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The Potential Activities of Two *Bacillus thuringiensis* Strains Against the Neonate Larvae of *Pectinophera gossypiella*

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ABSTRACT

The current study was conducted to investigate the biological activities of the two strains of *Bacillus thuringiensis* (*Bacillus thuringiensis* var. *Kurstaki* 1(*Bt K*1) and *Bacillus thuringiensis* var. *Kurstaki* 2 (*Bt K*2)) against the newly hatched (neonate) larvae of the pink bollworm, *Pectinophera gossypiella*.

The two strains exhibited their toxicity against the treated larvae. Also, the lethal effect was extended in the resulted stages, pupae and adults. Based on LC_{50} for total mortality, *Bt K*1 was more potent than *Bt K*2 where LC_{50} was $2.21x10^{10}$ and $3.11x10^{10}$, respectively. However, the two strains were revealed a reduction of pupation and adult emergence %. Irrespective of the strain, *Bt* significantly decreased larval duration and significantly increased pupal duration. No effect was recorded on morphogenesis.

In the present study, it was broadly that *Bacillus thuringiensis* showed its ability in the control of *Pectinophora gossypiella*.

INTRODUCTION

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is considered the main insect pests infested the cotton plants causing decreasing qualities and quantities of the cotton yield (Jaleel *et al.*, 2014; Parmar and Patel 2016; Moustafa *et al.*, 2019). This pest is difficult to control with insecticides (Lykouressis *et al.*, 2005).

Bacillus thuringiensis (*Bt*) is the most commonly used biopesticide worldwide (Osman *et al*., 2015). *Bt* can induce mortality, effects on growth and reproduction (Barker 1998; Erb *et al*., 2001; Huang *et al.*, 2018). Although many bacteria cause diseases to insects (Contwell 1974), only a few are used commercially as control agents. Some bacteria have been isolated from soil, insect habitats (Ohba *et al.*, 1979; McSpadden Gardner 2004), insect larvae (Abou El-Ela 1996), or stored products (Kares 1991). Despite most species of *Bacillus* are harmless saprophytes, two species viz., *B. thuringiensis* and *B. cereus* are considered important in the field of controlling some plant insects (Gray *et al*., 2006).

The use of entomopathogenic *Bt* as an insect biological control agent has received worldwide attention (Legwaila *et al.*, 2015; Opisa *et al.* ,2018). The aim of the present study is to evaluate the susceptibility of *P. gossypiella* to entomopathogenic bacteria.

MATERIALS AND METHODS

Rearing of Insect:

A culture of the pink bollworm, *P. gossypiella* (Sanders) (Lepidoptera: Gelechiidae) was reared under constant laboratory conditions of (27±1 °C and 65 % R.H) in the rearing room at the Bio Insecticides Production Unit, Plant Protection Research Institute, DoKki, Giza, Egypt. The neonate larvae were reared on an artificial diet described by (Rashad *et al*. 1993). The pupae were kept in clean glass villas without diet which was plugged with cotton until moths emerge.

Entomopathogenic Bacteria:

Bacillus thuringensis, kurstaki (K1 and K2) obtained from producing bioinsecticides, plant protection research institute Agriculture research center, Egypt.

Bioassay:

To study the toxicity of the entomopathogenic bacteria *B. thuringiensis* against the pink bollworm *P. gossypiella*, five concentrations of bacteria (10^8, 10^9, 10^{10}, 10^{11} and 10^{12} (bacteria/ml)) were prepared.

Insect Treatment:

Early 4th larval instar was immersed in five concentrations of bacteria for 30-60 Seconds and then transferred to sterile filter paper to dry. Four replicates (each replicate contained ten 4th larval instar). A control experiment was done, but larvae were immersed in distilled water. 4th larval instar was transferred by sterile forceps to glass tubes (2×7 cm) containing an untreated artificial diet. Tubes were plugged with cotton wool and incubated at 27±1 °C and 65 % R.H. The mortality was recorded daily until pupation and adult emergence.

Studied Criteria:

Mortalities, Pupation rate and adult emergence rate were expressed as %. The duration was recorded as mean days±SD.

Corrected Mortality:

The total mortality percentages were corrected against those of the control by Abbott's formula (Abbott, 1925) as follows:

$$\textbf{Corrected Mortality} = \frac{\textbf{Observed Mortality}\,\% - \boldsymbol{Control\ Mortality}\,\%}{\mathbf{100} - \boldsymbol{Control\ Mortality}\,\%} \times \mathbf{100}$$

LC$_{50}$ Calculation:

The corrected percentages of mortalities were plotted versus the corresponding concentrations on the logarithmic probability paper to obtain the corresponding Log-concentration probit lines. The lethal concentration of 50 % (LC$_{50}$) of treated insects was determined from the established regression lines (Finney 1971).

