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Toxicity and Biological Effects of Some Insecticides, IGRs and Jojoba oil on Cotton Leafworm Spodoptera littoralis (Boisd.)

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ABSTRACT

Toxicity of two chemical insecticides chlorpyrifos, es-fenvalerate; a bioinsecticide (protecto); an IGR (lufenuron) and jojoba oil against 2nd and 4th larval instars of cotton leaf worm, Spodoptera littoralis (Boisd.) and the effect of these compounds on some biological characters and fecundity were studied on 4th instar larvae. Es-fenvalerate proved as the most effective insecticide against the 2nd and 4th instar larvae of S. littoralis after a feeding period 24hrs on treated leaves, followed by chlorpyrifos, lufenuron, jojoba oil and protecto. As for the efficiency of the tested insecticides against the 2nd instar larvae of S. littoralis, their LC50 values were 0.15, 0.17, 0.31, 4.61 ml/L and 16.64 gm/L, respectively. Prolongation in larval and pupal periods resulted from treated 4th instar larvae. The larval durations were 16.9, 14.1, 13.7, 12.1 and 14.1 days for lufenuron, chlorpyrifos, es-fenvalerate, protecto, and jojoba oil with the higher concentrations, respectively. In addition protecto, and jojoba oil, at lower concentrations showed an increase in pupation percentage, being 85.9% and 81.2%, respectively compared to 99.6% for control. The female fecundity and fertility showed significant reduction of the number of eggs deposited by each female developed from 4th larval instar treated with es-fenvalerate, lufenuron, protecto and jojoba oil compared with the control. The obtained data indicated also that the tested compounds caused failure of the insects’ embryonic development, showing the lower egg hatchability percentage comparison with control.

Key words: chlorpyrifos- es-fenvalerate- protecto- lufenuron - jojoba oil - Spodoptera littoralis

Introduction

Cotton is one of the major economics crops in Egypt. Cotton plants are attacked by many pests, from the seedling stage to harvest causing different degrees and types of damage. Among these pests, the cotton leaf worm, S. littoralis (Boisd.) is one of the most injurious cotton pests in the world. It is found almost everywhere cotton and on several other crops (Matthews and Tunstall 1994). In the recent years, the toxicity of insecticides to humans and wildlife has caused much public concern and lead to the use of more selective pesticides. (Paoletti and Pimentel 2000). The intense and repeated applications of insecticides which are often from the same chemical group led to development of insects’ resistance to pesticides. To prevent the resistance phenomenon, there is a need for different compounds having different modes of action (Aydin & GÜrkan 2006). For this reason, it has become necessary to look for alternative means of pest control which can minimize the use of chemicals (El-Aswad, 2007).

Natural plant extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non target organisms (Sharma et al., 2006). There are more than 2400 plant species belonging to 189 plant families which are rich sources of bioactive organic compounds (Rao et al., 2005). Species from over 60 plant families have been identified as possessing insecticidal (Prakesh & Rao, 1997). Jojoba oil is a natural compound derived from the jojoba crop, Simmondsia chinensis L.

In Egypt, several laboratory studies were done on seed crude extracts of jojoba dealing with its pesticidal effect on various economic pests such as Pectinophora gossypiella, Bemisia tabaci, Empoasca discipiens, Agrotis ipsilon, Sesamia cretica, Ostrinia nubilalis and Schistocerca gregaria (Rofail et al., 2000; Salem et al., 2003; Yacoub, 2006 and Halawa et al., 2007) who stated that jojoba oil has the effect as toxic, antifeedant, growth and development inhibitors and oviposition inhibitor. A new approach to insect pest control is to use insect growth regulators (IGRs) which are considered to have little human toxicity (Schmutterer, 1985). The use of IGR compounds in insect control is known as insect developmental inhibitiors. These compounds have been tested successfully against several insect species (Pineda et al., 2007; El-barky et al., 2009 and Wang &Tian ,2009). More attention should be paid to the use of bioinsecticides such as compounds based on bacteria, fungi,

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and viruses (Rao et al., 1990). These groups have unique modes of action (Asher, 1993 and Thompson et al., 1999) and their properties may differ considerably from the conventional agents with which growers are familiar.

In Egypt, IPM strategy for protection of cotton plants based on rotation of insecticides. Biocides or IGRs has used for the first spray to control of newly hatched cotton leafworm, while, OP and Pyrethroids were used for the second, third and fourth sprays, respectively, against cotton leafworm and bollworms. (Sawicki and Denholm, 1987; Temerak, 2002 and Adel-Sattar et al., 2012).

