

Biochemical Studies of Some Pesticidal Formulations against *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae)

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Abstract: Some biochemical studies were carried out on 4th instar larvae of Pink bollworm (PBW) treated as neonate larvae with LC₅₀ for four tested compounds (Methomyl, Pyridalyl, Teflubenzuron and *Verticillium lecanii*). Results obtained indicated that all treatments decreased protein levels as compared with control, it recorded - 40.47, - 50.48, -55.02 and - 60.39 %. The total soluble lipid was estimated by 8.02, 7.94, 3.93 and 6.70, respectively. The percentage of high decrease was - 48.13% when larvae treated with (IGR) Teflubenzuron. An exception in Carbohydrate hydrolyzing enzymes activities was clearly obvious in invertase; it decreased by - 21.99% in *V. lecanii* treatment than in control. Great reduction in GOT and GPT transaminases activity in *P. gossypiella* treated with all tested compounds. Low increase in acid phosphates enzyme (1.85%), while high reduction percentage in alkaline phosphates (-59.46) in *V. lecanii* treatment than control. Chitinase enzymes were increased in moderate percentages in both Teflubenzuron and *V. lecanii* (23.66 and 29.84%, respectively).

Key words: *Pectinophora gossypiella* • PBW • Methomyl • Pyridalyl • Teflubenzuron • *Verticillium lecanii* • Toxicity • LC₅₀ • Biochemical studies

INTRODUCTION

Pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gellichidae) is considered as one of the major and important economic pests of cotton. It causes serious damage to cotton bolls and consequently considerable losses in yield and reducing the quality of lint [1-4]. The control of the PBW in Egypt depends mostly on the use of various synthetic insecticides. Often, the prolonged use of these chemicals has been accompanied by increased resistance. In addition, the release of these chemicals pollutes the environment and affects non-target organisms. The appearance of such problems has been accompanied by growing interest to use new safe bio-insecticide with a new mode of action [5]. For this reason, a microbial insecticide containing fungal spores of specific strain of *Verticillium lecanii* and contains 16.1% w/w) was evaluated in comparison to 3 other traditional pesticides against newly hatched larvae of PBW. The mycelium of this fungus produces insecticidal toxins which infect some insects and cause

death of the host. In this study newly hatched larvae of *P. gossypiella* was treated with LC50's of the 4 tested compounds to assess the biochemical changes in PBW larvae after treatment. The main metabolites levels of total proteins, total lipids and some vital enzyme activities of transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and the carbohydrate hydrolyzing enzymes (trehalase, invertase) and alkaline phosphatases were determined. The authors were interested to answer the question of whether the tested compounds have an effect on PBW metabolites or not to draw complete map of pesticides efficacy.

MATERIALS AND METHODS

Insect Used: The newly hatched larvae of pink bollworm, *P. gossypiella* used in this study originated from eggs obtained from a susceptible strain established in Bollworms Department, Plant Protection Research Institute, Dokki, Giza. This strain was reared in the laboratory for several generations away from any

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contamination with insecticides under constant conditions of ($26\pm1^{\circ}\text{C}$ and $75\pm5\%$ RH) on an artificial diet that previously described by Rashad and Ammar [6].

Experimental Insecticides: Three chemical insecticides, Methomyl (Nudrin 90% SP, DuPont), Pyridalyl (Pleo 50% EC, Sumitomo Chemical Co., Ltd.) and Teflubenzuron (Nomolt 15% SC, Agrochemical) as well as Fungal spores of *Verticillium lecanii* (Varsha: An entomopathogenic fungus *V. lecanii* and contains 16.1% w/w, Bioscience Technology, India, PVT LTD) were obtained from their respective manufacturers and used in the present studies.

Toxicological Studies: Pilot experiment was conducted to calculate LC₅₀ for the tested compounds using the proban software program according to Finney [7]. Serial dilutions were freshly prepared from the stock solution of Methomyl (Nudrin), Pyridalyl, Teflubenzuron (Nomolt) and *V. lecanii* (Varcha). In addition, replicates of control. The LC50's were 0.543, 698.11, 61.859 and 0.2254 ppm for Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii*, respectively.

