EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES

TOXICOLOGY & PEST CONTROL

F

ISSN
2090-0791

WWW.EAJBS.EG.NET

Vol. 13 No. 1 (2021)

www.eajbs.eg.net

Citation :*Egypt. Acad. J. Biolog. Sci.* (F.Toxicology &Pest control) *Vol.13(1)pp25-31(2021)*
DOI: 10.21608/EAJBSF.2021.138341

Egypt. Acad. J. Biolog. Sci., 13(1):25- 31(2021)

Egyptian Academic Journal of Biological Sciences
F. Toxicology & Pest Control
ISSN: 2090 - 0791
http://eajbsf.journals.ekb.eg/

Monitoring of Pesticides Resistance in *Spodoptera littoralis* from Some Governorates of Egypt: A Pilot Study Dependent on Biochemical Responses

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ARTICLEINFO
Article History
Received: 20/11/2020
Accepted: 19/1/2021

***Keywords*:**
Bioinsecticides; IGR's; *Spodoptera littoralis*; cotton fields; Egypt

ABSTRACT

Resistance ratio (RR) and biochemical alterations for seven bioinsecticides against *Spodoptera littoralis* in two Egyptian governorates were assayed. The values of LC_{50} have fluctuated from 42.9 to 0.01 ppm for the strain of Menofia governorate, but in Gharbia governorate the values have fluctuated from 0.004 to 0.1 ppm. On the other hand, RR for the examined insecticides: Radeiant®, Agrine®, DipelDF®, Dipel 2X®, Brotecto®, Radecal® and Biotect® during 2019 cotton season. Resistance ratio (RR) for seven insect growth regulators (IGR's) Demelen®, Tobron®, Demeron®, Kabres®, Kalegron® and Match® against *S. littoralis* were also assayed. LC_{50} values in Menofia governorate have fluctuated from 0.014 to 2.9 ppm, and RR values were very low. Finally these compounds still effective against *S. littoralis* during 2019 season. Alterations were induced in enzymes: acetylcholinesterase (AChE), glutathione-*S*- transferase (GST), acid and alkaline phosphatase as well as total protein, to field strains, with respect to laboratory strains. From all findings, the coupling of LC_{50}, RR values and-related enzymes may recognize a reliable tool to assess *S. littoralis* resistance. Moreover, bioinsecticides and IGR.s were very effective to control leafworm in cotton fields.

INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a polyphagous insect pest. It is considered to be a major pest of great economic importance in many countries since it attacks several host plants (~ 40 families). The damage mainly plants injuries are mainly due to feeding on the foliar pants by young larvae (Brown and Dewhurst, 1975). Due to the repeated application of conventional pesticides over seasons, a resistance phenomenon was developed against their actions. In Egypt, synthetic pesticides are still used to control such insects as a principle method (Hafez *et.al*.,2018). In a study by Mondall and Parween (2001), a resistance monitoring program of cotton leafworm against certain bioinsecicides during ton seasons at 8 governorates was done. This action provided the ideal to use alternative control measures. It was documented that, bio-insecticides and insect growth regulators (IGR's) have potential effects against Lepidopterous pests (Farag, 2001; Abdel-Aal, 2003; Seth *et al.*, 2004).

Coupling use of biochemical quantifications and bioassay methods provides a reliable tool to detect pest's resistance against pesticides action in field populations,

especially early stages of development. In fact, biochemical quantification provides activities of specific metabolic enzymes related to insect-resistance and changes may be made up at the target site (Roditakis *et al*., 2009). The study was designed to assess resistance monitoring of some bioinsecticides and IGR's in cotton 2019 season for *S. littoralis* in two governorates and evaluated some biochemical alterations attributing to resistance action.

MATERIALS AND METHODS

Field Strains:

Two field strains of the cotton leafworm were collected from the cotton field in several locations (Gharbia and Menofia) during 2019. After collection, the egg-masses were kept separately in a jar (400 ml), covered with muslin held in position by rubber band until the eggs hatched. The jars were provided with castor oil leaves for larval feeding and to provide the required humidity for hatching. Both field and laboratory strain were reared at 25 ± 2 °C and 70 ± 5 % relative humidity. The larvae were then used for bioassay study.

Insecticides Used:

Bio-insecticides and insect growth regulators (IGR's) were used in the study have potentiality against Lepidopterous insects. They were obtained from pesticide companies as listed in Table (1).

