Behavioral aspects of the pink bollworm, *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) field strain against some pesticides

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Abstract

The results of our study indicate the susceptibility of *P. gossypiella* adults to the three tested compounds including one IGR (Nomolt) and two bioinsecticides (Emacte, and Biover) with higher efficiency to the IGR than the other two bioinsecticides. The selected insecticides exhibited low to moderate toxicity to treated adults while inducing a high reduction in females' fecundity and hatchability precents. Results proved the impact of insecticides on the longevity of the exposed stage in addition to the latent effect on subsequent developmental stages. Treating adults of *P. gossypiella* with tested compounds fluctuates the incubation periods of eggs, and increases the duration of resulting immature stages and the total life cycle. Feeding *Chrysoperla carnea* on larvae of *P. gossypiella previously* treated as adults resulted in prolonged immature stages and egg incubation periods. Consequently, the total life cycle of the subsequent generation was significantly longer compared to the control group. Additionally, Emacte, Biover, and Nomolt decreased the percentages of pupation and adult emergence, causing malformation percentages with higher efficiency to the IGR compound (Nomolt) than the other two bioinsecticides.

Keywords: Emacte, Biover, Nomolt, *P. gossypiella, C. carnea*

Introduction

The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), is considered one of the most significant lepidopteran pests in various countries, including Egypt. According to (Kandil 2001), *P. gossypiella* is responsible for major infestations in Egypt. During early and mid-growing seasons, the pest targets fruiting parts and flowers (Hari, 1999 and Abd El-Mageed *et al*., 2007) [7, 1]. Severe infestations occurring from mid to late season on green bolls result in decreased quantity and quality of harvested lint and seeds, leading to substantial yield reduction estimated at 30-40% (Hamed and Nadeem, (2010) [6], Zaki, (2012) [30] and Vonzun, (2019).

Teflubenzuron, a member of the benzoyl phenyl urea group, is particularly effective against the immature stages of numerous lepidopterous insects demonstrating a relatively slow but potent action, (Kandil, *et al* 2013 and 20).

Eco-friendly benign bio-pesticide compounds have emerged as viable alternatives to conventional synthetic insecticides. These substances rely on various bioactive components that exhibit insecticidal properties. Their utilization is expanding rapidly, and they are extensively employed for diverse lepidopterous insects Moreover, bio-pesticide compounds are utilized in developing novel formulations to enhance efficacy (El-Bokl, 2016) [17].

Emamectin benzoate with a novel mode of action typically demonstrates higher selectivity and effectiveness at lower concentrations than conventional insecticides and has shown low to moderate impact on beneficial insects, (Khanzada *et al*., 2018) [11].

The objective of the present study was to evaluate the direct and latent effects of Emacte, Biover, and Nomolt compounds on pink bollworm adults and the resulting generation as well as the biological control agent *Chrysoperla carnea*.

The Chrysopidae family particularly *Chrysoperla carnea* (green lacewing), is a valuable biological control agent due to its predatory nature. While adults consume plant pollen and nectar, their larvae target soft-bodied insects such as aphids, thrips, whiteflies, and larvae of Lepidoptera (Rimoldi *et al*., 2008). Despite their benefits in controlling pests, lacewings and other beneficial insects have been harmed by the widespread use of non-selective insecticides. These pesticides, although effective against pests, negatively impact natural enemies.

Excessive pesticide use leads to two major issues: the emergence of insecticide-resistant pests and the elimination of natural predators (Croft and Brown 1975). As a result, it is crucial to evaluate the potential negative impacts of insecticides on beneficial insects and identify those compatible with biological pest control agents (Stapel *et al*., 2000).

However, lacewings have gained popularity in integrated pest management (IPM) programs, partly due to their tolerance to many insecticides, especially in their cocoon and larval stages (Medina *et al*., 2001) [12].

Materials and methods

Collecting of pink bollworm PBW (*Pectinophora gossypiella*)

Field strain larvae of *P. gossypiella* were collected from infested cotton bolls, at the end of the cotton season, during September-October 2022, Banha region, EL Qaliobia Governorate.

Collecting of the green lacewing (*Chrysoperla carnea)*

A patch of 60 to 70 eggs of *Chrysoperla carnea* was collected from cotton fields at the end of the cotton season. Collected eggs of *Chrysoperla carnea* were incubated under constant conditions of 26±1°C and 70±5% RH until hatching. Hatched larvae were allowed to feed on the field strains *P. gossypiella* larvae (treated and untreated) till cocoon formation. The resulting cocoons were kept in clean vials under the same constant conditions until adult emergence. Newly emerged adults of *Chrysoperla carnea* were kept in glass jars, and a hung cotton piece soaked in a 10% honey solution was provided for adult feeding.

