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

W.A. SHEHATA AND Feeby N. NASR

Plant Protection Research Institute, Agricultural Research Centre, Dokki, Giza Egypt.
(Manuscript received 23 March, 1999)

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 Bactospine, Bacillus thuringiensis (berliner) was higher than that of Bio-fly, Baeuveria bassiana in spite of the latter was applied with higher doses. Bactospine at the rate (2 cc/1 l.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 l.w.) reduced the infestation with the eggs, the larvae and the pupae by 65.35%, 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 Bactospine or Bio-fly gave good control of P. citri more than one spray. Bactospine at the rate (2 cc/1 l.w.) and Bio-fly at the rate (2 cc/1 l.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 Bactospine at a rate (2 cc/1 l.w.) is adequate effective to reduce the population of P.citri in lime orchards during the main flowering period of citrus, the citrus flower moth Prays citri Mill. (lep.: Hypomoneutidae) 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 Atta 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 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, Holland.

2. Bio-fly F.C.[a.i., * Beauveria bassiana* 3X10^7 spores/mg.] was supplied by El-Nasr Bioinsecticides 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.i.w. and Bio-fly at the rates of 2.0, 3.0 and 4.0cc/l.i.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 transferred separately into 1 X 4 cm specimen tubes covered with muslin. Upon moth emergence, pairs of moths were introduced 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 divided into 2 groups, each one consisted of 20 replicates. Bactospeine at the rate of 2 cc/l.i.w. was added to the feed-
ing solution of moths of the 1st cages group, while the 2nd 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 female 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) divided 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 Bacillus sp. i.e. 1.0, 1.5 & 2.0 cc/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/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/l. w., the last 5 dishes only received water and used for comparison. Petals 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 applications 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 Bacillus sp. at the rate (2 cc/l. w.) and Bio-fly at the rate (4 cc/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 divided into 3 sets (5 trees/set); the 1st and 2nd 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:

\[
\% \text{ reduction of infestation} = \left[ \frac{(c-t)\times 100}{c} \right]
\]

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 50% 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, Bacospine (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 Bacospine 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, Bacospine caused 61.70% reduction of no. of eggs per female.

Table 1. Effect of bacterial treatment of Bacospine 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.

<table>
<thead>
<tr>
<th>Period/days</th>
<th>No. of eggs/ female</th>
<th>% reduction of</th>
</tr>
</thead>
<tbody>
<tr>
<td>adult longevity</td>
<td>oviposition</td>
<td>adult longevity</td>
</tr>
<tr>
<td>female</td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>2.75 (1-6)</td>
<td>3.43 (1-5)</td>
<td>7.8 (7-9)</td>
</tr>
</tbody>
</table>

1.2. The larval stage: The efficacy of Bacospine on Prays citri larvae was shown in Table 2. Data revealed that the treatments of Bacospine 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/l.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/l.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/l.w.), followed by 53.48% reduction at the moderate concentration (3 cc/l.w.), while the least larval reduction was 29.95% at the diluted concentration (2 cc/l.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/l.w. and 2 cc/l.w., respectively. The moderate reduction rate of moth emergence was 47.06% when pupae exposed for a mid concentration (3 cc/l.w.)

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

3. Advanced effects of Bactosprene 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 Bactosprene at the rate (2.0 cc/l.w.) and Bio-fly at the rate (4 cc/l.w.) were applied on the larvae; at the same time, both the pupae and the adults were affected as Bactospino was applied at a rate (1.5 cc/l.w.) against the larvae, its effect continued on the pupae, caused 78.60% final reduction of infestation. Bio-fly used at the rate (3 cc/l.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.

<table>
<thead>
<tr>
<th>Bioinsecticide</th>
<th>Concentration (cc/1 l.w.)</th>
<th>Mean no. of alive larvae/100 flowers</th>
<th>% reduction of larval infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>treated</td>
<td>untreated</td>
<td>treated</td>
</tr>
<tr>
<td>Bactospeine</td>
<td>[Bacillus thuringiensis (berliner)]</td>
<td>2.0</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>8.0</td>
<td>26.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>11.6</td>
<td>26.2</td>
</tr>
<tr>
<td>(F) value</td>
<td>0.0</td>
<td>0.1</td>
<td>9.25**</td>
</tr>
<tr>
<td>L.S.D. (0.05)</td>
<td>0.0</td>
<td>0.2</td>
<td>3.09</td>
</tr>
<tr>
<td>L.S.D. (0.01)</td>
<td>0.0</td>
<td>0.3</td>
<td>6.22</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Bioinsecticide</th>
<th>Concentration (cc/1 l.w.)</th>
<th>Mean no. of individuals/100 flowers</th>
<th>% reduction of infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>hatched eggs</td>
<td>alive larvae</td>
<td>alive pupae</td>
</tr>
<tr>
<td></td>
<td>t.</td>
<td>c.</td>
<td>t.</td>
</tr>
<tr>
<td>Bio-fly</td>
<td>[Bacillus bassiana]</td>
<td>4</td>
<td>69.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>69.0</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>128.0</td>
<td>200</td>
</tr>
<tr>
<td>(F) value</td>
<td>14.06</td>
<td>17.05</td>
<td>17.04*</td>
</tr>
<tr>
<td>L.S.D. (0.05)</td>
<td>9.00</td>
<td>12.01</td>
<td>22.13</td>
</tr>
<tr>
<td>L.S.D. (0.01)</td>
<td>18.00</td>
<td>24.01</td>
<td>38.89</td>
</tr>
</tbody>
</table>

* t. = treated, c. = control
Table 4. Advanced effects of Bactospene and Bio-fly used against the larvae on the final reduction rate of *P. citri* infestation under laboratory conditions.