Table 1: Commercial formulations of the examined insecticides.

No	Common name	Formulation	Trade name/concentration	Souses
	Bio-insecticides			
1	***Bacillus thurigiensis***	**WP**	**Dipel2X® 6.5%**	**Watania**
2	***Bacillus thurigiensis***	**WP**	**Agreen® 6.5%**	**Ageri**
3	***Bacillus thurigiensis***	**WP**	**Brotecto® 9.4%**	**Kafr El-Zayat**
4	***Bacillus thurigiensis***	**WG**	**Dipel DF® 6.5%**	**My Trade**
5	***Bacillus thurigiensis***	**WP**	**Biotect® 9.4%**	**Kafr El-Zayat**
6	**Emamectin benzoate**	**EC**	**Radical® 0.5%**	**El-asraa**
7	**Spinetoram**	**EC**	**Radent® 12%**	**Shoura**
	Insect Growth Regulators (IGR's)			
1	**Chlorfluazuron**	**EC**	**Kabres® 5%**	**Help**
2	**Chlorfluazuron**	**EC**	**Toberon® 5%**	**Watania**
3	**Flufenoxuron**	**DC**	**Kalegron® 10%**	**Watania**
4	**Hexaflumuron**	**EC**	**Demeron® 10%**	**Syngentia**
5	**Lufenuron**	**EC**	**Match® 5%**	**Syngentia**
6	**Diflbenzuron**	**SC**	**Demelen® 48%**	**Dow**
7	**Demefron**	**WP**	**Demefron® 25 %**	**Watania**

Bioassay Tests:

Six aqueous concentrations for each formulation were prepared. Fresh castor–bean *Ricinus communis* leaves were dipped for 15 sec in each concentration, then left for one hr to dry. Then, the 4th instar larvae of each strain were fed on treated leaves and kept in plastic jars for 24 hr covered with muslin, and then the treated leaves were removed and provided with fresh untreated leaves in clean jars for another three days, respectively. A susceptible strain of *S. littoralis* was obtained from Central Agricultural Pesticides Laboratory, Dokki, Egypt, where it has been reared on fresh castor bean *R. communis* leaves for several generations without exposure to any insecticide. It served as a baseline reference strain for

comparisons between the studied strains. Three replicates of two larvae were tested for each concentration. Mortality percentages were recorded for 5 days from larvae transfer onto untreated leaves and corrected according to Abbot, (1925). To estimate LC_{50} values, the corrected mortality percentages were subjected to probit analysis according to the method of Finney (1971). The level of resistance in the field strains was calculated as follows:

RR = LC_{50} of field strain/LC_{50} of the susceptible strain

Biochemical quantifications:

1. AChE.

The enzymatic activity was determined according to Ellman *et al*. **(1961)** using acetylthiocholine iodide (ASChI) as a substrate. In a 5 ml test tube, 2.9 ml of 0.1M phosphate buffer pH 8.0, 0.1 ml of 0.075M 5, 5'-dithiobis 2-nitrobenzoic acid (DTNB) were added. Then, 20 µl of the prepared tissue supernatant was added. To the above mixture, the same volume of ASChI (0.075 M) was added. The optical density of the developed yellow colour was recorded after 10 min against the blank, which contained the entire reagent, except the substrate at 412 nm. The activity was calculated as µM of substrate hydrolysed per mg protein per min.

2. GST.

The activity was determined by the spectrophotometric method of Habig and Jakoby (1981) by using 1-Chloro, 2-4 dinitrobenzene (CDNB). Enzyme source was mixed with 500 µl of potassium phosphate buffer (50mM; pH 6.5). Incubation was done at 25 °C for 5 min, followed by adding 100 µl of 0.2 M CDNB and 150 µl of 10 mM reduced glutathione (GSH). After 1 min, the change of absorbance was recorded every 30 sec for 6 min at 340 nm. The enzyme activity was expressed as nM mg^{-1} min^{-1}. The unit will reduce 1.0 µM of oxidized glutathione per min at pH 7.6 at 25℃.

3. Acid Phosphatase (ACP).

The enzyme activity was measured according to the method of Kind and King (1954) by using specific kits (Bio Diagnostic Co., Germany). The absorbance of the sample and standard against the reagent blank was recorded at 510 nm. The activity was expressed as U/L.

