CONTROL OF THE EGYPTIAN COTTON LEAFWORM<br>Spodoptera littoralis (BOISD.) BY USE OF FORMULATED BACTERIA.<br>Youssef, L.A. 1; M. Mariy Faiza 1; S.A. Ibrahim 2 and I. Gamal Walaā. 1<br>1- Plant Protection Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Khelma, Cairo, Egypt<br>2- Genetics Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt

ABSTRACT

The efficacy of two pathogenic bacteria Bacillus thuringiensis var. kurstaki HD129 and Serratia marcescens were evaluated on 3rd and 5th larval instars of Spodoptera littoralis. These bacterial products were isolated by the Ain Shams Center of Genetic Engineering and Biotechnology. The commercial product Bacillus thuringiensis var. kurstaki "Proecto" was considered as a standard for comparison. Although, both two tested bacterial isolates had a high LC50 than the commercial product Proteclo, both exhibited higher accumulative mortality. This effect was more apparent for Serratia marcescens than HD129 i.e. 66.5% and 62% respectively for treatment of 3rd instar. Furthermore, the time required to kill 50% of insects (LT50) was the lowest when Serratia marcescens was tested, it was 4 days for both larval instars treated. Meanwhile, this period was 5 and 8 days for 3rd and 5th instar larvae treated with HD129 and it averaged 7 days for both treated instars when Proteclo was used. Percentage of malformation was higher and number of larvae pupation was lower when the two tested bacterial isolates were tested than commercial product.

INTRODUCTION

Microbial control is alternatives to chemical control agents to insect pests and is often species specific. Microbial control agents although may not meet the speed of action of chemical insecticides. They have been generally shown to have no negative impacts on plants and mammals or even non-target insects. Bacterial biopesticides are dominated by Bacillus thuringiensis strains, meanwhile the virus nuclear polyhedrosis virus (known as NPV) generally plays a significant role.

The Egyptian cotton leaf worm S. littoralis is an insect pest of an economic importance with a wide range of host plants. This species has acquired resistance to many insecticides and the use of other control measures is essential to aid in an over all IPM program Many lepidopteran species have been successfully controlled by microbial agents, e.g control of S. littoralis by B. thuringiensis (El-Hamaeky et al., 1990; Salama et al., 1993; Salem 1995; El- Gahr et al. 1995; Salama and Foda 1982; Salama et al. 1984) or control by using NPV (Salama et al. 1993; Harapaz and Wysoki 1984). The present study was conducted to evaluate the control effect of B. thuringiensis var. kurstaki HD129 and a strain of the bacterium Serratia marcescens on larvae of the cotton leaf worm Spodoptera littoralis.
MATERIAL AND METHODS

The original colony of the cotton leaf worm *S. littoralis* was obtained from a well-established culture, maintained at the Department of Plant Protection Faculty of Agriculture, Ain Shams University. Insect rearing was conducted in the laboratory as described by Youssef (1991).

Bacterial Cultures: -

The potency of two bacterial isolates were evaluated towards 3rd and 5th instar larvae of *S. littoralis*. The following isolates of bacteria were tested:

(i) *Bacillus thuringiensis* var. *kurstaki* (HD129)

(ii) *Serratia marcescens*.

These two isolates were kindly supplied as slants from Ain Shams Center of Genetic Engineering and Biotechnology to evaluate the efficiency of these two isolates. The commercial product *B. thuringiensis* var. *kurstaki* (Protecto) was used as a standard for comparison. This product was obtained as a wettable powder from the Plant Protection Research Institute, Ministry of Agriculture, Cairo.

Maintenance of *B. thuringiensis* var. *kurstaki* (HD129): -

Subcultures from the bacteria *B. thuringiensis* var. *kurstaki* (HD129) were made by inoculation in a defined media of Pepton Yeast Extract as described by Mohammed (2002). The inoculated flasks were incubated at 30± 1°C for 24h on a shaker set at 100-150 rpm. Pepton yeast extract agar plates were streaked by inoculate of the grown bacteria in the cultured test tubes using the streaking dilution method to obtain solitary pure colonies. Plates were incubated for 24h. at 30± 1°C. Solitary colonies grown on the agar surfaces were selected and subcultured on agar slant and kept until needed for the experimental work.

Maintenance of the bacteria *S. marcescens*: -

Subcultures from the bacterial samples *S. marcescens* were made by inoculation of Pepton Glycerol media. The inoculated flasks were incubated at 30± 1°C for 24h on shaker (set 100-150 rpm.) to obtain solitary pure colonies. Pepton Glycerol agar plates were streaked by inoculate of the grown bacteria in the cultured test tubes and incubated for 24h. at 30± 1°C. Solitary colonies grown on agar surface were selected and subcultured on agar slants and reserved until required for the experimental work.