Experimental site

The study was performed under laboratory conditions at Bollworm Department Research, of Plant Protection Research Institute, Agriculture Research Center (ARC).

Tested compounds

Tested compounds	Compounds information			
	Trade name	**Common name**	**Application rate (Recommend in field)**	**Treatment rate (Half recommend)**
Avermectin	Emacte 15% EC	Emamectin	150 cm3/100 L	0.75 cm3/ 1 L
Beauveria	Biover 10%	*Beauveria bassiana*	200 g / 100 L	1 g / 1 L
Benzoyl phenyl urea	Nomolt 15% SC	Teflubenzuron	50 cm3/ 100 L	0.25 cm3/ 1 L

Experimental design

Host treating (*Pectinophora gossypiella)*

Three groups of *Pectinophora gossypiella* each of 30 pairs of freshly adult moths divided into 3 replicates (10 females X 10 males/ replicate), were tested. moth pairs were placed in a chimney glass cage supported by a piece of cotton soaked in half concentration/ each compound for 48 hours. tested moths were allowed to feed on cotton pieces soaked in the tested concentration of each tested compound (Emacte, Biover, and Nomolt). A similar group of control Untreated moths were allowed to feed on cotton pieces soaked in a 10 % sugar solution and used as a control check. After 2 days, the treated cotton pieces were replaced by others soaked in a 10 % sugar solution (untreated). All chimneys were examined daily to determine the mortality, pre-ovipostion, ovipostion, and post-ovipostion periods; females, and the number of laid eggs.

Additional batches of treated and untreated moths were prepared for predator nutriment to facilitate the completion of biological studies on the subsequent developmental stages.

The resulting generation

The newly laid eggs of treated females for each compound were gathered and maintained in an incubator at 26±1°C and 70±5% RH. The eggs' number, hatchability percentages, and incubation times were evaluated.

Three sets of 30 tubes (2 X 7.5 cm) containing untreated diet were utilized to examine the development periods of immature stages. Newly hatched larvae from eggs produced by treated females were individually transferred into these tubes using a camel hair brush. The tubes were sealed with cotton plugs and kept in an incubator at 26±1°C and 70±5% RH. Daily inspections were conducted until pupation occurred. The resulting pupae from each strain were moved to clean tubes until adult emergence. Subsequently, the percentage of egg hatchability, egg incubation time, larval and pupal development duration, and the percentage of adult emergence were determined.

Effect of *P. gossypiella* treated larvae on (*Chrysoperla carnea*):

larvae of *Chrysoperla carnea* were allowed to feed on the field strains larvae of *P. gossypiella* treated with the half-recommended concentration of tested compounds (Emacte, Biover, and Nomolt) under constant conditions of 26±1°C and 70±5% RH until adult emergence. The predator's initial larval stage was predator's initial larval stage was fed on the pest's first larval instar. In contrast, the predator's subsequent instars consumed the pest's second and third larval instars

A similar group of *Chrysoperla carnea* larvae was allowed to feed on untreated larvae of *P. gossypiella* and used as a control check. Newly emerged adults of *Chrysoperla carnea* were sexed immediately after emergence, and kept in glass jars, with a hugged cotton piece soaked in a 10% honey solution for adult feeding. For each treatment, three replicates were prepared. all treatments and control checks were kept under the same constant conditions, till egg laying. The duration of eggs, larvae, and pupae added to the Total immature were recorded. Also, the percentages of Cocoon, adults, as well as malformation percent, were estimated

Statistical analysis

One-way analysis of variance (ANOVA) and Duncan's multiple range tests of means were used Duncan's, (1955).

Results

Adult stage

Insecticide treatment of newly emerged adult *P. gossypiella* moths resulted in considerable mortality rates (Table 1). Two days post-treatment, death rates reached 50%, 40%, and % for Emacte, Biover, and Nomolt treatments respectively, compared to 0% in the untreated control group.

Table 1: Mortality rates of *Pectinophora gossypiella* treated adults along life span

Tested compounds	Initial number	Adults' mortality % post-treatment by			
		(0-2) days	(3-4) days	(7-11) days	(12-26) days
Emacte	30	50	16.7	33.3	0.0
Biover	30	40	13.3	36.7	10
Nomolt	30	10	10	43.3	36.7
Untreated	30	0.0	0.0	10	90

These rates continued to rise throughout the adult lifespan. On the third and fourth days after treatment, adult mortality rates of 16.7%, 13.3%, and 10% were observed, with no deaths in the untreated group. Furthermore, 33.3%, 36.7%, and 43.3% OF mortality were recorded between 7 to 11 days post-treatment for the aforementioned compounds, respectively, compared to 10% in the untreated group. The total lifespan for treated and untreated moths ranged from 11 to 26 days. By the end of the lifespan, 10% and 36.7% of adult mortality were reported for Biover and Nomolt compounds respectively, in contrast to the 90% observed in the control group.

