Combined effects of *Bacillus thuringiensis* and *Serratia marcescens* on cotton leaf worm, *Spodopteralittoralis*

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Abstract: In this study evaluated insecticidal activity of ***Bacillus thuringiensis*** and *Serratia marcescens* against cotton leaf worm, *Spodopteralittoralis*. Biopesticides (*Bacillus thurinogensis* (4QSTR1) + *Serratia marcescens*) appeared significant increase in duration by days of larval stage in three concentrations (20, 25 and 75 ppm). Whereas, *Bacillus thurinogensis* (4QSTR1) appeared the same trend in 75 ppm. The biopesticides (*Bacillus thurinogensis* (4QSTR1) + *Serratia marcescens*) showed significant increase in pupation percentage in all treatments about 75 ppm. The highest effect in emergence of *S. littoratis* by bioinsecticid *Bacillus thurinogensis* (4QSTR1) + *Serratia marcescens* in 25,50 and 75 ppm concentration.
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1. Introduction

Insect pest management in agriculture is important to safeguard crop yields and productivity. The cotton leaf worm, *Spodopter alittoralis* (Boisd.) is a highly destructive insect pest. The cotton leaf worm is a key pest for wide range of economical pests on cotton. Controlling larval stage with recommended pesticides became insufficient (**Badr *et al.*, 1995**). The extensive use of insecticides to control *S. littoralis* larvae has led to several problems as development of resistance and residual effects **(Frank *et al*., 1990).** Chemical insecticides have negative potential on environmental pollution. They can kill non-target organisms and cause human intoxication. Insecticides caused to important need to use new environmentally acceptable products (**Tirado Montiel *et al.*, 2001**). Many insecticidal proteins and molecules are available in nature, which are effective against agriculturally important pests but innocuous to mammals, beneficial insects and other organisms.

The use of bacteria as biological control agents may minimize the problems caused by the excessive useof chemicals.Microbial pesticides are becoming recognized as an important factor in crop and forest protection and in insect vector control (**Khetan, 2001**). *Bacillus thuringiensis* (Berliner) (Bt) is the most widely used biopesticide (**Glare & Callaghan 2000**). B. *thuringiensis* produces an insecticidal protein toxin during sporulation called endotoxin proteins. This toxin is effective against three orders of insect pests (Lepidoptera, Diptera, and Coleoptera) (**Vidyarthi *et al.*, 2002**).The produce of insecticidal protein crystal has made this organism the most successful commercial biopesticide and the development of new toxin delivery systems **(Martens *et al*., 1995).**

Serratia marcescens, a gram-negative bacillus classified as a member of the Enterobacteriaceae. *Serratia marcescens* produces chitinolytic enzymes, which causes degradation of chitin in cell walls, physiological and morphological effects on pupal and adult stages where it caused a significant increase in the proportion of pupal mortality, adult malformation and sterility with treated moths and also affected some enzymes activity (**El-Sheikh, 2006**).The increase of the demand of the bioproduct in comparison to chemical products is due to many advantages of the bioproduct **Azevedo *et al.* (2002**) .

This investigation aimed to evaluate insecticidal activity of combined effects of *Bacillus thuringiensis* and *Serratia marcescens* against larvae of *Spodopteralittoralis*.

2. Materials and Methods

Microbial Strains

Bacillus thurinogensis(4QSTR1) was obtained from Bacillus Genetics Stock Center, Biochemistry Dept., Ohio University, Columbus, USA and *Serratia marcescens* was obtained from Department of Agriculture microbiology, Soil, water and Environmental Institute, Agriculture Research Center, Giza, Egypt. The strains were maintained on L.B medium, containing: 1% tryptone, 0.5% yeast extract and 0.5% NaCl, pH 7.5 (**Sambrook *et al*., 1989**).

Plants:

Fresh leaves of cotton were collected daily, squares and middle leaves were used for the

experiments. Leaves were cleaned and three grams were weighted and placed in clean containers.

Cotton leafworm.

Spodopteralittoralis: wild type strain of *S. littoralis* used in this study. *Spodoptera Littoralis* (Boisd) larvae obtained from the laboratory culture of plant protection research institute, agricultural research center**.** Egg masses were kept in glass jars (500 ml) with untreated castor bean leaves till hatching covered with cotton cloth and supplemented according to **(Klanfonand De Barjac, 1985).**

Methods

Separation of Crystals and Endospores

Crystals and endospores were collected and purified according to **Karamanlidou *et al*. (1991).** Pellets were resuspended in small volumes of distilled water and stored at -5°C.

