Assessment the Resistance Levels in Field Strain of Cotton Leafworm to some Insecticides

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ABSTRACT

This study was performed to assess the resistance levels in a cotton leafworm (CLW), Spodoptera littoralis (Boisd) field strain in comparison with a laboratory strain toward alpha-cypermethrin, chlorpyrifos, methoxfenozide, chlorantraniliprole, and chlorfluazuron. Their Ovicidal activity and residual toxicity against neonates were also evaluated. Glutathione S-transferases (GST) and total esterases activities of 2nd and 4th larval instars treated with these insecticides were determined. The field strain exhibited different level of resistance where cypermethrin, chlorpyrifos, and methoxfenozide ratios (RR) were 12.7, 48.0, and 13.8, respectively for 2nd instar, while 4th instar is only resistant to cypermethrin, chlorpyrifos with RR of 120.8 and 19.0 compared to lab strain. Concerning the ovicidal and residual toxicity at the recommended field rate, cypermethrin, chlorpyrifos, chlorantraniliprole, and chlorfluazuron achieved mortality of treated eggs and neonates of lab strain 95.3, 98.9, 93.3, and 96.8%, respectively as well as 75.6, 70.0, 85.2 and 91.6%, respectively against field strain. However, methoxyfenozide showed a non-significant difference between lab and field strains. GST and esterases activities were significantly higher in the field strain compared with that of lab strain. It can be concluded that monitoring of insecticide resistance is a crucial step for the plant protection and resistance management programs.

Keywords: Cotton leafworm; Insecticide resistance; Glutathione S-transferases; total esterases.

INTRODUCTION

Cotton leafworm (CLW), Spodoptera littoralis, is a major destructive and polyphagous insect, attacking a wide range of field and vegetable crops (Kandil et al., 2003). It spreads in Mediterranean regions and temperate zones in Asia and Africa (Jones et al., 1994). Larvae occurs during the whole cycle of cotton, feeds on leaves, fruiting points, flower buds and also on bolls, causing an extensive economic loss (Hatem et al., 2009). Therefore, several insecticide applications were required for CLW control (Abou-Taleb, 2016).

In Egypt, many insecticide groups and applications were used to combat CLW and preserving the crop yield. The extensive and unwise use of insecticides, multiple generations of CLW and the wide host range per year, resulted in the development of resistance (Abo-Elghar et al., 2005; Tabashnik et al., 2014). The continuous monitoring of resistance is the first and essential step to resistance management programs. Moreover, it is necessary to know the insecticide resistance mechanisms in insect pests for development of resistance management strategies. Enhanced metabolism, nerve insensitivity, reduced penetration and target site insensitivity are identified mechanisms of insecticides resistance in CLW (Attia, 1999; Abo Elghar et al., 2005).

More attention needs to be given to the management of insect pests at other stages of its development, when it may be more susceptible to the insecticides (Renkleff et al., 1995). Many studies had been achieved to evaluate the ovicidal activity against many insect species (El-Guindy et al., 1983; Canela et al., 2000). In addition, avoiding selection pressure of the insect population to insecticides requires searching for an effective alternatives and/or pest control strategies. Therefore, this study focused on the monitoring of insecticide resistance in the field strain of CLW (collected from Alamrya, Alexandria government, Egypt). Also, the activities of GST and total esterases in the field and laboratory strains were compared.

MATERIAL AND METHODS

Laboratory strain of CLW

A susceptible strain of the S. littoralis has been reared for many years in the Plant Protection Research Station, Alexandria, Egypt. Larvae were fed on castor bean leaves under controlled laboratory conditions (25 ± 2 °C, RH 65%) for several years avoiding exposure to any pesticides according to the method of Eldefrawi et al. (1964).
Field strain of CLW

Cotton leafworm egg masses were collected from cotton fields of Alamrya, Alexandria government during 2021 cotton season and transferred to the laboratory. Larvae for experimental purposes were reared in the laboratory on castor bean leaves at the aforementioned conditions.

