

Toxicological and Biological Studies of Some Pesticidal Formulations against *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

¹Ezzat F. El- Khayat, ²Amira, M. Rashad, ¹Tahany R. Abd-El Zaher,
¹Ali M. Shams El-Din and ²Hanady S. Salim

¹Faculty of Agriculture (Moshtohor), Benha University, Egypt

²Plant Protection Research Institute Agricultural Research Center (ARC) Dokki, Giza, Egypt

Abstract: In the present study, newly hatched larvae laboratory strain of *Pectinophora gossypiella*, were treated with estimated LC₅₀ values of Methomyl, Pyridalyl, Teflubenzuron and *Verticillium lecanii* 80% at concentration of 0.543, 698.11, 61.859 and 0.2254 ppm respectively, to study their effects on duration of larval, pupal, total immature stages, longevity, fecundity and fertility of the resulting adults. The three tested compounds (Methomyl, Teflubenzuron and *V. lecanii*) prolonged the larval duration by 18.81, 25.87 and 18.41 days/larvae, respectively, oppose to 15.10 days in control. Also, the aforementioned compounds increased the pupal period and the duration of total immature stages especially in case of the treatment with Teflubenzuron the period increased 2 times than control. In addition, the tested compounds caused considerable increase in female pre-oviposition and post oviposition periods. The Methomyl and *V. lecanii* were significantly affected the oviposition period as it was shortened to 6.10 days and 10.6 days, respectively, while, in case of Pyridalyl and teflubenzuron prolonged it to 20.8 and 14.16 days, respectively, compared to 14.8 days for control. Also, the results obtained indicated reduction in the fecundity of the female and the percentage of hatchability.

Key words: *Pectinophora gossypiella* • Pink bollworm (PBW) • Methomyl • Pyridalyl • Teflubenzuron and *Verticillium lecanii* • Toxicity • LC₅₀ • Biological studies

INTRODUCTION

Cotton bollworms are the most destructive pests infesting cotton plants. The pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) is the key pest of cotton, (*Gossypium spp.*) in many cotton producing areas of the world. It causes serious damage in cotton bolls resulting in high reduction in quantity and quality of cotton yield. In Egypt, cotton control programs including different sprays with conventional insecticides are recommended by Egyptian Ministry of Agriculture to combat these pests. So, many investigators studied the effect of different insecticides in rotations against bollworms infesting cotton plants during successive seasons under different levels of infestation to determine the best sequence for pests' control [1-5]. Also, some authors, Kandil *et al.* [6, 7] and El-Shennawy [8] on *P. gossypiella* studied the effect of many pesticides on developmental periods of insect, they found that it

prolonged larval, pupal periods and the latent effect appears on the longevity, fecundity of adult stage and fertility of eggs of Lepidopteron pests. The present study was carried out to determine the toxicity of Methomyl, Pyridalyl, Teflubenzuron and *Verticillium lecanii* against *Pectinophora gossypiella* (Saunders). The study was extended to investigate the effect of these compounds on some biological aspects for immature and adult stages of PBW resulted from treated newly hatched larvae.

MATERIALS AND METHODS

Insect Used: Newly hatched larvae of pink bollworm *P. gossypiella*, used in this study was obtained from laboratory colony of Bollworm Department, Plant Protection Research Institute, Agricultural Research Center (ARC), reared for several generations away from any contamination with insecticides on an artificial diet that previously described by Rashad and Ammar [9].

Pesticides Used:

- Methomyl (Nudrin 90% SP): Chemical name (E, Z) methyl N-[(cmethylamino) carbonyl] oxy] ethanimidothioate.
- Pyridalyl (Pleo 50% EC): Chemical name: 2-[3-(2, 6 dichloro-4-[(3, 3-dichloro-2- propenyl) oxy] phenoxy] propoxy]-5- (trifluoromethyl) pyridine.
- Teflubenzuron (Nomolt 15% SC): Chemical name: N-[(3, 5-dichloro- 2, 4- difluorophenyl) amino carbonyl] - 2, 6 - difluorobenzamide.
- *Verticillium lecanii* (Varcha 16.1% w/w' fungal spores)

Type: An entomopathogenic fungus. Microbial insecticide containing fungal spores of specific strain of *Verticillium lecanii* and contains 16.1% w/w. the mycelium of this fungus produces insecticidal toxins which infect some insects and cause death to the host.

