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

Gamal Omar¹, Ahmed Ibrahim² and Khalid Hamadah¹*

1- Faculty of Science, Al-Azhar University, Zoology and Entomology Department, Madinat Nasr, Cairo, Egypt

2- Plant Protection Research Institute, Agricultural Research Center, Dokki. Giza, Egypt

E-mail*: khalid_hamadah@azhar.edu.eg

---

**ARTICLE INFO**

**ABSTRACT**

The current study was conducted to investigate the biological activities of the two strains of *Bacillus thuringiensis* (*Bacillus thuringiensis* var. *Kurstaki* 1 (*Bt K1*) and *Bacillus thuringiensis* var. *Kurstaki* 2 (*Bt K2*)) against the newly hatched (neonate) larvae of the pink bollworm, *Pectinophora 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 LC₅₀ for total mortality, *Bt K1* was more potent than *Bt K2* where LC₅₀ was 2.21x10⁴ and 3.11x10⁴, 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.*, 2015).

*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 thuringiensis, 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 (10⁸, 10⁹, 10¹⁰, 10¹¹ and 10¹² (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:

Corrected Mortality = \[ \frac{\text{Observed Mortality} \% - \text{Control Mortality} \%}{100 - \text{Control Mortality} \%} \times 100 \]

LC₅₀ 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% (LC₅₀) of treated insects was determined from the established regression lines (Finney 1971).

Statistical Analysis of Data:
All obtained data were statistically 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 K1):*
*Bacillus thuringiensis var. Kurstaki 1 (Bt K1)* 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
The Potential Activities of Two Bacillus thuringiensis Strains

8.3% at 10^{12}, 10^{11} and 10^{10} (bacteria/ml) compared to 0.0% of control pupae; adult mortality 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                 | 55.0         | 36.4                | 87.5                | 2.9                 | 87.5                | 86.49                  |
| 10^{11}                     | 50.0                 | 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         | 0.00                | 100.0               | 3.2                 | 25.0                | 18.92                  |
| 10^{8}                      | 10.0                 | 90.0         | 0.00                | 100.0               | 0.00                | 10.0                | 2.70                   |
| control                     | 07.50                | 92.50        | 0.00                | 100.0               | 0.00                | 07.50               | 0.00                   |

B) Bacillus thuringiensis var. Kurstaki 2 (Bt K2):

The highest concentration of Bacillus thuringiensis var. Kurstaki 2 (Bt K2) 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 K2 exhibited the same trend of Bt K1.

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                 | 55.0         | 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 K1 was more potent than Bt K2 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 K1 and Bt K2 on the larval and pupal development after-treatment of the newly hatched larvae (Neonate) of P. gossypiella. 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 K1 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 K1 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** LC₅₀ values of the Bacterial isolates, Bt K1 and Bt K2 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 K1*                   | 2.21x10^{10}         | 1.12x10^{10}                | 4.52x10^{10}                |
| *Bt K2*                   | 3.11x10^{10}         | 1.44x10^{10}                | 7.38x10^{10}                |

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

| Concentrations (bacteria/ml) | *Bt K1*                | *Bt K2*                |
|-----------------------------|------------------------|------------------------|
|                            | 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  | 12.24±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 a  | 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 (Schneef 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. gossypiella* after-treatment of the newly hatched larvae. However, LC₅₀ was 2.21x10^{10} and 3.11x10^{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, *Sylete 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 LC50: 88.5 (1st instars larvae) and 200 (4th instars larvae). P. gossypiella was found to have low sensitivity with regard to LC50 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 Staphylococcus 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 2x (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 K1 and Bt K2 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 2x) 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 Staphylococcus 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. gossypiella 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 LC50 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 K1 and Bt K2 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.

REFERENCES

Abbas A.A., Nada M. A., El-Sayed A.A.A., Abd-El-Hamid N.A., El-Shennawy R.M.A. (2017). Toxicity and Latent Effects of Some Control Agents on Pink Bollworm Pectinophora gossypiella (Saunders). *Egyptian Academic Journal of Biological Sciences, A. Entomology*, 10(1): 25–34. DOI: 10.21608/EAJBSA.2017.12688.

Abbott W.S. (1925). A method for computing the effectiveness of insecticides. *Journal of Economic Entomology*, 18: 265-267.

Abdel-Aziz S.H. (2000). Physiopathological studies on bacterial infection of cotton leaf worm, *Spodoptera littoralis*. Master Science Thesis, Faculty of Science, Ain Shams University, Egypt.

Abdel-Rahim E.F.M. (2011). Latent effect of microbial insecticides against the second instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.). *Egyptian Journal of Agricultural Research*, 89 (2):477-498.

Abou El-Ela A.A. (1996). Pathogenic studies on some Lepidoptera insects. Master Science Thesis, Faculty of Science, Cairo University, Egypt.