4. Alkaline Phosphatase (ALP).

The enzyme activity was measured by using phenyl phosphate as a substrate (N.S. Bio-Tec., kits, UK). So, the complex colour of *P*-nitrophenyl phosphate was measured at 405 nm against the blank. The activity was expressed as U/L (Bowers and McComb, 1966).

5. Protein Content.

Protein level was determined according to the method of Lowry *et al.* (1951). The intensity of the developed blue colour was measured at 750 nm against the blank. Bovine serum albumin (BSA) was used as a standard.

Statistical Analysis:

Data are presented as mean ±SE. Significant differences between the mean values of the laboratory strain and the two field populations were calculated by one-way analysis of variance (ANOVA) at $P \geq 0.05$.

RESULTS AND DISCUSSION

Data in Table (2) show LC_{50}, slope and resistance ratio (RR) for seven bioinscecticides against *S. littoralis*. LC_{50} value has fluctuated from 42.9 to 0.01 ppm for Menofia governorate, but in Gharbia governorate the value fluctuated from 1004 to 0.01 ppm. On the other hand, RR value was very low the value did not appear any resistance in the tow strains for bio-insecticides: Radeiant®, Agrien®, DipelDF®, Dipel 2X®, Brotecto®, Radecal®, and Biotect®, respectively.

Table 2: LC_{50}, slope and resistance ratio (RR) values of certain bioinsecticides from two governorates against *S. littoralis* during 2019 cotton season**.**

Bioinsecticides	Susceptible		Menofia			Gharbia		
	LC_{50}	slope	LC_{50}	Slope	RR	LC_{50}	Slope	RR
Radeiant®	2.83	1.91	0.061	0.55	0.02	0.36	0.17	0.12
Agrien®	54.51	0.989	22.93	0.68	1.12	197.3	0.16	3.61
Daipel-2X®	118.47	0.97	11.23	0.43	066	336.4	0.25	2.84
Dipel-DF®	305.71	0.882	42.98	1.02	2.35	1004.1	0.18	3.28
Brotecto®	196.60	0.915	3.33	0.42	0.12	164.7	0.14	0.83
Radical®	0.0124	1.91	0.011	0.63	0.91	0.16	0.18	12.9
Biotect®	14.99	0.989	3.16	0.84	0.21	177.4	0.25	0.12

Data in Table (3) show LC_{50}, slope and RR values for seven IGR's Demelen®, Tobron®, Demeron®, Kabres®, Kalegron® and Match® against *S. littoralis* in the two governorates. LC_{50} value in Menofia Governorate fluctuated from 0.28 to 6.73 ppm for all tested IGR's. In Gharbia governorate LC_{50} values have fluctuated from 0.014 to 2.9 ppm. On the other hand, RR values in both governorates were very low. Finally, these compounds still effective in *S. ilttoralis* during this season.

In this study, RR values were as follows: Agrien® (1.12 and 3.61) Dipel2X® (0.66 and 2.84), DipelDF® (235 and 3.28), Brotecto® (0.12 and 0.83), Radical® (0.63 and 12.9) and Biotect® (0.21 and 0.12), respectively, for Menofia and Gharbia samples. This finding is in accordance with that obtained by El-Hadek *et. al*. (2020), where RR and efficacy values of some bioinsecticides on-field strain of *S. littoralis* in governorates: Sharkia, Dakahlia, Behera, Kafr-El shek, Fayoum and Beni-swif were fluctuated from very low to low resistance. Another investigation in the present work illustrates RR and efficacy values of IGR's used (Table 3). The data of RR were as follows: Demelen® (2.39 and 0.16), Tobron® (0.29 and 0.18), Demeron® (0.26 and 0.01), Kabres® (0.63 and 0.25), Kalegron® (0.50 and 0.06), Match® (0.02 and 0.99) and Demefron® (1.30 and 0.99), respectively, for Menofia and Gharbia. In a similar finding by El-Hadek *et. al*. (2020) monitoring of resistance in the above governorates showed no resistance for all IGR's in *S. littoralis*.