Toxicological Studies: This study was carried out to evaluate the toxic effect of Methomyl, Pyridalyl, Teflubenzuron and Varsha compounds against newly hatched larvae of the pink bollworm susceptible strain under constant conditions of $26\pm 1^\circ\text{C}$ and $75\pm 5\%$ R.H. Pilot experiment was conducted to calculate LC_{50} for each compound. Serial concentration dilutions, prepared ranged as a followed: (1.32, 0.659, 0.33296, 0.1648 and 0.0824 were freshly prepared from the stock solution of compound (1 m/l liter water) for Methomyl' (62.50, 31.25, 15.625, 7.8125 and 3.906) for Pyridalyl (37.5, 18.75, 9.375, 4.688, 2.344 and 1.171) were freshly prepared from the stock solution of compound (1 m/l liter water) for Teflubenzuron and (0.50, 0.25, 0.125, 0.0625 and 0.0313) for *V. lecanii*. Four replicates of 50 tubes/concentration were used in addition to four replicates of 50 other tubes containing untreated diet as control. Two grams of artificial diet was poured into each glass tube (2 x 7cm). The tested concentrations were spread using micropipette on the upper surface of the diet. The untreated tubes treated with distilled water only. All tubes were held uncapped for an hour to allow dryness and individual neonate larvae of PBW was placed into each tube using fine hair brush and capped by cotton wool, then incubated under controlled conditions ($26\pm 1^\circ\text{C}$ and 65-70% R.H). After 24 h percentage of mortality for each tested concentrations of Methomyl and Pyridalyl were estimated and after three days for Teflubenzuron and

V. lecanii and then LC_{50} and LC_{90} were calculated using the Proban software program according to Finney [10].

Biological Studies: Latent Effect of Different Compounds at LC_{50} on Some Biological Aspects of *P. gossypiella*: To study the latent effects of the four tested compounds on certain biological aspects of *P. gossypiella*, the LC_{50} concentrations of 0.543, 698.11, 61.859 and 0.2254 ppm of Varsha and 0.000732 ppm of Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii* were applied, respectively on newly hatched larvae of *P. gossypiella*. The LC_{50} concentration of each compound was spread using micropipette on the upper surface of the diet poured in the glass tubes. The control tubes treated with distilled water only. Four replicates of 50 tubes were used for each compound in addition to four replicates of 50 tubes containing untreated diet as control. All tubes were held uncapped for an hour to allow dryness and individual neonate larvae of PBW was placed into each tube using fine hair brush and capped by cotton wool, then incubated under the control conditions. Larval and pupal durations, weight of both full grown larvae and pupae (one-day old) and pupation percent were estimated. The emergence percent and sex ratio measured as percent of females from the total number of emerged adults were also estimated. The obtained data were statistically analyzed with one-way analysis of variance (ANOVA) ($P < 0.05$) according Snedecor and Cochran [11] and Duncan's multiple range test means was used Duncan's [12].

Adult Longevity, Fecundity and Fertility of the Pink Bollworm Moths Resulted from Treated Newly Hatched Larvae with Different Compounds: The newly emerged moths of each treatment and control were placed in glass chimney cage for mating. Three replicates were used for each compound and untreated ones. Each replicate had five pairs. The upper and lower surfaces of each cage were covered with muslin cloth held in position with rubber bands. Moths were fed on sucrose solution 10 % by providing each cage with soaked piece of cotton wool. The cages were examined daily until the death of moths. The number of eggs laid by females was counted on the upper and lower covers. Fecundity (eggs number) per female and fertility were calculated. In addition, female longevity as pre-oviposition, oviposition and post-oviposition periods and male longevity were determined. The following equation was used for calculating the percent of fecundity and percent of egg viability (control or deficient of fecundity).

$$\text{Control of fecundity or egg viability} = \frac{C-T}{C} \times 100$$

Where:

C: the estimated parameter in check

T: the same parameter in treatment

Statistical Analysis: The obtained data were statistically analyzed using F-test at 0.05 of probability according to computer program (COSTAT).