Biochemical Studies: For the biochemical studies, 50 newly hatched larvae of *P. gossypiella* were transferred individually to glass tubes (2 x 7 cm) containing 2.0 g. of treated diet / each compound and untreated diet and kept under the aforementioned controlled conditions. Ten days later larvae/each treatment and untreated control were collected and kept in clean tubes in a refrigerator ($7\pm1^{\circ}\text{C}$) for chemical analysis. Total protein, total lipids and

determine activity of trehalase, invertase enzymes, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) and esterase, chitin enzyme activities were determined colorimetrically according to Reitman and Frankle [8], Knight *et al.* [9], Bradford [10] and Ishaaya and Swiriski [11].

RESULTS AND DISCUSSION

Larvae treated with Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii*, as well as the untreated check (control) was chemically analyzed and the results were as follows:

Total Soluble Protein and Lipids in *P. gossypiella*

Treated Larvae: Total proteins, carbohydrates and lipids are considered essential biochemical components necessary for growth, development and reproduction. Thus the 4th larval instar of PBW treated as newly hatched larvae with LC50 of the 4 tested compounds were evaluated. The obtained data in Table 1 show the concentration of total soluble protein (TSP), total soluble lipid (TSL) and the changes as a percentage from the control in larval supernatants of the pink bollworm *P. gossypiella* treated with LC₅₀ concentrations of Methomyl, Pyridalyl Teflubenzuron and *V. lecanii*. The protein level decreased in all treated larvae in comparison to the untreated control, it recorded -40.45, -50.48, -55.02 and -60.39 % for the above mentioned pesticides, respectively. This decrease may be due to that the proteins are among the most important compounds of

Table 1: Chemical composition of *Pectinophora gossypiella* larvae resulted from treated neonate larvae with LC50 of four compounds under controlled conditions ($27\pm1^{\circ}\text{C}$ and $75\pm5\%$ R.H.).

Treatments	LC50 ppm	Total soluble protein (TSP)mg/ml		Total soluble lipid (TSL) mg/ml		Enzymes					
		Conc.	C%	Conc.	C%	SA	RA%	SA	RA%	Transaminase enzymes IU/L	
										GOT	GTP
Methomyl	0.5436	31.18 ± 1.8441	- 40.45	8.02 ± 0.5749	5.86	3189.0± 121.5072	61.96	337.33±25. 1064	47.09	58.67 ± 3.5119	77.0±2.6457
Pyridalyl	698.11	25.93 ± 1.5409	- 50.48	7.94± 0.3874	4.81	3090.67± 89.5228	56.93	497.0± 24.98	116.72	57.33 ± 5.5076	72.67±4.0415
Teflubenzuron	61.85	23.55 ± 1.3501	- 55.02	3.93± 0.2597	- 48.13	3904.0± 116.0517	98.27	95.67± 9.2916	-58.28	108.0 ± 7.5498	1033.37±7.7675
<i>Verticillium lecanii</i>	0.2254	20.74 ± 1.1226	- 60.39	6.70± 0.8056	- 11.56	1536.67± 68.4787	- 21.96	211.0± 12.7067	-79.99	75.33±7.5719	61.0±2.6457
Control	--	52.36 ± 2.7732		7.58 ± 0.5033		1969.0± 62.5540		229.33± 15.3080		121.0±7.9373	147.0±19.7

Concentration expressed as (mg/ml)

Treatment- Control

$$\text{C\%} = (\text{Change \%}) = \frac{\text{Treatment- Control}}{\text{Control}} \times 100$$

SA (Specific activity) as (μg pyruvate /ml)

