Effect of Some Biocides on the Biological and Prediction Parameters of the Pink Bollworm, Pectinophora gossypiella (Saund.) (Lepidoptera: Gelechiidae)

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ABSTRACT

The biological parameters of the pink bollworm, *Pectinophora gossypiella* (Saund.) were affected when treated as newly hatched larvae with LC50s of the biocides; Protecto (*Bacillus thuringiensis*), Biover (*Beauveria bassiana*) and Protecto + Biover. There was prolongation in pupal duration, female adult longevity especially in oviposition period except for with Protecto + Biover treatment. Also, the biocides decreased hatchability and increased sterility (observed and corrected) addition, larval duration, male adult longevity, egg laying rate, mating frequency and percentages of egg hatchability, Fecundity and mating ability were decreased as compared with the control. On the other hand, the predictive (life table) parameters of the pink bollworm were also affected after biocides treatments. The progeny/female (Mx) decreased in case of Protecto + Biover treatment only, while the same parameter increased with the other treatments. Survival rate (Lx) decreased with all treatments. Also, there was a decrease in the net reproductive rate (Ro), increase rate (intrinsic rate of natural increase (r_m) and finite rate of increase (e^{rm})) compared with the control. The biocides also increased the generation period (T) and doubling time (DT). Sex ratio (female/total) decreased in Protecto treatment; whereas, it increased in Protecto + Biover treatment and it had nearly the same value of the control with Biover treatment.

Key Words: Biocides, Biological parameters, Pectionophaora gossypiella.

INTRODUCTION

The newly hatched larvae of the pink bollworm, *Pectinophora gossypiella* (Saunders) is the most destructive stage for buds, flowers and bolls of cotton, okra, althea, hibiscus and jute.

Bacillus thuringiensis must be ingested by a susceptible insect in order to be effective. The microorganism produces both spores (resting stage) and crystalline protein (an endotoxin) which becomes activated and binds to certain receptors in the insect gut causing pores through which gut contents can enter to the body cavity and bloodstream of the insect. The insect ceases feeding and dies within a few days (Tabashnik, et al., 2003).

Beauvaria bassiana, is a pathogenic fungus with a large host range and it is used for insect biological control. B. bassiana infects and kills the pest when it comes in contact with the fungal spores. Once the fungal spores attach to the cuticle of insect, they germinate sending out structures (hypha) that penetrate and proliferate in the body of the insect. It may take 3-5 days for insects to die, but infected cadavers may serve as a source of spores for secondary spreading of the fungus. Insects can also spread the fungus through mating (Long et al., 2000). Wraight and Ramos (2002) found that in areas infested with the diamondback moth, the insects have become resistant to B.t., the B. bassiana can be used as part of an integrated control program by organic growers, depending on the population pressure and application methods. Thus, mixture of the biocides, protecto (B.t.) and biover (B. bassiana) against the pink bollworm was suggested.

The biological parameters of the pink bollworm as larval, e.g. pupal, adult durations and mortalities.....etc. assessed the effect of the biocides used and gave indication on insect development if it was exposed to the tested compounds. Assessment of the biocide effects on the fecundity, sterility and mating may shed light on their potency.

The life table parameters can be used as a guide to investigate the pest population development. This is a valid method for assessment of the efficacy of the compounds used (Mohamed, 1987) and to clarify the effect of external factors on the growth, survival, reproduction, intrinsic and finite rate of increase for the pest population (Abou-Setta, et al. 1986; Wittmeyer&Coudron, 2001 and El-Gemeiy, 2002).

The present study aimed to evaluate the effect of biocide compounds, protecto and biover either singly or in combination on some biological and prediction (life table) parameters of the pink bollworm treated as newly hatched larvae.

MATERIALS AND METHODS

Materials:

A- The insect:

The newly hatched larvae of the pink bollworm were reared in the Bollworms Department, Plant Protection Research Institute, Agriculture Research Center on a semi artificial diet as described by Rashad and Ammar (1985). Rearing conditions were controlled at 27±1°C and 65-75% RH.

