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Larvicidal activity assessment for anticholinesterase insecticides against laboratory and field strains of Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

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Abstract
Anticholinesterase insecticides are widely applied in crop protection programs for the chemical control of Egyptian cotton leafworm (*Spodoptera littoralis*). Therefore, the continuous evaluation of the insecticidal activity against sensitive (laboratory) and resistance (field) strain is of great importance to reconsider their applicability. Herein, the larvicidal activity of carbamate esters (methomyl) and organophosphorus (chlorpyrifos) was evaluated *via* feeding bioassay technique under laboratory conditions against second instar larvae of the Egyptian cotton leafworm (*Spodoptera littoralis*). The results revealed that the laboratory strain's larvicidal activity was higher than the field strain for both methomyl (LC_{50} = 413.0 ppm, LC_{50} = 581.49 ppm) and chlorpyrifos (LC_{50} = 219.73 ppm, LC_{50} = 275.94 ppm) insecticides. Moreover, the resistance of chlorpyrifos (RR = 1:1.26) was lower than that of methomyl (RR = 1:1.41) in laboratory and field strains, respectively.

Keywords: *Spodoptera littoralis*, insecticide resistance, anticholinesterase insecticides, methomyl, chlorpyrifos

1. Introduction

Egyptian cotton leafworm, *Spodoptera littoralis* is extremely polyphagous pest with numerous hosts causing economically important losses. In Egypt, Egyptian cotton leafworm is considered one among the main pests attacking the foremost important crops. Chemical control strategy for the Egyptian cotton leafworm through the utilization of synthetic insecticides represents the most effective tool. However, the high rate of application raises the resistance to most of the presently used insecticides [1-3].

Anticholinesterase insecticides are one among the foremost widely applied agrochemicals in crop protection programs despite their harmful effects on the environment and therefore the human health. Anticholinesterase insecticides inhibit acetylcholinesterase (AChE) within the *Systema nervosum* of the treated insects. The subsequent increase within the concentration of acetylcholine (ACh) in cholinergic neural connections causes hyperstimulation of various cholinergic receptors and exhaustion. Carbamate esters and organophosphorus (OP) are the two main classes of anticholinesterases insecticides utilized in the protection programs from Egyptian cotton leafworm Richardson [4-6]. Therefore, the larvicidal activity of carbamate esters (Methomyl) and organophosphorus (chlorpyrifos) insecticides was evaluated against laboratory and field strains of the Egyptian cotton leafworm, *Spodoptera littoralis* to estimate their susceptibility.

2. Materials and Methods

2.1 Laboratory strain

A Sensitive (laboratory) strain of Egyptian cotton leafworm was cultivated under optimum conditions (Temperature 25±2 °C, Relative humidity 65±5%, and photoperiod 8 h: 16 h, light: darkness. The leaves of castor bean plant (*Ricinus communis* L.) were used for the cultivation during larvae stage till pupation and emergence. Emerged adults were supplied by a small piece of cotton saturated with a 10% sugar solution for feeding and as well by leaves of Tafla (*Nerium oleander*) for egg-laying [7].

2.2 Field strain
One field strain of all the eggs masses was collected from different cotton fields in Sohag Governorate, Egypt, and reared for one generation under the same laboratory conditions of laboratory strain [7].

2.3 Insecticides
Insecticides used in the larvicidal activity assessment used as received from Sigma-Aldrich Ltd., Germany (Table.1).

2.4 Bioassay
The Larvicidal activity of methomyl and chlorpyrifos was evaluated *via* feeding bioassay technique under the recommended conditions [8]. Briefly, different concentrations of methomyl and chlorpyrifos were prepared (50, 100, 200, 400, and 600 ppm). Then, equivalent sizes of castor-bean plant leaves were washed and dipped into the solutions. Triton X_{100} (0.1%) surfactant was added to the solutions to increase the adhesion of insecticides with the leaf [9]. The treated and untreated (control) leaves were introduced into a drying container containing equal numbers (20 larva/replica) of new molting 2nd instar larvae of *Spodoptera littoralis*. The containers were maintained and covered with a muslin cloth to allow aeration. To control the bioassay experiment, each treatment has three replicas.

2.5 Statistical analysis
The acute mortality of treated insets was estimated after one day of treatment (Equation1). The natural mortality was corrected by utilizing *Abbott's* formula (Equation 2), where C and T are the numbers of live larvae after treatment in control and treated groups [10]. The sub-lethal (LC_{25}) and lethal (LC_{50}) concentrations were calculated by using the probit analysis program Finney [11].

$$\text{Mortality percentage} = \frac{\text{No. of dead larvae}}{\text{No. of larvae inserted}} \times 100 \quad (1)$$

$$\text{Corrected mortality percentage} = \left[\frac{C-T}{C}\right] \times 100 \quad (2)$$

3. Results and Discussion
3.1 Methomyl larvicidal activity
The larvicidal activity of methomyl insecticide was investigated against 2nd instar larvae of laboratory and field strains of Egyptian cotton leafworm (*Spodoptera littoralis*) after one day of treatment. The mortality rates showed a positive correlation with methomyl insecticide concentrations (Fig.1a). The sub-lethal (LC_{25}) and lethal (LC_{50}) concentrations were calculated as recommended for pesticide formulations using the probit analysis program (Fig.1b) [11]. Interestingly, the laboratory strain exhibited higher larvicidal activity with LC_{25} = 139.06 ppm, and LC_{50} = 413.0 ppm while, filed strain showed a lower activity with LC_{25} = 195.14 ppm, and LC_{50} = 581.49 ppm with resistance ratio of 1:1.41 for lab and field strains, respectively (Table 2) [5, 6].