Treatment- Control

$$\text{RA (Relative activity \%)} = \frac{\text{Treatment- Control}}{\text{Control}} \times 100$$

insects that bind with foreign compounds [12]. Moriya *et al.* [13] observed rapid and significant inhibition of protein synthesis when they examined the effect of cytotoxicity (LC_{50}) pyridalyl on the cellular protein synthesis in cells derived from *S. frugiperda*. In addition, Elbarky *et al.* [14] found that the total protein in the 4th instar larvae of *S. littoralis* treated with the LC_{50} of r radiant was significant decreased by -69.87% compared to control group. They concluded that this reduction may be due to inhibition of DNA and RNA synthesis. However, the decrease of the total protein may reflect the decrease in the enzymatic activities of various enzymes. Data in Table 1 revealed that the mean total lipid was estimated by 8.02, 7.94, 3.93 and 6.70 mg/ml in Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii* treatments, respectively compared to 7.58 mg/ml in control. However, the high percentage of decrease was - 48.13% when larvae treated with Teflubenzuron (IGR) followed by moderate reduction in *V. lecanii* treatment (-11.56%). The present result is in agreement Abdel-Aal [15], who found that chlorfluazuron caused significant decrease of total lipid and carbohydrates of the treated *S. littoralis* larvae. Hamadah *et al.* [16] found a predominant inhibitory in lipid content of *S. gregaria* nymphs that treated with pyriproxyfen.

Carbohydrate Hydrolyzing Enzymes: In many organisms, changes in trehalase activity are closely linked to alteration in physiological conditions or development, indicating that this enzyme plays an important role in such biological functions as homeostasis and developmental events [17]. In case of treatment of newly hatched larvae with LC_{50} of methomyl, pyridalyl and teflubenzuron the results represented in Table 1 show to the changes in the activity of carbohydrate hydrolyzing enzymes of *P. gossypiella*. The activity of Invertase enzymes highly increased reaching 3189.0, 3090.67 and 3904.0for the pesticides mentioned, respectively. On contrary, invertase activities decreased to 1536.0when larvae treated by *V. lecanii*. In addition, trehalase is known as an important enzyme found in hymolymph and fat bodies [18] in which insect degrade trehalose to glucose for internal energy supply [19]. The obtained results in Table 1 show that the significant highest activity of trehalase enzyme was noticed in pyridalyl treatment (116.72%) followed by methomyl (47.09%) above the control level. While, reaching 95.67 μ g pyruvate /ml (R= -58.28%) in Teflubenzuron and nearly low effect in *V. lecanii* 211.0 μ g pyruvate/ml (R= -79.93%). Khedr [20] recorded that the treated 4th instar larvae of *S. littoralis* with Dipel 2 x

(*B. thuringiensis*) caused significant decrease in the activity of amylase, trehalase. On the contrary, there was an increase in the activity of trehalase which ranged between (1.5-2 times) 337.33 and 497.0 in Methomyl and Pyridalyl, respectively, compared to 229.33 in control. These findings supported our results.

Transaminase Enzymes (GOT and GPT or ALT and AST): Data in Table 1 shows the transaminase enzymes activity on the 4th larval instar of *P. gossypiella* resulted from newly hatched larvae treated with Methomyl, Pyridalyl, Teflubenzuron and *V. lecanii*. The levels of GOT and GPT were highly decreased to 58.67, 57.33, 108.0 and 75.33 IU/L for AST, respectively compared with (121.0 IU/L) in the control. Also, the Alanine aminotransferase (ALT) were 77.0, 72.67, 105.33 and 61.0 IU/L compared with (147.0 IU/L) the control.. Data indicated that, Methomyl, Pyridalyl and *V. lecanii* induced significant inhibition effect on the total ALT and AST activity on the full grown larvae of PBW treated as newly hatched larvae. This may be due to the imposition of stress on larvae during treatment. Teflubenzuron was the least effective. Katumuma *et al.* [21] reported that the GPT enzyme acts as a catalytic factor in the metabolism of carbohydrate. Assar *et al.* [22, 23] found that Match induced inhibitory effect on the house fly, *Musca domestica* at 1000 ppm and Consult had no effect on the total activity of AST. With respect to the total ALT activity, match and consult elicited inhibitory effect on the total ALT activity. Similarly, Assar *et al.* [23] recorded that the tested IGR elicited inhibitory effect on alanine amino transferase (ALT) and aspartate aminotransferase (AST) at 0.1 ppm, while induced significant stimulatory effect at 1 ppm in *Culex pipiens*.

