International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 13-05-2019; Accepted: 18-06-2019
www.entomologyjournals.com
Volume 4; Issue 4; July 2019; Page No. 127-131

Influence of three insecticides from three different groups on *Pectinophora gossypiella* (Saund.) (Lepidoptera: gelechiidae)

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Abstract
Under laboratory conditions, toxicological evaluation of three insecticides, profenofos, alpha-cypermethrin & flufenoxuron against newly hatched larvae of *Pectinophora gossypiella* (Saund.). The obtained results show a prolongation in larval and pupal developments resulted from treated larvae by the three compounds. Also, they prolonged the total immature period; flufenoxuron was the highest with 31.43 days compared with untreated which was 22.33 days. Also, effect of the three insecticides on fecundity and longevity of adults was done. Study of biochemical parameters; the results accentuated that the treated larvae with profenofos, alpha-cypermethrin & flufenoxuron reduced the total soluble protein, lipid and carbohydrate contents which play a key role in the metamorphosis and developmental process in body of *P. gossypiella* larvae stage. The levels of GOT was highly increased in alpha-cypermethrin treatment. At the same time, the lowest reduction in GPT activity of treated *P. gossypiella* occurred in profenofos, while the highly reduction recorded in flufenoxuron compared with untreated.

Keywords: *Pectinophora gossypiella*, profenofos, alpha-cypermethrin and flufenoxuron, biology, biochemical studies

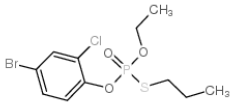
Introduction
The pink bollworm, *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae) is a worldwide pest of cotton and considerable an economically important pest. In Egypt, this insect cause damages in cotton bolls were resulting high reduction in quantity and quality arising to one million kantar annually (Khidr *et al*., 1996 and Kandil *et al*., 2012) [13, 10]. Cotton infestation generally controlled with different groups of insecticide. After more than 30 years on the market, the role of pyrethroids or phosphorus in modern pest control is still significant today. Some authors studied the susceptibility of the pink bollworm *P. gossypiella* to some insecticides in different Governorates and laboratory (Abbas *et al*., 1996, Abdel–Sattar and Guindy, 1988) [1, 2].
Insect Growth Regulators IGRs are low risk insecticides, which have a relatively minor detrimental effect on the environment and its inhabitants and become important components in IPM programs (Horowitz and Ishaaya 2004) [9]. (IGRs) are compounds which interfere with break the life-cycle of arthropod development and metamorphosis of insects. Its effect on several insect species (Khebeb *et al*. 1997) [12], such as *P. gossypiella*, Earias insulana, Spodoptera litturales and other insects (Kandil *et al*., 2013 and Said *et al* 2017) [11, 19].
According to used insecticides from different chemical groups; the present work was carried out to study the toxicity and efficacy of profenofos, alpha-cypermethrin & flufenoxuron from three different insecticide groups on *P. gossypiella* newly hatched larvae, followed by some biological aspects for immature and adult stages as well as biochemical aspect for larvae resulted from treated larvae.

Material and Methods
Insect Used: The susceptibility strain of pink bollworm *P. gossypiella*, newly hatched larvae used in this study were reared in Bollworms Department laboratory, Plant Protection Research Institute; Agriculture Research Center (ARC), away from any treated with insecticides on an artificial diet described by

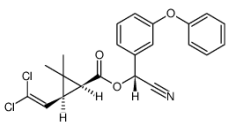
Rashad and Ammar (1985) [16].

Pesticides used
Three different pesticides were experimentally used in this study:

1. **Common name:** profenofos 50% EC
   Trade name: Agricron (50% E.C.)
   Chemical name: 4-bromo-2-chloro-1-[Ethoxy (propylsulfanyl) phosphoryl] oxybenzene
   Stucture formula:

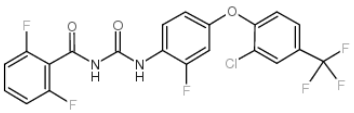


2. **Common name:** Alpha-Cypermethrin 10%EC
   Trade name: Alphacyper (10% EC)
   Chemical name: (1S, 3S)-(R)-Cyano (3 Phenoxyphenyl) methyl3-(2, 2-dichlorovinyl)-2, 2-Dimethylcyclopropanecarboxylate
   Structure formula:



3. **Common name:** flufenoxuron 10% DC
   Trade name: Floxate (10% DC)
   Chemical name: N-({[4-[2-chloro-4-(trifluoromethyl) Phenoxy]-2-fluorophenyl} carbamoyl)-2, 6-Difluorobenzamide.