RESULTS AND DISCUSSION

Toxicity of four Compounds on Newly Hatched Larvae of *P. gossypiella*: Data presented in Table 1 show the LC₅₀ and LC₉₀ values resulted from newly hatched larvae of *P. gossypiella* treated with the Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii*. The LC₅₀ values were 0.543, 698.11, 61.859 and 0.2254 ppm, respectively. Pyridalyl and *V. lecanii* are considered the highest potent than Teflubenzuron and Methomyl. On contrast there was more variation with LC₅₀ values of Teflubenzuron and Pyridalyl, whereas Teflubenzuron had values of 61.859 ppm, but Pyridalyl had a highly variable value of 698.11 ppm. The results of our study almost are in agreement with those obtained by Swamy *et al.* [13], who reported that LC₅₀ value for indoxacarb was 0.0144 ppm for treated pink bollworm larvae in the laboratory. Eissa *et al.* [14] indicated that the LC₅₀'s values of deltamethrin and chlorpyrifos among the treated newly hatched larvae of *P. gossypiella* laboratory strain were 3.9 and 60 ppm, respectively.

Larval and Pupul Stages: Data in Table 2 illustrates the LC₅₀ latent effect of Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii* on PBW larval and pupal period and weigh resulted from treated compared with untreated eggs. The three tested compounds prolonged the duration of larval stage, significantly. These periods estimated by 18.81, 25.87 and 18.41 days/larvae treated with Methomyl, Teflubenzuron and *V. lecanii* on contrast when larvae treated with Pyridalyl this period decreased to 13.6 days/larvae oppose to 15.10 days in control. Also, the used compounds caused high significant increase in pupal period, the presented duration were 9.60, 11.37, 16.72 and 11.10 days/ pupa, respectively, compared to 8.9 days in control. The total immature stage of PBW resulted from treated eggs highly elongated to 28.41, 24.97, 42.69 and 29.51 days for Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii*, respectively, compared with 24.00 days in control (Table 2). In addition, the average weight of untreated larva was 0.0447g/larva. This average decreased insignificantly to reach 0.0275, 0.0258 and 0.0371 g/larva for Methomyl, Pyridalyl and Teflubenzuron tested, respectively, while, it highly significantly decreased to 0.0217g/larvae resulted from treated by *V. lecanii*. Also, the average weight of untreated pupae was 0.0414g. This average decreased insignificantly to reach 0.0154, 0.0230 and 0.0301 g/pupae for, Methomyl, Pyridalyl and Teflubenzuron tested, respectively, while, it highly significantly decreased to 0.0128 g/pupae resulted from treatment with *V. lecanii* compared to control 0.0414g/pupae in control. Yasir *et al.* [16] found that Lufenuron caused high significant effects on larval mortality, larval weight and larval duration of *T. castaneum*. Succeeding development of pupae and emergence of adults were seriously prohibited.

