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Toxicological and Biochemical Aspects of *Bacillus thuringeinsis var. kurstaki* on Fall Armyworm, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae).

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae), causes direct damage by feeding on many crops. parts of the host plant. The aim of the study is to estimate the effect of *Bacillus thuringeinsis var. kurstaki* on *S. frugiperda*. Our results indicate that the value of LC_{50} was 0.125 mg/ml. Treatment of second instar larvae of *S. frugiperda* by LC_{50} of *B. thuringeinsis kurstaki* caused a prolongation of larval duration and pupal duration, and a significant reduction in pupation%. The biochemical studies on 6th instar larvae of *S. frugiperda* after treatment as 2nd instars by LC_{50} of *B. thuringeinsis kurstaki* showed a significant decrease in the activity of Amylase enzyme and Trehalase compared to untreated larvae, while Invertase activity was significantly increased compared to control. The results showed a general disturbance in three carbohydrate enzymes in larvae treated as 2nd instars of *S. frugiperda* with LC_{50} of *B. thuringeinsis kurstaki*.

INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) is a voracious pest, further threatens many crops production and poses a risk to food and nutrition security (Huesing *et al.* 2018). *Spodoptera frugiperda* is a polyphagous pest that attacks different cultivated crops and causes serious damage (Montezano, *et al.* 2018). The larval stage is the destructive stage, whose caterpillars feed on vegetative and reproductive parts of the host plants and cause high grain yield loss (Sarmento *et al.* 2002). The lepidopterous pests have resistance against many registered pesticides. Bio-insecticides are an alternative strategy for the integrated pest management (IPM) program (Abd El-Samei *et al.* 2019). Bacterial insecticides are eco-friendly, easily degradable, target specific and safer insecticides (Chattopadhyay *et al*. 2017).

The aim of the present studies is to evaluate the effect of *Bacillus thuringeinsis var. kurstaki* on second instar larvae of *Spodoptera frugiperda* and its effects on some biological and biochemical aspects of the 6th larval instar homogenate.

MATERIALS AND METHODS

1-Rearing Technique of Fall Armyworm, *Spodoptera frugiperda*:

The Fall armyworms, *Spodoptera frugiperda* were obtained from the division of the Cotton Leaf Worm, Plant Protection Research Institute Agriculture Research Center. Larvae were maintained at 25 °C ± 1 °C, 60% ± 5% (RH) and a 14:10-h light: dark. The larvae were reared on fresh castor leaves (*Ricinus communis*) and larvae were reared separately to avoid cannibalism.

2-Compounds Used:

Protecto®: *Bacillus thuringeinsis var. kurstaki*, (32000 I.U. /mg) WP was applied at the rate of 300 gm/feddan. The commercial biopesticides were obtained from the Bioinsecticide Production Unit, Plant Protection Research Institute, Agriculture Research Centre, Giza, Egypt

3-Toxicological Studies on Fall Armyworm, *Spodoptera frugiperda*:

These experiments were carried out on the second larval instars of *S. frugiperda* fed on castor bean leaves using the leaf dipping technique. One hundred larvae were divided into four replicates; every 25 larvae were used with five serial aqueous concentrations of *B. thuringiensis*. Each concentration was replicated three times with 30 larvae for each test. Fresh and clean leaves of *R. communis* were dipped for 30 sec in each concentration per Abo El-Ghar *et al.*, (1994). Clean castor leaves treated only with distilled water were served to control larvae. Mortality percentage was made after 48 hours. The percentages of concentration mortality were calculated daily and corrected according to Abbott's equation (1925). LC_{50} value was calculated by using the probit-analysis method of Finney (1971).

4-Effects the Sublethal of *Bacillus thuringeinsis var. kurstaki*, on Biological Aspects of *Spodoptera frugiperda*:

LC_{50} of *B. thuringeinsis kurstaki*, treated of newly ecdysed 2nd instars larvae of *S. frugiperda*. Prepared three replicates of treatment, with 50 larvae in each, and 50 as control. Refresh leaves of castor bean for remaining larvae, to study the parameters such as larval duration, pupal duration, pupation% and adult emergence %.