Tested insecticides

Alpha-cypermethrin (Alpha-cypermethrin® 10% EC) was produced by Tagros Chemicals India Limited. Chlorpyrifos (Dursban 48%EC) and methoxyfenozide (Runner® 24%SC) were supplied by Dow Agrosciences Co. Chlorantraniliprole (Coragen 20% SC) was provided by DuPont Du Nemours Company. Chlorfluazuron (Atabron®5%EC) was supplied by Syngenta. Field rates of the tested insecticides were 250 cm<sup>3</sup>, 1 liter, 120 cm<sup>3</sup>, 60 cm<sup>3</sup>, 120 cm<sup>3</sup> per fed. for Alpha-cypermethrin, chlorpyrifos, methoxyfenozide, chlorantraniliprole and chlorfluazuron, respectively. Feddan is sprayed by total volume 150 Liter using CP3 Kynapsk sprayer.

Bioassay studies

Toxicity of tested insecticides against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of CLW laboratory and field strains was carried out. Homogenous pieces of the castor bean leaves were dipped in a series of each insecticide concentrations for 10 sec., held vertically to allow excess solution to drip off and dried at room temperature. Treated castor bean leaf pieces were transferred to a plastic cups, and the appropriate number and weight of starved larvae for 6 hrs were added. Each concentration was replicated four times. Mortality percentages were recorded after 24 hrs of treatment for cypermethrin and chlorpyrifos, and after 72 hrs for chlorantraniliprole, methoxyfenozide and chlorfluazuron. Mortality percentages were corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971). Median lethal concentrations (LC<sub>50</sub>) values were calculated and compared for the laboratory and field strains.

Ovicidal activity

Ovicidal activity of tested insecticides on the laboratory and field strain of CLW egg masses was determined. The upper layers of egg masses were removed with a fine hair brush. The lower layer in each egg mass was counted by the binocular. The counted egg masses were dipped for 5 seconds in recommended field rate of each tested insecticide in comparison to control (dipped in water) according to Dittrich (1967). Each treatment was replicated three times. Treatments and control were held in a plastic cups (9x4 cm) at 27±2°C, 65-75% RH and observed until hatching. The number of un-hatched eggs, dead neonates and live larvae were counted, and the mortality percentages were calculated.

Assay of GST and esterases activity

Whole larvae of the 2<sup>nd</sup> instar and the midguts of the 4<sup>th</sup> instar larvae (laboratory and field strains) were rinsed in ice-cold 100 mM phosphate buffer pH 7 and homogenized in glass homogenizer (1: 10 w/v) in the same buffer. The homogenate was centrifuged at 15,000 rpm for 30 min at 4°C using Cryofuge 20-3, Heraeus Christ centrifuge. The supernatant was served as the enzymes source.

Glutathione S-transferase was determined using 1-chloro, 2,4-dinitrobenzene (CDNB) as a substrate (Kao et al., 1989). The enzyme activity was determined at 340 nm as the change in the optical density per hr per mg enzyme protein. Bovine serum albumin (BSA) was used as a standard for the protein determination according to Lowry et al., 1951.

Esterase activity was determined according to the method of Van Asperen (1962) using α-naphthyl acetate as a substrate. Enzyme activity was measured as changes in optical density at 450 nm and calculated as Δ OD min<sup>-1</sup> mg protein<sup>-1</sup>.

Statistical analysis

Means were compared for significance using LSD test at probability 0.05 (SAS Statistical software, 1999).

RESULTS AND DISCUSSION

RESULTS

Toxicity of tested insecticides:

Susceptibility of 2<sup>nd</sup> and 4<sup>th</sup> larval instars of laboratory and field strains to selected insecticides is presented in Tables 1 and 2. Data showed that, 2<sup>nd</sup> instar larvae of the field strain demonstrates varied resistance ratios to the tested insecticides. Field strain 2<sup>nd</sup> instar larvae showed high resistance towards cypermethrin, chlorpyrifos and methoxyfenozide with resistance ratio 12.7 and 48.0 and 13.8, respectively. On the other hand, field strain was tolerant to chlorantraniliprole and chlorfluazuron with resistance ratio 4.6 and 5.0 (Table 1). Regarding 4<sup>th</sup> instar larvae, the field strain exerted high resistance levels to cypermethrin with resistance ratio 120.8. A moderate resistance is recorded to chlorpyrifos, where resistance ratio was 19.0. The 4<sup>th</sup> instar larvae of the field strain exhibited tolerance to chlorantraniliprole, methoxyfenozide and chlorfluazuron with resistance ratios 4.5, 7.3 and 3.3, respectively (Table 2).