B- Tested compounds:

- **1- Protecto**, is a commercial formulation of *Bacillus thuringiensis kurstaki* and it is a product of the Special Unit for Producing Bioinsecticides, Plant Protection Research Institute, Agriculture Research Center, Egypt, with 32000 international toxicity units. The active ingredient was 6.4% and the application rate was 300gm/feddan.
- **2- Biover**, a commercial formulation of *Beauvaria bassiana* and it is a product of the same Unit with 32000 viable spores per mg. The active ingredient was 10% and the recommended application rate was 200gm per 100 liter water/feddan.

Methods:

A- Bioassay of the tested biocides:

The pink bollworm, *P. gossypiella* was treated as newly hatched larvae as follows: Fifty gm of semi artificial diet were mixed with 2 cm of each concentration from the tested biocides and divided between three petri-dishes (9 cm diameter). The petri- dish used as control was treated with 2 cm distilled water mixed with 50gm artificial diet. The concentrations of the Protecto were 2,1, 0.5, 0.25 and 0.125 g/L, while; the Biover were 3, 2, 1, 0.5 and 0.25 g/L.

Thirty newly hatched larvae of P. gossypiella were left starving for 4 hours, then exposed for about 6 hours to each replicate\concentrate\tested biocide and kept at $27\pm1^{\circ}$ C and 65-75% R.H. Then the numbers of alive and dead larvae were calculated according to Abbot (1925). The LC₅₀ used for treatments for each biocide was determined using the computer software program Proban. The LC₅₀ of protecto was 0.3408 g/L, Biover was 1.0200 g/L and a mixture of both bioagents was prepared by adding the LC₅₀ of each bioagent to one liter water.

The following biological parameters were investigated as follows:

The durations of larvae or pupae surviving per replicate per treatment were recorded and averaged. Adult longevity was determined based upon cumulative number of males and females remaining alive each day. Pre-oviposition, oviposition and post-oviposition periods were determined by placing 2-5 pairs of emerged moths in a clean glass cages (17 cm height and 7-12 cm in diameter) till death of adult females.

Mating frequency and mating ability percentage were determined by placing the adult moths (1-day old) in 3 replicates; each replicate contained five males and five females. The mating frequency was determined by dissection of the females to estimate the presence of spermatophores after 24, 48 and 72 hours and immediately after death. Evaluation of the mating frequency was determined by counting the number of spermatophores per mated female under binocular.

While, mating ability percentage was calculated as follows:

Mating ability
$$\% = \frac{\text{Number of mated females}}{\text{Total number of experimental females}} \times 100$$

Egg laying (total number of eggs per female) calculated from daily counts of deposited eggs on piece of paper. Each treatment yielded data about the daily egg production and on the differential survival of females. Egg hatchability percentage was counted as follows:

Control of hatchability percentage calculated according to Zidan and Abdel-Megeed (1987) as follows:

Fecundity percentage calculated according to Crystal and Lachance (1963) as follows:

Sterility observed percentage calculated according to Zidan and Abdel-Megeed (1987) as follows:

% Sterility observed = 100 – Egg hatchability percentage

Corrected sterility percentage calculated according to Zidan and Abdel-Megeed (1987) as follows:

% corrected sterility =
$$\frac{\text{% Sterility observed - Check}}{100 - \text{Check}} \times 100$$

Life cycle extended from egg deposition till adult emergence (days).

All the previous biological parameters were statically analyzed using a computer software program costat.

B- Prediction parameters:

The data of prediction (life table) study were analyzed by using life 48 basic computer program of (Abou-Setta, et al. (1986).

The input data for the program includes insect name, temperature used, number of observations, the time intervals between observations, the developmental time from egg to adult female as the number of observation intervals, initial number of female, fraction of eggs laid reaching maturity, sex ratio as females per total, number of eggs laid for each interval.

The program has output data which includes information for each interval of adult female age: total progeny per interval (egg laying rate) (M), number of females alive at age x (L), mean female age at each interval mid-point (X), female progeny per female produced during the day x (Mx), rate of survival (Lx), the product of [(Mx)(Lx)] as (MxLx), and the final values of RML (the product of (Mx)(Lx) is then divided by the value of e (the base of natural logarithm to the power of (r_m)).