Table 2: Effect of tested compounds on ovipositional period fecundity and fertility percentages of *Pectinophora gossypiella*-treated adults

Tested compounds	Ovipositional period (days ± SE)			Fecundity		% Fertility
	Pre-oviposition	Oviposition	Post-oviposition	Total eggs/ female	No. hatched eggs/ female	
Emacte	4.7b	5.0b	1.0b	50.7b	10c	19.7
Biover	2.7c	7.0b	1.7b	63b	40b	63.5
Nomolt	7.3a	13.3a	4.7a	30c	13c	43.3
Untreated	2.3c	12.0a	1.7b	150a	123a	82.0
LSD 0.05	1.345	2.114	1.279	20.107	6.982	-
P	.0001 ***	.0000 ***	.0009 ***	.0000 ***	.0000 ***	

Oviposition duration, egg production, and fertility

Table (2) illustrates the impact of administering Emacte, Biover, and Nomolt to adult *Pectinophora gossypiella* on their oviposition periods, female egg production, and egg viability.

The data revealed variations in pre-oviposition (4.7, 2.7, and 7.3 days), oviposition (5.0, 7.0, 13.3 days), and post-oviposition durations (1.0, 1.7, 4.7 days) for Emacte, Biover, and Nomolt treatments respectively, compared to the control group. Additionally, the average number of eggs laid (fecundity) by treated females was 50.7, 63.0, and 30 eggs/female for Emacte, Biover, and Nomolt respectively in contrast to 150 eggs/female in the untreated group.

Furthermore, Table (2) shows a significant effect of treatments on egg fertility. Emacte had the most pronounced influence (19.7% viability), followed by Nomolt (43.3%), and Biover (63.5%), compared to 82.0% hatchability in the untreated control.

Duration of the first generation

Data presented in Table (3) illustrates the duration of various pink bollworm developmental stages following the treatment of adults with three compounds: Emacte, Biover, and Nomolt. The findings indicate that these compounds had a significant delayed effect on the embryonic development period (egg stage incubation). The embryonic development for the examined treatments took 4.3, 3.6, and 4.7 days, respectively compared to 2.6 days for the untreated group.

Table 3: Adverse assay of the three tested compounds on the first resulting generation

Tested compounds	Duration (days ± SE)				
	Eggs incubation time	Immature durations			Life cycle
		Larvae	Pupae	Total immature	
Emacte	4.3a	17.0b	8.7ab	25.7b	30b
Biover	3.6b	16.7b	8.3bc	25b	28.6b
Nomolt	4.7a	19.7a	9.7a	29.4a	34.1a
Untreated	2.6c	14.3c	7.3c	21.6c	24.2c
LSD	0.810	1.952	1.1099	1.517	1.961
P	0.001**	0.0017 **	0.0079 **	.0000 ***	0.0001 ***

The data also shows that the average larval durations were 17.0, 16.6, and 19.7 days, while the pupal stage lasted 8.7, 8.3, and 9.7 days, respectively. Statistical analysis revealed highly significant differences among the developmental stages across the three treatments, as shown in Table (3).

The duration of *P. gossypiella* immature stage resulting from the three treatments, is recorded in Table (3). Results demonstrate that the average developmental period required for different immature stages increased significantly to 25.7, 25.0, and 29.4 days for Emacte, Biover, and Nomolt respectively. compared to 21.6 days in the untreated control.

Results in Table (3) also demonstrated the difference in the estimated period from eggs to adult emergence (life cycle) for the resulting stages in each treatment. The life cycle was prolonged gradually to 30.0, 28.6, and 34.1 days in Biover, Emacte, and Nomolt treatments, respectively with a significant difference from the untreated groups (24.2 days).

Effect of rearing *Chrysoperla carnea* on *P. gossypiella* treated larvae

Larval and cocoon duration

The data presented in Table (4) revealed a notable increase in the duration of both larval and cocoon stages, extending the total immature period of *Chrysoperla carnea* when reared on *P. gossypiella* larvae that had been treated. The application of Emacte, Biover, and Nomolt resulted in larval durations of 12.3, 11.6, and 13.3 days, respectively, while cocoon durations were 7.6, 6.3, and 8.3 days, respectively. These figures contrast with the control group, which exhibited a larval duration of 8.3 days and a cocoon duration of 5.6 days. As a result, the total immature period was prolonged to 19.9, 17.9, and 21.6 days for the treated groups, respectively, compared to 13.9 days observed in the untreated control group.

