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Effect of insect growth regulators on *Pectinoghora gossypiella*, *Spodoptera littoralis* (Lepidoptera: Gelechidae: Noctuidae) eggs and a predator spider *Thanatus albini* (Arachnida: Philodromidae) egg-sacs with some biological aspects of predator

El-Gabaly, A.R.; Aiman , M. Adly; Dalia, A. Abdel-Salam and Hassan, M.I.

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

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Abstract:

Under the laboratory condition of 27±1°C and 70- 75% RH. two experiments were conducted under the laboratory conditions of 26±1°C and 65-70% RH. to study the effect of the insect growth regulator, diflurobenzeron (Dimilin) ; 48% on toxicity, of Pectinophora gossypiella (Saundars) , *Spodoptera littoralis* (Boisduval) (Lepidoptera: Gelechidae: Noctuidae) eggs and spider predator *Thanatus albini* (Audouin) (Arachnida: Philodromidae) egg-sacs as well as on some biological aspects of predator. Results were concluded that the egg incubation period averaged 6.3 days/eggs of S. littoralis treated, while it decreased to 4.4 days/ eggs in untreated, *P. gossypiella* was (6.5 days) in treated than (3.6 days) in untreated and *T. albini* predator egg-sacs was 18.3 days in treated compared with 13.3 days in untreated also, predatory spiderling was able to develop and reproduce better on *P. gossypiella* and *S. littoralis* untreated than treated. The spider passes through seven spiderlings during its life. The total period of the first and second instar spiderlings lasted 24.0 and 17.9 days when spiderlings resulted from eggs treated and untreated, respectively, and fed on moving stages of the *Tetranychus urticae* Koch (Acari: Tetranychidae) . While, the total period from 3rd to 7th spiderlings longer to 119.0 and 133.0 days when resulted from eggs treated and shorted to 79.76 and 81.4 days when the spider resulted from eggs untreated (control) and fed on *P. gossypiella* and *S. littoralis*, respectively. The total food consumption of the predator was 348.0 and 378.7 preys/PBW treated and untreated respectively, while it was 248.6 and 279.7preys *S. littoralis* /predator, treated and untreated, respectively.

Introduction

The pink bollworm (PBW) *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is one of the most serious insect pests infesting the cotton, (*Gossypium* spp.) in many cotton producing areas in Egypt or in the world. It causes serious damage in cotton from flowers to bolls and great loss as in both quality and quantity of cotton yield (Kandil, 2001).

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) is an economically important insect pest known to attack various agricultural crops and is widely distributed in Egypt. It is reported to potentially cause 35% to 55% yield losses at the cotton and vegetative stages of the crops (Rao *et al.*, 2014).

The true spiders are one of the most important biological control agents against of different pests infesting different crops (Huseynov, 2007). True spiders hardly play a major role in controlling insect pests; also, most spiders are generalists with respect to their diet but for efficient pest control, the spiderling *Thanatus albini* (Audouin) belong to family Philodromidae were recorded as a biological control agent against certain agricultural pests such as two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), aphids, *S. littoralis* and/ or *P. gossypiella* (Hendawy and El-Mezayyen, 2003 and Pfannenstiel, 2008).

Insecticide application is considered the best method to manage all pests such a *T. urticae*, aphids, *S. littoralis* and/ or *P. gossypiella*. Due to its economic importance and widely known losses to agricultural crops, however, repeated applications and extensive use of insecticides have resulted in ecological imbalances such as toxic effects on natural enemies and humans (Ahmad *et al.*, 2007 and Abbas *et al.* , 2012).

Insect growth regulators (IGRs) have a more specific mode of action on some Lepidoptera and are not highly toxic to non-target organisms when compared to many conventional insecticides (Desuky *et al*., 2012). Interestingly, most of the IGRs the rapid death of the insect through failure of a key regulatory process to operate or function. that have shown effectiveness against insect pests cause the rapid death of the insect through failure of a key regulatory process to operate or function. In the treated eggs stages the insecticide caused serious malformations of the eggshell. The exposure caused other types of clearly abnormal development of eggs: two micropylar regions were noticed. Hence, even a low concentration of IGRs in the diet can lead to serious disturbances in reproduction and thus possibly at the population level.