Table 3: LC_{50}, slope and resistance ratio of certain IGR's from two governorates against *S. littoralis* during 2019 cotton season

IGR's	Susceptible		Menofia			Gharbia		
	LC_{50}	slope	LC_{50}	slope	RR	LC_{50}	slope	RR
Demelen®	17.3	0.77	2.78	1.28	2.39	2.9	0.19	0.16
Tobron®	0.80	1.11	0.28	1.49	0.29	0.15	0.23	0.18
Demeron®	1.95	0.82	0.52	1.15	0.26	0.02	0.14	0.01
Kabres®	5.14	1.04	0.82	1.16	0.63	1.31	0.21	0.25
Kalegron®	7.14	0.64	1.25	1.51	0.5	0.014	0.16	0.061
Match®	2.50	0.28	6.73	0.79	0.02	0.083	0.21	0.99
Demefron®	1.74	0.69	1.68	0.91	1.30	1.73	0.14	0.99

Biochemical Studies.

Data in Table (4) show activities of some enzymes samples of Menofia, Gharbia and susceptible strain of *S. littoralis* during 2019 cotton season.

As shown in Table (4), total protein content in both Menofia and Gharbia collected from cotton fields was significantly reduced as compared with the laboratory strain. Results

showed that the lowest value of protein content was formed in samples of Menofia 159 mg/g tissue.

Regarding AChE activity, data showed that field strains exhibited much higher levels of AChE activity than in the laboratory strain, representing 200 µM/mg/min for Gharbia, followed by 100 µM/mg/min for Menofia compared with laboratory strain (2µM/mg/min). Regarding GST, the laboratory strain exhibited increased activity 3 and 15 times greater than that in Menofia and Gharbia. Data also in Table (4) indicated that there is slightly increased activity in ACP in the laboratory strain (17.6 U/L) compared to Menofia (16.6 U/L). In contrast, Gharbia had slightly elevated levels of ACP (20.0 U/L) related to the laboratory one. A similar trend was observed in case of ALP activity, where the lowest activity was recorded for the larvae collected from Gharbia governorates (93.0 U/L) compared to susceptible strain (99 U/L). Slight activity increase was obtained in Menofia strain (105.0 U/L).

Table 4: Determination of enzyme activity and total protein in 3 populations of *S. littoralis* field strains.

Insect population	Total protein content (mg/g tissue)		AChE (µM/mg/min)		GST (mM/mg/min)		ACP (U/L)		ALP (U/L)	
	Mean	% change	Mean	% change	Mean	% change	Mean	% change	Mean	% change
Menofia	159.32 ±18.51	- 8.9	100 ±0.001	3.33	0.0149 +0.003	-66.3	16.59 ±0.119	5.4	104.97 ±1.67	574
Gharbia	168.75 ±8.98	-8.2	200 ±0.006	1.026	0.0030 ±0.001	-93	19.86 ±0.319	13.79	93.05 ±2.01	-619
Lab strain (Control)	191.67 ±1.81	-----	2.00 ±0.001	-------	0.0443 ±0.03	------	17.55 ±0.213	-----	99.23 ±1.00	------

Each value represents the mean of 3 replicates±SE.

Results on biochemical parameters suggested that much higher elevated activity of AChE measured in the two field populations could be involved in resistance occurrence in larvae collected from the two governorates. These findings obtained no significant difference in GST, ACP and ALP activities between the two populations as compared to the susceptible strain, indicating that these detoxifying enzymes may not be responsible for developing resistance in both field populations. Induced by bioinsecticide and IGR's used. These findings agree with Ibrahim, A. and Ali, (2016) who evaluated the resistance toward emamectin benzoate and the cross-resistance toward three insecticides from different chemical groups encouraged studying the biochemical mechanism of resistance. Megahed *et al.* (2013) insecticidal activities of three bioinsecticides (emamectin benzoate (Proclaim®), abamectin, Romacten® and Tracer® were evaluated on the 4th larval instar of the cotton leafworm, *Spodoptera littoralis* by leaf dipping technique as well as determining the biochemical changes in treated insects. These bioinsecticides showed immediate effects with 24 hr-LC_{50} values of 0.17, 0.23 and 38 ppm for emamectin benzoate (Proclaim®), abamectin (Romacten®) and spinosad (Tracer®), respectively. Marked biochemical changes were recognized in treated insects such as reduction of ALP and AChE activities, total protein, total lipids and glucose contents. On other hand, there were significant increases of AST and ALT activities. Finally, we can conclude this study that biocides and IGR's were very

effective in the Control of *S. littoralis* and they recorded Lack and low levels of resistance after 2019 cotton season. Therefore, in order to maximize the negative effects of the chemicals on the environment and natural enemies in the management of pests, natural insecticides could be Integrated into IPM programs.

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