Statistical Analysis of Data:

All obtained data were statically analyzed by Student's t-distribution by using (SPSS) computer program to test the significance of the difference between means ± SD.

RESULTS

Insecticidal Activities After Treatment the Newly Hatched (Neonate) Larvae of *P. Gossypiella* by Feeding*:*

***a) Bacillus thuringiensis* var. *Kurstaki 1* (*Bt K*1):**

Bacillus thuringiensis var. *Kurstaki 1* (*Bt K*1) exhibited progressive mortality against the larvae of *P. gossypiella* after treatment the newly hatched larvae (Table 1). The highest concentration caused 72.5% larval mortality vs. 7.5% of control larvae. The lethal effect was extended in the resulted stages, pupae and adults viz. pupal mortality % was 36.4, 27.8 and

8.3% at 10^{12}, 10^{11} and 10^{10} (bacteria/ml) compared to 0.0% of control pupae; adult mortlaity was 2.9, 7.7, 4.5 and 3.2% at 10^{12}, 10^{11}, 10^{10} and 10^{9} (bacteria/ml) compared to 0.0% of control adults. Total mortality was increased gradually with the increased concentrations (bacteria/ml) where total mortality was 87.5, 70.0, 47.5, 25.0 and 10.0% at 10^{12}, $10^{11,}$ 10^{10}, 10^{9} and 10^{8} vs. 7.5% of control insects. All bacterial concentrations reduced the pupation % to 27.5, 45.0, 60.0, 77.5, 90.0 at 10^{12}, $10^{11,}$ 10^{10}, 10^{9} and 10^{8} (bacteria/ml) vs 92.50 % of control pupae. Also, the highest three concentrations decreased the adult emergence % to 63.6, 72.2 and 91.7% at 10^{12}, 10^{11} and 10^{10} (bacteria/ml) vs. 100 % of control adults.

Table 1 Biological activity of the entomopathogenic bacteria, *Bt K1* against the newly hatched (neonate) larvae of *P. gossypiella*.

Concentrations (bacteria /ml)	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergency (%)	Adult mortality (%)	Total mortality (%)	Corrected mortality (%)
10^{12}	72.5	27.5	36.4	63.6	2.9	87.5	86.49
10^{11}	55.0	45.0	27.8	72.2	7.7	70.0	67.57
10^{10}	40.0	60.0	8.3	91.7	4.5	47.5	43.24
10^{9}	22.5	77.5	00.00	100.0	3.2	25.0	18.92
10^{8}	10.0	90.0	00.00	100.0	0.00	10.0	2.70
control	07.50	92.50	00.00	100.0	0.00	07.50	0.00

***B) Bacillus thuringiensis* var. *Kurstaki* 2 (*Bt K*2):**

The highest concentration of *Bacillus thuringiensis* var. *Kurstaki 2* (*Bt K*2) induced 60.0% larval mortality of *P. gossypiella* after-treatment of the newly hatched larvae whereas other concentrations exhibited a slight lethal effect (Table 2). Extended mortalities were recorded in the pupal and adult stage. Pupal mortalities were recorded at the highest three concentrations by 31.3, 27.3 and 14.8% at 10^{12}, 10^{11} and 10^{10} (bacteria/ml) compared to 0.0% for control pupae. On the other hand, 27.30 and 18.75% of adult mortality were recorded at 10^{12} and 10^{11} (bacteria/ml) vs. 0.0 % of control adults. The total mortality % was in a concentration-dependent manner viz. 80.0, 67.5, 42.5, 20.0 and 12.5% at 10^{12}, $10^{11,}$ 10^{10}, 10^{9} and 10^{8} (bacteria/ml) compared to 5.0% of control insects. With respect to Pupation and adult emergence %, *Bt K*2 exhibited the same trend of *Bt K*1.

Table 2 Biological activity of the entomopathogenic bacteria, *Bt K2* against the newly hatched (neonate) larvae of *P. gossypiella*.

Concentrations (bacteria/ml)	Larval mortality (%)	Pupation (%)	Pupal mortality (%)	Adult emergency (%)	Adult mortality (%)	Total mortality (%)	Corrected mortality (%)
10^{12}	60.0	40.0	31.3	68.75	27.3	80.0	78.95
10^{11}	45.0	55.0	27.3	72.7	18.75	67.5	65.79
10^{10}	32.5	67.5	14.8	85.2	0.0	42.5	39.47
10^{9}	20.0	80.0	0.0	100.0	0.0	20.0	15.79
10^{8}	12.5	87.5	0.0	100.0	0.0	12.5	7.89
control	5.0	95.0	0.0	100.0	0.0	5.0	0.00

c) LC_{50}:

Depending on the data of LC_{50} for the total mortality of *P. gossypiella* after-treatment of the newly hatched larvae, *Bt K*1 was more potent than *Bt K*2 where LC_{50} was 2.21x10^{10} and 3.11x10^{10}, respectively (Table 3).