The objective of the present study was to investigate toxicity of two chemical insecticides chloropyrifos, esfenvalerate and the bioinsecticide protecto, IGRs, lufenuron and jojoba oil against 2nd and 4th larval instars of cotton leaf worm, S. littoralis (Boisd.) and the effect of these compounds on certain biological characteristics of the 4th instar larvae, under laboratory conditions.

Materials And Methods

1. Test insect:

A laboratory strain of S. littoralis was obtained from Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza, Egypt. Insects were reared on castor bean leaves at 27±2°C and 65±5 % R.H. The bioassay tests were carried out using freshly moulted 2nd and 4th instars. The formed pupae were collected and placed in clean jars with moist saw dust placed at the base to provide as pupation site. Adults were provided with 10% sucrose solution, for nutrition and small oleander branches as oviposition sites.

2. Insecticides and chemicals used:

2.2 Conventional insecticides:

- Chlorpyrifos (Dursban 48%EC) as organophosphorus insecticide: (0.0-diethyl 1-0) 3,5,6, trichloropyridin-2-yl) phosphorothioate.
- Esfenvalerate (Sumi-alpha 5%EC) pyrethrid comp., (S)cyano-3-phenoxybenzyl (s)-2-(4-chlorophenyl)-3methylbutrate.

2.3. The bioinsecticide Protecto:

W.P. based on Bacillus thuringiensis subsp. kurstaki (32x10^3 I.U/mg). Active ingredient 9.4% inert ingredient (Carrier) 90.6%.

2.4. Insect Growth Regulator (IGR):

- Lufenuron 5% EC: N-[2.5-dichloro-4-(1,1,2,3,3,3- hexafluoro-prop-oxy)-phenylaminocarbonyl] 2.6-difluoro-benzamide.

2.5. Jojoba oil (81 Nat-1): CH₃ (CH₂)₇ CH=CH-(CH₂)₁₁ CH₂-O-CO(CH₂)₉ CH=CH(CH₂)₁ CH₃ [C₂₂H₄₃COOC₁₉H₃₇]

3. Toxicity tests:

Serial concentrations of tested compounds were prepared by dissolving in distilled water. In the bioassays, 2nd and 4th larval instars of S. littoralis were treated by feeding. Five replicates (each of 20 larvae/ concentration) were used. Control larvae were treated with distilled water only. Five concentrations (1.5, 1, 0.75, 0.5 and 0.25 ml/L) for chlorpyrifos and es-fenvalerate while, IGRs, lufenuron were (1, 0.8, 0.4, 0.2 and 0.1 ml/L), jojoba oil concentrations were (20, 10, 5, 2.5 and 1.25 ml/L) and concentrations (20, 10, 5, 2.5 and 1.25 gm/L) were used for protecto. All the castor bean leaf discs were dipped into solution containing different concentrations of tested compounds for 5 seconds and then they were air dried with laboratory for one hour. Control discs were dipped for the same period into distilled water. Mortality percentages were measured after 24 hrs. Although, protecto is a bioinsecticid depending upon entomopathgic bacteria, B. thuringiensis subsp. kurstaki ; mortality data on larvae were recorded only 24 hours after treatment in order to be the same as occurred with the remaining materials. Mortality rates were corrected according to Abbott’s formula (Abbott, 1925) and plotted against concentrations as log/probit regression lines. LC₅₀, LC₉₀ values and the toxicity index as well as the slope of the lines were calculated (Finney, 1971) using “LdPLine®” software. http://embakr.tripod.com/ldpline/ldpline.htm.
4. Biological activity:

Effects of tested compounds on some biological aspects of *S. littoralis* were determined using the same five concentrations used in toxicity test for each tested compounds. The treated instar and its subsequent developmental stages were determined as follows: hundred newly moulted 4th instar larvae of *S. littoralis* were used for each compound. Five replicates were tested for each compound, twenty larvae in each replicate, they were fed on castor bean leaves treated with the different concentrations of chlorpyrifos, es-fenvalerate and the bioinsecticide protecto, IGRs, lufenuron and Jojoba oil. In control test, leaves were treated with distilled water only. All larvae were allowed to feed for 24 hrs on the treated leaves then to complete their life-cycle on fresh castor bean leaves. Some biological aspects such as: percentage of larval mortality, larval malformations, larval duration, pupal duration, percentage of adults emergence, adults malformation and sex ratio, fecundity and fertility were determined.

5. Statistical analysis:

All experimental data were statistically analyzed using analysis of variance and F-test (ANOVA) using software computer program.