The role of insect chitin synthesis resulting (IGRs) in all stages developmental has been extensively reviewed (Tasei, 2001). Due to their mode of action these types of different (IGRs) by disrupting the molting process, or cuticle formation, during specific developmental stages in insects are likely to pose greater hazard to larval stages than to adult insects Prabhaker and Toscano (2007). However, there are few studies on the effects of these types of compounds on the true spider

Therefore, this work aimed to study the effect of recommended compounds (IGRs) on eggs of *P. gossypiella* and *S. littoralis* and latent effect on some biological aspects of spiderling, *T. albini* under laboratory conditions

Materials and methods

1. Insect used:

The pink bollworm *P. gossypiella*, used in this study was obtained from laboratory colony of Bollworm Department, Plant Protection Research Institute; Agriculture Research Center (ARC), reared for several generations away from any contamination with insecticides on an artificial diet that previously described by Rashad and Ammer (1985). While, first instar larvae of *S. littoralis* were reared on the castor bean leaves under the laboratory conditions of 26±1°C and 65-70% RH.

2. Culture techniques of *Tetranychaus urticae*:

The two spotted spider mite, *T. urticae* were collected from castor bean leaves infested with different stages of the spider mite *T. urticae* from Giza Governorate and transferred to the laboratory to use in the feeding of the spider, it reared according to Dittrich (1962).

Predaceous spider specie (sac eggs and different stages of spider- ling) were collected from cultivated cotton in Qaliobia Governorate, by using the hand picking and then brought to the laboratory for identification. The spiders found on foliage, on different the plant cotton ages and associated with flower.

3.Pesticides used:

An insect growth regulator (IGR) was experimentally used in this study:

Common name: Diflurobenzeron (benzoylurea)

Trade name: Dimilin 48%

Rate of application: 125cm/fed.

4. Prepared compounds:

Different concentrations of the tested compounds were prepared as followed:

Dimilin: 480, 240, 120, 60, 30, 15 and 7.5 mg/L.

5. Toxicity on eggs of two pests *Pectinophora gossypiella* and *Spodoptera littoralis*:

Samples of eggs for different pests eggs *P. gossypiella* or *S. littoralis* (1-3 days' old eggs) treatment were done by dipping a piece of paper containing eggs on the different tested concentrations of the compound. Three replicates from each eggs of *P. gossypiella* or *S. littoralis* were used; each replicate was 100 eggs of *P. gossypiella* and 70 eggs for *S. littoralis* the both eggs on paper was dipped in each concentration of the compound.

6. Toxicity on eggs predator spider:

The toxicity of the tested compound against eggs of *T. albini* spiderling sacs was studied. Samples of eggs for *T. albini* spiderling sacs treatment were done by dipping a piece of paper containing egg sacs on the different tested concentrations of the compound. Three replicates from each egg sacs were used; each replicate from 2-3 eggs sacs (each eggs sac contend 70-110 eggs) on paper was dipped in each concentration of compound. After that the papers were left until dried. Other three replicates of similar eggs were dipped in water and left as control. Then, the treated and untreated (control) eggs were kept in an incubator under constant conditions 25±1°C and 75±5% RH. The numbers of hatchability daily and percentages of hatchability were estimated. Percentages of mortalities were corrected according to Abbott's formula (1925) as follows:

$$\%\text{Corrected mortality} = \frac{T - C}{100 - C} \times 100$$

Where; T: %mortality in treatment

C: %mortality in check

Data were corrected LC_{25} and LC_{50s} of diflubenzuron, were calculated by using proban software.

7. Biological studies of the spider predator *Thanatus albini*:

The eggs sacs of spider *T.albini* predator were treated with LC_{50} of tested compound; it transferred to glass vials (2 X 7cm) and kept under constant conditions of 26±1°C and 70-75 % RH. A control check was done in distilled water for the same time. The numbers of hatchability daily, percentages of hatchability and the incubation period of eggs sacs were estimated. The first instar *T. albini* spiderling hatchability from eggs treated and untreated was then placed singly in glass tubes containing with the individual prey. The tubes were stoppard with cotton wool and held until adults' stage.

Feeding capacity: To rearing the spiderling predator; two groups used; each group 60 individuals' spiderling were reared from hatchability eggs sacs treated or untreated to maturity on different prey. The newly emerged *T. albini* spiderling were placed individually in glass tubes (15 cm high X1.5cm wide) with enough numbers of the two spotted spider mite *T. urticae* were offered daily until (the end) developmental period of 1st and 2nd instar *T. albimii* spiderling. After that, these tubes were divided in four groups, each of 30 tubes. The first and 2nd groups resulted from eggs sacs treated were enough numbers of eggs and newly hatched larvae of PBW, it was offered daily to first group, while, the second group was offered the 2nd instar larvae of *S. littoralis*. At the same time the third and fourth groups from hatchability eggs sacs untreated fed on the numbers offered from each prey; the two preys were increased as the predator's spiderling old. The numbers of consumed prey from each PBW and *S. littoralis* were recorded daily and the total consumptions were assessed.