Ovicidal activity of tested insecticides against CLW:

One of our objectives in this study was to compare between the ovicidal activity and the residual toxic effect to the new hatched neonates of the tested
insecticides against the laboratory and field strains of CLW (Figure 1). It was obvious that, there was a significant difference between the ovicidal and residual toxicity of cypermethrin, chlorpyrifos, chlorantraniliprole and chlorfluazuron against both strains. For the laboratory strain, cypermethrin, chlorpyrifos, chlorantraniliprole and chlorfluazuron at the recommended field rates achieved 95.3, 98.9, 93.3 and 96.8%, respectively, mortality of treated eggs and neonates of the laboratory strain. The same treatments achieved 75.6, 70.0, 85.2 and 91.6%, respectively, for the egg masses of field strain. On the other hand, there was no significant difference between the ovicidal and residual toxicity against neonates of methoxyfenozide against the laboratory and field strains (Figure 1).

Activity of GST and esterases in the laboratory and field strains of CLW

Activity of GST in the 2nd instar larvae of the field strain (2.08 ΔOD / mg protein / hr) was 2.0-folds the laboratory strain (1.04 ΔOD / mg protein / hr). In respect of the 4th instar larvae, activity of GST in the field strain (3.24 ΔOD / mg protein / hr) was 2.12-fold of laboratory strain (1.53 ΔOD / mg protein / hr) (Table 3). Table (4) shows the activity of esterases in the laboratory and field strains. Esterases activity were 0.18 and 0.23 ΔOD / mg protein / min in the 2nd and 4th larval instars of laboratory strain, while it was 0.68 and 0.89 ΔOD / mg protein / min in the 2nd and 4th larval instars of field strain. Esterases activities were 3.78 and 3.87 fold in the 2nd and 4th instar larvae of the field strain compared to the laboratory strain, respectively.

Table 1. Comparative toxicity of some insecticides against laboratory and field strains of Spodoptera littoralis 2nd instar larvae

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Strain</th>
<th>LC₅₀ᵃ (mg L⁻¹)</th>
<th>Confidence limits (mg L⁻¹)</th>
<th>Slopeᵇ ± SE</th>
<th>RRᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypermethrin</td>
<td>Lab.</td>
<td>0.048</td>
<td>0.036 - 0.063</td>
<td>0.920 ± 0.075</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>0.608</td>
<td>0.441 - 0.844</td>
<td>0.781 ± 0.069</td>
<td>12.7</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Lab.</td>
<td>1.083</td>
<td>0.846 - 1.391</td>
<td>1.158 ± 0.098</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>51.93</td>
<td>42.57 - 64.45</td>
<td>1.281 ± 0.110</td>
<td>48.0</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>Lab.</td>
<td>0.135</td>
<td>0.102 - 0.182</td>
<td>0.985 ± 0.092</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>0.621</td>
<td>0.432 - 0.901</td>
<td>0.680 ± 0.067</td>
<td>4.6</td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>Lab.</td>
<td>0.594</td>
<td>0.485 - 0.723</td>
<td>1.292 ± 0.106</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>8.21</td>
<td>6.14 - 11.76</td>
<td>0.909 ± 0.098</td>
<td>13.8</td>
</tr>
<tr>
<td>Chlorfluazuron</td>
<td>Lab.</td>
<td>0.702</td>
<td>0.571 - 0.864</td>
<td>1.235 ± 0.105</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>3.53</td>
<td>2.70 - 4.63</td>
<td>1.055 ± 0.094</td>
<td>5.0</td>
</tr>
</tbody>
</table>

ᵃThe concentration causing 50% mortality.
ᵇSlope of the concentration-mortality regression line ± standard error.
ᶜResistance ratio equals LC₅₀ of field strain / LC₅₀ of laboratory strain.

