

**TOXICOLOGICAL AND BIOLOGICAL EFFECTS OF BACTERIA, *Bacillus thuringiensis* KURSTAKI ON *Pectinophora gossypiella* (SAUND.), AND ENTOMOPATHOGENIC FUNGI, *Beauveria bassiana* ON *Earias insulana* (BOISD.)**

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**ABSTRACT**

Laboratory studies were conducted for evaluate the effect of Dipel 2x, (*Bacillus thuringiensis* Kurstaki) against pink bollworm *Pectinophora gossypiella* (Saund.), and Biover® (*Beauveria bassiana*) (Balsamo) against spiny bollworm, *Earias insulana* (Boisd.).The accumulated mortalities of both pink bollworm, *Pectinophora gossypiella* and spiny bollworm, *Earias insulana* larvae, after six days of treatment, were represented as the acute toxicity were, Dipel 2x attained 17.18±0.63 % larval mortality at 32x10<sup>6</sup>IU concentration against *P. gossypiella* larvae while Biover® resulted in 15.55±0.59% larval mortality against *Earias insulana* larvae comparing with 0.00% larval mortality in untreated check.

At the same time the tested biocide *Btk* (Dipel 2x) caused different influences on all biological aspects of pink bollworm which decreasing larval duration, pupal weight, pupation percentage, adult emergence, oviposition periods, adult longevity, female fecundity decreased strongly which recorded 149.75±5.23 egg/female comparing with that 362.00±2.31 egg/female in untreated check. The tested biocide *Btk* (Dipel 2x) also decreasing hatchability percentage that exhibited 73.16±1.15 % as compared with 88.09±0.57 % that achieved in untreated check. Also, results cleared that the effect of Biover® fungi attained decreasing in all biological aspects, except the male and female longevity and oviposition periods compared with untreated check. Female fecundity was moderately influenced when spiny bollworm larvae treated by Biover® that achieved 60.33±11.66 eggs compared with 78.33±5.78 eggs laid /female deposited by check females. Hatchability percentage of spiny bollworm also decreased vigorously which attained 41.12±6.45% as compared with that 82.33±7.51% in untreated once.

**Keywords:** *Bacillus thuringiensis* Kurstaki, Dipel 2x, *Pectinophora gossypiella*, *Beauveria bassiana*, Biover®, *Earias insulana*, concentration, biocides.

**INTRODUCTION**

In Egypt, during the late cotton-season, cotton plants suffer from the infestation with pink bollworm, *Pectinophora gossypiella* and the spiny bollworm, *Earias insulana*. Both bollworms are of the most serious insects that constitute a major part of the pest complex on cotton in Egypt. The loss caused by *P. gossypiella* to cotton arises to one million kentar annually. Although new insecticides give excellent control to resistant strains of insects, no one can accurately predict how long resistant insect populations will take

to develop (Metwally *et al*, 1980). In Egypt studied the effect of Dipel 2x, (*Bt kurstaki*) and Xantari, (*Bt aizawia*) against pink bollworm larvae to determine the LC 50 by feeding neonate larvae on artificial diet. Results revealed that Dipel 2x (powder) and Xantari were more effective against pink bollworm than their mixture (s), i.e. *Bt. sub sp. kurstaki* and *Bt aizawia* were not compatible (Karima, El-Lebody 2003). (*Bt kurstaki*) and Xantari caused significant inhibition of growth of pink bollworm (Ibargutxi *et al.* 2006). Entomopathogenic fungi in common with other insect natural enemies can be employed for biocontrol strategies (Shah and Pell 2003). Laboratory studies reported that Dipel 2x compound was the most toxic biocide against pink and spiny bollworms neonate larvae (Hegab, 2008).

Therefore, the present study aims to throw some light on the toxic and latent effect of Dipel 2x, (*Bacillus thuringiensis kurstaki*) and entomopathogenic fungi Biover®, (*Beauveria bassiana*) against pink and spiny bollworms, under laboratory conditions.