3.2 Chlorpyrifos larvicidal activity
The larvicidal activity of chlorpyrifos insecticide was investigated on 2nd instar larvae of laboratory and field strains of Egyptian cotton leafworm (*Spodoptera littoralis*) after one day of treatment. The mortality rates showed a positive correlation with chlorpyrifos insecticide concentrations (Fig.2a). The sub-lethal (LC_{25}) and lethal (LC_{50}) concentrations were calculated as recommended for pesticide formulations using the probit analysis program (Fig.2b) [11]. Interestingly, the laboratory strain exhibited higher larvicidal activity with LC_{25} = 81.35 ppm, and LC_{50} = 219.73 ppm while, filed strain showed a lower activity with LC_{25} = 102.06 ppm, and LC_{50} = 275.94 ppm with resistance ratio of 1:1.26 for lab and field strains, respectively (Table 3) [5, 6].

Table 1: IUPAC name, chemical group, and chemical formula of methomyl, and chlorpyrifos insecticides.

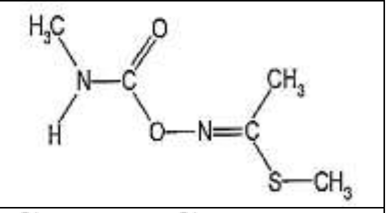
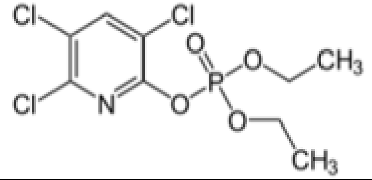
Insecticide	IUPAC name	Chemical group	Chemical formula
Methomyl	Methyl -N-(methylcarbamoyloxy) ethanimidothioate	Carbamate esters	
Chlorpyrifos	Diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl)oxy-lambda-5-phosphane	Organophosphate	

Table 2: Toxicity data of 2nd instar larvae of laboratory and field strains of Egyptian cotton leafworm, *Spodoptera littoralis* exposed to different concentrations of methomyl insecticides.

Insect	(Confidence Limit at 95%)		Slope	X^2	Index	RR[a]
	LC_{25} (ppm)	LC_{50} (ppm)				
Lab Strain	139.06 (121.57-180.19)	413.00 (339.-561.38)	1.52	5.19	100	1.0
Field Strain	195.14 (180.14-269.76)	581.49 (482.28-880.81)	1.52	0.77	71.4	1.41

[a]Resistance Ratio

Table 3: Toxicity data of 2nd instar larvae of laboratory and field strains of Egyptian cotton leafworm, *Spodoptera littoralis* exposed to different concentrations of chlorpyrifos insecticides.

Insect	(Confidence Limit at 95%)		Slope	X^2	Index	RR[a]
	LC_{25} (ppm)	LC_{50} (ppm)				
Lab Strain	81.35 (72.48 - 119.51)	219.73 (206.13 - 297.12)	1.62	7.63	100	1.0
Field Strain	102.06 (96.95-149.14)	275.94 (270.63-397.30)	1.62	0.204	79.86	1.26