Finally, the program prints the precise life table sheet parameters of that study as the sum of RML, the generation time (T) was calculated by $[\Sigma((X)(Lx)(Mx))/Ro]$, the net repro ductive rate (Ro) was calculated by $[\Sigma((Lx)(Mx))]$, the doubling time(DT) resulted from dividing the normal logarithm on r_m , the intrinsic rate of natural increase (r_m) was calculated by $[\ln(Ro)/T]$ and the finite rate of increase (e^{rm}) is the natural antilogarithm of the intrinsic rate of increase and gives the number of times which the population multiplies in a unit time (doubling time, DT). Also, the sex ratio was estimated.

RESULTS AND DISCUSSION

A-The biological parameters of the P. gossypiella treated by some biocides:

1-Larval and pupal durations:

The larval duration of the pink bollworm, P. gossypiella averaged 21.2 days in the non treated groups. Slight significant decreases to 19.13 and 19.17 days were obtained when newly hatched larvae were treated with LC₅₀'s of Protecto and Protecto + Biover, respectively. While reduced duration due to Biover treatment (19.42 days) was found insignificant (Table 1 and Figure 1).

On the other hand, the pupal duration revealed a significant increase after treatment being 14.54, 14.14 and 11.48 days for the three treatments, respectively compared with the control (8.9 days) (Table 1 and Figure 1).

Table (1): Effect of tested biocides on some biological parameters of *P.gossypiella* treated as newly hatched larvae.

Disaidas	Larval	Pupal	Adult long	evity (days)	Female adult longevity (days)			
Biocides (LC ₅₀)	duration (days)	duration (days)	ै	\$	Pre-oviposition period	oviposition period	Post-oviposition period	
Protecto	19.13 ^b	14.54 ^a	16.75 ^b	22.75 ^b	3ª	16.75°	3 ^b	
Biover	19.42 ^{ab}	14.14 ^a	16.6 ^b	26.40 ^a	3 ^a	18.4ª	5ª	
Protecto+Biover	19.17 ^b	11.48 ^b	15.6 ^b	20.60 ^c	3ª	12.6 ⁶	5 ^a	
Control	21.2ª	8.90°	21.8ª	20.20 ^c	2 ^b	13.2 ^b	5ª	
LSD _{0.05}	1.883	2.187	2.081	1.988	0.002	1.998	1.331	

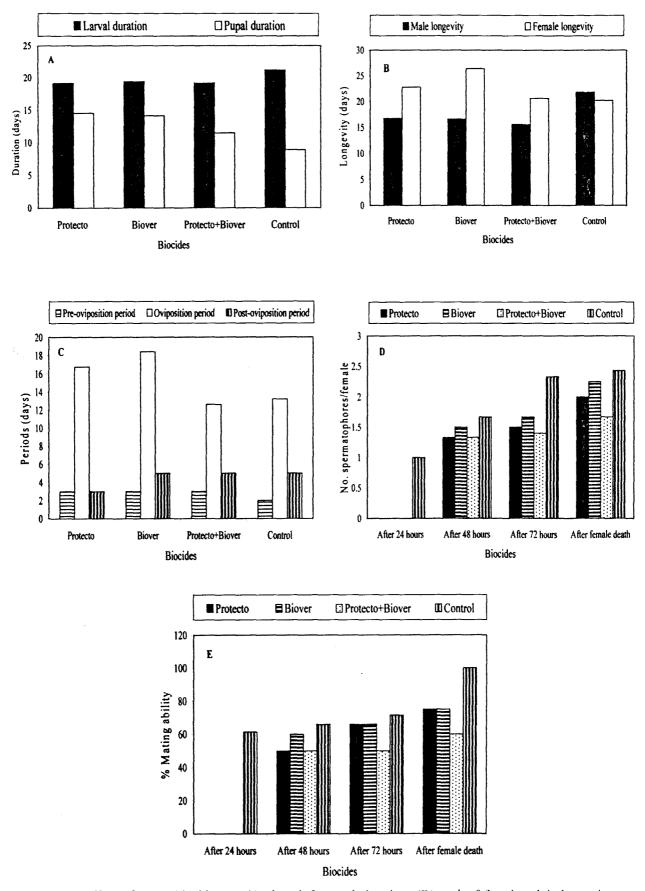


Fig. (1): Effect of some biocides on (A) larval & pupal duration, (B) male &female adult longevity, (C) periods of pre-oviposition, oviposition & post-oviposition, (D) mating frequency and (E) mating ability percentage of the pink bollworm.