Abou-zeid N.M., El-Lebody K.A., Mahmoud N. A., ElSharkawy M.A. (2015). Identification, Insecticidal Effect and Antibiosis Studies of some Bacteria Isolated from Naturally Infected *Pectinophora gossypiella* (SAUND.) collected from cotton fields in Egypt. *Journal of Plant Protection and Pathology*, 6(12): 1685–1696

Alsaeed G., Ashouri A., Talaei-Hassanloui R. (2017). Evaluation of Bacillus thuringiensis to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions. *Agricultural Sciences*, 8: 591–599. DOI: 10.4236/as.2017.87045

Barker J. (1998). Effect of Bacillus thuringiensis subsp. kurstaki toxin on the mortality and development of the larval stages of the banded sunflower moth (Lepidoptera: Cochyliidae). *Journal of Economic Entomology*, 91(5): 1084–1088. https://doi.org/10.1093/jee/91.5.1084.

Bouslama T., Chaieb I., Rhouma A., Laarif A. (2020). Evaluation of a Bacillus thuringiensis isolate-based formulation against the pod borer, Helicoverpa armigera Hübner (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 30:16. https://doi.org/10.1186/s41938-020-00218-z

Contwell G.E. (1974). Insect diseases. Vols I & II, Marcel Decker Inc. New York.

Dutton A., Romeis J, Bigler F (2005). Effects of *Bt* maize expressing crylAb and *Bt* spray on *Spodoptera littoralis*. Entomologia Experimentalis et Applicata, 114: 161 – 169. https://doi.org/10.1111/j.1570-7458.2005.00239.x

Erb S.L., Bourchier R.S., van Frankenhuyzen K., Smith S.M. (2001). Sublethal effects of Bacillus thuringiensis Berliner subsp. kurstaki on Lymantria dispar (Lepidoptera: Lymantriidae) and the Tachinid parasitoid Compsilura concinnata (Diptera: Tachinidae). *Environmental Entomology*, 30(6): 1174–1181. https://doi.org/10.1603/0046-225X-30.6.1174
The Potential Activities of Two Bacillus thuringiensis Strains

Finney D. J. (1971). Probit analysis 3rd ed., Cambridge Univ. press, London UK.
Fite T., Tefera T., Negeri M., Damta T., Sori W. (2019). Evaluation of Beauveria bassiana, Metarhizium anisopliae, and Bacillus thuringiensis for the management of Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) under laboratory and field conditions. Biocontrol Science and Technology, 30: 278-295. https://doi.org/10.1080/09583157.2019.1707481

Gahramanova G., Mamay M., Mammadov Z. (2020). Biological characteristics and efficacy of Bacillus thuringiensis var. thuringiensis against the cotton leaf roller, Syleptes derogate (Fabricius, 1775) (Lepidoptera: Crambidae). Egyptian Journal of Biological Pest Control, 30: 85. https://doi.org/10.1186/s41938-020-00289-y.

Gonzalez-Cabrera J., Molla O., Monton H., Urbaneja A. (2011). Efficacy of Bacillus thuringiensis (Berliner) in controlling the tomato borer, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae). BioControl, 56:71–80. DOI: 10.1007/s10526-010-9310-1

Gray, E.J., Lee, K.D., Souleimanov, A.M., Di Falco, M.R., Zhou, X., LY, A., Charles, T.C., Driscoll, B.T., & Smith, D.L. (2006). A novel bacteriocin, thuricin 17, produced by plant growth promoting rhizobacteria strain Bacillus thuringiensis NEB17: isolation and classification. Journal of Applied Microbiology, 100(3): 545-554. doi: 10.1111/j.1365-2672.2006.02822.x.

Hanen B., Sameh S., Sonia K., Najeh B.H., Tahya S.B., Slim T., Lobna A. M. (2016). Isolation and characterization of a new Bacillus thuringiensis strain with a promising toxicity against Lepidopteran pests. Microbiological Research, 9(15):186–187.

Hegab M.E., Zaki A.A. (2012). Toxicological and biological effects of bacteria, Bacillus thuringiensis KURSTAKI on Pectinophora gossypiella (SAUND.). and entomopathogenic fungi, Beauveria bassiana on Earias insulana (BOISD.). Journal of Plant Protection and Pathology, 3 (3): 289 – 297. DOI: 10.21608/jppp.2012.83762

Huang S., Li X., Li G., Jin D. (2018). Effect of Bacillus thuringiensis CAB109 on the growth, development, and generation mortality of Spodoptera exigua (Hübner) (Lepidoptera:Noctuidae). Egyptian Journal of Biological Pest Control, 28: 19. doi:10.1186/s41938-018-0101-9.

Imam I. (2018). Histological Effect of Bacillus thuringiensis Isolate against Pink Bollworm Larval Midgut. Pectinophora gossypiella (Saund.). Journal of Plant Protection and Pathology Mansoura University, 9 (12): 803 – 806. DOI: 10.21608/jppp.2018.44070

Jaleel W., Saeed S., Naqqash, M.N., Zaka S.M. (2014). Survey of Bt. cotton in Punjab Pakistan related to the knowledge, perception and practices of farmers regarding insect pests. International Journal of Agriculture and Crop Sciences, 14(7): 10-20.

