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# COMPARATIVE TOXICITY OF CERTAIN INSECTICIDES AGAINST LARVAE AND EGG STAGES OF COTTON LEAFWORM LABORATORY AND FIELD STRAINS

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## ABSTRACT

**Resistance to major insecticide classes was diagnosed in the cotton leafworm (CLW) *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). This study was carried out to monitor the resistance levels in field strain collected from Abou- Homos, El-Behira government to some insecticides in comparison with laboratory strain. In addition, ovicidal activity of the formulated tested insecticides against laboratory and field strain of CLW egg masses was determined. The activities of glutathione *S*-transferases (GST) and total esterases were determined in both strains. The results revealed that 2$^{nd}$ instar larvae of the field strain exhibited different levels of resistance to cypermethrin, chlorpyrifos, and methoxfenozide with resistance ratios (RR) 12.7, 48.0, and 13.8, respectively, while 2$^{nd}$ instar larvae of field strain showed tolerance ratios 4.6 and 5.0 to chlorantraniliprole and chlorfluazuron, respectively. Fourth instar larvae exhibited high resistance levels to cypermethrin (120.8) and moderate resistance to chlorpyrifos (19.0). On the other hand, 4$^{th}$ instar larvae of field strain showed tolerance ratios to chlorantraniliprole (4.5), methoxfenozide (7.3) and**

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**chlorfluazuron (3.3). Concerning the ovicidal and residual toxicity, cypermethrin, chlorpyrifos, chlorantraniliprole and chlorfluazuron at the recommended field rate achieved 95.3, 98.9, 93.3 and 96.8% mortality of treated eggs and neonates of the laboratory strain, respectively. 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 is no significant difference between the ovicidal and residual toxicity of methoxfenozide against neonates of both strains. The field strain exerted elevated GST and esterases activity compared to the laboratory one. Therefore, monitoring of insecticide resistance is the first step for the resistance management programs.**

**Keywords:** Cotton leafworm; Insecticide resistance; Glutathione *S*-transferases (GST); 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 (**El-Sheikh, 2015**). The extensive and unwise use of insecticides, multiple generations of CLW and

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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 **(Prabhaker *et al.*, 1996)**. Moreover, studying the mechanisms of resistance development in insect pests against insecticides may help in management of resistance. Different mechanisms of resistance to insecticides have been identified in CLW, including enhanced metabolism, nerve insensitivity, reduced penetration and target site insensitivity **(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 Abou-Homos, El-Behira governorate, Egypt). Also, the activities of GST and total esterases in the field and laboratory strains were compared.

## MATERIALS 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 castor oil 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)**.

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**Field strain of CLW**

Cotton leafworm egg masses were collected from cotton fields of Abou-Homos, El-Behira governorate 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 methoxfenozide (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.

**Bioassay studies**

Toxicity of tested insecticides against 2$^{nd}$ and 4$^{th}$ instar larvae of CLW laboratory and field strains was carried out. Homogenous pieces of the castor oil 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 oil leaf pieces were transferred to a plastic cups, and the appropriate number and weight of starved larvae 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

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analysis **(Finney, 1971)**. Median lethal concentrations ($LC_{50}$) 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 (5 seconds) in recommended field rate of each tested insecticide, while the control was 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**

Total larvae of the 2nd instar and the collected midguts of the 4th 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 assay mixture consisted of 50 mM CDNB in 95% ethanol, 50 mM GSH and 20 µl of enzyme source in 2.5 ml of 50 mM phosphate buffer (pH 7.5). Changes in absorbance were measured at 340 nm for up to 3 min and the enzyme

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activity in terms of µM of CDNB conjugated $min^{-1}$ mg of enzyme $protein^{-1}$ was calculated using the extinction coefficient of 9.6 $mM^{-1}$ $cm^{-1}$. Protein concentration was measured according to **(Lowry *et al.*, 1951)** using bovine serum albumin (BSA) as a standard.

