Potential Evaluation of Certain Conventional Pesticides on Fourth Instar Larvae of Cotton Leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) under Laboratory Conditions

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ABSTRACT

Background: The Cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered as serious pest in Egypt which causes remarkable losses of cotton yields. Objective: to evaluate the toxicity of certain pesticides related to 4 pesticide groups on fourth instar larva of *Spodoptera littoralis* after 24-h exposure under laboratory conditions. Results: Chlorpyrifos is the most potent pesticide among the selected compounds (LC$_{50}$ = 4.7 µg/ml) followed by methomyl (LC$_{50}$ = 121.3 µg/ml), pyrethrins (LC$_{50}$ = 1381.8 µg/ml), and the least toxic one was spinosad (LC$_{50}$ = 1530.4 µg/ml). However, according to the toxicity index for LC$_{50}$ values, The values were 100, 3.87, 0.34, and 0.31 for chlorpyrifos, methomyl, spinosad, and pyrethrins, respectively, whereas for the LC$_{90}$ values were 100, 7.90, 0.77, and 0.89 for chlorpyrifos, methomyl, spinosad, and pyrethrins, respectively. Conclusion: Synthetic chlorpyrifos is most toxic pesticide. However, field evaluation should be carried out to illustrate the efficacy of the selected pesticides on *Spodoptera littoralis* with the suggestion of using of the best mix of selected pesticides and other methods of integrated pest management (IPM) on *Spodoptera littoralis* to maximize the advantages.

KEYWORDS: *Spodoptera littoralis*, Chlorpyrifos, Pyrethrins, Spinosad, Methomyl, Toxicity index

INTRODUCTION

The Cotton Leafworm, *Spodoptera littoralis* (Boisd.), is considered to be the key pest in Egypt that reduce or compromise both the yield and quality of cotton production which results in causing great economic losses [1-6]. Therefore, choosing the best method to control *Spodoptera littoralis* is considered paramount. Chemical control is one of a common method to control *Spodoptera littoralis*. Further, chemical control has many advantages and disadvantages. For instance, it enables to kill pests within the shortest time, and preventing pests’ resurgence. Furthermore, the major advantage of chemical control is its efficiency. Most pesticides act very fast in that when selected properly they are highly effective in suppress pest populations below the economic injury level (EIL) [7-9].

On the other side, the disadvantages in that it was promoted the evolution of pesticide resistance, and it has harmful effects on non-target organisms [10-14].

Thus, using efficient pesticides from different classes and mode of actions may result an appropriate control of *Spodoptera littoralis*. In this regard, chlorpyrifos and methomyl exert their toxic action by inhibiting specific
enzymes of the nervous system, acetyl cholinesterases (AChE) [15-19]. However, Mode of action of spinosad acts by disrupting binding of acetylcholine to nicotinic acetylcholine receptors at the postsynaptic cells of the nervous system [20]. Further, pyrethrins bind to sodium channels that occur along the length of nerve cells causing disruption on the nervous system [21].

In our study, we evaluated four pesticides, chlorpyrifos, methomyl, pyrethrins, and spinosad on the fourth instar larvae after 24-h exposure under laboratory conditions.

MATERIAL AND METHODS

Strain:
A laboratory susceptible strain of Cotton leafworm, Spodoptera littoralis (Boisd.) reared in the laboratory of Plant Protection Department Research building, Faculty of Agriculture, Assiut University. The rearing regime was described before by El-Defrawi et al., (1964) and Ahmed, (2014) [22, 2]. Briefly, insects were reared under controlled conditions in an incubator at 26 ± 2°C and 65 ± 10% RH with 8:16 L:D photoperiod. Larval jars were supplied daily with fresh Castor leaves, Ricinus communis L., as a source of food which was provided daily. The adult were kept separately and mated on the third day of emergence in clean jars (250 g), adults were fed on 10% honey solution, fresh green leaves of Tafla, Nerium oleander (L.) were provided for egg laying.