Structure formula:



Toxicological studies

Serial concentrations from the stock solution of profenofos and alpha-cypermethrin were prepared as follows: 0.27, 0.13, 0.065, 0.032 & 0.016 ppm. And serial concentrations from flufenoxuron were prepared as follows: (12.5, 6.25, 3.12, 1.56 & 0.39 ppm) were tested against newly hatched larvae of *P. gossypiella*.

Determine the toxicity of tested compounds on 1st instar larvae of *P. gossypiella*

To determine the toxicity of three tested compounds against 1st instar larvae of *P. gossypiella*, the different concentrations of each tested compound were sprayed on the surface of an artificial diet in Petri dishes. Three replicates for each concentration, each replicate had 30 of 1st instar larvae of the PBW was allowed to feed on the treated diet for each compound and kept under constant conditions of 26□1□C and 75□5 %RH. After one day, for profenofos and alpha-cypermethrin and the 3 days for flufenoxuron the dead larvae were counted to represent acute toxicity of the three tested compounds. The LC50 and LC90 values with their fiducially limits estimated by Probit (proban) analysis software according to (Finny 1971).

Biological studies

For some biological studies, alive larvae of PBW resulted from treated with LC50 of profenofos, alpha-cypermethrin & flufenoxuron were transferred individually to glass tubes (2 x 7.5 cm) using camel hair brush on artificial diet without any treatment and the same for the untreated larvae. The tubes were capped with cotton stopper and inspected daily until pupation.

Larval, pupal durations and weights & adult emergence were determined. Newly emerged moths resulted from the three treatments and the untreated were sexed and transferred to glass cage. Each treatment was replicated three times. The moths were fed on 20% sucrose solution. Cages were inspected daily to evaluate the oviposition period, numbers of eggs laid, % hatchability and the longevity of adult females and males for each treatment.

The eggs hatchability percentages were calculated according to following equation:

% Egg hatchability =
(No. hatched eggs/ No. deposited eggs) X 100

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

% Fecundity =
(eggs No. of treated female/
eggs No. of untreated female) X 100.

The recorded data were statistically analyzed with one-way analysis of variance (ANOVA) (P< 0.05) (Snedecor, 1952) and Duncan's multiple range test means was used (Duncan's, 1955).

Biochemical analysis

Samples of *P. gossypiella* larvae were collected after 14 days from treated the 1st instar larvae with the three compounds and the untreated and kept in clean tubes for biochemical analyses. Larvae were homogenized in distilled water. The homogenates were centrifuged at 5000 r. p. min. at 5˚C in refrigerated centrifuge. The supernatants' were kept in deep freezer at -20˚C till use for biochemical assays. Larvae were analyzed chemically for each compound with untreated check in Physiological Dept. of plant Protection Researches Institute, (P.P.R.I.).

Determination of enzyme activity

Total protein, total lipids, total carbohydrates, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined colorimetrically according to Koller (1984), Drevon and Schmitt (1964) Cromppton and Birt (1967) and Trinder (1969).

Resulted and Discussion

Toxicity of three compounds on *P. gossypiella* 1st instar larvae:

The susceptibility of *P. gossypiella* 1st instar larvae to three tested insecticides of profenofos, alpha-cypermethrin & flufenoxuron was presented in Table (1).