Bioassay of Toxicity

Three bioinsecticide (*B.thuringiensis*, *Serratia marcescens* and combined of *Bacillus thuringiensis* and *Serratia marcescens***)** containing endospores and crystals were applied on 250 ml bottles as well as mixed with 3 grams of leaves as diet for larvae. Five concentrations were prepared in distilled water which were as follows (0.0, 20, 25, 50 and 75 ppm). The food for larvae was prepared by soaking three gram of fresh leaves of cotton in 10 ml of each bacterial suspension using three fold**.** The leaves were removed after 24 hrs and replaced by another treated ones after the jars were cleaned and dried. Larval mortality was recorded daily up to pupation developed. Mortality percentage was corrected by abbott formula (**Abbott, 1925**). Percentage of pupation and moth (butter fly) emergence were based on the number of normal pupae or moths (butter flys) obtained.

Statistical analysis:

The obtained data of mite numbers were subjected to the analysis of variance test (ANOVA) with mean separation at 5% level of significance according to the method of **Snedecor and Cocharn (1967).**

3. Results and discussion

As shown from the results presented in the Table 3, biopesticides(*Bacillus thurinogensis* (4QSTR1) + *Serratia marcescens*) appeared significant increase in Duration by days of larval stagein three concentrations (20, 25 and 75 ppm). Whereas, *Bacillus thurinogensis* (4QSTR1) appeared the same trend in 75 ppm. This result agreed with **Mohamed (2006)**, also found that the development time of larvae and pupae were extended as well as adult emergence after treatment with bacterial or viral agents.

Table 1. Duration by days of larval stage treated with different concentrations of bioinsecticide.

Treatment	Bioinsecticide concentrations(ppm)			
	20	25	50	75
Control	11	11	11	11
Bacillus thurinogensis (4QSTR1)	6	10	9	3
Serratia marcescens	10	9	7	4
Bacillus thurinogensis (4QSTR1) + *Serratia marcescens*	12	8	6	6
F- test	NS	*	*	*
LSD 5%		3.03	3.5	5.8

NS,*,** = Insignificant differences , significance at 0.05 and 0.01 probability levels, respectively.

The data presented in Table 2 showed that biopesticides (*Bacillus thurinogensis* (4QSTR1) + *Serratia marcescens*) showed significant increase in pupation percentage in all treatments about 75ppm. This result agreed with **El-Khateeb and El-Sabagh, (2008)** found that a low reproductive capacity in the cotton leaf worm moths treated with bioagent.

Table 2. Pupation percentage of *S. littoratis* treated with different concentrations of bioinsecticide.

Treatment	Bioinsecticide concentrations(ppm)			
	20	25	50	75
Control	1.00	1.00	1.00	1.00
Bacillus thurinogensis (4QSTR1)	0.15	0.12	0.13	0.02
Serratia marcescens	0.18	0.14	0.09	0.02
Bacillus thurinogensis (4QSTR1) + *Serratia marcescens*	0.11	0.14	0.07	0.04
F- test	NS	*	NS	*
LSD 5%		0.054		0.061

NS,* = Insignificant differences, significance at 0.05 probability levels, respectively.

Data presented in Table 5 showed the highest effect in emergence of *S. littoratis* by bioinsecticid *Bacillus thurinogensis* (4QSTR1) + *Serratia marcescens*in 25,50 and 75 ppm concentration. This result agreed with **Abdel-Aal *et al.* (2009)** found that some chitin synthesis inhibitors (CSI) increased chitinase activity of the late 6th instar larvae of *S. littoralis* and recorded that chitinase and protease are essential for digestion of old endocuticle in the moulting process. So, any changes in these enzyme activities may attribute to the interference of the (CSI) in moulting process. Whereas, **El- Shershaby *et al.* (2008)** indicated that, B. thuringiensis var. kurstaki resulted in a great reduction in protein content of *S. littoralis* larvae and these toxins of *B. thuringiensis* are responsible for the inhibition of protein synthesis by forming a protein complex.

Table 3. Emergence of *S. littoratis* treated with different concentrations of bioinsecticide .

Treatment	Bioinsecticide concentrations(ppm)			
	20	25	50	75
Control	1	1	1	1
Bacillus thurinogensis (4QSTR1)	0.12	0.11	0.05	0.02
Serratia marcescens	0.08	0.10	0.05	0.02
Bacillus thurinogensis (4QSTR1) + *Serratia marcescens*	0. 14	0.13	0.04	0.04
F- test	*	*	NS	*
LSD 5%	0.068	0.164		0.061

NS,*,** = Insignificant differences , significantce at 0.05 and 0.01 probability levels, respectively.

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