Table 2. Comparative toxicity of some insecticides against laboratory and field strains of Spodoptera littoralis 4th instar larvae

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Strain</th>
<th>LC₅₀ᵃ (mg L⁻¹)</th>
<th>Confidence limits (mg L⁻¹)</th>
<th>Slopeᵇ ± SE</th>
<th>RRᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypermethrin</td>
<td>Lab.</td>
<td>0.072</td>
<td>0.052 - 0.097</td>
<td>0.894 ± 0.090</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>8.70</td>
<td>6.50 - 11.43</td>
<td>0.927 ± 0.079</td>
<td>120.8</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Lab.</td>
<td>8.80</td>
<td>7.15 - 10.75</td>
<td>1.38 ± 0.13</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>167.07</td>
<td>131.79 - 213.17</td>
<td>1.04 ± 0.10</td>
<td>19.0</td>
</tr>
<tr>
<td>Chlorantraniliprole</td>
<td>Lab.</td>
<td>2.82</td>
<td>2.07 - 3.90</td>
<td>0.825 ± 0.071</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>12.61</td>
<td>9.61 - 16.74</td>
<td>1.02 ± 0.09</td>
<td>4.5</td>
</tr>
<tr>
<td>Methoxyfenozide</td>
<td>Lab.</td>
<td>6.35</td>
<td>5.26 - 7.78</td>
<td>1.47 ± 0.14</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>46.05</td>
<td>37.25 - 57.13</td>
<td>1.33 ± 0.12</td>
<td>7.3</td>
</tr>
<tr>
<td>Chlorfluazuron</td>
<td>Lab.</td>
<td>3.62</td>
<td>2.77 - 4.77</td>
<td>1.04 ± 0.09</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Field</td>
<td>12.01</td>
<td>9.33 - 15.59</td>
<td>1.12 ± 0.10</td>
<td>3.3</td>
</tr>
</tbody>
</table>

ᵃThe concentration causing 50% mortality.
ᵇSlope of the concentration-mortality regression line ± standard error.
ᶜResistance ratio equals LC₅₀ of field strain / LC₅₀ of laboratory strain.
Fig. 1. Ovicidal and residual toxicity of some insecticides at field rate against neonates of *Spodoptera* laboratory and field strains egg masses. Error bars represent standard deviation (SD) of three replications. Columns within a group with a letter in common are not significantly different according to Student-Newman Keuls (SNK) test (LSD at P < 0.05)

Table 3. Activity of glutathione S-transferases in the field and laboratory strains of cotton leafworm

<table>
<thead>
<tr>
<th>Larval instar</th>
<th>Specific activity (ΔOD / mg protein / hr) ± SE</th>
<th>Field / Lab. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Field strain</td>
<td>Laboratory strain</td>
</tr>
<tr>
<td>2nd</td>
<td>2.08 a ± 0.08</td>
<td>1.04 b ± 0.05</td>
</tr>
<tr>
<td>4th</td>
<td>3.24 a ± 0.04</td>
<td>1.53 b ± 0.07</td>
</tr>
</tbody>
</table>

Means within a row with a letter in common are not significantly different according to analysis of variance (ANOVA) test (LSD at P < 0.05).

Table 4. Activity of *S. littoralis* esterases in the field and laboratory strains of cotton leafworm

<table>
<thead>
<tr>
<th>Larval instar</th>
<th>Specific activity (ΔOD / mg protein / min ± SE)</th>
<th>Field / Lab. ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Field strain</td>
<td>Laboratory strain</td>
</tr>
<tr>
<td>2nd</td>
<td>0.68 a ± 0.01</td>
<td>0.18 b ± 0.01</td>
</tr>
<tr>
<td>4th</td>
<td>0.89 a ± 0.01</td>
<td>0.23 b ± 0.01</td>
</tr>
</tbody>
</table>

Means within a row with a letter in common are not significantly different according to analysis of variance (ANOVA) test (LSD at P < 0.05).

**DISCUSSION**

Insecticide resistance is a serious worldwide problem, where many different insect species became resistant to about 400 different compounds (Whalon *et al.*, 2008). Monitoring of insecticide resistance in any insect pest is very important step for the insect management and resistance management programs (Zhang *et al.*, 2016). The present study investigated the levels of resistance in the field strain of CLW to some conventional and non-conventional insecticides. Field strain exhibited different levels of resistance to the tested insecticides compared to the laboratory strain. While field strain exerted high resistance levels to cypermethrin and chlorpyrifos and it exhibited tolerance to chlorantraniliprole, methoxyfenozide and chlorfluazuron. In addition, *Spodoptera* sp. has the ability to develop resistance to wide range of insecticides (Tong *et al.*, 2013).