8. Statistical analysis:

The relation between duration of different stages also, total of consumption for each prey examined, data were subjected to the analysis of variance test (ANOVA) (one ways classification ANOVA) followed by a least significant difference, LSD at 5% (Costat Statistical Software 1990).

Results and dissection

1.Toxicity of IGR on 1-3 days eggs of *Pectinophora gossypiella*:

The toxicity of the diflurobenzeron against the *P. gossypiella*1-3 days' old eggs expressed as hatchability number and percentage at different concentrations was presented in Table (1). These results obviously showed that number hatchability was positively correlated with different concentrations which depend on the efficacy of IGR compound; it showing that the total number hatchability increased with decreased the concentrations (6, 26, 44, 36, 61 and 82 eggs hatchability, respectively, at the concentrations of 7.5,15, 30, 60, 120 and 240 ppm, compared to 94 total number hatchability in control and the percent hatchability reductions decreased with decreased the concentrations 94, 74, 56, 64, 39, and 18% eggs reduction at the concentrations of 240, 120, 60, 30, 15 and 7.5 ppm, respectively, compared to 6 % hatchability in control.

Table (1): Effect of diflurobenzeron compound on hatchability *Pectinophora gossypiella* 1-3 days' old eggs.

Tested eggs	Con. (ppm)	Initial number of eggs	No. of hatchability after				Total hatchability	% hatchability reduction
			4 (day)	5 (day)	6 (day)	7day)		
1-3 days	**240**	100	0	0	0	6	6	94
	120	100	0	0	17	9	26	74
	60	100	0	0	31	13	44	56
	30	100	0	7	39	11	36	64
	15	100	0	3	31	37	61	39
	7.5	100	3	23	49	7	82	18
	Control	100	20	73	1	0	94	6

Also, the results presented in Table (2) summarized that the efficacy of IGR at different concentrations against the hatchability of *S. littoralis* 1-3 days old ' eggs. It's cleared that the different concentrations high effected on number of eggs hatchability, which, decreasing gradually with an increase the tested concentrations. It recorded by (64, 62, 57, 43, 27 and 11 eggs hatchability, respectively, at the concentrations of (7.5,15, 30, 60,120, and 240 ppm) and the percent hatchability reductions decreased with decreased the concentrations.

Table (2): Effect of diflurobenzeron compound on hatchability of *Spodoptera littoralis* 1-3 old days eggs.

Tested eggs	Con. (ppm)	Initial number of eggs	No. of hatchability after				Total hatchability	% hatchability reduction
			4 (day)	5 (day)	6 (day)	7day)		
1-3 days	**240**	70	0	0	11	---	11	84.3
	120	70	0	5	22	---	27	61.4
	60	70	0	9	31	3	43	61.43
	30	70	0	7	24	26	57	38.5
	15	70	0	15	31	16	62	88.57
	7.5	70	6	30	25	3	64	91.43
	Control	70	20	41	5	0	66	94.3

2. Effect of diflurobenzeron compound on 1-3 days eggs of the *Thanatus albini* predator:

The toxicity of the diflurobenzeron against the *T. albini* predator days' old eggs sacs expressed as number and percentage of hatchability at different concentrations was presented in Table (3). These results obviously showed that number hatchability was positively correlated with different concentrations which depend on the efficacy and penetration of compound to the eggs inside the sacs recorded the total number hatchability decreased with increased the concentrations (82, 74, 62, 54, 47 and 11) eggs hatchability from sacs treated at the concentrations of (7.5, 15, 30, 60,120 and 240 ppm) and the percent hatchability reductions decreased with decreased the concentrations

Table (3) Effect of diflurobenzeron compound on hatchability of *Thanatus albini* 1-3 old days eggs sacs.

Tested eggs sacs	Con. (ppm)	Initial number of eggs sacs	No. of hatchability after from treated				%Total hatchability	%hatchability reduction
			13 (day)	17 (days)	19 (days)	18-25 (days)		
1-3 days	**240**	2-3 (90)	0	0	0	29	11	89
	120	2-3 (85)	0	0	21	26	47	53
	60	2-3 (87)	0	10	12	32	54	46
	30	2-3 (90)	0	3	29	30	62	38
	15	2-3 (87)	0	13	31	30	74	14.9
	7.5	2-3 (93)	0	16	19	47	82	11.8
	Control	2-3 (70)	0	20	53	12	67	4.82

(2-3 eggs sacs) contents average number from 60-80 eggs inside the eggs sacs.