Table 1: Comparative toxicity of some pesticides against newly hatched larvae of *P. gossypiella*

Pesticides used	LC ₅₀	LC ₉₀	Toxicity index*			Confidence limits for LC ₅₀	
			LC ₅₀	LC ₉₀	Slope " S.D.	Upper	Lower
Methomyl	0.543	69.735	41.51	4.012	0.6072*0.2706	0.2019	1.463
Pyridalyl	698.11	255777.49	0.032	0.0011	0.4992*0.326	3.506	138978.42
Teflubenzuron	61.859	985.47	0.364	0.284	1.0647*0.36	22.495	170.107
<i>Verticillium lecanii</i>	0.2254	2.798	100	100	1.1701*0.456	0.0955	0.532

C₅₀ or LC₉₀ of the efficient compound

*Toxicity index, Sun [15] = ----- x 100

LC₅₀ or LC₉₀ of the other compound

Table 2: Biological aspects of *P. gossypiella* immature stages resulted from treated newly hatched larvae with different compounds under controlled conditions (26° 11C and 75% 5% RH)

Treatments	Larval stage				Pupal stage				Total immature duration	
	LC50	Mean numbers of accumulated mortality after 10- 15 days	%	Duration (days)	Weight of larvae (g)	% Pupation	% Mortality and malformed	Duration (days)		Weight (g)
	Conc. (ppm)									
Methomyl	0.5436	55.00c	5.60b	18.81"1.24b	0.0275"0.01c	75.00b	6.50d	9.60"0.60c	0.0154"0.004d	28.41"1.84b
Pyridalyl	698.11	63.30a	3.9c	13.6"0.66d	0.0258"0.001c	66.00c	9.40c	11.37"0.36 b	0.0230"0.001c	24.97"0.66b
Teflubenzuron	61.859	59.00b	7.30a	25.87"0.51a	0.0371"0.01b	63.00cd	14.38a	16.72"0.33a	0.0301"0.07b	42.69"0.84a
<i>Verticillium lecanii</i>	0.2254	63.10a	3.60c	18.41"0.426b	0.0217"0.002d	61.00d	11.30b	11.10"0.50b	0.0128"0.0020d	29.51"2.026b
Control	--	5.00d	2.40d	15.10"0.20c	0.0447"0.008a	94.00a	3.75e	8.90"0.10d	0.0414a	24.00"0.3c
LSD 0.05	--	2.234	0.631	1.212	0.0014	3.044	1.753	0.921	0.003	4.676

Table 2: continued

Treatments	Adult stage				
	% Adult emergence	% Malformation	% Sex Ratio		
			♀	♂	
Methomyl	57.0d	7.9c	41d		59a
Pyridalyl	73.6b	11a	70.3a		28.6d
Teflubenzuron	65.0c	9.5b	53c		47b
<i>Verticillium lecanii</i>	51.0e	6.8c	62b		38c
Control	94.1" 0.65	2.0d	52.46c		47.54b
LSD 0.05	1.652	1.268	1.566		1.566

Table 3: Latent effect of LC₅₀ of tested compounds on newly hatched larvae stage of *P. gossypiella* under controlled conditions (26°1EC and 75% 5% RH)

Compound	Concentration ppm	Oviposition (period day□ s "SE)*			Fecundity		Longevity	
		Pre-oviposition	Oviposition	Post-oviposition	Total eggs /♀	% egg hatchability	♀	♂
Methomyl	0.5436	3.20"0.4c	6.10"0.7d	3.30" 0.45c	69.6 " 3.5 e	50.47 d	12.77" 0.7d	11.17" 66
Pyridalyl	698.11	3.8" 0.1b	20.8 " 0.71a	3.8 " 0.12b	329.6 " 6.17a	47.4 d	28.5" 2.4 a	18.9" 0.67 a
Teflubenzuron	61.85	4.37"0.588a	14.16"0.15b	6.60" 0.166a	129.72 " 3.51 d	58.80 c	25.10" 1.65 b	19.95" 0.59a
<i>Verticillium lecanii</i>	0.2254	4.67"0.62b	10.60"0.95c	3.43" 0.25c	193.0 " 8.59c	68.66 b	18.79" 3.8c	17.00b
Control	--	2.67+0.1d	14.8 =3.05b	2.9 +0.38d	219.22 " 4.5b	94.27 a	20.34"0.55c	17.96b
LSD 0.05	--	0.495	2.581	1.64	9.86	4.17	2.653	1.853