<table>
<thead>
<tr>
<th>Bioinsecticide</th>
<th>Concentration (cc/t l.w.)</th>
<th>Mean no. of alive individuals</th>
<th>Final % reduction of infestation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>larvae*</td>
<td>pupae**</td>
</tr>
<tr>
<td>Bactospene</td>
<td>2.0</td>
<td>2.6</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>8.0</td>
<td>5.6</td>
</tr>
<tr>
<td>Bio-fly</td>
<td>4.0</td>
<td>11.8</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>17.4</td>
<td>17.4</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Season</th>
<th>Bioinsecticide</th>
<th>Concentration (cc/t l.w.)</th>
<th>Mean no. of alive larvae/200 flowers</th>
<th>% reduction of infestation</th>
<th>P.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>one spray</td>
<td>two sprays</td>
<td>one spray</td>
</tr>
<tr>
<td>1996</td>
<td>Bactospene</td>
<td>2</td>
<td>12.0</td>
<td>45.2</td>
<td>4.6</td>
</tr>
<tr>
<td>1997</td>
<td>Bactospene</td>
<td>2</td>
<td>11.6</td>
<td>39.6</td>
<td>2.6</td>
</tr>
<tr>
<td>1996</td>
<td>Bio-fly</td>
<td>4</td>
<td>10.2</td>
<td>22.8</td>
<td>5.4</td>
</tr>
<tr>
<td>1997</td>
<td>Bio-fly</td>
<td>4</td>
<td>11.8</td>
<td>27.4</td>
<td>5.8</td>
</tr>
</tbody>
</table>

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 Bactospine (bacterial treatment) and Bio-fly (fungal treatment) which were applied at the doses of 2 cc/l.lw. and 4 cc/l.lw., respectively, on the reduction percentages of P.citr in lime orchards throughout the two successive main flowering seasons of 1996 and 1997. Data elucidated that two sprays of Bactospine at a concentration (2 cc/l.lw.) 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/l.lw.) 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/l.lw.) afforded the utmost reduction percentage of infestation followed by a fungal treatment at a rate (4 cc/l.lw.), 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 aforementioned results it could be recommended that two sprays of Bactospine at a dose of 2 cc/l.lw. are quite effective to control P.citr on lime trees, the 1st spray begins mid-April and the 2nd spray takes place 7 days after the 1st one.
REFERENCES


تقييم معملي وتطبيق حقيقة لبعض المبيدات الحيوية
على فراشة أزهار الموالح في حدائق الليمون بمصر

وجهة أبوب شماتة، فهفي دُبية نصر

معهد بحوث ومقاومة الديدان- مركز البحوث الزراعية - الدقي- الجيزة.

تم دراسة تأثير المبيد السيكولي: بيكسيسين والمبيد البكيولي: بيكسيسين-

أظهرت النتائج العملية أن المبيد المكترفي كان أكثر تأثيراً على الخرزوة مقاومة للبيد
القطري، هذا بالرغم من أن المبيدات المستعملة في الأخير كانت أعلى. وجد أيضاً أن المبيد
البكيولي بتركيز 2 سم/3 للتر، له فعالية أكبر على الفراشة مقاومة بالخزوة لكلفة. أما
استخدام المبيد الواثقي بتركيز 4 سم/3 للتر، له فعالية الأفضلية بالخزوة، البهارات،
وال耧اطية بتركيز 0.85 و1.5 و2.77 للتر، تم أيضاً دراسة التأثير المقدم للمعايدة
الخزوة بالمبيدات الحيوية على أطر الفراشة والخزوة الكاملة.

أوصى نتائج التطبيق أن إعطاء دُبيشتين متتابعتين للمبيدات الحيوية كان له تأثيراً
معنوي كبيرًا على تخفيض معدل الإصابة فوق استخدام رشة واحدة. أظهرت المعايدة البتريدي
بتركيز 2 سم/3 للتر، والمادة الخزوة بالكترفي 4 سم/3 للتر، له فعالية تقلد الإصابة بقدر
الأフォー بتركيز مشابه في جميع المراحل التي تبين أن إعطاء معد
المادة الخزوة من تركيز عادل فعالاً بفعل الإصابة.

يمكن بوجه عام التوصية بإعطاء دُبيشتين متتابعتين للمبيد البكيولي بتركيز 2 سم/3 للتر، خلال
فصول المزرعة الرئيسي لإشجار الفراشة لتكشف على مكاسب جيدة لفراشة أزهار الموالح دون
ال_NEED إلى استخدام المبيدات المشرفة الكيميائية.