2- Adult longevity:

The mixture of biocides Protecto and Biover, followed by each of them alone showed significant reduction in adult males developed from newly hatched treated larvae. The decreases ranged between 15.6 and 16.75 days. While, the control value was 21.8 days (Table 1 and Figure 1A).

On the other hand, the longevity of adult females developed from Biover or Protecto treated larvae showed significant increase as compared with control or biocides mixture (Table 1 and Figure 1).

3- Pre-oviposition, oviposition and post-oviposition periods:

Table (1) and figure (1B) showed that there were no differences between the biocides effects on preoviposition period. But at the same time, they prolonged this period by one day.

Furthermore, the natural oviposition period was 13.2 days; this value was insignificantly decreased to 12.6 days in the females developed from newly hatched larvae treated by Protecto + Biover. When the biocides were used alone, the values significantly increased to 16.75 and 18.4 days for Protecto and Biover, respectively.

The biocide, Biover when used alone or mixed with Protecto at their LC_{50} 's did not affect post-oviposition period (5 days) (Table 1 and Figure 1C), but this value was significantly decreased by 2 days in case of Protecto treatment.

4- Mating frequency and ability percentage:

Table (2) and Figure (1D & E) illustrated that spermatophores of the normal dissected females of the pink bollworm ranged between zero to two spermatophores/mated female after 24 hours, while the dissected females initiated from biocides treated larvae had not register any spermatophores.

After two days, the mating frequency of the dissected females in the control averaged 1.67 spermatophores/mated female. On the other hand the dissected females developed from newly hatched larvae treated by Protecto alone or when mixed with Biover registered 1.33 spermatophores/mated female (Table 2 and Figure 1D). While, when Biover was used alone, the spermatophores were 1.5/mated female.

After 72 hours, the average number of spermatophores in normal dissected females increased to 2.33 (Table 2 and Figure 1D), this average number decreased to 1.67 spermatophores/ mated female developed from newly hatched larvae treated by Biover, followed by Protecto (1.5 spermatophores/mated female) and then Protecto + Biover (1.40 spermatophores/mated female).

The spermatophores in the control from 1-day old until death were 2.43 spermatophores/ mated female. The dissected females of the pink bollworm developed from newly hatched larvae treated by Protecto+Biover showed 1.67 spermatophores/mated female, the last number increased to two spermatophores/mated female in Protecto treatment, followed by Biover treatment (2.25 spermatophores/mated female).

The mating ability of the adult female in the check (Table 2 and Figure 1E) reached 61.4% within 24 hours, then gradually increased to 66% after 48 hours, 71.4% after 72 hours and at the end of their life, all dissected dead females showed 100% mating abilities.

Adult females developed from treated hatched larvae had no mating ability within the first 24 hours, while the percentages reached to 50% mating ability after 48 hours, 66% after 72 hours and continue to increase until become 75% when the females passed their life from 1-day old until death.

The mating ability percentage of the females treated as newly hatched larvae by Biover had the same result of the protecto treatment except at the 48 hours interval, the adult females showed 60% mating ability in case of Biover treatment (Table 2 and Figure 1E).

Table (2): Effect of some biocides on the mating frequency and ability percentage of the pink bollworm.

Biocides		Mating ability %						
(LC_{50})	24 h.	48 h.	72 h.	After death	24 h.	48 h.	72 h.	After death
Protecto	0.0(0-0)	1.33 (0 – 2)	1.5(0-2)	2 (0 – 4)	0.0^{b}	50°	66 ^b	75 ^b
Biover	0.0(0-0)	1.5 (0 – 2)	1.67 (0 – 2)	2.25 (0 - 4)	0.0 ^b	60 ^b	66 ^b	75 ^b
Protecto+Biover	0.0(0-0)	1.33 (0 – 2)	1.40 (0 – 2)	1.67 (0 – 2)	0.0^{b}	50°	50°	60°
Control	1 (0-2)	1.67 (0 – 3)	2.33 (1 – 4)	2.43 (2 – 4)	61.4ª	66ª	71.7ª	100 ^a
LSD _{0.05}	-	-	-	-	0.941	1.883	1.988	1.631