3. Toxicological effect of diflurobenzeron on eggs of ***Pectinoghora gossypiella*, *Spodoptera littoralis* and *Thanatus albini* :**

Data in Table (4) showed that LC_{50} values of diflurobenzeron was more effective against *P. gossypiella* and *S. littoralis* than *T. albini*. The LC_{50} values were 28.23 and31.4 ppm for *P. gossypiella* and *S. littoralis* eggs, respectively, and increased approximately to two times with *T. albini*, the LC_{50} value estimated by 63.24 ppm. Kandil *et al.* (2012) recorded that the toxicological of lufenuron, chlorfluazuron and chromafenozide (IGR) against *P. gossypiella* eggs.

Table (4): Toxicological effect of diflurobenzeron on eggs of *Pectinoghora gossypiella, Spodoptera littoralis* and *Thanatus albini.*

Comp.	Stage used	LC values				Slop ± SE	
		Instars used	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)		
Difluroben zeron	1-3 old days eggs	*P. gossypiella*	8.75	28.23	493.5	1.75	0.15
		S. ***littoralis***	11.57	31.4	571.5	1.85	0.13
		T. albini	21.38	63.24	889.7	1.43	0.19

4. Incubation periods of eggs:

Data presented in Table (5) showed that the egg incubation period averaged 6.3 days/eggs of *S. littoralis* treated, while it decreased to 4.4 days/ eggs in untreated, on the other hand, in, *P. gossypiella* the incubation period increased approximately two time (6.5 days) in treated than (3.6 days) in untreated and *T. albini* predator the incubation period of eggs sacs was 18.3 days in treated compared with 13.3 days/ egg sac in untreated.

Table (5): Percent of hatchability and incubation period treated eggs of *Spodoptera littoralis*, *Pectinoghora gossypiella* and *Thanatus albini* with IGR compound under laboratory condition (25±1°C and 65-70 % RH.).

Compound used	Insect and predator used		Conc. (ppm)	% of hatchability treated	% of hatchability control	Incubation period treated	Control
IGR	Egg stage	*S. littoralis*	28.23	51	94.3	6.3.3±1.2	4.4±**0.3**
		P. gossypiella	31.4	50	94	6.5±0.6	3.6±**0.5**
		T. albimii	63.24	56	98	18.3±1.3	13.3±**1.3**

T. albini was able to develop successfully from egg to adult stage when fed on *P. gossypiella* and S. *littoralis*; it had seven instars spiderling; the first and 2nd instar spiderlings after hatching from the eggs sacs treated or untreated fed on immature stages of *T. urticae*; but from the 3rd to 7th instar spiderling of *T. albini* fed on treated and untreated *S. littoralis* or *P. gossypiella.*

Data in Table (6) recorded that the total duration from 1st and 2nd instars spiderling of *T. albini* was significantly shorter (17.9 days) when resulted from eggs untreated and fed on *T. urticae* during this period spiderling of *T. albini* consumed 58.3 fed on immature stages of *T. urticae*, while it was longer (24.6 days) in case of spiderling resulted from treated eggs and consumed 48.9 preys of immature stages of *T. urticae*. Data recorded in Table (7) show that the duration from the 4th to 7th instar was longer duration than other instars in predacious, but the 7th instar decreased in time duration. The duration of different instars of *T. albini* was affected obviously by treatment and two different food sources. The total developmental periods from 3rd to 7th instar of *T.albini*, when fed on *P. gossypiella* eggs treated and untreated were 94.4 and 61.86 days/ respectively, while, being decreased to 84.4 and 63.5 days when provided by *S. littoralis* treated and untreated , respectively.

Table (6): Durations of developmental stages and prey consumption of *Thanatus albini* resulted from eggs treated and fed of *Tetranychaus urticae*, at 25±1°C and 65-70 % RH.

Developmental stages *T. albini*	Duration time *Thanatus albini* resulted from treated and untreated			Prey consumption of *Tetranychus urticae*		
	Duration (in days) ±S.E.	Duration (in days) ±S.E.	LSD	Treated ±S.E.	Untreated ±S.E.	LSD
1st spiderling	11.6±1.2	8.6±0.3	1.521	22. 3±1.3	20.0±1.6	0.643
2nd spiderling	13.0±1.4	9.3±0.3	3.110	26.6±3.5	38.3±1.5	6.127
Total 1st and 2nd instars spiderling	24.6±1.8	17.9±1.3	3.244	48.9±4.3	58.3±5.2	5.110

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).

Table (7): Duration (in days) *Thanatus albini* resulted from treated eggs with IGR compound and reared under laboratory condition (25±1°C and 65-70 % RH.).