## **MATERIALS AND METHODS**

### **Acute and chronic Effects, of some biocides, on the pink and spiny bollworms.**

#### **Mass Rearing:**

Newly hatched larvae of Pink bollworm, *Pectinophora gossypiella* (Sunders) susceptible strain (PBW) which reared in culture in bollworm research Department, Plant Protection Research Institute, (Sharkia branch) have been described by (Abd El-Hafez *et al.* 1982). In case of SBW, full-grown larvae were collected from infested green cotton bolls obtained from the field at the end of cotton growing seasons and a mass rearing was prepared in incubator at  $26 \pm 1^{\circ}\text{C}$  and  $80 \pm 5\%$  R.H. at Plant Protection Research Institute, Sharkia branch Lab. then reared on artificial diet described previously by Amira, M. Rashad and Ammar (1985).

#### **Tested compounds:**

##### **A. Dipel 2X**, a commercial formulation of *Bacillus thuringiensis kurstaki*

(Berliner) (*Btk*) it is a product of the Special Unit for producing bioinsecticides, plant protection research institute Agriculture research center, Egypt, with  $32 \times 10^6$  international toxicity units, based on rate of application per feddan of 200g./400 Liter water.

##### **B. Biover®**, a commercial formulation of *Beauveria bassiana* (Balsamo) it is

a product of the same unit with  $32 \times 10^6$  viable spores per mg. based on rate of application per feddan of 200g./400 Liter water.

#### **Test trials:**

Five grams of kidney bean artificial diet were putted in each Petri dish (7cm diameter x1.5cm in height). The concentration tested (0.5g. of each biocides were diluted in one liter water). One ml of the tested concentrations of both Dipel 2X and Biover® were distributed on the surface of the diet then were left to dry. Twenty newly hatched larvae of PBW or SBW were transferred to the surface of treated media in Petri dishes after drying. Petri dishes were covered by fine and soft paper below the glass cover and placed in an electrical incubator running at  $26 \pm 1^{\circ}\text{C}$  and  $80 \pm 5\%$  R.H. Three

replicates for every treatment were done. After 24 hr of exposure and feeding, alive larvae of both pink and spiny bollworms were transferred individually to glass tube, (2x7.5 cm), containing about four grams of untreated media and the glass tubes were covered with a piece of absorbent cotton and held in incubator under the above mentioned conditions. The treated larvae were inspected daily and the dead larvae were recorded till pupation. Percent of larval mortalities were deduced after 6 days (which considered as a toxic effect) of Dipel 2X and Biover® tested biocides. To record the biological aspects i.e. larval duration, weight of 4<sup>th</sup> instar larvae, pupal duration and pupation percentage. Emerged moths were coupled (for five replicates for every treatment). Moths of two tested pests were caged in a glass Jar (250 ml in size) covered with muslin clothes, and secured by rubber bands. Moths were fed on 10 % sucrose solution, using soaked cotton wool and changed by new one daily. Paper piece was putted over the upper opening of the Jar serving as oviposition site. Each Jar was inspected daily to record the number of deposited eggs/ female till death. Fecundity, oviposition periods as female, male and female longevities, and hatchability percentages were calculated and recorded.

**Statistical analysis:**

The obtained results of each mortality and biological parameters were subjected to analysis of variance to clear Standard Error values of insect's toxicity and latent effect parameters. The analysis of variance was computed using Costat computer program Cohort Software. P. O. Box 1149, Berkeley CA 9471 (Costat program methods 1990).

## **RESULTS AND DISCUSSION**

### **Acute and chronic Effects, of some biocides on the pink and spiny bollworms.**

**Acute effect:**

The accumulated mortalities of both *Pectinophora gossypiella* and *Earias insulana* larvae, after six days of treatment, were represented as the acute toxicity.

Data in Table (1) indicated that Dipel 2X recorded mortality percentage of (17.18±0.63 %) on *Pectinophora gossypiella*, While Biover® recorded (15.55±0.59%) against spiny bollworm larvae after 6 days from treatment as compared with control treatment that recorded (0.00%) .