Many studies had reported high resistance levels in CLW against organophosphates, pyrethroid and carbamate insecticides (Attia, 1999; Abo Elghar *et al.*, 2005) which is compatible with results of the present study. Many pyrethroid and organophosphorus insecticides have been used for CLW control, with appearance of resistance and cross resistance (Abdallah, 1991; Rashwan *et al.*, 1992; Abou-Taleb *et al.*, 2016).
More recent, a significant intra-regional variation in susceptibility of different CLW populations has been reported in Nile Delta Egypt through 2002-2004 seasons (Abo-Elghar et al., 2005). Abou-Taleb (2010) also recorded differences in the susceptibility of CLW from different governorates to chlorpyrifos and cypermethrin.

Information about the biochemical mechanisms conferring resistance to certain insecticides has been shown to be very important for resistance management programs. One of the most important factors of insect resistance is the increase in metabolic activity resulting in higher detoxification of insecticides by enzymes such as monooxygenases, GSTs and esterases (Denholm and Rowland, 1992; Chen et al., 2007).

In the present study, measurements of esterases and GSTs activities in the field and laboratory strains were compared. Data showed that field strain exerted elevated esterases and GST activity compared to the laboratory one. In previous studies, higher esterases and GST activities are associated with organophosphate and pyrethroid resistance in CLW and other lepidopteran species (McCaffery, 1998; Abo Elghar et al., 2005; Abou-Taleb, 2010). Also, Yu et al. (2003) showed that, detoxification enzyme activity such as GST and hydrolases were higher in field strains of *S. frugiperda* (has high resistance levels to carbamate, organophosphate and pyrethroid insecticides) than in the susceptible strain.

Cotton leafworm completes its life cycle from egg, larvae, pupae and adult stages, therefore managing insect pests in various stages; through application of larvicidal and ovicidal chemicals is very useful. Eggs may be more susceptible to the insecticides used for control more than other insect stages (Canela et al., 2000). If insects are managed at the egg stage, vegetables and crops can be protected from marketable economic losses (Pavunraj et al., 2020). In this study, it was obvious that, there was a significant difference of the ovicidal and residual toxicity for cypermethrin, chlorpyrifos, chlorantraniliprole and chlorfluazuron between the laboratory and field strains. Abou-Taleb (2010) recorded high ovicidal activity for chlorpyrifos and esfenvalerate against CLW. Finally, alternation between insecticides with different modes of action will reduce increasing selection pressure of CLW populations and delay the resistance development to insecticides (Tikar et al., 2009; Pu et al., 2010).

**CONCLUSION**

Both the larval and egg stages of CLW field strain showed a different resistance levels to the tested insecticides compared to the laboratory strain. Thus it is very important to apply a proper strategy for countermeasure of resistance such as rotation between insecticides with different mode of action may prevent or delay the development of resistance in this insect. In addition, the continuous monitoring of resistance is crucial for plant protection and resistance management program.

**REFERENCES**


تقييم مستويات المقاومة في السلالة الحقلية لدودة ورق القطن لبعض المبيدات الحشرية

تم إجراء هذه الدراسة لتقييم مستويات المقاومة في سلالة حقلية لدودة ورق القطن لمبيدات السيبرميثرين، الكلوربيريفوس، الكلورأنتريتليبيرول، الميثوكسيفينوزيد، والكلورفلوزيرون مقارنة بالسلالة المعملية. أيضا تم تقييم نشاط وضع البيض وتقدير السمية على الفقس الناتج. نشاط إنزيمات الجلوتاثيون إس ترانسفيريز والإستريز الكلي في كل من الطور اليرقي الثاني والرابع المعامل بهذه المبيدات تم تقديرها. أظهرت السلالة الحقلية مستويات مختلفة من المقاومة. حيث كانت نسبة المقاومة (RR) لمبيدات السيبرميثرين والكلوربيريفوس والميثوكسيفينوزيد 12.7، 48.0، 13.8 على التوالي للطور اليرقي الثاني. بينما اظهر الطور اليرقي الرابع مقاومة فقط لمبيد السيبرميثرين و الكلوربيريفوس بنسبة مقاومة (RR) 120.8. و كانت نسبة الموت في السلالة الحقلية 75.6، 65.2، 91.6%، 93.3%، 98.9، 95.3%، 98.9، 95.3% في السلالة المعملية على التوالي. بينما كانت نسبة الموت في السلالة الحقلية 75.6، 65.2، 91.6% على الترتيب.

الكلمات المفتاحية: دودة ورق القطن، مقاومة المبيدات، جلوتاثيون إس ترانسفيريز، الاستريز الكلي.