Pesticides:
The commercial formulations of Methomyl (Lannate 90% SP, E. I. du Pont de Nemours, Wilmington, DE, USA), Spinosad (SpinTor 24% SC, DowAgroSciences, Indianapolis, IN, USA), Pyrethrins (Pyrethrum 5% EC, Agropharm Ltd., UK), and Chlorpyrifos (Dursban 48% EC, DowAgroSciences, Indianapolis, IN, USA) were used in the bioassay. These materials were obtained from Central Agricultural Pesticides Laboratory (CAPL) in Dokki, Giza, Egypt (Table 1).

Table 1: Selected pesticides used in the laboratory experiments

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Common name</th>
<th>Group</th>
<th>Chemical Name</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lannate 90% SP</td>
<td>Methomyl</td>
<td>carbamate</td>
<td>(E,Z)-methyl N-(((methylamino)carbonyl)oxy)ethanimidiothioate</td>
<td><img src="H3CNO.png" alt="Structure" /></td>
</tr>
<tr>
<td>SpinTor 24% SC</td>
<td>Spinosad</td>
<td>spinosyns</td>
<td>{[2R,3aS,5aR,5bS,9S,13S,14R,16aS,16bR]-13-{{[(2R,5S,6R)-5-(Dimethylamino)-6-methyltetrahydro-2H-pyran-2-yl]oxy}-9-ethyl-14-methyl-7,15-dioxo-2,3,5a,5b,5h,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-1H -as-indaceno[3,2-d]oxacyclododec-2-yl} 6-deoxy-2,3,4-tri-O-methyl-α-L-mannopyranoside}</td>
<td><img src="SpinTor.png" alt="Structure" /></td>
</tr>
<tr>
<td>Pyrethrum 5% EC</td>
<td>Pyrethrins</td>
<td>pyrethroid</td>
<td>{[1(15S)-2-methyl-4-oxo-3-[(2Z)-penta-2,4-dienyl]cyclopent-2-en-1-yl] (1R,3R)-2,2-dimethyl-3-O-methylprop-1-eny)cyclopropane-1-carboxylate}</td>
<td><img src="Pyrethrum.png" alt="Structure" /></td>
</tr>
<tr>
<td>Dursban 48% EC</td>
<td>Chlorpyrifos</td>
<td>organophosphate</td>
<td>O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate</td>
<td><img src="Dursban.png" alt="Structure" /></td>
</tr>
</tbody>
</table>

Bioassay Test:
One set of three replicates each contain 10 newly molted 4\textsuperscript{th} instar larvae for each concentration of each tested pesticide were used. The pesticide concentrations were dissolved in distilled water. Treatment of larvae was conducted by the leaf dipping technique [23-24]. Fresh and clean castor leaves, Ricinus communis L., were immersed for 10 second in the prepared concentrations of the tested compounds. The treated leaves were then left to dry at room temperature before being offered to the 4\textsuperscript{th} instar of Spodoptera littoralis larvae. Larvae were left to feed on treated leaves for 24-h exposure. The same numbers of larvae was used for control experiments in which larvae were offered fresh clean castor leaves dipped in distilled water only. The percentages of mortality
of larvae were recorded at 24-h. Mortality percentage was estimated and corrected according to Abbott’s formula [25].

Bioassay data were pooled and analyzed (the LC$_{50}$, LC$_{90}$, and 95% Confidence limits values) by using IBM SPSS statistics 23 program (SPSS Inc., Chicago, IL, USA). Figures were done by using GraphPad Prism 6.01 software (San Diego, CA, USA) and by using SigmaPlot 11 (Systat Software, Inc., San Jose, CA, USA).

Toxicity index was calculated as follows:

Toxicity index = \[(LC \text{ value of the most toxic tested pesticide} / LC \text{ value of the tested pesticide}) \times 100\].