Development Effects After Newly Hatched (neonate) Larvae of *P. gossypiella:*

Table (4) reveals the effect of *Bt K*1and *Bt K*2 on the larval and pupal development after-treatment of the newly hatched larvae (Neonate) of *P. gossypeilla*. *Bt,* irrespective of the strain, significantly decreased larval duration and significantly increased pupal duration.

For the larval duration, the highest reduction was recorded at the highest concentration of *Bt K*1 by 9.90±0.78 at 10^{12} (Bacteria/ml) vs. 14.23±0.33 of control larvae. Also, the same highest concentration of *Bt K*1 induced the highest increased of pupal duration as 10.25±0.29 compared to 7.24±0.31 of control pupae. No effect was recorded on morphogenesis.

Table 3 LC_{50} values of the Bacterial isolates, *Bt K*1 and *Bt K*2 after-treatment of the newly hatched larvae (Neonate) of *P. gossypiella***.**

Entomopathogenic bacteria	LC50 (bacteria / ml)	Lower limit (bacteria / ml)	Upper limit (bacteria / ml)
*Bt K*1	2.21×10^{10}	1.12×10^{10}	4.52×10^{10}
Bt K2	3.11×10^{10}	1.44×10^{10}	7.38×10^{10}

Table 4 Effect of the Bacterial isolates on larval and pupal duration (mean days±SD) of *P. gossypeilla* after-treatment of the newly hatched larvae (Neonate).

Concentrations (bacteria/ml)	*Bt K*1		*Bt K*2	
	Larval duration	Pupal duration	Larval duration	Pupal duration
10^{12}	9.90±0.78 d	10.25±0.29 d	11.42±0.32 d	8.46±0.16 d
10^{11}	11.55±0.57 d	9.54±0.16 d	12.2±0.23 d	7.63±0.14 d
10^{10}	12.4±0.29 c	8.73±0.26 d	12.63±0.09 d	7.28±0.26 d
10^{9}	13.07±0.83 a	8.19±0.22 d	13.58±0.50 b	6.82±0.12 a
10^{8}	13.67±0.58 a	7.66±0.20 a	14.5±1.0 a	6.86±0.10 a
Control	14.23±0.33	7.24±0.31	14.79±0.09	6.87±0.1

Conc.: concentration; mean ± SD followed with the letter (a): is not significantly different ($P > 0.05$), (b): significant ($P < 0.05$), (c): very significant ($P < 0.01$), (d): extremely significant ($P < 0.001$)**.**

DISCUSSION

Bt is the most commonly used bio-pesticide worldwide (Osman *et al*., 2015). *B. thuringiensis* is very well-known as a bio-control agent especially its crystal protein against many insects (Schnepf 1998). Despite most species of *Bacillus* are harmless saprophytes, two species viz., *B. thuringiensis* and *B. cereus* are considered medically and environmentally important especially in the field of controlling some plant insects (Gray *et al.*, 2006).

The use of *Bt* became a vital component in integrated pest management. *Bt* proved to be the best alternative to pesticides (Gonzalez *et al*., 2011).

a- *Bt* Toxicity:

In the current study, *Bacillus thuringiensis* var. *Kurstaki* 1 and *Kurstaki* 2 exhibited their toxic effect against *P. gosyypiella* after-treatment of the newly hatched larvae. However, LC_{50} was 2.21×10^{10} and 3.11×10^{10} for *Bt K1* and *Bt K2, respectively*. The obtained data were in conformity with other several studies that have proven the toxicity of different strain of *Bt* against some insects as *Bt* var. *thuringiensis* against the cotton leaf roller, *Syllepte derogata* (Gahramanova *et al*., 2020), *Bacillus thuringiensis* CAB109 on *Spodoptera exigua* (Huang *et al*., 2018), *Bt* against the pod borer, *Helicoverpa armigera* (Bouslama *et al*., 2020; Fite *et al.*, 2019), *Bt against P. gossypiella* (Abbas *et al.*, 2017). The LC50 values for *Bt* 4D1, Bt 4D4 and Bt 4G1 were 6.10, 6.62 and 8.18 μg/ml for the 2nd instar; 9.90, 10.20 and 11.12 μg/ml for the 3rd instar; and 19.82, 23.16 and 24.54 μg/ml for the 4th instar, respectively, while the Bt 4K5 and Bt 4XX4 were not toxic to *Tuta absoluta* (Sandeep Kumar *et al.*, 2020).