Effect of Teflubenzuron and *V. lecanii* on Acid and Alkaline Phosphatase, Esterases and Chitinase Enzymes Activities in *P. gossypiella*:

Acid and Alkaline Phosphatase Activities: As shown in Table 2, after ten days from treatment of newly hatched larvae with Teflubenzuron and *V. lecanii* acid phosphates enzyme activity fluctuated up and down than control in negligible values (+1.85 and -1.85, respectively). But an increase of alkaline phosphatase enzymes activity was observed in case of Teflubenzuron. The percentage of increasing was 13.56% (5534.67±95.12 μ g pyruvate/ml) than control (4873.66±62.47). On the contrary, the treatment of *V. lecanii* produced a significant decrease in the alkaline phosphatase enzymes activity, it was by-

Table 2: Changes in activities of acid, alkaline phosphatase, esterases and chitinase enzymes in *P. gossypiella* larvae treated with Teflubenzuron and *Verticillium lecanii*

<i>Verticillium lecanii</i>					Teflubenzuron			
Activity	Acid phosphatase	Alkaline phosphatase	Esterases	Chitinase enzymes	Acid phosphatase	Alkaline phosphatase	Esterases	Chitinase enzymes
SA	311.66±2.84	1975.67±60.3020	843.33±29.5969	1183.67±38.2134	300.33±6.3	5534.67±164.7584	5534.67±164.7584	1127.33±29.7377
RA%	1.85	-59.46	-10.32	29.84	-1.85	13.56	13.56	23.66
Control	306±3.214	4873.67±62.47	940.00±31.0	911.67±41.5010	306.0±3.214	4873.67±108.2143	4873.67±108.2143	911.67±41.5010

SA (Specific activity) as (μ g pyruvate /ml)

$$\text{RA (Relative activity \%)} = \frac{\text{Treatment- Control}}{\text{Control}} \times 100$$

59.46% lower than in control. This result is in conformity with the findings of Dahi *et al.* [24], who reported that the activity of alkaline phosphatase enzymes significantly decreased in *S. littoralis* 4th instar larvae after 24 hours of treatment at the LC₅₀ of radiant.

Esterases and Chitinase Enzymes in *P. gossypiella*: Data in Table 2 show the esterase and chitinase enzymes activity on full grown larvae of *P. gossypiella* resulted from treated newly hatched larvae treated with Teflubenzuron and *V. lecanii*. The levels of esterases in Teflubenzuron treatment chitinase was increased to 1094.33 (16.42%) while it decreased to reach 843.33 IU/L (-10.28%) in *V. lecanii* compared to (940.00 ±) in the control. The chitinase enzymes activity increased to 1127.33 U/L (23.66%) and to 1183.77 IU/L (29.84%) in Teflubenzuron and *V. lecanii* treatments, respectively, compared to (911.67.00 and 911.66 IU/L) in the control. Similar results were reported by Abdel-Aal [15], who found that treatment of *Spodoptera littoralis* larvae with either chlorfluazuron or two biocides caused significant increase in chitinase activity compared to control. Generally, according to the results of the biological and the biochemical studies directed in sequence using bio-insecticide *V. lecanii* proves to be effective to developmental stages of PBW where it led to significant reduction in soluble protein, lipids with disturbances in carbohydrate hydrolyzing enzymes activities (Invertase and trehalase) and can be a possible candidate to be applied on cotton plants against PBW by the Ministry of Agriculture after successful field experiments.

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