Table 1: Toxicological evaluation of profenofos, alpha-cypermethrin & flufenoxuron against newly hatched larvae of pink bollworm *P. gossypiella*

Insecticide	LC_{50}	LC_{90}	Slope
profenofos	0.036(0.02-0.06)	0.29(0.15-0.056)	1.42±0.31
alpha-cypermethrin	0.046(0.028-0.067)	0.28(0.03-0.07)	1.63±0.34
flufenoxuron	1.79(1.19-2.49)	11.60(7.28-25.47)	1.58±0.25

The LC50s values for one day old larvae were nearly similar for both profenofos and alpha-cypermethrin, with an LC50s of 0.036 and 0.046, on contrast there was more variation with LC50 value of flufenoxuron, the LC50 estimated by 1.79 ppm. This data revealed that the 1st instar larvae less susceptible to flufenoxuron than two compound profenofos and alpha-cypermethrin.

Biological activity:

Table (2) showed that the duration, weight of larvae & pupae and total immature stage, for newly hatched larvae resulted from treatments with LC50 value of profenofos, alpha-cypermethrin & flufenoxuron.

Larval and Pupal stages

Data in Table (2) showed that the LC50 latent effect of profenofos, alpha-cypermethrin & flufenoxuron on PBW larval & pupal period and weight resulted from treated newly hatched larvae compared with untreated. The three tested compounds prolonged the duration of larval stage, significantly. These periods estimated by 18.1, 19.77 and 21.33 days/larvae oppose to 14.33 days in untreated. Also, the used insecticides caused high significant increase in pupal period, by 9.43, 10.63 and 10.10 days/ pupa, respectively, compared to 8.00 days in the untreated. The total immature stage of PBW were 27.53, 30.40 and 31.41 days for profenofos, alpha-cypermethrin & flufenoxuron, respectively, compared with 22.33 days in untreated. In addition, the average larval weight decreased significantly to reach 0.023, 0.025 and 0.019 g/larva for the three insecticides, respectively, while it was 0.037 g/larva in

untreated. Also, the pupal weight resulted from treated eggs decreased significantly in all treatments than untreated.

Mortality and malformation

As shown in Table (2) and Fig. (1-9) the percentage of larval mortality estimated by 69.33, 63.67 and 62.33% mortality resulted from 1st instar larvae treated with profenofos, alpha-cypermethrin & flufenoxuron, respectively, compared with 6% in untreated, and the malformed larvae resulted from the three treatments were 7.0, 6.67 and 7.7 % respectively compared with 2% in untreated.

Also, the high malformed percentage appeared in pupae resulted from larvae treated with alpha-cypermethrin & flufenoxuron estimated by 5.67 and 5%, respectively and the lowest percentage of malformation recorded by 4.33 % treated by profenofos.

Adult stage

Data in Table (3) show that the three tested insecticides elongate significantly the pre-ovipositiion period of emerged females from treatment to reach 2.87, 3.83 and 3.60 days, respectively, compared with 2.67 in untreated. In contrast, the oviposition period of emerged females from flufenoxuron treatment increased than untreated to be 13.80 days, while no significant increase was recorded with profenofos and alpha-cypermethrin treatments compared with untreated.

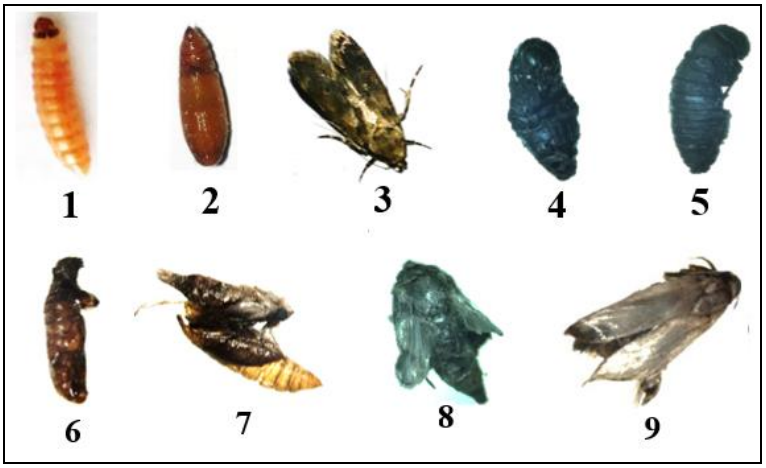


Fig (1-9): Morphological deformations of pupae and adult resulted from *P. gossypiella* treated newly hatched larvae with flufenoxuron and control. **Fig 1,2&3:** Normal larvae, pupae and adult. **Fig 4&5:** Shape intermediate between larvae and pupae resulted from newly hatched larvae treated with flufenoxuron. **Fig 6:** Pupae resulted from newly hatched larvae treated with flufenoxuron. **Fig 7, 8&9:** Adult resulted from

Also, data in Table (3) clear that flufenoxuron elongated the post-oviposition period of *P. gossypiella* significantly from 2.33 days in untreated to 5.43 days/female.