These results coincide the moderately effect of both Ecotch, *Btk* and Dipel 2x at different concentrations against *Spodoptera littoralis* newly hatched larvae and a low degree of efficiency inhibiting 10.50% mortality (Al-Shannaf, 2002). *Bacillus thuringiensis Kurstaki* strain exhibiting high toxicity against *S. exigua* while retaining its high toxicity against *Helicoverpa armigera*. The toxicity of *Btk*, D1-23 against *S. exigua* and *Helicoverpa armigera* was improved by 15.4 and 25.9%, respectively (Zhang *et al.* 2009). The effects of *Beauveria bassiana* Bb21 strain had strong pathogenicity on *Myzus persicae*, at laboratory condition with the LD<sub>50</sub> of 97 conidia x mm (-2) (45-191, 95% confidence interval), but was less pathogenic to the second instar nymph of *Chrysoperla carnea*, with the LD<sub>50</sub> of 1089 conidia x mm (-2).

The pathogenicity of Bb21 to *Harmonia axyridis* was very weak, with a low infection rate of 13% even at a high concentration  $5 \times 10^8$  conidia x mL (-1). When applied at the high concentration  $5 \times 10^8$  spores x mL(-1), Bb21 shortened the larval stage of *H. axyridis* averagely by 1.4 d and decreased the adult emergence rate and fecundity by 33% and 14%, respectively (Zhu *et al.* 2011).

**Latent effect:**

A Latent effect of Dipel 2x and Biover® tested biocides on pink and spiny bollworms larvae was expressed as some biological aspects including, larval duration, weight of 4<sup>th</sup> instars larvae, pupation percentage, pupal weight, pupal duration, percent of adult emergence, oviposition periods of emerged females, longevity of males and females, female fecundity and hatchability percentage.

**larval duration:**

Data summarized in Table (1) revealed that Dipel 2x, *Btk* caused shortening in larval duration up to  $12.62 \pm 0.59$  days compared with untreated larvae which recorded ( $14.89 \pm 0.041$  days). While Biover® *B. bassiana* entomopathogenic fungi caused highly decreased in larval duration ( $10.42 \pm 0.29$  days) compared with ( $16.13 \pm 0.16$  days) which recorded in untreated larvae.

**Weight of the 4<sup>th</sup> instar larvae:**

Data presented in Table (1) showed that, the influence of Dipel 2x, *Btk* tested concentration caused slight decreased in larval weight where attained ( $0.0483 \pm 0.0058$  g.) at  $32 \times 10^6$  Unit concentration, comparing with ( $0.0488 \pm 0.006$  g.) which recorded in untreated check. Meanwhile in case of spiny bollworm treated larvae by Biover®, *B. bassiana* the larval weight was ( $0.0783 \pm 0.0014$  g.) as compared with ( $0.0786 \pm 0.0015$  g.) in untreated check.

**Table (1): Effect of the tested biocides on larval, pupal and adult emergence of pink and spiny bollworms under laboratory conditions**

Insects	Compounds	Concentrations	Rate of application /feddan	Acute toxicity ***	Larval duration /day	Larval weight/g.	Pupal weight/g.	Pupation percentages %	Pupal duration/day	Adult emergence percentages%
Pink bollworm	* Dipel 2x	$32 \times 10^6$ Unit	200g.	17.18	12.62	0.0483	0.0384	77.21	25.50	77.21
				± 0.63	± 0.59	± 0.0058	± 0.0006	± 4.05	± 0.64	± 4.05
Control				0.00	14.89	0.0488	0.0398	85.55	10.69	85.55
					± 0.041	± 0.006	± 0.0004	± 2.92	± 0.56	± 2.92
Spiny bollworm	** Biover®	$32 \times 10^6$ Unit viable spores/mg	200g.	15.55	10.42	0.0783	0.0533	35.00	8.93	83.34
				± 0.59	± 0.29	± 0.0014	± 0.0004	± 4.81	± 0.043	± 1.93
Control				0.00	16.13	0.0786	0.0684	96.43	10.89	85.85
					± 0.16	± 0.0015	± 0.0005	± 1.73	± 0.14	± 0.084

\* Dipel 2x, *Bacillus thuringiensis Kurstaki*

\*\*\* Larval mortality % after 6 days

\*\* Biover®, *Beauveria bassiana*

± =Standard Error

Generally the two tested bio-compounds revealed slight decrease in the weight of the 4<sup>th</sup> instar larvae of the two tested insects.