**RESULTS AND DISCUSSION**

The potential toxicity of selected conventional pesticides on fourth instar larvae of *Spodoptera littoralis* after 24-h was presented in Table 2 and Figure 1. The most toxic pesticide was chlorpyrifos with LC$_{50}$ and LC$_{90}$ values of 4.7 and 22.4 µg/ml. Methomyl showed moderate toxicity and the LC$_{50}$ and LC$_{90}$ values were 121.3 and 283.7 µg/ml. However, pyrethrins and spinosad demonstrated the lowest toxicity pesticides among the tested compounds (the LC$_{50}$ and LC$_{90}$ values were 1381.8 and 2903.5 µg/ml for pyrethrins and 1530.4 and 2513.5 µg/ml for spinosad).

Table 2: Toxicity of selected pesticides on 4th instar larvae of *Spodoptera littoralis* after 24-h exposure

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Conc. (µg/ml)</th>
<th>Mortality %</th>
<th>LC$_{50}$ (µg/ml)</th>
<th>95% CL Lower</th>
<th>Upper</th>
<th>LC$_{90}$ (µg/ml)</th>
<th>95% CL Lower</th>
<th>Upper</th>
<th>Slope (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methomyl</td>
<td>50</td>
<td>6.7</td>
<td>121.3</td>
<td>100.4</td>
<td>145.3</td>
<td>283.7</td>
<td>225.9</td>
<td>398.0</td>
<td>3.5 (± 0.46)</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>33.3</td>
<td>70.0</td>
<td>93.3</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>70.0</td>
<td>93.3</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>33.3</td>
<td>93.3</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>33.3</td>
<td>93.3</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinossad</td>
<td>800</td>
<td>0</td>
<td>1530.4</td>
<td>1381.4</td>
<td>1693.1</td>
<td>2513.5</td>
<td>2198.9</td>
<td>3078.0</td>
<td>5.9 (± 0.78)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>16.7</td>
<td>63.3</td>
<td>73.3</td>
<td>93.3</td>
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<td></td>
<td></td>
</tr>
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<td></td>
<td>1600</td>
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<td>73.3</td>
<td>93.3</td>
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<td>2000</td>
<td>63.3</td>
<td>73.3</td>
<td>93.3</td>
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<td></td>
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<td>3000</td>
<td>63.3</td>
<td>73.3</td>
<td>93.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrethrins</td>
<td>500</td>
<td>6.7</td>
<td>1381.8</td>
<td>667.3</td>
<td>2142.4</td>
<td>2903.5</td>
<td>1926.0</td>
<td>3193.6</td>
<td>4.0 (± 0.52)</td>
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<tr>
<td></td>
<td>1000</td>
<td>26.7</td>
<td>86.7</td>
<td>100</td>
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<td>2200</td>
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<td>100</td>
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<tr>
<td>Chlorpyrifos</td>
<td>1</td>
<td>10.0</td>
<td>4.7</td>
<td>3.5</td>
<td>6.4</td>
<td>22.4</td>
<td>14.8</td>
<td>43.1</td>
<td>1.9 (± 0.26)</td>
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<tr>
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<td>1.5</td>
<td>23.3</td>
<td>4.7</td>
<td>3.5</td>
<td>6.4</td>
<td>22.4</td>
<td>14.8</td>
<td>43.1</td>
<td>1.9 (± 0.26)</td>
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<td>53.3</td>
<td>4.7</td>
<td>3.5</td>
<td>6.4</td>
<td>22.4</td>
<td>14.8</td>
<td>43.1</td>
<td>1.9 (± 0.26)</td>
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<tr>
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<td>10</td>
<td>70.0</td>
<td>4.7</td>
<td>3.5</td>
<td>6.4</td>
<td>22.4</td>
<td>14.8</td>
<td>43.1</td>
<td>1.9 (± 0.26)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>100</td>
<td>4.7</td>
<td>3.5</td>
<td>6.4</td>
<td>22.4</td>
<td>14.8</td>
<td>43.1</td>
<td>1.9 (± 0.26)</td>
</tr>
</tbody>
</table>

According to the slope value of the LC$_P$ lines of the 4 tested pesticides, *Spodoptera littoralis* showed relative high homogeneity response to the spinosad (5.9) and low homogeneity to chlorpyrifos (1.9). However, moderate homogeneity response was found with methomyl (3.5) and pyrethrins (4.0).