Table 4: Effect on *Chrysoperla carnea* reared on *P. gossypiella*-treated larvae

Tested compound	Immature durations (days ± SE)			Resulting stages %			
	Larvae	Cocoon	Total immature	Cocoon	Malformed Cocoon	Adults	Adults malformed
Emacte	12.3*a*	7.6ab	19.9b	79.4	17.6	77.3	***13***
Biover	11.6*a*	6.3ab	17.9ab	84.3	7.6	89.6	10
Nomolt	13.3*a*	8.3a	21.6a	67.8	23.2	67.6	27
Untreated	8.3*b*	5.6b	13.9c	97.8	0.0	100	3.0
LSD	2.029	2.055	3.463	-			
P	0024 **	.0604 ns	.0047 **				

Impact on cocoon formation

Data in Table (4) indicated a reduction in percentages of *Chrysoperla carnea* pupae due to Emacte, Biover, and Nomolt treatments. The pupal percentages were 79.4%, 84.3%, and 67.8% respectively, compared to 97.8% in the untreated control. Furthermore, these treatments demonstrated significant efficacy in inducing pupal malformation, with rates of 17.6%, 7.6%, and 23.2% respectively, while no malformations were observed in the untreated control.

Impact on adult emergence and malformation

As shown in Table (4), all pupae successfully developed into adults (100%) with no reduction in the untreated control. However, this percentage decreased to 67.6%, 77.3%, and 89.6% for the Nomolt, Emacte, and Biover treatments, respectively. Furthermore, the percentages of adult malformation recorded in Table 5 were estimated at 13, 10, and 27% for the three treatments compared to 3% in the untreated control.

Discussion

Effect of treating *P. gossypiella* adults with tested compounds

Treatment of newly emerged adults of *Pectinophora gossypiella* with LC_{50} of Emacte, Biover, and Nomolt resulted in substantial cumulative mortality rates. This treatment also induced a minor fluctuation in female longevity and a significant reduction in egg production, with treated females ovipositing 50.7, 63.0, and 30.0 eggs per female for the previous compounds, compared to 150 eggs per female in untreated moths. Furthermore, egg hatchability decreased to 19.7%, 63.5%, and 43.3% for the tested compounds, compared to 82.0% in the untreated. Results also revealed various delayed effects, including extended embryonic development periods of 4.3, 3.6, and 4.7 days for the respective treatments. Significant increases in immature stage duration were observed, with average larval periods of 17.0, 16.7, and 19.7 days, and pupal duration of 8.7, 8.3, and 9.7 days for the respective compounds. Consequently, the life cycle was prolonged to 30.0, 28.6, and 34.41 days for Biover, Emacte, and Nomolt treatments, exhibiting significant differences from the untreated group's 24.2 days. These findings corroborate El-Barkey *et al*., (2009) [4], who demonstrated extended larval and pupal development in *P. gossypiella* following treatment with Radiant and Hexaflumuron. Our results were also coordinated with some authors who tested different IGRs against lepidopterous insects, e.g., *P. gossypiella*, El-Shennawy (2009) [18]; *Earias insulana*, El-Shennawy and Kandil *et al*., (2017) [19] as they recorded a prolonging in larval and pupal durations with negative latent effect on the emerged adults. Similar results were reported by Reda *et al*., (2010) [24], on *Spodoptera littoralis* and Shaurub *et al*., (2018) [27], on *Agrotis ipsilon*, where significant increases in larval and pupal duration were observed after treatment with flufenoxuron (Cascade). Additionally, Said *et al*, (2017) [25] recorded that, the IGR compound namely, Teflubenzuron caused long-term inhibition effects with a defect in the molting process at different stages of *P. gossypiella* insect. In addition, Moustafa and Salem (2019) [15], noted prolonged larval, pupal, and total immature durations in *Pectinophora gossypiella* (Saund.) larvae treated with Flufenoxuron. In a laboratory study, Omar *et al*., (2021) [23] demonstrated the effectiveness of *B. bassiana* against various developmental stages of *P. gossypiella*. Also, El-Shennawy and Kandil (2022) [20] observed that Flufenoxuron and Spinetoram extended the duration of both larval and pupal stages of *P. gossypiella*, resulting in significantly prolonged total immature periods when compared to the control group. Similarly, Taha and Radwan (2023) [28] approved the susceptibility of pink bollworms to insect growth regulator insecticides. Consistently, El-Shennawy and Kandel (2024) [21] demonstrated mortality percentages with developmental disturbances of different stages of *P. gossypiella* treated as eggs with Emacte and Nomolt compounds moreover a strong negative effect on eggs' hatchability and adult emergence percentages.

