for 7.80 (7-9) days and 6.00 (5-7) days in comparison of treated ones elapsed 2.75 (1-6) days and 3.43 (1-5) days. Therefore, Bactospeine (under the present conditions) reduced female and male longevities by 64.74% and 42.83%, respectively.

1.1.2. Oviposition period: The oviposition periods of untreated and treated females were 6.60 (5-8) days and 1.53 (0-4) days, respectively. Thus, bacterial treatment of Bactospeine resulted in 76.82% reduction of oviposition period of treated moths.

1.1.3. Egg-laying capacity: The egg-laying capacity was the most biological aspects affected with bacteria. No. of eggs of untreated female ranged 95-313 eggs/female with a mean of 181.60 eggs; while ones deposited 0-84 eggs/female with a mean of 33.47 eggs. That means, Bactospeine caused 81.70% reduction of no. of eggs per female.

Table 1. Effect of bacterial treatment of Bactospeine at the rate of 2 cc/1 l.w. on some biological aspects of *P.citri* moths under mean laboratory conditions of 25.6 ± 0.1 °C and 65% R.H.

Period/days						No. of eggs/ female		% reduction of			
adult longevity				oviposition				adult longevity		oviposition period	No. of eggs/ female
t.		c.		t.	c.	t.	c.				
female	male	female	male					female	male		
2.75 (1-6)	3.43 (1-5)	7.8 (7-9)	6.00 (5-7)	1.53 (0-4)	6.60 (5-8)	33.47 (0-84)	181.60 (95-313)	64.74	42.83	76.82	81.70

t. = treated, c. = control

1.2. The larval stage: The efficacy of Bactospeine on *Prays citri* larvae was shown in Table 2. Data revealed that the treatments of Bactospeine at the concentrations of 2.0,1.5 and 1.0 cc/1 l.w. produced reduction of larval infestation by 90.08, 69.48 and 55.73%, respectively. Statistical analysis showed that the differences between the concentrations used resulted highly significant effect on the reduction rates of larval infestation.

2. Effect of Bio-fly on immature stages: Lists in Table 3 declare the role of Bio-fly tested at the dosages of 4.0,3.0 and 2.0 cc/1l.w. on the egg hatchability, the larval infestation and the adult emergency of *P.citri*. Statistical analysis showed significantly effects between concentrations applied and the reduction percentages of eggs, larvae and pupae infestation.

2.1. The egg stage: Reduction percentages of eggs infestation reached 65.83,55.50 and 36.00% when the eggs were treated with Bio-fly at the concentration rates of 4.0,3.0 and 2.0 cc/1l.w., respectively.

2.2. The larval stage: Various percentages of larval reduction were obtained as Bio-fly was sprayed with different concentrations. The most larval reduction was 68.45% at the highest concentration (4 cc/1l.w.), followed by 53.48% reduction at the moderate concentration (3 cc/1l.w.), while the least larval reduction was 29.95% at the diluted concentration (2 cc/1l.w.).

2.3. The pupal stage: The reduction rates of adult emergence ranged between 72.55% and 13.73% as pupae were treated with Bio-fly at the rates of 4 cc/1l.w. and 2 cc/1 l.w., respectively. The moderate reduction rate of moth emergence was 47.06% when pupae exposed for a mid concentration (3 cc/1l.w.)

Howere, the immature stages of *P. citri* treated with Bio-fly at the highest rate (4 cc/1l.w.) could be arranged according to their susceptability of fungal pathogenic into: the pupae were most susceptible followed by the larvae and finallythe eggs were least affected.

3. Advanced effects of Bactospeine and Bio-fly on the final percentage reduction of *P.citri* infestation : Results recorded in Table 4 reveal the efficiency of microbial agents used against the larvae of *P. citri* and its extended effect on the pupae and the adults formed from them on the final percentage reduction of infestation. Data emphasized that the final percentages reduction of infestation reached 97.71% and 78.61% when Bactospeine at the rate (2.0 cc/1l.w.).and Bio-fly at the rate (4 cc/1l.w.) were applied on the larvae; at the same time, both the pupae and the adults were affected as Bactospeine was applied at a rate (1.5 cc/1l.w.) against the larvae, its effect continued on the pupae, caused 78.60% final resuction of infestation. Bio-fly used at the rate (3 cc/1l.w.). had limited effect only on the larvae, reduced 53.48% of infestation.

Table 2. Effect of Bactospeine on the rate of *P.citri* larval infestation under laboratory conditions.

Bioinsecticide	Concentration (cc/1 l.w.)	Mean no. of alive larvae/100 flowers		% reduction of larval infestation
		treated	untreated	
Bactospeine [*Bacillus thuringiensis* (berliner)]	2.0	2.6	26.2	90.08
	1.5	8.0	26.2	69.48
	1.0	11.6	26.2	55.73
(F) value			9.25**	
L.S.D. (0.05)			3.99	
L.S.D. (0.01)			6.22	

Table 3. Effect of Bio-fly on the immature stages of *P.citri* infestation under laboratory conditions.

Bioinsecticide	Concentration (cc/1 l.w.)	Mean no. of individuals/100 flowers						% reduction of infestation		
		hatched eggs		alive larvae		alive pupae		eggs	larvae	pupae
		t.	c.	t.	c.	t.	c.			
Bio-fly (*Baeuveria bassiana*)	4	68.3	200	11.8	37.4	5.6	20.4	65.83	68.45	72.55
	3	89.0	200	17.4	37.4	10.8	20.4	55.50	53.48	47.06
	2	128.0	200	26.2	37.4	17.6	20.4	36.00	29.95	13.73
F value =		17.04*		5.28*		66.23**				
L.S.D. (0.05) =		22.13		8.31		1.95				
L.S.D. (0.01) =		38.89		12.94		3.03				

t. = treated, c. = control

Table 4. Advanced effects of Bactospeine and Bio-fly used against the larvae on the final reduction rate of *P. citri* infestation under laboratory conditions.