![Fig. 1: LC$_P$ lines of selected pesticides tested against 4th instar larvae of *Spodoptera littoralis* after 24-h exposure](image-url)
The toxicity index of selected pesticides was shown in Figure 2. In this interim, for LC\textsubscript{50} values, the values were 100, 3.87, 0.34, and 0.31 for chlorpyrifos, methomyl, spinosad, and pyrethrins, respectively, whereas for the LC\textsubscript{90} values were 100, 7.90, 0.77, and 0.89 for chlorpyrifos, methomyl, spinosad, and pyrethrins, respectively. Chlorpyrifos was more toxic than methomyl, pyrethrins, and spinosad by 25.8-, 294-, and 325.6-fold, respectively. Methomyl was more potent than pyrethrins and spinosad by 11.4- and 12.6-fold, respectively.

![Toxicity index graphs](image)

**Fig. 2:** Toxicity index (A) from LC\textsubscript{50} values and (B) from LC\textsubscript{90} values of selected pesticides tested against 4\textsuperscript{th} instar larvae of *Spodoptera littoralis* after 24-h exposure. Toxicity index = [(LC value of the most toxic tested pesticide / LC value of the tested pesticide) × 100].

Plentiful studies in harmony with our study. In this regard, using the feeding leaf bioassay after 48-h post treatment, Ezz El-Din *et al.* [26] estimated the LC\textsubscript{50} values of chlorpyrifos, methomyl, and spinosad, the values were 13.78, 70.78, and 101.87 mg/l, respectively. Further, El-Khayat *et al.* [27] demonstrated that chlorpyrifos was the most effective insecticide among the tested insecticides (telbufenuron, tebufenozide, methoxyfenozide, and spinosad) that recorded 0.1 and 0.809 ppm for 2\textsuperscript{nd} instar larvae and 0.472 and 6.838 ppm for 4\textsuperscript{th} instar larvae, respectively after 24-h exposure. In another study, Abaza [28] revealed that The LC\textsubscript{50} value of chlorpyrifos was 30.85 ppm after 24-h post treatment. However, Tong *et al.* [29] found that chlorpyrifos showed moderate toxicity among the ten tested pesticides and the LC\textsubscript{50} value was 4.18 mg/l on a field resistance strain of *Spodoptera litura* after 48-h exposure in China. At the same trend, Ghoneim *et al.* [30]concluded that chlorpyrifos was shown moderate toxicity on 4\textsuperscript{th} instar larvae of *Spodoptera littoralis* after 24-h exposure and the LC\textsubscript{50} values on lab and field strains were 6.3 and 93.4 ppm, respectively. In contrast, Saeed *et al.* [31] assessed the toxicity of chlorpyrifos along with cypermethrin, lufenuron, and emamectin benzoate on 2\textsuperscript{nd} instar larvae of *Spodoptera exigua* field population after 72-h exposure, they found that chlorpyrifos was the least toxic pesticide (LC\textsubscript{50}= 175 mg/l) and the most potent pesticide was emamectin benzoate (LC\textsubscript{50}= 0.005 mg/l).

In general, chlorpyrifos showed significant toxicity on 4\textsuperscript{th} instar larvae of *Spodoptera littoralis* under laboratory conditions. However, to magnify its toxicity with the respect to surround environment, it’s much better to insert chlorpyrifos in IPM programs to minimize the risks to non-target organisms and the environment, plus, to reduce the presence of pesticide resistance towards *Spodoptera littoralis*.

**REFERENCES**