5- Biochemical Studies:

5.1. Determination of Amylase, Invertase and Trehalase Activity:

Larvae were collected after six days following the treatment of the second instar and placed in ice containers. Digestive enzymes were determined according to the method described by Ishaaya and Swirski (1976) using trehalose, sucrose, and soluble starch as substrates for trehalase, invertase and α-amylase, respectively. 20µl of diluted enzyme solution was incubated for 10min at 30˚c with 250µl 1%starch (soluble potato starch, Lintner grade, Sigma Chemical Co.) in 50mM acetate buffer pH 5.0 containing 20mM NaCl and 0.1mM $CaCl_2$.

The reaction was stopped by adding 250µl dinitrosalycylic acid (DNS) reagent (1g of 3, 5 dinitrosalycylic acids with 20ml of 2N NaOH and 30g NaK-tartrate made up to 100mL with distilled water) to each tube in boiling water for 5min. Samples were cooled, diluted with 2.5ml H_2O, and read at 550nm on Spectronic 1201 (Beckman, USA). Glucose was used as a standard. Appropriate dilutions of enzyme supernatant were used to obtain a linear production of glucose equivalents.

For each test, enzyme activity was determined from triplicate analyses of three groups of Insects. The enzyme activity was expressed as µg glucose released /min/gm fresh weight.

6. Statistical Analysis Procedure:

Statistical analysis using a student t-test of the obtained data was performed by COSTAT program, for Windows.

RESULTS AND DISCUSSION

1-Toxicological Studies of *Bacillus thuringeinsis var. kurstaki*, on *Spodoptera frugiperda*:

Table (1) recorded the efficiency of *B. thuringeinsis kurstaki*, on the 2nd instar larvae of *S. frugiperda*. The LC_{50} values were recorded (0.125 mg/ml). The treatment by *B. thuringeinsis kurstak* caused significant toxicity in the bioassay test with an 83.72 % of accumulative mortality percentage, at the end of the larval stage. The slope value was (1.433 ± 0.272). The present results agree with Massochin *et al.*, (2010), which showed that the tested *B. thuringiensis thuringiensis*, was highly toxic in the bioassays with 100% of corrected mortality to *S. frugiperda* larvae and the LC_{50} results indicated that the expressed protein was toxic too, with an LC_{50} of 10.88 µg/ml. Also, agreed with the results of Ricardo *et al.*, (2000), which found that the *in vivo* activities of *Bt* strains tested on second instar *S. frugiperda* larvae are shown the pathogenic causing 80, 40 and 100% of mortality. And Abdel-Salam *et al.* (2018), found a high rate of mortality in *S. littoralis* larvae treated with *B. thuringiensis.*

Table 1: Toxicity values of *B. thuringeinsis kurstaki* against 2nd instars *S. frugiperda* larvae.

Treated compound	LC_{50} (gm/ml)	Confidential limit (95%) LC_{50} (gm/ml)		Slope±S.E.	Accumulative mortality% (At the end of larval stage)
		Lower	Upper		
B. thuringeinsis kurstaki	0.125	0.092	0.184	1.433 ± 0.272	83.72

2-Effects the sublethal of *Bacillus thuringeinsis var. kurstaki*, on Some Biological Aspects of *Spodoptera frugiperda*:

When *S. frugiperda* larvae ingest the toxin of *B. thuringeinsis kurstaki* inserted into the insect gut cell membrane, paralyzing the digestive tract, and forming a pore, so *S. frugiperda* stops eating and starves to death. The results in Table (2) indicate that the use of LC_{50} of *B. thuringeinsis* against *S. frugiperda* caused a prolongation of mean larval duration and pupal duration, compared to control and a significant reduction in pupation% and adult emergence %. Where the duration of the larval instar of *S. frugiperda* was 23.2 days, which was more than that of the control by 1.2 days, i.e., prolongation of mean larval duration than the control. Meanwhile, the pupal duration was 15.3 days as compared to 12.6 days in the control, which means it increase than the control by 2.7. The pupation % was reduced to 48.9 % which was a reduction of nearly half its value in untreated insects 95.4%. While the adult emergence % was a reduction of 14.8% than the control, where it was 83.4% and in control was 98.2%.