**Pupal weight:**

Data in Table (1) indicated Dipel 2x, *Btk* decreased pupal weight which achieved (0.0384±0.0006 g.) comparing with (0.0398±0.0004g.) obtained in untreated once. Results also, proved that entomopathogenic fungi Biover® exhibited (0.0533±0.0004g.) as compared with (0.0684±0.0005) which attained with untreated check.

**Pupation Percentages:**

The data in Table (1) indicated that, the tested concentration of Dipel 2x, *Btk* reduced the pupation percentage by 77.21±4.05% compared with (85.55±2.92 %) which was recorded with check experiment. Also results in the same Table showed that Biover®, *B. bassiana* caused strongly decreased pupation percentage that attained (35.00±4.81 %) as comparing with that of (96.43±1.73 %) which recorded in untreated check.

**Pupal duration:**

Also present results proved that Dipel 2x, *Btk* tested compound caused highly prolongation in pupal duration showing 25.50±0.64 days compared with 10.69±0.56 days which attained in control. Also, results in the same Table proved that the influence of Biover® fungi tested against spiny bollworm caused mediate decrease in pupal duration which achieved 8.93±0.043 days compared with that of 10.89±0.14 days that exhibited in untreated larvae.

**Adult emergence percentages:**

Also present results indicated that Dipel 2x, *Btk* tested biocide was resulted in emergence percentage of 77.21±4.05 % compared with 85.55±2.92 % for control. Also results tabulated in the same Table showed that the entomopathogenic fungi Biover® tested biocide attained (83.34±1.93%) compared with that of 85.85±0.084 % in untreated check.

**Oviposition periods of emerged females:**

**Pre-oviposition period:**

Also data showed that Dipel 2x, *Btk* had slightly effect the tested parameter and attained (2.00±0.58 days), compared with (2.25±0.59 days) for untreated check. Meanwhile the other tested biocide Biover® caused prolongation in pre oviposition period of spiny bollworm which attained (4.33±0.66 days) compared with (2.17±0.69 days) for untreated once.

**Oviposition period:**

As shown in Table (2) the effect of Dipel 2x, *Btk* on pink bollworm female moths, which caused prolongation in oviposition periods that recorded (21.50±0.69 days), comparing with that (15.25±0.56 days) of untreated check. Also, results tabulated in the same Table showed that the entomopathogenic fungi Biover® tested against spiny bollworm resulted in prolongation this period up to 9.33±1.33 days as compared with 8.00±0.58 day that recorded in untreated once.

**Post-oviposition period:**

Also data presented revealed that, the post-oviposition period for pink bollworm female were (1.50±0.69 days) when treated by Dipel 2x, *Btk* comparing with untreated check that exhibit 1.38±0.57 days. But the influence

of Biover® tested biocide against spiny bollworm was  $7.00 \pm 1.53$  days compared with that ( $4.66 \pm 0.88$  days) recorded in untreated check as a post-oviposition period.