Effect of rearing *Chrysoperla carnea* on *P. gossypiella* treated larvae

Regarding the impact on *Chrysoperla carnea* fed on *P. gossypiella*-treated larvae, the study found that exposure to half the recommended concentration of Emacte, Biover, and Nomolt increased both larval and pupal durations to 12.3, 11.6, and 13.3 days for larva, and 7.6, 6.3, and 8.3 days for pupa, respectively, compared to control durations of 8.3 and 5.6 days for larvae and pupae. This resulted in significantly extended total immature durations of 19.9, 17.9, and 21.6 days for the respective compounds, in contrast to 13.9 days in the control. Moreover, Emacte, Biover, and Nomolt reduced pupation percentages (79.4%, 84.3%, and 67.8%) and adult emergence (77.3%, 89.6%, and 67.6%). They also induced malformation percentages in pupae (17.6%, 7.6%, and 23.2%) and adults (13%, 10%, and 27%) compared to 0.0% and 3.0%, for control, respectively. Our research findings were consistent with Vogt's (1994) study, which demonstrated that certain insect growth regulators, including Cascade (flufenoxuron), Dimlin, Nomolt, and Insegar, had moderate to severe negative impacts on *Chrysoperla carnea* under field conditions. In addition, Medina *et al*. (2003) [13], reported the innocuous effect of the insect growth regulator on green lacewing adults. However, Nasreen *et al* (2007) [16] indicated that lufenuron exhibited no detrimental impacts on eggs and adults but resulted in substantial larval mortality.

The findings of our study align with those of Dutton *et al*. (2003) [3] who discovered that *C. carnea's* developmental period was extended when larvae consumed Bt-contaminated *S. littoralis* larvae. Hussain *et al* (2012) [8] demonstrated that Spinosad displayed lower toxicity and was deemed safer for the third-larval instar of *C. carnea*. Likewise, Karthik and Gunasekaran (2015) [10] noted that over 70% of adult emergence occurred with *Emamectin benzoate*. However, Zia *et al.* (2017) [31] found the bioinsecticide spinosad relatively harmless to *C. carnea* larvae and adults. Furthermore, Kandil *et al* (2018) [9] reported that feeding *C. carnea* on *P. gossypiella* eggs treated with recommended and half-recommended doses of chromafenozide extended the duration of larval instar by approximately twice that of the control. Additionally, Khanzada *et al*., (2018) [11] observed minimal larval mortality in *C. carnea* when treated with the bioinsecticide Spinosad, followed by *Emamectin benzoate*. It has been considered IPM-compatible due to its relatively low toxicity to predatory mites compared with spider mites (Ibrahim and Yee 2000) [22].

Their research also noted a low reduction in pupation and adult emergence rates for these compounds. Conversely, Shaalan and Kandil (2010) [26] reported that Radiant 12% reduced the lifespan of predatory *C. carnea*.

Moreover, Giolo *et al*., 2009 [5], stated that Pesticides can more easily damage predator larvae than adults because larvae walk on treated surfaces and cannot fly. Consequently, they are generally considered the most susceptible stage. They demonstrated that abamectin had no toxic effects on adult *C. carnea*. However, Pesticides can more easily damage predator larvae than adults because larvae walk on treated surfaces and cannot fly. Consequently, they are generally considered the most susceptible stage. Although laboratory toxicity studies have provided adequate data for determining insecticide use in IPM (when mortality rates were low in laboratory experiments) (Barrett *et al.* 1994), additional semi-field and field studies are necessary. More research is needed to investigate the effects of insecticides on lacewings and other predators and parasitoids under various conditions to determine optimal chemical usage in IPM programs.

Conclusion

In conclusion, the results indicate the susceptibility of *P. gossypiella* adults to the three tested compounds with higher efficiency to the IGR compound (Nomolt) than the other two bioinsecticides (Emacte and Biover). Tested compounds exhibited low to moderate toxicity to treated adults while inducing a significant reduction in females' fecundity and egg hatchability, in addition to the latent effect on subsequent developmental stages. Furthermore, results proved the impact of insecticides on *Chrysoperla carnea* fed on *P. gossypiella*-treated larvae by significantly extending both larval and pupal durations, consequently prolonging total immature durations. Exposure of *Chrysoperla carnea* to the tested compounds resulted in fluctuations in egg incubation periods, increased durations of resulting immature stages and total life cycle, reduced pupation and adult emergence rates, and induced malformation percentages in both pupae and adults.

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