**Results And Discussion**

1- Toxicity and efficacies of tested compounds:

Data in Table (1) and figures (1, 2, 3, 4&5) showed that es-fenvalerate proved as the most effective insecticide against the 2nd instar larvae of *S. littoralis* after 24hrs a feeding period on treated leaves, followed by chlorpyrifos, lufenuron, jojoba oil and protecto, showing the medium lethal concentration (LC₅₀) values of 0.15, 0.17, 0.31, 4.61 ml/L and 16.64 gm/L, respectively.

Almost, the same results were obtained also against the 4th larval instar the LC₅₀ value was 0.18ml/L followed by chlorpyrifos (0.26ml/L), lufenuron (0.37ml/L) while jojoba oil and protecto were considered to be less toxic insecticides, their LC₅₀ values were 6.99ml/L and 21.52gm/L, respectively. The corresponding LC₉₀ reached 1.98, 4.42, 63. 82 ml/L and 1185.47gm/L for chlorpyrifos, lufenuron, jojoba oil and protecto, respectively, while their toxicity index being 69.23, 48.65, 2.58 and 0.84% (Based on LC₅₀ of es-fenvalerate 100%), respectively.

<table>
<thead>
<tr>
<th>Tested compound</th>
<th>2nd instar larvae</th>
<th>4th instar larvae</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Slope ±SE</td>
<td>LC₅₀ in lim at 95%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC₉₀ in lim at 95%</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>Chlorpyrifos (ml/L)</td>
<td>1.38±0.24</td>
<td>0.17</td>
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<tr>
<td></td>
<td></td>
<td>0.09-0.25</td>
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<tr>
<td>Es-fenvalerate (ml/L)</td>
<td>1.30±0.24</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06-0.23</td>
</tr>
<tr>
<td>Lufenuron (ml/L)</td>
<td>1.16±0.16</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.24-0.39</td>
</tr>
<tr>
<td>Protecto (gm/L)</td>
<td>0.73±0.14</td>
<td>16.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.64-37.38</td>
</tr>
<tr>
<td>Jojoba oil (ml/L)</td>
<td>1.21±0.18</td>
<td>4.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.30-5.83</td>
</tr>
</tbody>
</table>

Toxicity index = LC₅₀ of the most effective compound/LC₅₀ of the tested compound × 100

These results agree with those obtained by (Abd El-Kareem et al., 2010) reported that Protecto was the most potent bioinsecticide compared with Viruset, Profect, and Bioranza. The mortality rates increased at the termination of the larval stage. Second larval instar showed higher susceptibility to all the tested compounds than the 4th larval instar. This might be due to differences in sizes and defense mechanisms between instars. It is well documented that older instars of the cotton leaf worm are able to tolerate the toxic effect of these bioagents. Similar observations were reported by (Mabrouk, 2001; Mabrouk and El-Abbas, 2002, Hanafy et al., 2005; Abdel-Aziz, 2007; and Abd El-Kareem, 2007).
Fig. 1: Toxicity line of chlorpyrifos against 2\textsuperscript{nd} and 4\textsuperscript{th} larval instars of \textit{Spodoptera littoralis}.

Fig. 2: Toxicity line of esfenvalerate against the 2\textsuperscript{nd} and 4\textsuperscript{th} larval instars of \textit{Spodoptera littoralis}.

Fig. 3: Toxicity line of lufenuron against 2\textsuperscript{nd} and 4\textsuperscript{th} larval instars of \textit{Spodoptera littoralis}.
2- Effect of tested compounds on some biological parameters of 4th larval instar of the cotton leaf worm, S. Littorals:

Summarized results in Tables (2,3,4,5 and 6) showed the latent effects of treating 4th larval instar of S. littoralis by different tested compounds (chlorpyrifos, es-fenvalerate, lufenuron, protecto, and jojoba oil) on the biology and fecundity of the survived larvae.

2.1. Larval stage:

It is clear that all tested compounds, significantly, prolonged the duration of the larval stage than that of the untreated check. Tables (2, 3,4,5 and 6) revealed that larval duration was 16.9, 14.1,13.7,.12,1and 14,1 days for lufenuron,chlorpyrifos, es-fenvalerate, protecto, and jojoba oil with the higher concentrations, respectively. These results agree with Dean et al., (1998) and Moriello et al.,(2004) who reported that lufenuron is known as an insect development inhibitor/ insect growth regulator. It is active against larval developmental stages ,causing cuticular lesions and interfering in the chitin biosynthesis.

2.2. Larval mortality:

The percentage of larval mortality had increased by increase of concentration and tested compound. Data in Tables (2, 3,4,5 and 6) indicated that the es-fenvalerate was proved to be the most effective insecticide ( 89%) followed by chlorpyrifos (88%), jojoba oil (73%), lufenuron (69%) and protecto (51%), respectively.
2.3. Pupal stage:

The pupal duration was affected in all treatments compared with the untreated check. The averages ranged between 12.8 and 8.2 days of the higher and the lower concentrations in case of chlorpyrifos, while it was 6.8 days in control. These results may be due to the delaying of moulting process. In addition, there was an increase in pupation percentage (85.9% and 81.2%) in case of pupae resulted from 4th instar larvae treated by protecto, and jojoba oil at lower concentrations.