Our results showed that *B. thuringeinsis* caused a significant prolongation in the larval duration and pupal duration of *S. frugiperda* and a reduction in the percentage of pupation attributed to the slower metabolic rate of these larvae as a direct effect of *B. thuringeinsis* application. These results were supported by Al-Jamil and Hassan (2019), who studied the effect of *Bacillus thuringensis* (Berliner) on the biological aspect of *S. littoralis* (Boisd.), found, the slow movement of the larvae and the lack of feeding and the larvae affected by the loss of the body weight before mortality and then vomiting of brown fluid from its bodies this was the symptoms of infection with bacteria. The results agreed with EL-Sabagh *et al.*, (2017) who showed the effect of *Bacillus thuringiensis* as bioinsecticides on the 2nd and 4th instar larvae of *S. littoralis* and found a decrease in both larval and pupal duration after larvae treatment and a decrease in the pupation and the adult emergence percentage compared to the control. Also, agreement with María *et al.*, (2022), showed high toxicity towards the fall armyworm, *Spodoptera frugiperda* (J.E.

Smith), fourth instar larvae in bioassays using the microdroplet ingestion technique of *Bacillus thuringiensis.*

Table 2: The effect of LC_{50} of *Bacillus thuringeinsis kurstaki* on 2nd instar larvae of *S. frugiperda*.

Treated compound	Mean larval duration (days ± S.E.)	Mean pupal duration (days ± S.E.)	Pupation (%)	Adult emergence (%)
B. thuringeinsis kurstaki	23.2 ns ±0.2	15.3 ** ±0.06	48.9	83.4
Control	22.4 ±0.09	12.6 ±0.1	95.4	98.2

**moderately significant (P<0.01) and ns not significant, (student-test).

3-Biochemical Studies:

Treated 2nd instars of *S. frugiperda* by LC_{50} of *B. thuringeinsis kurstaki* detected the disturbance in three carbohydrates enzymes, Amylase, Invertase, and Trehalase. Data in Table (3) showed that Amylase activity was 34.91 in untreated larvae, this level was a significant reduction in treated larvae with LC_{50} of *B. thuringeinsis kurstaki* to 23.00 µg glucose/min/g.b.wt. Also, Trehalase activity was a highly significant reduction in treated larvae compared to the control, where in control was 36.15 and in treatment was 26.03 µg glucose/min/g.b.wt. On the other hand, Invertase activity was significantly increased from 48.14 in untreated larvae to 67.31 µg glucose/min/g.b.wt. in larvae treated.

The results showed a general disturbance in three carbohydrate enzymes in larvae treated as 2nd instars of *S. frugiperda* with LC_{50} of *B. thuringeinsis kurstaki.* Treatment by *B. thuringeinsis kurstaki* caused disruption in the midgut tissue which, therefore, must have caused disturbance of its digestive carbohydrase enzymes. The present study agrees with those obtained by Abd-El Wahed *et al.*, (2011) and with Hamama *et al*., (2015) who reported that changes in enzymatic activities after treatment with bioinsecticides indicated that changes in the physiological balance of the midgut affect these enzymes. Our results are supported by similar observations by Ishaaya *et al.,* (1971) who found generally a reduction in larval digestive enzymes because of their binding to inactive (zymogens) or active digestive enzymes. El-Sheikh (2012), studied the effects of *B. thuringiensis* on *S. littoralis* and detected, the carbohydrates hydrolyzing enzymes as amylase insignificantly decreased compared to the untreated one, and trehalase was significantly decreased. Trehalase is present in large amounts in the haemolymph of most insects and its production of glucose is needed for chitin build-up in the newly synthesized cuticle; its activity might be an indicator of energy reserves, Wyatt, (1967). The decrease in trehalase activity was similar to El-Ghar et al. (1995) who stated that *B. thuringiensis* reduced trehalase activity by 53% after 2 days of treatment.

Table 3: Activities of Amylase, Invertase, and Trehalase in *S. frugiperda* larvae treatment as 2nd instar larvae with LC_{50} values of *B. thuringeinsis kurstaki.*

Treated compound	Digestive carbohydrase enzymes (µg glucose/min/g.b. wt)		
	Amylase	Invertase	Trehalase
B. thuringeinsis kurstaki	23.00**±1.03	67.31***±2.32	26.03**±1.04
Control	34.91±1.41	48.14±1.89	36.15±1.72

Each value represents the average of three replicates ± S.E

***: highly significant (P<0.001) and **moderately significant (P<0.01), (student-test).

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