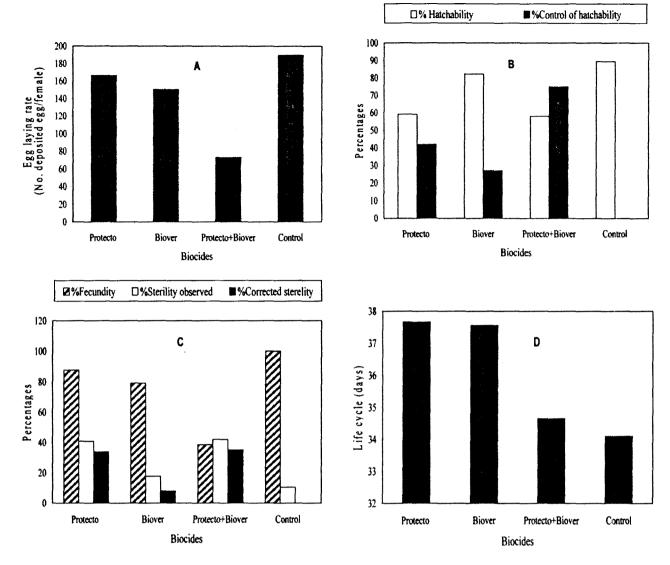


Fig. (2): Effect of some biocides on (A) the egg laying rate, (B) hatchability, (C) percentages of fecundity & sterility and (D) life cycle of the pink bollworm.

When the biocides, Protecto and Biover were combined, the mating ability percentage was 0.0% after 24 hours for the females developed from the newly hatched larvae treatment. While, the percent was 50% after 48 and 72 hours.

5- Egg laying rate and hatchability percentage:

The egg laying rate of the normal females was 189.8 eggs/female as shown in Table (3) and Figure (2A), this value decreased to 73 eggs/female for those individuals developed from newly hatched larvae treated with Protecto+Biover, followed by Biover (150.4 eggs/female) and Protecto (166.5 eggs/female).

The deposited eggs by the females developed from newly hatched larvae treated with Biover had the highest hatchability percentage (82.31%) compared to 89.5% in the control. While, the Protecto + Biover treatment had 58.1% hatchability compared to 59.2% after Protecto treatment.

6- Hatchability percentage:

Table (3) and figure (2B) described that the hatchability of deposited eggs by the females, treated as newly hatched larvae by the Protecto and Biover mixture, was 75.03%. This value decreased to 41.99% and 27.09% when the Protecto and Biover were used alone, respectively.

7- Fecundity percentage:

Protecto and Biover mixture gave the lowest fecundity percentage (38.46%), followed by Biover (79.24%) and Protecto (87.72%) (Table 3 and Fig. 2C).

Table (3): Effect of tested biocides on the egg, fecundity, sterility and life cycle of P. gossypiella treated as newly hatched larvae.

Biocides	Egg laying rate (No. of egg/female)	Egg Hatchability %	Control of hatchability %	Fecundity %	Sterility observed %	Corrected sterility %	Life cycle (days)
Protecto	166.5 b	59.00°	41.99 ^b	87.72 ^b	40.8 ^a	33.85 ^a	37.66 ^a
Biover	150.4 ^c	82.31 ^b	27.09°	79.24°	17.69 ^в	8.034 ^b	37.46 ^a
Protecto+Biover	73.0 ^d	58.10°	75.03 ^a	38.46 ^d	41.9 a	35.08 a	34.65 ^b
Control	189.8 ª	89.50 ª	0.00^{d}	100.0°	10.5 °	0.000°	34.10 ^b
LSD _{0.05}	1.883	1.987	1.631	1.363	1.883	1.633	2.089

Table (4): Life table parameters of P. gossypiella treated as newly hatched larvae with LC₅₀'s of tested biocides.

Biocides	T (days)	(Ro)	Increase rate		– DT (days)	Sex ratio
Biocides			r _m	e ^{rm}	DI (days)	SCA Fatio
Protecto	44.27	45.53	0.086	1.090	8.059	0.462
Biover	42.98	63.76	0.097	1.101	7.146	0.514
Protecto + Biover	39.49	10.02	0.058	1.060	11.95	0.634
Control	35.35	71.05	0.121	1.128	5.747	0.54
(T) = The generation time	(Ro) = The net representation (Ro)		oductive rate $(DT) = The doubling$		ng time	

⁽T) = The generation time (r_m) = The intrinsic rate of natural increase

8- Sterility observed and corrected percentages:

The observed sterility of the control was 10.5% (Table 3 and Figure 2C), this value increased to 17.69% after treatment with Biover, followed by Protecto treatment (40.8%) and reached to 41.9% after treatment with combination of Protecto and Biover.