2.4. Pupal malformations:

The data in Tables (2, 3,4,5 and 6) indicated that the used chlorpyrifos, es-fenvalerate, lufenuron and jojoba oil caused high increase in malformation of pupae than protecto. The malformation increased by increasing the concentrations.

2.5. Adult stage:

Tables (2, 3,4,5 and 6) showed that the percentages of adults emergence was highly reduced from 94.1 to 32%, 39.5 %, 50.1, 60.3% and 73.3 after larval feeding with the higher concentration of es-fenvalerate, chlorpyrifos, lufenuron, jojoba oil and protecto, respectively.

2.6. Adult malformations:

As shown in Tables (2, 3,4,5 and 6) the highest percentages of adults malformation recorded with es-fenvalerate, chlorpyrifos, lufenuron, and jojoba oil were 18.8%, 16.9%, 13.2% and 10.8%, respectively compared to 5.7% for the bioinsecticide protecto.

2.7. Sex-ratio:

Illustrated data in Tables (2, 3,4,5 and 6) showed that the treated 4th instar larvae with different concentrations of chlorpyrifos shifted the sex-ratio as it increased the female and decreased the male ratio than that of control. This ratio was 4♀:2♂ with concentrations 0.75 and 0.5 ml/L compared to 3♀:2♂ for control. On contrast, in case of es-fenvalerate the sex ratio was 3.6♀:2.2♂ (nearly the same ratio) for the control, the same results were observed with lufenuron. The es-fenvalerate showed highly significant reduction in adult males and females developed from treated 4th larval instars. The decrease was 6.4 and 6.6 days for males and females with lower concentrations, while the control value was 10.2 and 11.4 days, respectively Table (3). While, lufenuron showed highly significant increase in adult females longevity. The increase was 10.1 days, with higher concentration compared to 6.7 days for control Table (4).

2.8. Female fecundity and fertility:

Data in Tables (2, 3,4,5 and 6) showed significant reduction of the number of eggs laid by each female developed from treated 4th larval instars compared with the control. Table (2) described the hatchability of deposited eggs by female developed from treated 4th larval instars with concentrations (0.75, 0.5 and 0.25ml/L) of chlorpyrifos. The hatchability percentages were 26.4, 34.9 and 45.9%, respectively compared with 96.6% in control. Tables (3,4,5&6) showed the same significant reduction of the number of eggs laid by each female developed from treated 4th larval instar treated with es-fenvalerate, lufenuron, protecto and jojoba oil compared with the control. These data indicate that the tested compounds caused failure of the insects embryonic development, showing the lower egg hatchability percentage compared with control. These results agree with (Sammour et al., 2008) who found a reduction in fecundity and egg hatchability of cotton leaf worm after treatment of larval instars with chorfluazuron and leufenuron and failure of egg hatchability may be due to the penetration of insecticide into the eggs and prevent hatchability by interfering with embryonic cuticle synthesis so the new hatch probably cannot use its muscles to free itself from egg chorion.

Table 2: Effect of chlorpyrifos on some biological parameters on 4th larval instar of Spodoptera littoralis.

<table>
<thead>
<tr>
<th>Tested conc. (ml/L)</th>
<th>Larval stage</th>
<th>Pupal stage</th>
<th>Adult stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean duration (days)</td>
<td>% mortality</td>
<td>% Malformed</td>
</tr>
<tr>
<td>1.5</td>
<td>14.10±0.2</td>
<td>98</td>
<td>27.3</td>
</tr>
<tr>
<td>1.0</td>
<td>13.80±0.5</td>
<td>80</td>
<td>29.9</td>
</tr>
<tr>
<td>0.75</td>
<td>11.70±0.2</td>
<td>70</td>
<td>32.7</td>
</tr>
<tr>
<td>0.5</td>
<td>10.30±0.3</td>
<td>60</td>
<td>32.2</td>
</tr>
<tr>
<td>0.25</td>
<td>8.10±0.0</td>
<td>40</td>
<td>42.6</td>
</tr>
<tr>
<td>Control</td>
<td>9.90±0.4</td>
<td>0</td>
<td>88.6</td>
</tr>
</tbody>
</table>

* Significant at (p < 0.05), ns: not significant  **: moderately significant (p < 0.01)  ***: highly significant (p < 0.001).
References