[a]Resistance Ratio

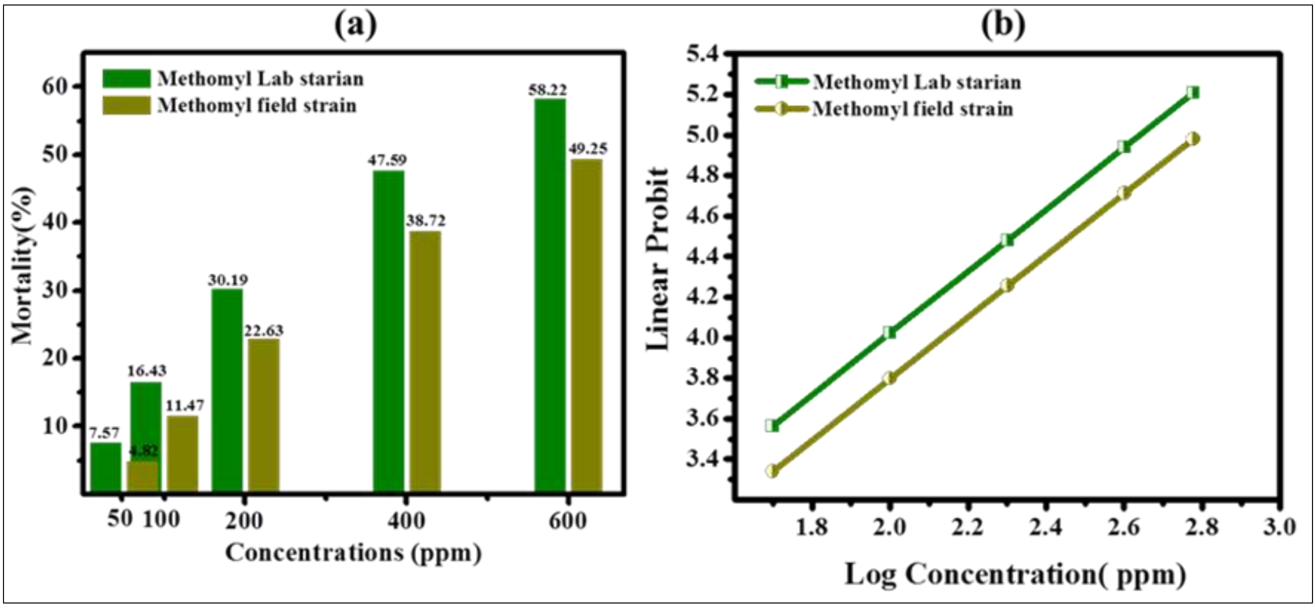


Fig 1: Mortality for 2nd instar larvae of laboratory and field strains of Egyptian cotton leafworm, *Spodoptera littoralis* exposed to different concentrations of methomyl insecticide (a), and their corresponding toxicity lines (b).

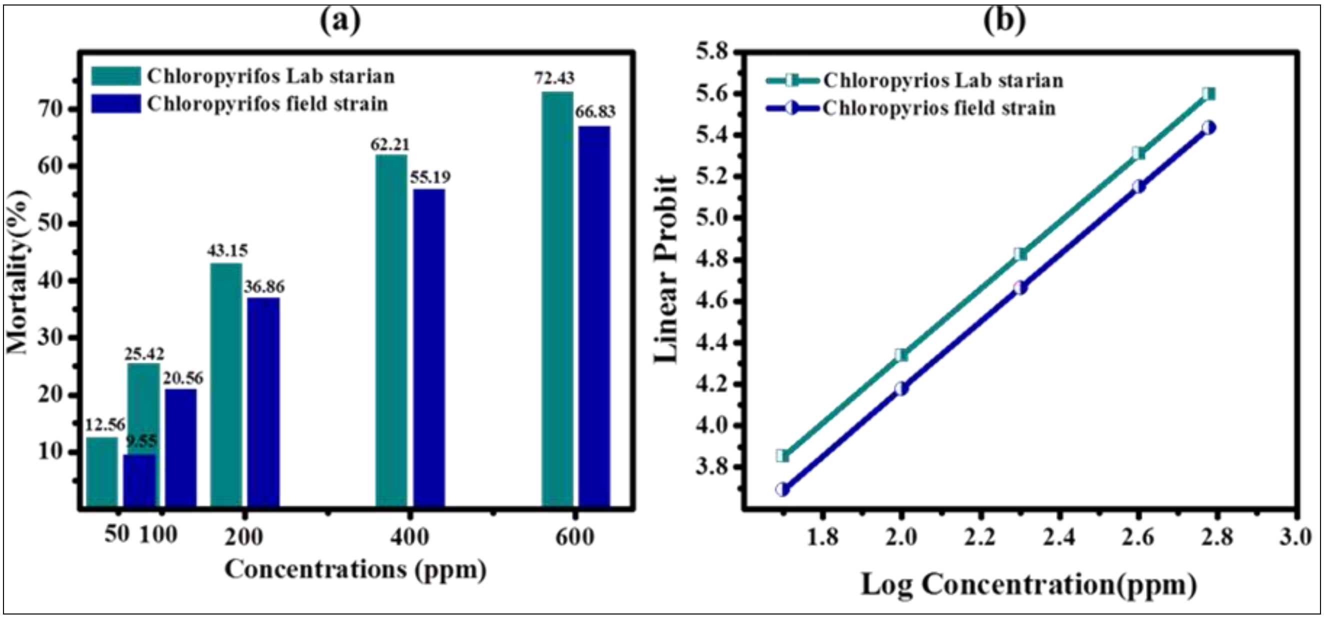


Fig 2: Mortality for 2nd instar larvae of laboratory and field strains of Egyptian cotton leafworm, *Spodoptera littoralis* exposed to different concentrations of chlorpyrifos insecticide (a), and their corresponding toxicity lines (b).

4. Conclusion

Anticholinesterase insecticides are widely applied in crop protection programs despite their harmful effects. Therefore, the continuous evaluation for their insecticidal activity against sensitive (Laboratory) and resistance (field) strain is very important. Thus, the larvicidal activity of methomyl and chlorpyrifos insecticides was investigated against 2nd instar larvae of laboratory and field strains of Egyptian cotton leafworm, *Spodoptera littoralis.* The results revealed that the larvicidal activity for laboratory strain was higher than the field strain for both methomyl and chlorpyrifos insecticides, and the activity for organophosphorus (chlorpyrifos) higher than carbamate esters (methomyl) for laboratory and field strains.

5. References

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