Female's longevity was highly significant affected by flufenoxuron, the adult females longevity was 25.50 days/♀ compared to 18.21 days/ ♀ in untreated. Female longevity increased in treatments mostly due to the increase in post oviposition period. Also, the male's longevity resulted from PBW flufenoxuron treatment was longer than the untreated. It was 17.77 days compared with 15.42 days/ ♂ in untreated.

Fecundity (eggs laid and hatchability)

Data presented in Table (3) shows the main numbers of laid eggs value for profenofos, alpha-cypermethrin & flufenoxuron were 174.67,

Table 2: Biology of *P. gossypiella* immature stages resulted from treated larvae with three different compounds.

Pesticides	Larval stage				Pupal stage				Total immature
	% Mortality	Malformed	Weight	Duration	%Pupation	Malformed	Weight	Duration	
profenofos	69.33	7.00	0.023^{c}	18.1^{c}	73.67	4.33	0.022^{b}	9.43^{b}	27.53^{b}
alpha-cypermethrin	63.67	6.67	0.025^{c}	19.77^{b}	77.33	5.67	0.020^{bc}	10.63^{a}	30.40^{a}
flufenoxuron	62.33	7. 7	0.029^{b}	21.33^{a}	80	5.00	0.018^{c}	10.10^{a}	31.43^{a}
Untreated	6	2	0.037^{a}	14.33^{d}	92	1.00	0.032^{a}	8.00^{c}	22.33^{c}
LSD			0.002	0.61			0.003	0.40	1.01
P			0.000***	0.000***			0.000***	0.000***	0.000***

Table 3: Effect of three different compounds on longevity and fecundity of PBW females resulted from treated larvae.

Pesticides	Oviposition period			Fecundity		Longevity	
	Pre-oviposition	oviposition	Post-oviposition	Total egg/♀	%hatchability	♀♀	♂♂
profenofos	2.87^{c}	11.20^{c}	1.97^{c}	174.67^{c}	59.33^{c}	16.73^{c}	9.97^{d}
alpha-cypermethrin	3.83^{a}	9.80^{d}	2.27^{b}	164.33^{d}	47^{d}	15.57^{d}	11.83^{c}
flufenoxuron	3.60^{b}	13.80^{a}	5.43^{a}	190^{b}	63.67^{b}	25.50^{a}	17.77^{a}
Untreated	2.67^{d}	12.33^{b}	2.33^{b}	224.33^{a}	96.67^{a}	18.21^{b}	15.42^{b}
LSD	0.18	0.43	0.36	0.04	0.83	0.49	0.35
P	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

163.33 And 190 egg/female, respectively, compared with 224.33 eggs/ ♀ in untreated. As shown in Table (3) the percentages of eggs hatchability were 59.33, 47.00 and 63.67% on profenofos, alpha-cypermethrin & flufenoxuron, respectively, compared with 96.67 % in untreated.

In this respect, author's works on pink bollworm as (Rashad *et al*. 2006 and Said *et al*. 2017) they recorded that *P. gossypiella* adults resulted from treated larvae with diflubenzuron or teflubenzuron caused high reduction in female fecundity and fertility. Also, Sammour *et al*. (2008), they found a reduction in fecundity and egg hatchability of cotton leaf worm after treated larval instar with Chlorfluazuron and Leufenuron. And, Sabry (2013) showed that lambda-cyhalothrin was the most effective pesticide against the pink bollworm larvae when compared to thiamethoxam and Buprofezin when applied in field. Also, Radwan *et al*., (2018) found that larval duration of *P. gossypiella* increased in lambda-cyhalothrin followed by chloropyrifos compared with control.