Biover gave the lowest corrected sterility (8.034%) after treatment of newly hatched larvae, this value increased to 33.85% with Protecto treatment and to 35.08% after treatment with Protecto mixed with the Biover (Table 3 and Figure 2C).

9- Life cycle:

As shown in Table (3) and Figure (2D) when the newly hatched larvae of the pink bollworm treated by Protecto + Biover, the value was 34.65 days which was similar to the control (34.1 days), but in case of the Protecto and Biover when used alone, the life cycle was prolonged by three days.

The obtained results were coinciding with that of Amer (2007) who mentioned that Dipel-2x increased pupal duration, adult longevity, life cycle, the percentages of larval & pupal mortality and sterility of the pink bollworm, Pectinophora gossypiella (Saunders) when treated as newly hatched larvae. On the other hand, it decreased egg laying and egg hatching.

B- The prediction (Life table) parameters:

1-Female progeny/female (Mx) and rate of survival (Lx):

Figure (3) illustrated that female progeny/female (Mx) of the control ranged between 0.18 to 14.84, the last values increased in the females developed from newly hatched larvae treated with Protecto where it ranged between 0.69 to 21.46 females progeny/female. Moreover, it ranged between 1.80 to 28.35 females progeny/female in Biover treatment. The opposite was found in Protecto + Biover treatment where the Mx values were reduced compared with the previous two biocides and the control, it ranged between 0.42 to 6.29 females progeny/female developed from newly hatched larvae treated with a combination of Protecto + Biover.

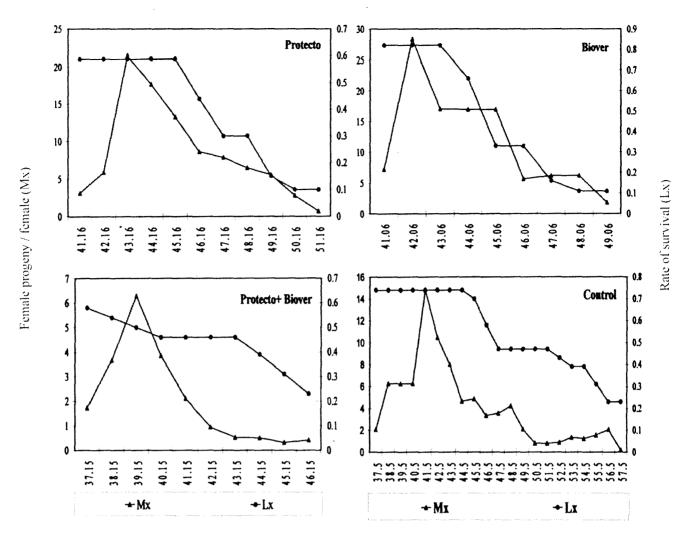
The (Lx) parameter (rate of survival) ranged between 0.29 and 0.93 times in the normal females. The females treated as newly hatched larvae with Protecto had survival rate that ranged between 0.10 and 0.59 times, while in Biover treatment it ranged between 0.11 and 0.82 times. The females developed from newly hatched larvae treated with Protecto + Biover had survival rate which ranged between 0.23 and 0.58 times.

2-Generation period (T):

The generation period lasted 44.27 days in case of Protecto treatment (Table 4), followed by Biover (42.98 days). While, the mixture of Protecto+Biover caused the best reduction (39.49 days) as compared with that in the control (35.35 days).

⁽DT) = The doubling time

 $⁽e^{rm})$ = The finit rate of increase



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Fig. (3): Effect of tested biocides on the female progeny/ female (Mx) and survival rate (Lx) of the pink bollworm.

Actual female age x (days)

3- Net reproductive rate (Ro):

The tested biocides caused high reduction of net reproductive rate (Ro) when the pink bollworm treated as newly hatched larvae as shown in Table (4). The biover treatment was 63.76 females/female in one generation, the last value decreased to 45.53females/female in Protecto treatment. The biocide mixture (Protecto + Biover) induced high reduction to net reproductive rate reaching 10.02 females/female compared with the control (71.05 females/female).