**Table (2): Effect of *Bacillus thuringiensis Kurstaki* and *Beauveria bassiana* on immature stages of the pink and spiny bollworms.**

Insects	Compounds	Concentration	Rate of application /feddan	Oviposition period/female/day			Adult longevity in days		Female fecundity Eggs/Female	Hatchability %
				Pre-oviposition period	Oviposition period	Post-oviposition period	Female	Male		
Pink bollworm	* Dipel 2x	$32 \times 10^6$ IU	200g.	2.00	21.50	1.50	25.00	22.63	149.75	73.16
				$\pm$ 0.58	$\pm$ 0.69	$\pm$ 0.69	$\pm$ 1.20	$\pm$ 0.59	$\pm$ 5.23	$\pm$ 1.15
Control				2.25	15.25	1.38	18.88	23.88	362.00	88.09
				$\pm$ 0.59	$\pm$ 0.56	$\pm$ 0.57	$\pm$ 0.60	$\pm$ 0.57	$\pm$ 2.31	$\pm$ 0.57
spiny bollworm	** Biover®	$32 \times 10^6$ viable spores/mg	200g.	4.33	9.33	7.0	20.67	20.00	60.33	41.12
				$\pm$ 0.66	$\pm$ 1.33	$\pm$ 1.53	$\pm$ 3.18	$\pm$ 1.53	$\pm$ 11.66	$\pm$ 6.45
Control				2.17	8.00	4.66	14.83	15.67	78.33	82.33
				$\pm$ 0.69	$\pm$ 0.58	$\pm$ 0.88	$\pm$ 0.35	$\pm$ 0.88	$\pm$ 5.78	$\pm$ 7.51

\* Dipel 2x, *Bacillus thuringiensis Kurstaki*

\*\* Biover®, *Beauveria bassiana*  $\pm$  =Standard Error

#### Adult longevity:

##### A- Female:

Also data showed that Dipel 2x, *Btk* achieved prolongation in female longevity ( $25.00 \pm 1.20$  days), as compared with ( $18.88 \pm 0.60$  days) that recorded with untreated one. On the other hand, Biover® tested biocide against spiny bollworm also, attained prolongation in female longevity  $20.67 \pm 3.18$  days as compared with  $14.83 \pm 0.35$  in untreated check.

##### B-Male:

Also data indicated that, the Dipel 2x, *Btk* tested biocide lead to slight shortening in male longevity ( $22.63 \pm 0.59$  days) comparing with  $23.88 \pm 0.57$  days in untreated check. While the effect of Biover® entomopathogenic fungi tested against spiny bollworm achieved  $20.00 \pm 1.53$  days compared with  $15.67 \pm 0.88$  days which exhibited in untreated check.

**Female fecundity:**

Also present results indicated that the tested bacteria, *Btk* achieved a highly noticeable reduction in the female fecundity which recorded (149.75±5.23 %) comparing with (362.00±2.31 %) that attained in untreated check. Also, results proved that Biover® tested fungi attained highly decreasing in female fecundity 60.33±11.66 eggs laid/female as comparing with that 78.33±5.78 eggs laid/female in untreated once.

The present results agree with those obtained by (Moawad *et al.* 1996) who found that increasing the used concentration of biocide *Bt* caused an elongation of female longevity of *Spodoptera littoralis*. Increasing the used concentrations of Ecotch, Dipel 2x and Bioclean caused a shortness adult longevity (Al-Shannaf, 2002). Similar results were obtained by (Salama and Zaki 1986) and (Moawad *et al.* 1996). Interactive effect of *Bacillus thuringiensis* var. *Kurstaki* HD-1 and proteinase inhibitors in the larvae of *Helicoverpa armigera* depended upon the quality and quantity of proteinase inhibitors, which vary widely in different plants (Gujar *et al.* 2004).

**Hatchability of eggs:**

The tested bacteria Dipel 2x decreased hatchability percentage of pink bollworm where recorded 73.16±1.15% comparing with 88.09± 0.57% in untreated check. Meanwhile the tested Biover® caused highly decreasing in spiny bollworm hatchability percentage which attained 41.12±6.45% as compared with 82.33± 7.51 % achieved in untreated check.