Biochemical analysis

Larvae resulted from treated larvae with LC50 of profenofos, alpha-cypermethrin & flufenoxuron, as well as in control were chemically analyzed and results were as follows:

Total soluble protein

Data in Table (4) revealed that profenofos, alpha-cypermethrin caused reduction in soluble protein of treated larvae. But there was increase in flufenoxuron treatment. The total soluble protein were 17.20, 12.33 and 27.60 mg/gbw in profenofos, alpha-cypermethrin and flufenoxuron treatments, respectively.

All treatment; profenofos, alpha-cypermethrin and flufenoxuron caused lipid reduction by -48.18, -27.38 and 56.80%. The total lipid content were 21.30, 15.20 and 12.67 mg/gbw, respectively compared to 29.33 mg/gbw in untreated. The results presented in Table (4) show that total carbohydrate decreased in PBW larvae resulted from treated by profenofos, alpha- cypermethrin and flufenoxuron by (14, 13.70 and 10 mg/gbw, respectively) compared to 17.5 mg/gbw in untreated.

Table 4: Changes in activities of some biochemical parameter in *P. gossypiella* larvae treated with three compounds.

Biochemical aspects	profenofos		alpha-cypermethrin		flufenoxuron		Untreated
	Mean±S.D.	Mean±S.D.	Mean±S.D.	% Change	Mean±S.D.	% Change	
Total protein (mg/g.b.wt)	12.33^{d}±1.25	-42.84	17.20^{c}±0.83	-20.26	27.60^{a}±1.21	27.96	21.57^{b}±1.22
Total lipid (mg/g.b.wt)	15.20^{c}±1.10	-48.18	21.30^{b}±0.80	-27.38	12.67^{d}±1.25	-56.80	29.33^{a}±0.47
Total carbohydrate (mg/g.b.wt)	14^{b}±1.63	-20	13.70^{b}±0.92	-21.71	10^{c}±0.82	-42.86	17.5^{a}±0.41
ALT	249.56^{c}±4.37	-13.81	465.67^{a}±1.47	60.82	211^{d}±1.41	-12.28	289.56^{b}±5.97
AST	117.70^{c}±1.75	-44.74	198^{b}±1.63	-7.04	113^{d}±1.63	-46.95	212.99^{a}±1.47

Means followed by the same letters are not differ significantly (ANOVA, Duncan's multiple range tests, $P < 0.05$)

Change (%) = [(Mean values of treatment- Mean values of control)/ Mean values of control] ×100 A positive value means increase in enzyme activity and the negative values means a decrease.

Data in Table (4) shows the transaminase enzymes activity on larvae of *P. gossypiella* resulted from treated with alpha-cypermethrin, profenofos and flufenoxuron. The levels of GOT was highly increased to 465.67 IU/L on alpha-cypermethrin treatment compared with the untreated which was 289.56IU/L, while the activity of GOT was decreased in profenofos and flufenoxuron than untreated. There was reduction in GPT activity of *P. gossypiella* treated with alpha-cypermethrin, profenofos and flufenoxuron compared with the untreated.

in this respect, Radwan *et al*., (2018) found that total protein content was increased in chlorpyrifos treatment than lambda-cyhalothrin than untreated larvae of pink bollworm. Kandil *et al*., (2012) reported that tested IGRS against *P. gossypiella*, reduced protein and total carbohydrate contents and have an inhibitory effect on ALT and AST. The result agree with (Kandil, *et al*. 2013 and Said *et al*. 2017) who recorded that (IGR's) caused high different in ALT and AST for *P. gossypiella* larvae than control. Also, Elgohary (2013) revealed that chlorfluazuron gave the highest reduction in AST activity, but flufenuxoron, chlorfluazuron, chlorpyrifos and beta-cyfluthrin caused significant reduction in ALT activity and total lipid.

Conclusion

In conclusion, the important of treated pink bollworm with insecticides from different groups as; organophosphorus, pyrethroids and insect growth regulator leads to reduce percentages of infestation.

Acknowledgement

To Dr. Mervat Kandil for statistical analyzed of all data.

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