4- Increase rate

4.1- Intrinsic rate of natural increase (r_m) :

Table (4) showed that intrinsic rate of natural increase (r_m) i.e. the ability of inheriting increase for the normal female was 0.121 times/female/day. While, the females treated as newly hatched larvae with Protecto and Biover had intrinsic rate reduction compared with the control to become 0.086 and 0.097 times/female/day, respectively. On the other hand, Protecto + Biover caused the highest reduction from intrinsic rate to reach 0.058 times/female/day.

4.2- Finite rate of increase (e^{rm}):

The daily population of the normal pink bollworm increased to 1.128 times/female/day (Table 4). Also, the females initiated from newly hatched larvae treated with Biover had capacity (1.101 times/female/day) near from the control, followed by Protecto treatment (1.090 times/female/day) and then Protecto + Biover treatment which had the lowest population capacity (1.060 times/female/day).

5- Doubling time (DT):

The calculated time for population becomes twice that means doubling time (DT) depend on the intrinsic rate of natural increase (r_m) which was affected by many factors as the rate of survival, generation time, female in progeny and fecundity.

The pink bollworm population in control multiplies every 5.747 days (Table 4). This increased to 7.146 and 8.059 days when the pink bollworm was treated as newly hatched larvae with Biover and Protecto, respectively. While, the treatment with Protecto +Biover had the longest time (11.95 days) for multiplication.

6- Sex ratio:

In control, the sex ratio was 0.54 (%females). Also, the pink bollworm developed from newly hatched larvae treated with Biover had nearly the same ratio (0.514). The ratio was 0.462 with Protecto and increased to 0.634 with a combination of Protecto and Biover.

The aforementioned results were in agreement with Amer (2006) who reported that Dipel-2x ($B.\ thuringiensis$, Kurstakie.) decreased the rate of survival (Lx), r_m and sex ratio when it used in newly hatched larvae of the pink bollworm treatment. In the same time, it increased the generation period.

Generally, the combination of Protecto and Biover had potentiating effects in relation to most biological and prediction parameters of the pink bollworm than when the two biocides were used singly.

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الملخص العربي

تأثير بعض المبيدات الحيوية على القياسات البيولوجية والتنبؤية لدودة اللوز القرنفلية Pectinophora gossypiella (Saund.) (Lepidoptera: Gelechiidae)

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تهدف هذه الدراسة إلى معاملة يرقات الفقس الحديث لدودة اللوز القرنفلية بالتركيز النصفى المميت لبعض المركبات الحيوية [بروتكتو ببيوفار]. تأثرت بعض القياسات البيولوجية لدودة اللوز القرنفلية كما يلى: حدثت زيادة في فترة الطور العذرى - فترة حياة الإناث البالغة خاصة فترة وضع البيض ما عدا معاملة البروتكتو ببيوفار حيث افتربت القيمة من الكونترول -حدثت زيادة أيضا في مدة دورة الحياة بالإضافة إلى زيادة النسبة المئوية من مدى تحكم المركبات في الفقس -العقم (الظاهرى -المصحح). أثرت نفس المركبات الحيوية بالخفض لكل من فترة الطور اليرقى خترة حياة الذكور البالغة -معدل وضع البيض -عدد مرات التزاوج كما أدت المركبات الحيوية إلى خفض النسب المئوية لكل من فقس البيض -الخصوبة -القدرة على التزاوج وذلك مقارنة بقيم الكونترول. تأثرت القياسات التنبؤية (جداول الحياة) بمعاملات المركبات الحيوية كما يلى: إنخفضت عدد الإناث الناتجة لكل أنثى (Mx) في حالة المعاملة بالبروتكتو +البيوفار فقط بينما أدت باقى المعاملات إلى زيادة السركبات الديوية المستخدمة بالخفض على معدل البقاء (Lx) وذلك في جميع المعاملات .كما حدث خفض في معدل الزيادة اليومى (((وات) و العكس صحيح أدت المركبات الحيوية السابق ذكرها إلى زيادة فترة الجيل (T) – وفترة تضاعف الجيل (DT) . انخفضت النسبة الجنسية (عدد الإناث بالنسبة للعدد الكلى) في معاملة البروتكتو + البيوفار كما إقتربت من قيمة الكونترول في معاملة البيوفار.