Bioinsecticide	Concentration (cc/1 l.w.)	Mean no. of alive individuals			Final % reduction of infestation
		larvae*	pupae**	adults**	
Bactospeine	2.0	2.6	1.0	0.6	97.71
	1.5	8.0	5.6	5.6	78.63
Bio-fly	4.0	11.8	9.2	8.0	78.61
	3.0	17.4	17.4	17.4	53.48

* Larvae treated with bioinsecticides.
** Pupae and adults resulted from treated larvae.

Table 5. Efficacy of Bactospeine [*Bacillus thuringiensis* (berliner)] and Bio-fly [*Baeuveria bassiana*] on the reduction rate of *P.citri* infestation in lime orchards during 1996 and 1997 seasons.

Season	Bioinsecticide	Concentration (cc/1 l.w.)	Mean no. of alive larvae/200 flowers				% reduction of infestation		P.
			one spray		two sprays		one spray	two sprays	
			t.	c.	t.	c.			
1996	Bactospeine	2	12.0	45.2	4.6	45.2	73.45	89.82	0.05
1997	Bactospeine	2	11.6	39.6	2.6	39.6	70.71	93.43	0.01
1996	Bio-fly	4	10.2	22.8	5.4	22.8	55.26	76.32	0.01
1997	Bio-fly	4	11.8	27.4	5.8	27.4	56.93	78.83	0.01

t. = treated, c. = control

Field Application

Data in Table 5 showing the efficacy of one spray and two sprays of the two commercially products of Bactospeine (bacterial treatment) and Bio-fly (fungal treatment) which were applied at the doses of 2 cc/1l.w. and 4 cc/1l.w., respectively, on the reduction percentages of *P.citri* infestation in lime orchards throughout the two successive main flowering seasons of 1996 and 1997. Data elucidated that two sprays of Bactospeine at a concentration (2 cc/1l.w.) gave considerable reduction of infestation ranged 89.82-93.43%, while one spray reduced 70.71-73.45% of infestation. On the other hand, one spray with Bio-fly at a rate (4 cc/1l.w.) resulted 55.26-56.93% reduction of infestation, in the case of two sprays applied, reduction of infestation raised into 76.32-78.83% during 1996 and 1997 experiments. However, a bacterial treatment at a rate (2 cc/1l.w.) afforded the utmost reduction percentage of infestation followed by a fungal treatment at a rate (4 cc/1l.w.), consequently, a bacterial treatment was active pathogenic agent rather than a fungal treatment in spite of the later was used at a higher concentration. Moreover, statistical analysis showed significant differences between the number of sprays used and the reduction percentages of infestation.

From the forementioned results it could be recommended that two sprays of Bactospeine at a dose of 2 cc/1l.w. are quite effective to control *P.citri* on lime trees, the 1st spray begins mid-April and the 2nd spray takes place 7 days after the 1st one.

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تقييم معملى وتطبيق حقلى لبعض المبيدات الحيوية على فراشة أزهار الموالح فى حدائق الليمون بمصر

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تم دراسة تأثير المبيد الحيوى البكتيرى بكتوسبين والمبيد الحيوى الفطرى بيوفلاى ضد فراشة أزهار الموالح على أشجار الليمون البلدى تحت الظروف المعملية والحقلية خلال موسمى ١٩٩٦ و١٩٩٧ .

أظهرت النتائج المعملية أن المبيد البكتيرى كان أكثر تأثيراً على الحشرة مقارنة بالمبيد الفطرى، هذا بالرغم من أن التركيز ات المستعملة فى الأخير كانت أعلى. وجد أيضا أن المبيد البكتيرى بتركيز ٢ سم ٣ / لتر ماء ذو فعالية أكبر على اليرقات مقارنة بالحشرات الكاملة . أدى إستخدام المبيد الفطرى بتركيز ٤ سم ٣ /لتر ماء إلى تخفيض معدل الإصابة بالبيض ، اليرقات ، والعذارى بنحو ٥٦,٨٣ و ٦٨,٤٥ و ٧٢,٥٥ ٪ ، على الترتيب . تم أيضا دراسة التأثير المتقدم لمعاملة اليرقات بالمبيدات الحيوية على أطوار العذارى والحشرات الكاملة .

أوضحت نتائج التطبيق الحقلى أن إعطاء رشتين متتاليتين بالمبيدات الحيوية كان له تأثيراً معنويا كبيراً على تخفيض معدل الإصابة يفوق إستخدام رشة واحدة . أظهرت المعاملة البكتيرية بتركيز ٢ سم ٣ /لتر ماء ، والمعاملة الفطرية بتركيز ٤ سم ٣ /لتر ماء تخفيضاً لمعدل الإصابة بمقدار (٨٩,٨٢ - ٩٣,٤٣٪) و (٧٦,٣٢ - ٧٨,٨٣٪) ، على الترتيب . فى جميع الحالات تبين أن زيادة معدل التركيز ينتج عنه تخفيضاً أعلى لمعدل الإصابة .

يمكن بوجه عام التوصية بإعطاء رشتين من المبيد البكتيرى بمعدل ٢ سم ٣ /لتر ماء خلال موسم التزهير الرئيسى لأشجار الموالح للحصول على مكافحة جيدة لفراشة أزهار الموالح دون الحاجة إلى إستخدام المبيدات الحشرية الكيماوية .