Bioassay for bacteria: -

One ml. of each of subculture HD129 and *Serratia marcescens* was placed in 100ml distilled water. As described by Schlegel (1986), series of dilutions were prepared [1%, .01%, 1x10⁻⁴%, 1x10⁻⁵%, 1x10⁻⁶%, 1x10⁻⁷%] from which the number of colony forming unit (cfu) were determined.

The commercial product Protecto (obtained as a wettable powder) series of dilutions were prepared from 1gm of the product [1%, .01%, 1x10⁻
4%, 1x10^{-3}%, 1x10^{-6}%, 1x10^{-8}%, 1x10^{-10}%). Also the number of colony forming unit (CFU) were counted according to Schlegel (1986).

The larvicidal activity of the bacterial strains was evaluated on newly moulted 3rd and 5th instar of *S. littoralis* larvae. Fresh Castor oil leaves were cut in leaf discs, measuring 3 cm in diameter. These discs were immersed in each of the prepared dilution of each tested strain and then left to dry at room temperature before being offered to the 3rd and 5th instar larvae confined in plastic cups. Larvae well fed on contaminated leaf discs for 3 days and then provided with uncontaminated leaf discs for the subsequent duration of the larval instars. Each treatment was comprised 25 larvae and was replicated 5 times. The same numbers of larvae were considered as a control, which was offered castor oil leaves immersed in distilled water. Larval development as well as pupal survival and level of infection were considered, any malformation and sequence of infection were recorded. The sequences of symptoms of infection were recorded as well as larval development. Mortality was calculated daily and an accumulative larval mortality was determined at the end of the larval stage. Mortality percentages were corrected according to Abbott (1925) formula. Results were presented graphically as log/probit regression lines and LC_{50} values calculated by computer program Sigma plot for Windows (version 21). Furthermore, any malformation of larvae or pupa was recorded. As the standard commercial product Protecto is of known potency, the LC_{50} of the two tested bioagents HD129 and *Serratia marcescens*. Potency was calculated by the following formula, as described by Salama and Foda (1982).

\[
\text{Potency sample (IU/mg)} = \frac{\text{LC}_{50} \text{ standard} \times \text{potency of standard (IU/mg)}}{\text{LC}_{50} \text{ sample}}
\]

**RESULTS**

A range of concentrations was prepared from *(HD129)* and *S. marcescens*. These preparations were tested on 3rd and 5th instar larvae of *S. littoralis* (Boisd) HD129 and *S. marcescens*. Toxicity was exhibited in a dose dependent phenomenon. Generally, the symptoms of the toxins to treated larvae could be summarized in the following sequence: -

(i) Loss of appetite as insect food consumption decreases as denoted by smaller castor oil leaves surface area eaten.

(ii) Decrease response to stimulation.

(iii) Diarrhea and larvae regurgitating vomiting of some fluids.

Furthermore, toxicity of HD129 was exhibited by the appearance of spots on the prolegs that then extend as dark brown on the abdomen then to the entire body. The larvae's body contents were soft to touch and the integument with a firm texture. Meanwhile, infection with *Serratia marcescens* toxins causes the appearance of a reddish pink pigmentation first on the prolegs that then extend on the whole integument, the infected larvae become very soft and the integument ruptures easily. Both tested bioagents lead to subsequent larval paralysis and death. From the plotted regression
lines the LC$_{50}$ values of the tested toxins were determined, results are shown in Fig (1,2). Also LT$_{50}$ and a cumulative percentage mortality of larvae are exhibited in Table (1,2).

Table (1): Potency of at LC$_{50}$ values on 3$^{rd}$ instar larvae of *Spodoptera littoralis*.

<table>
<thead>
<tr>
<th>Bioagents</th>
<th>LC$_{50}$ (cfu)</th>
<th>Slope</th>
<th>Potency (IU/mg)</th>
<th>LT$_{50}$ (days)</th>
<th>Accumulative % mortality (at the end of larval stage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protecto</td>
<td>40*10$^5$</td>
<td>0.37273</td>
<td>32000</td>
<td>7</td>
<td>56%</td>
</tr>
<tr>
<td>HD129</td>
<td>65*10$^5$</td>
<td>0.312715</td>
<td>52000</td>
<td>5</td>
<td>62.1%</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>105*10$^7$</td>
<td>0.299390</td>
<td>84000</td>
<td>4</td>
<td>66.5%</td>
</tr>
</tbody>
</table>

(F) between treatments = 0.29327 (sign.)  
LSD = 11.724

Table (2): Potency of at LC$_{50}$ values on 5$^{th}$ instar larvae of *Spodoptera littoralis*.