* Means within the same column followed by the same letter are not different according to Duncan's test at 0.05 level

Adult Stage: Data in Table 3 show that all tested compounds Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii* elongate significantly the pre-oviposit period of emerged females from treated newly hatched larvae to reach 3.20, 3.8, 4.37 and 4.67days, respectively, compared with 2.67 in control. The oviposition period of emerged females from Methomyl and *V. lecanii* treatments was shortened to be 6.10 and 10.60 days, while high significant increase was recorded in treatment of Pyridalyl (20.8 days) compared to 14.8 day in the control. Also, data in Table 3 clear that the tested compounds elongated the post-oviposition period of *P. gossypiella* significantly from 2.9 days in control to 6.6 and 3.8 days/female for Teflubenzuron and Pyridalyl treatments, respectively.

Adult Longevity: Data in Table 3 show that adult females and males' longevity were shortened significantly affected by treating neonate with Methomyl of LC₅₀. These periods

were 12.77 days for females and 11.17 days, for males respectively. In contrast the longevity of females and males in case of Teflubenzuron, *V. lecanii* and Pyridalyl compounds were longer than control. These periods were 25.10, 18.79 and 28.5 days for females and 19.95, 17.00 and 18.9 days for males, respectively, compared with 20.34/? and 17.96/? days in control. Luna *et al.* [17] found that methoxyfenozide reduced the longevity of *Spodopetera exigua* males up to 17% compared with females. Hegab and Zaki [18] indicated that no effect of biover (*Beauveria bassiana*) fungi on the spiny bollworm, *Earias insulana* longevity of both sexes.

Reproductive Potential: Data in Table 3 show high significant decrease in numbers of eggs laid by females resulted from all treatments. The main numbers of laid eggs value for Methomyl, Teflubenzuron and *V. lecanii* was 69.5, 129.72 and 193 egg/female, respectively,

compared with 219 eggs/ female in control. Data presented in Table 3 showed high reduction in percentage of eggs hatchability which were 50.47, 58.8, 47.4 and 68.66% on Methomyl, Pyridalyl and Teflubenzuron and *V. lecanii*, respectively, compared with 94.27 % in control. Abdel-Aal [19] and Rashad *et al.* [20] reported that fecundity and egg- hatchability percent of treated cotton leaf worm *Spodopetra littoralis* and *P. gossypiella* adults with IGRs compounds decreased, as compared with control. Generally, according to the foregoing results it could be concluded that *V. lecanii* proves to be the effective pesticide against the developmental stages of PBW, followed by Methomyl and can be applied on cotton plants as bio control component but it needs more experiments efforts in large scale sequence to earn its advantage in certain periods.