Two larval instars of *P. gossypiella* were markedly affected with *B. cereus* spore-crystal by LC_{50s}; 88.5 (1st instars larvae) and 200 (4th instars larvae). *P. gossypiella* was found to have low sensitivity with regard to LC_{50} after treatment by *B. cereus* MA7 supernatant where it showed 284.8 and 277.5 for the 1st and 4th instars, respectively (Mahfouz and Abou El-Ela 2011). *Bacillus thuringiensis* var. *kurstaki* exhibited its effect against *S. exigua* and *Helicoverpa armigera* (Zhang *et al.,* 2009), *Plutella xylostella* (Legwaila *et al.,* 2015). Abou-zeid *et al.* (2015) revealed that *Staphyloccus sciuri* and *Micrococcus luteus* were the most effective against 1st instar larvae of *P. gossypiella*.

Many studies have reported the susceptibilities of lepidopteran larvae to *Bt* toxins (Alsaedi *et al.*, 2017; Hanen *et al*., 2016). Hegab and zaki (2012) recorded that Dipel 2× (*Bacillus thuringiensis Kurstaki*) caused 17.18±0.63 % larval mortality at 32×106IU concentration against *P. gossypiella* larvae. While the biocide Protecto from *Bacillus thuringiensis* Subsp *Kurstari* alone against *S. littoralis* had the least effect, it induced mortalities 10, 10 and5% at the three tested doses (Abdel-Rahim 2011). In addition, Abdel-Aziz (2000) and Dutton *et al*. (2005) recorded high susceptible larvae of *S. littoralis* toward the *B. thuringiensis* var *kurstaki* (Dipel- 2x) represented by higher mortality compared to control.

b- *Bt* and Disturbance of Development and Metamorphosis:

However, the current study recorded the effect of *Bt K*1 and *Bt K*2 on reduction of pupation and adult emergence %. Also, *Bt* irrespective of the strain significantly decreased larval duration and significantly increased pupal duration. These data were in harmony with other studies as *Bt.* significantly prolonged the larval duration of *P. gossypiella* and insignificant increase the pupal duration (Abbas *et al.*, 2017). The tested biocide *Btk* (Dipel 2×) caused different influences on all biological aspects of pink bollworm which decreasing larval duration, pupation percentage and adult emergence (Hegab and zaki 2012). Furthermore, their latent effect caused the lowest pupation % resulted from treated *P. gossypiella* larvae by *Staphyloccus sciuri* and *Micrococcus luteus* (Abou-zeid *et al.*, 2015). The percentages of pupation and adult emergence of *P. gossypiella* were negatively correlated with the increase of spore-crystal concentration and positively with the increase in the concentration of the supernatant of *B. cereus* (Mahfouz and Abou El-Ela 2011). The biocide Protecto from *Bacillus thuringiensis* Subsp *Kurstari* alone significantly increased the larval and pupal period of *S. littoralis.* It significantly decreased pupation and adult emergence %. (Abdel-Rahim 2011). The effects of *Bt* on larval, pupal and adult durations and adult emergence of *H. armigera* were significantly different (Fite *et al.,* 2019).

Disturbance in development, metamorphosis and inducing mortalities of *P. gosyypiella* after treatment by *Bt* may result from its mode of action. After ingestion of *Btk*, the active toxin is known to bind to and destroy the midgut epithelium, resulting in rapid gut paralysis, which causes the larva to stop feeding within hours in the most sensitive species (Talekar 1992). *Btk*-affected larvae die from starvation, which may take several days. Since *Btk* does not kill rapidly, users may incorrectly assume that it is ineffective if treatments are assessed a day or two after application (Legwaila *et al.*, 2015). However, Imam (2018) proved the effect of bacterial isolate *Bacillus thuringiensis* on the midgut of the 4th larval instar of the pink bollworm, treated with LC_{50} CFU/ml. The study showed several histological changes; some epithelial cells were disintegrated, vacuolated and their cell boundaries were destructed and separated from the basement membrane.

On the other hand, larval mortality, according to (Yoshinori and Kaya 1993), is probably due to either the septicemia in which the bacterial spores invade the hemocoel, multiply, produce toxin and subsequent kill the insect; or due to the toxemia in which the bacteria produce toxin and confined to the gut lumen. Mortality in infected larvae may also be due to the deficiency in the excretory system due to Malpighian tubules infection (Lotfy 1988).

CONCLUSIONS

Bacteria are one of the microbial insect pathogens and are considered a non-chemical alternative for the suppression of insect pests. The current study broadly showed that *Bt K*1and *Bt K*2 have a toxic potential against *P. gossypiella.* However, the bacteria-induced developmental disturbance to the immature stages. Further study is needed to show some light about the mode of action of bacteria.

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