<table>
<thead>
<tr>
<th>Bioagents</th>
<th>LC$_{50}$ (cfu)</th>
<th>Slope</th>
<th>Potency (IU/mg)</th>
<th>LT$_{50}$ (days)</th>
<th>Accumulative % mortality (at the end of larval stage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protecto</td>
<td>35*10$^7$</td>
<td>0.3171522</td>
<td>32000</td>
<td>7</td>
<td>62%</td>
</tr>
<tr>
<td>HD129</td>
<td>46*10$^7$</td>
<td>0.285920</td>
<td>42057</td>
<td>8</td>
<td>56%</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>112*10$^7$</td>
<td>0.278338</td>
<td>102400</td>
<td>4</td>
<td>64%</td>
</tr>
</tbody>
</table>

(F) between treatments = 1.32871 (sign.)  
LSD = 8.310

Protecto, the commercial *B. thuringiensis* var. *kurstaki* was used as a standard in a range of concentration and LC$_{50}$ determined under conditions of the present work [Fig (3)]. Its LT$_{50}$ and accumulative percentage are shown in Tables (1,2), it was obvious that this commercial product was the most toxic to *S. littoralis* larvae either treated as 3$^{rd}$ or 5$^{th}$ instars. The LC$_{50}$ was 40*10$^5$ and 35*10$^7$ cfu. respectively HD129 was more toxic than *S. marcescens*. Its LC$_{50}$ was 65*10$^5$ and 46*10$^7$ cfu for 3$^{rd}$ and 5$^{th}$ larval instars respectively. Meanwhile, for *S. marcescens* these values were 105*10$^5$ and 112*10$^7$ cfu for the respective mentioned instars. However, the LT$_{50}$ of *S. marcescens* was slightly more rapid than HD129, as 50% of treated 3$^{rd}$ instar larvae died after 4 days approximately.

Meanwhile, LT$_{50}$ was 5 days when HD129 was used, but was extended to 7 days when Protecto was used. As expected 5$^{th}$ instar larvae were much more tolerant than 3$^{rd}$ instars. This was evident for the two tested bioagents as well as the standard commercial Protecto. The accumulative percentage mortality (at the termination of the larval stage) was higher when *S. marcescens* was tested than for the use of HD129 or Protecto, (Tables 1,2). It was found to be 66.5% and 64% for 3$^{rd}$ and 5$^{th}$ instars respectively as compared to 62% and 56% when HD129 was tested for the respective mentioned larval instars.

The potency sample of *S. marcescens* was much higher than that of HD129, as this potency was 84000 and 102400 IU/mg for the treatment of 3$^{rd}$ and 5$^{th}$ instar larvae respectively, (Table 1,2). This value could be expressed a ratio increase of 1:16 and 1:24 than that of HD129. Some larvae of *S. littoralis* recovered from the toxins up on transfer to control diet.
Fig (1) Effect of HD129 on 3\textsuperscript{rd} & 5\textsuperscript{th} instar larvae of \textit{S. littoralis}

- HD129 3\textsuperscript{rd} instar larvae (LC50=65*10\textsuperscript{5} cfu)
- HD129 5\textsuperscript{th} instar larvae (LC50=46*10\textsuperscript{5} cfu)

Fig (2) Effect of \textit{S. marcescens} on 3\textsuperscript{rd} & 5\textsuperscript{th} instar larvae of \textit{S. littoralis}

- \textit{S.m.} 3\textsuperscript{rd} instar larvae (LC50=105*10\textsuperscript{5} cfu)
- \textit{S.m.} 5\textsuperscript{th} instar larvae (LC50=112*10\textsuperscript{5} cfu)
Meanwhile, other larvae appeared with some abnormalities, which was more evident upon moulting (Fig 4). When 5th instar larvae were infected with LC50 of the tested HD129 and S. marcescens the duration of the subsequent instars of the larvae that survived was not significantly different than those of the control. Meanwhile, for the treatment of 3rd instar larvae, only the duration of the 6th instar was slightly impaired, the period of this last instar was shortened by 36-48 hours than the control. Following treatment with the two tested bioagents at LC50 value, the number of surviving larvae pupating was reduced between 30-36 % for both instars treated. Meanwhile, it was between 26-28 % when Protecto was used. The percentage of malformed pupae was more evident when 5th instar were treated, especially with treatment by LC50 of HD129 as it reached 12% as compared to 4 and 8 % when 3rd instar were fed on castor oil leaves contaminated with LC50 of S. marcescens and Protecto respectively.