REFERENCES

1. Nassef, M.A. and W.M. Watson, 1999. Sequential spray schedules of insecticides to control bollworms as target pests in addition to certain sapsuckers as non-target pests in cotton fields. Egypt. J. Agric. Res., 77(3): 1155-1161.
2. Hegab, M.E.A., 2002. Studies on the bollworms infesting cotton in Sharkia Governorate, Egypt. M.Sc. Thesis, Fac. Agric. Zagazig Univ.
3. Al-Shannaf, H.M., Hala M. Mead and A.H. Sabry, 2002. Toxic and Biochemical Effects of Some Bioinsecticides and Igrs on American Bollworm, *Helicoverpa armigera* (Hüb.) (Noctuidae: Lepidoptera) in Cotton Fields. J. Biofertil. Biopestici., 3: 118.
4. Amer, A.E.A., 2004. Ecological and Physiological studies on bollworms. Ph. D. Thesis. Fac. Agric., Zagazig Univ., Moshtohor, pp: 213.
5. Zaki, A.A.T., 2006. Toxicological and biological studies on bollworms. Ph.D. Thesis Fac. Agric., Benha Univ.
6. Kandil, M.A.A., T.R. Abd El-Aziz and A.M. Rashad, 2005. Some biological and biochemical effects of chitin synthesis inhibitors on pink Bollworm *Pectinophora gossypiella* (Saunders). Ann. Agric. Sci., Moshtohor, 43(4): 1991-2002.
7. Kandil, M.A.A., A.F. Ahmed and H.Z. Moustafa, 2012. Toxicological and biochemical studies of lufenuron, chlorfluzuron and chromafenozide against *Pectinophora gossypiella* (Saunders). Egypt. Acad. J. Biol. Sci., 4(1): 37- 47.
8. El-Shenawy, A.M.R., 2009. Evaluation of some pesticides against pink bollworm *P. gossypiella* (Saunders) M.Sc. Thesis, Faculty of Science, Al-Azhar University, pp: 166.
9. Rashad, A.M. and E.D. Ammar, 1985. Mass rearing of the spiny bollworm, *E. insulana* (Boisd.) on semi-artificial diet. Bull. Ent. Soc. Egypt, Eco. Ser., 65: 239-244.
10. Finney, D.J., 1971. Probit-analysis. 3rd Ed., Cambridge University Press, London.
11. Snedecor, G.W. and W.G. Cochran 1980. Statistical Methods. 7thEd. The Iowa State Univ. Press. Ames, Iowa, U.S.A., pp: 507.
12. Duncan, D.B., 1955. Multiple Range and Multiple F test. Biometrics, 11: 1-42.
13. Swamy, S.V.S.G., N.H.P. Rao and V.H. Rao, 2000. Publishing year is not available LC₅₀ values of insecticides for the control of pink bollworm *Pectinophora gossypiella* (Saund.). Plant Protection Bulletin (Faridabad), 52(3/4): 24-25.
14. Eissa, I.S., S.M. Elawady, S.M. Najeeb and A.M.M. Adly, 2005. Effect of some insecticides deltamethrin, (CPY) clorpyrophos (COP) and demsisa extract (*Ambrosia maritime*) Fam.: Compositae on certain biological aspects of alive insects of *Pectinophora gossypiella* (Saunders) and *Eaias insulana* (Boisd.). Ann. Agric. Sci., Moshtohor, 43(2): 943-953.
15. Sun, Y.P., 1950. Toxicity index. An improved method of comparing the relative toxicity of Insecticides. J. Econ. Entomol., 43: 45-53.
16. Yasir, M., M. Sagheer, M.U. Hassan, S.K. Abbas and W. Muhammad, 2012. Impairment of growth, development and reproduction in *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) due to larval exposure to lufenuron-treated diet. Abstract of 32nd Pakistan congress 158 of zoology.
17. Luna, L.C., V.A. Robinson, A.M. Martinez, M.S. Schneider, J. Figueroa, G. Smahe, E. Vinuela, F. Budia and S. Pineda, 2011. Long-term effects of methoxyfenozide on the adult reproductive processes and longevity of *Spodoptera exigua* (Lepidoptera: Noctuidae). Entomol., Society, America, 104(4): 1229-1235.

18. Hegab, M.E.A. and A.A.A. Zaki, 2012. Toxicological and biological effects of bacteria, *Bacillus thuringiensis kurstaki* on *Pectinophora gossypiella* (Saund.) and entomopathogenic fungi, *Beauveria bassiana* on *Earias insulana* (Boisd). *J. Plant Prot. and Path.*, Mansoura Univ., 3(3): 289-297.
19. Abdel-Aal, A.E., 2006. Effect of chlorofluazuron, on some biological and enzymes activity of cotton leaf worm *Spodoptera littoralis* (Boisd.). *Bull. Ent. Soc. Egypt, Econ. Ser.*, 32: 171-185.
20. Rashad, A.M., M.A.A. Hewady and M.A.A. Kandel, 2006. Effect of neemazal, spinosad and dimiln on some biological and physiological activities of pink bollworm. *Pectinophora gossypiella* (Saund.). *Annals of Agricultural Science, Moshtohor*, 44(1): 309-319.