The obtained results are in agree with (Al-Shannaf, 2002) who found that the influence of Dipel 2x, Ecotch and Bioclean biocides on the fecundity of emerged females decreased significantly. The average number of egg-mass deposited by emerged female was decreased with increasing the used concentration of each biocide. The percent of hatching eggs of pink bollworm was significantly reduced by Chinimix and Spintor the influence of Biorepel had insignificant effect. Increasing the concentration of all tested compounds induced a gradual decreased in hatchability of eggs (Amer, 2004). Fungal endophytes negative effects on the fitness of *H. armigera* first generation. The reduction in fitness parameters of *H. armigera* across two generations is caused indirectly via an endophyte-triggered reduction in plant quality (Lara and Stefan 2009). Laboratory experiments on the evaluation of infectivity of *Beauveria bassiana* (Bals.) Vuill. to tobacco caterpillar *Spodoptera litura* as influenced by two okra varieties (Suganya and Selvanarayanan 2010).

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**التأثيرات السامة والبيولوجية لبكتريا باسيليس ثيورينجنسس على دودة اللوز  
القرنفلية وفطر البيوفاريا باسيانا على دودة اللوز الشوكية  
محمد السيد محمد على حجاب وأحمد عطا عبد الله توفيق زكى  
معهد بحوث وقاية النباتات-مركز البحوث الزراعية-الدقى-الجيزة -مصر**

أجريت تجربة معملية لتقييم كفاءة كل من المركب الحيوى باسيليس ثيو رينجنسس (دايبل ×٢) وفطر البيوفاريا باسيانا ( البيوفار) تحت ظروف معملية عند درجات حرارة ورطوبة ثابتة على سلالة حساسة ليرقات حديثة الفقس لدودة اللوز القرنفلية وسلاله حقلية لدودة اللوز الشوكية حيث كانت النسبة المئوية لموت يرقات دودة اللوز القرنفلية بعد ٦ ايام من المعاملة بالمركب البكتيرى دايبل ×٢ ( $0.63 \pm 17.18$  %) مقارنة بالغير معاملة ( $0.00$  %). في حالة معاملة دودة اللوز الشوكية بمركب البيوفار كانت النسبة المئوية للموت بعد ٦ ايام ( $0.09 \pm 15.55$  %) مقارنة بالغير معاملة ( $0.00$  %). كما حسب معدل تكوين العذارى وخروج الفراشات، كذلك دراسة التأثير المتأخر للمركبات المختبرة على أطوار الحشرة المختلفة وعلى معدل وضع البيض ونسبة الفقس للإناث الناتجة من اليرقات المعاملة حديثة الفقس. أوضحت النتائج ان المركب البكتيرى كان له تأثيراً على جميع الصفات البيولوجية المدروسة، حيث إنخفض كل من فترة العمر اليرقى ووزن كل من طورى اليرقات والعذارى والنسبة المئوية للتعدير ونسبة خروج الفراشات الكاملة. كما كان له تأثيراً معنوياً على فترات وضع البيض وطول فترة حياة الذكور الناتجة من اليرقات المعاملة وكذلك على الكفاءة التناسلية للإناث الفراشات حيث تسبب فى خفض معدل وضع البيض  $0.23 \pm 149.75$  بيضه/أنثى مقارنة بـ  $2.31 \pm 362.00$  بيضة/أنثى فى الكنترول. وكذلك أنخفضت نسبة فقس البيض وكانت ( $0.15 \pm 73.16$  %) مقارنة بالغير معاملة كانت ( $0.57 \pm 88.09$  % بيضه للأنثى) فى حالة معاملة دودة اللوز القرنفلية. أيضاً أوضحت النتائج أن معاملة دودة اللوز الشوكية بفطر البيوفار كان له تأثيراً كبيراً على كفاءة الإناث فى وضع البيض حيث كانت ( $11.66 \pm 60.33$  % بيضه) مقارنة بالغير معاملة والتي كانت ( $5.78 \pm 78.33$  % بيضه). كذلك المعاملة بالفطر خفضت نسبة فقس البيض للأنثى حيث كانت ( $6.45 \pm 41.12$  %) مقارنة بالإناث الغير معاملة والتي كانت ( $7.51 \pm 82.33$  %).

قام بتحكيم البحث

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