Spiderling	Reared on *Pectinoghora gossypiella*	control	LSD at 5%		*Spodoptera littoralis*	control	LSD at 5%	
Duration (in days) ±S.E.	Duration (in days)±S.E.	Duration (in days)±S.E.	LSD	P	Duration (in days)±S.E.	Duration (in days)±S.E.	LSD	P
3rd spiderling	15.6±0.6	10.6±0.66	3.106	**	13.6±0.5	11.3±0.3	0.855	***
4th spiderling	18.3±0.6	11.3±0.9	2.615	***	16.3±1.1	13.6±0.5	1.462	***
5th spiderling	19.3±0.6	12.0±0.66	4.211	***	17.3±1.3	11.0±0.6	4.213	**
6th spiderling	23.6±0.33	16.36±1.5	3.509	**	21.6±2.1	15.6±1.5	3.557	***
7thspiderling	17.6±1.3	11.6±0.66	2.11	**	15.6±1.6	12.0±0.7	1.980	***
Total from 3rd to 7th	94.4±6.11	61.86±3.9	6.744	***	84.4±5.9	63.5±4.6	6.471	**
Total immature stage	119.0±6.9	79.76±4.21	7.182	**	133.3±6.33	81.4±5.4	7.991	***
Life cycle	137.3±6.5	93.06±6.9	11.254	***	151.16±10.33	82.7	12.211	**

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).

The total immature stage (Total developmental periods) of *T. albini*, when spiderling resulted from untreated eggs and fed on *P. gossypiella* eggs untreated were 79.76 days/ while, being longer 119.0 days when spiderling resulted from treated eggs (Table, 7). In contrast, the total immature stages lasted a longer time (133.3 days) when they spiderling resulted from treated eggs and fed on *S. littoralis* compared with 81.4 days in control.

5. Food consumption of the immature stages of *Thanatus albini* :

T. albini, the 1st and 2nd spiderling had the least prey consumption rates than the older stages because of their small sizes. They consumed Values gradually increased where increased the ages. The 3rd spiderling consumed an average of 40.0and 32.6 preys/ *T. albini* /day on PBW eggs and 22.6 and 18.7 preys, respectively, when they were provided by *S. littoralis* (Table, 8). *T. albini* consumed of *P. gossypiella* more preys during the duration than *S. littoralis* during all stages of the predator. The total food consumption of the predator was 348.0±21.8 and 378.7±32.3 preys from PBW treated and untreated respectively, while that was 248.6±17.6and 279.7±23.9 preys *S. littoralis* /predator, treated and untreated respectively.

Table (8): Food consumption *Thanatus albini* resulted from treated eggs with IGR diflurobenzeron and reared under laboratory condition (25±1°C and 65-70 % RH.).

Spiderling	Reared on *Pectinoghora gossypiella*	Control	LSD) x= 0:05		*Spodoptera littoralis*	Control	LSD) x= 0:05	
Duration (in days) ±S.E.	**Food consumption ±S.E.**	**Food consumption ±S.E.**	**LSD**	**P**	**Food consumption ±S.E.**	**Food consumption ±S.E.**	**LSD**	**P**
3rd spiderling	40.0±3.2	32.6±2.7	2.310	**	22.6±2.7	18.7±0.8	2.184	**
4th spiderling	56.0±3.5	65.1±3.6	5.142	***	44.0±2.3	54.0±3.1	4.681	**
5th spiderling	78.0±5.1	85.0±5.7	3.171	**	58.0±3.2	64.0±5.2	4.921	***
6th spiderling	104.0±6.3	116.0±4.5	6.325	***	71.0±4.5	83.0±3.6	6.122	***
7thspiderling	70.0± 4.9	80.0±6.3	5.114	***	53.0±4.3	60.0±4.8	4.51	**
Total consumption	348.0±21.8	378.7±32.3	7.158	***	248.6±17.6	279.7±23.9	7.722	***

Values are mean ± SE of three replicates.
Values within the same column having the same letters are not significant different (ANOVA, Duncan's multiple range tests, P < 0.05).

Under the laboratory condition some experiments were conducted to study the effect of diflurobenzeron (Dimilin) compound on two insects eggs and spiderling. The results showed that the percent of hatchability increased with increased the concentrations of compound after treated with dimilin, LC_{50} values dimilin was more (toxicity) effective against *P. gossypiella* and *S. littoralis* than *T. albini*. The LC_{50} values increased approximately to two times with *T. albini* spiderling predator than two insects. Also, the *T. albini* predator consumed of *P. gossypiella* more preys during the duration than *S. littoralis* during all stages of the predator

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