Malformation of pupa was mainly observed as shortening of their length and appearance of larval-pupal intermediates, (Fig 5), in all treatment adult eclosion was totally inhibited as insect failed to emerge as moths or died as pupa.
Fig (4): Malformed larvae of *Spodoptera littoralis* following treatment by LC$_{50}$ of HD129 as 5$^{th}$ instar.

Fig (5): Malformed pupa of *Spodoptera littoralis* following treatment by LC$_{50}$ of HD129 as 5$^{th}$ instar.
DISCUSSION

New isolates of bacteria have to be established so as to avoid the building of resistant of *S. littoralis* to this bacterial bioagents. In the present work a new isolates of *B. thuringiensis var. kurstaki* was tested i.e. (HD129) as well as the bacterium *S. marcescens*.

Potency of these two bioagents were compared with a standard commercial *B. thuringiensis var. kurstaki* product Protecto widely used for the control of many lepidopterous insects [El-Hamaeky et al. 1990, Vandenberg & Shimanuki 1990]. This commercial product proved to have a higher toxic effect than the other two bioagents investigated, as exhibited by it's much lower LC50 value. This is somehow expected as it being a commercial product it must has a longer persistence or activity as probable results of the addition of adjuvants or additives to achieve high efficacy. Meanwhile, HD129 and *S. marcescens* are newly prepared isolates. However the potency of the two tested bioagents was quite comparable, LC50 and potency sample of HD129 were much lower than that of *S. marcescens* which in contrast exhibited the highest LC50 value calculated. Meanwhile, Farrar et al. (1998) reported that the bacterial isolate *S. marcescens* killed the corn earworm with a very low oral dose. However, LT50 of *S. marcescens* was the lowest exhibiting 4 days for larvae treated either as 3rd or 5th instars. This period was the maximum-recorded i.e. 7 days when Protecto was used. It is a well-known fact that older larvae are usually much more tolerant to the toxic effect of many bioagents [Mohamed et al. 2000, Romellah and Abdel-Mageed 2000].

This site of action of *B. thuringinese* toxin is the insect mid gut epithelium Gill et al. (1992) and one of the symptoms of poisoning is gut paralysis Gould and Anderson (1991). The bacterial bioagents do not cause a rapid kill, therefore their effect becomes apparent after a few days as infected insects eat little, later leading to starvation and death. Form the obtained results, it seems that *S. marcescens* caused much more rapid toxic effect, one or more factors are probably responsible for the potency of this toxin. Furthermore, the potency of *Serratia marcescens* when used at LC50 values was superior to Protecto and also slightly higher than HD129 to 3rd and 5th instars. This was exhibited in a higher accumulative mortality percentage as well as a higher reduction in larvae entering the pupal stage. Although, with higher LC50 value HD129 was more efficient for the control of *S. littoralis* larvae than Protecto. The binding characteristics of HD129 to the mid gut epithelium of the infected larvae could be involved as suggested by Herreo et al. (2001) and Gilliand et al. (2002).
REFERENCES


El-Hamasyky M.; AF. Rafaeli; MA. Heagazy and NM. Hussein (1990). Knock-down and residual activity of certain insecticides Bacillus thuringiensis and their binary mixtures against the cotton leaf worm Spodoptera littoralis (Boisd) in cotton fields. Mededelingen-van-de-Faculteit-Landbouwweten schappen, Rijks Universiteit. Gent. 55.2b, 593-599.


Mohamed Manal S. (2002). Genetic improvement of the efficiency of some bacterial biopesticide by gene manipulation techniques. B.Sc. Faculty of Agriculture, Ain Shams University, Shoubra El- Kheima, Cairo, Egypt.


Youssef, L. A. et al.


Salama H.S.; A. Sharaby and MM. El-Din (1993). Mod. of action of Bacillus thuringiensis and nuclear polyhedrosis virus in the larvae of Spodoptera littoralis (Boisd). Insect Science and it’s application 14 (4): 537-543.

Salama HS.: A.Sharaby and MM. EI-Din (1993). Mod of action of Bacillus thuringiensis and nuclear polyhedrosis virus in the larvae of Spodoptera littoralis (Boisd) . Insect Science and its application 14 (4) : 537·543.


Vandenberg J.D. and U. Shimanuki (1990) . Application methods for Bacillus thuringiensis used to control larvae of the greater wax moth (Lepidoptera: Pyralidae) on stored bee wax combs . J. Econ. Entomol. 83(3) : 766·771.