Kares A.E. (1991). Effect of mixture of Bacillus thuringiensis (Berliner) and chemical insecticides against larvae of pink bollworm Pectinophora gossypiella (Lepidoptera: Gelechiidae). Egyptian Journal of Biological Pest Control, 1: 15-23.

Legwaila M.M., Munthali DC, Kwere Pe B, Obopile M (2015). Efficacy of Bacillus thuringiensis (var. kurstaki) against diamond-back moth (Plutella xylostella L.) eggs and larvae on cabbage under semi-controlled greenhouse conditions. International Journal of Insect Science, 7: 39–45. DOI: 10.4137/IJIS.S23637

Lotfy M.N. (1988). Pathogenesis of Bacillus thuringiensis toxic crystals in larvae of the silkworm, Bombyx mori. Philosophy Doctor Thesis, Faculty of Science., Ain Shams University, Egypt.

Lykouressis, D., Perdikis D., Samartzis D., Fantinou A., Toutouzas S. (2005). Management of the pink bollworm Pectinophora gossypiella (Saunders) (Lepidoptera:
Gelechiidae) by mating disruption in cotton fields. *Crop Protection*, 24: 177-183. https://doi.org/10.1016/j.cropro.2004.07.007.

Mahfouz S.A., Abou El-Ela A.A. (2011). Biological Control of Pink Bollworm *Pectinophora gossypiella* (Saunders) by *Bacillus cereus* MA7. *Journal of Microbial and Biochemical Technology*, 3(2): 030-032. DOI: 10.4172/1948-5948.1000047

McSpadden Gardner B.B. (2004). Ecology of *Bacillus* and *Paenbacillus spp* in agricultural systems. *Phytopathology*, 94 (11): 1252-1258. doi: 10.1094/PHYTO.2004.94.11.1252.

Moustafa H.Z., Lotfy D.E., Karim A.H. (2019). Effect of entomopathogenic fungi on *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) and *Earias insulana* (Lepidoptera: Noctuidae) and their predators. *Egyptian Journal of Plant Protection Research Institute*, 2 (1): 9-15.

Ohba M., Aizawa K., Furusawa S. (1979). Distribution of *Bacillus thuringiensis* serotype in Ehine perfecte Japan. *Applied Entomology and Zoology*, 14: 340-345.

Opisa S., du Plessis H., Akutse K.S., Fiaboe K.K.M., Ekesi S. (2018). Effects of Entomopathogenic fungi and *Bacillus thuringiensis* based biopesticides on *Spoladea recurvalis* (Lepidoptera: Crambidae). *Journal of Applied Entomology*, 142(6): 617–626. https://doi.org/10.1111/jen.12512.

Osman G.E.H., Already R., Assaeedi A.S.A., Organji S.R., El-Ghareeb D., Abulreesh H.H., Althubiani A.S. (2015). Bioinsecticide *Bacillus thuringiensis* a comprehensive review. *Egyptian Journal of Biological Pest Control*, 25(1): 271–288.

Parmar V.R., Patel C.C. (2016). Pink Bollworm: A Notorious Pest of Cotton: A Review. *AGRES - An International e-Journal*, 5 (2): 88-97.

Rashad A.M., Nada M.A., Abd El-Salam N.M. (1993). Effect of some factors on diapausing larvae and emerging moths of the pink bollworm, *Pectinophora gossypiella* (Saunders). *Egyptian Journal of Applied Sciences*, 8(1): 488-500.

Sandeep Kumar J., Jayaraj J., Shanthi M., Theradimani M., Venkatasamy B., Irulandi S., Prabhu S. (2020). Potential of standard strains of *Bacillus thuringiensis* against the tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Egyptian Journal of Biological Pest Control*, 30:123. https://doi.org/10.1186/s41938-020-00326-w

Schnepf E., Crickmore N., Van Rie J., Lereclus D., Baum J., Feitelson J., Zeigler D.R., Dean D.H. (1998). *Bacillus thuringiensis* and Its Pesticidal Crystal Proteins. *Microbiology and Molecular Biology Reviews*, 62(3): 775–806.

Talekar N.S. (1992). Diamondback moth and other crucifer pests: Proceedings of the second international workshop: December 10–14, 1990, Asian Vegetable Research and Development Center, Tainan, Taiwan, 92–368, 603.

Yoshinori T., Kaya H.K. (1993). Bacterial infections: Bacillaceae. In: “Insect pathology.” Published by Academic press INC., 83-146.

Zhang X., Liang Z., Siddiqui Z.A., Gong Y., Yu Z., Chen S. (2009). Efficient screening and breeding of *Bacillus thuringiensis subsp. kurstaki* for high toxicity against *Spodoptera exigua* and *Heliothis armigera*. *Journal of Industrial Microbiology & Biotechnology*, 815-820. F.