Esterase activity was determined using α-naphthyl acetate as a substrate according to the assay method described by **Van Asperen (1962)**. A total volume of 900 µl reaction mixture contained: 865 µl solution of 1.55 mM fast blue RR salt and 100 mM sodium phosphate buffer (pH 7.6), 30 µl of enzyme source and 5 µl of 90 mM α-naphthyl acetate in ethanol. The reaction mixture was vortexed and changes in absorption at 450 nm were monitored on Sequoia-Turner Model 340 spectrophotometer for up to 5 minutes. An assay mixture without enzyme was used as a blank. Enzyme activity was calculated as Δ OD $min^{-1}$ mg $protein^{-1}$.

### Statistical analysis

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

## RESULTS

### Toxicity of tested insecticides

Susceptibility of $2^{nd}$ and $4^{th}$ larval instars laboratory and field strains to selected insecticides is presented in Tables (1 and 2). Data showed that, $2^{nd}$ instar larvae of the field strain demonstrated varied resistance ratios to the tested insecticides. Field strain $2^{nd}$ 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

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was tolerant to chlorantraniliprole and chlorfluazuron with resistance ratio 4.6 and 5.0 (Table 1).

**Table (1): Comparative toxicity of some insecticides against laboratory and field strains of *Spodoptera littoralis* 2nd instar larvae**

| Insecticide | Strain | $LC_{50}$[a] (mg $L^{-1}$) | Confidence limits (mg $L^{-1}$) | Slope[b] ± SE | RR[c] |
|---|---|---|---|---|---|
| Cypermethrin | Lab. | 0.048 | 0.036 - 0.063 | 0.920 ± 0.075 | - |
| | Field | 0.608 | 0.441 - 0.844 | 0.781 ± 0.069 | 12.7 |
| Chlorpyrifos | Lab. | 1.083 | 0.846 - 1.391 | 1.158 ± 0.098 | - |
| | Field | 51.93 | 42.57 - 64.45 | 1.281 ± 0.110 | 48.0 |
| Chlorantraniliprole | Lab. | 0.135 | 0.102 - 0.182 | 0.985 ± 0.092 | - |
| | Field | 0.621 | 0.432 - 0.901 | 0.680 ± 0.067 | 4.6 |
| Methoxyfenozide | Lab. | 0.594 | 0.485 - 0.723 | 1.292 ± 0.106 | - |
| | Field | 8.21 | 6.14 - 11.76 | 0.909 ± 0.098 | 13.8 |
| Chlorfluazuron | Lab. | 0.702 | 0.571 - 0.864 | 1.235 ± 0.105 | - |
| | Field | 3.53 | 2.70 - 4.63 | 1.055 ± 0.094 | 5.0 |

[a] The concentration causing 50% mortality. [b] Slope of the concentration-mortality regression line ± standard error. [c] Resistance ratio equals $LC_{50}$ of field strain / $LC_{50}$ of laboratory strain.

Regarding 4th instar larvae, the field strain exerts high resistance levels to cypermethrin with resistance ratio 120.8. A moderate resistance is recorded to chlorpyrifos, where resistance ratio was 19.0. The 4th 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).

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**Table (2): Comparative toxicity of some insecticides against laboratory and field strains of *Spodoptera littoralis* 4th instar larvae**

| Insecticide | Strain | $LC_{50}$[a] (mg $L^{-1}$) | Confidence limits (mg $L^{-1}$) | Slope[b] ± SE | RR[c] |
|---|---|---|---|---|---|
| Cypermethrin | Lab. | 0.072 | 0.052 - 0.097 | 0.894 ± 0.090 | - |
| | Field | 8.70 | 6.50 - 11.43 | 0.927 ± 0.079 | 120.8 |
| Chlorpyrifos | Lab. | 8.80 | 7.15 - 10.75 | 1.38 ± 0.13 | - |
| | Field | 167.07 | 131.79 - 213.17 | 1.04 ± 0.10 | 19.0 |
| Chlorantraniliprole | Lab. | 2.82 | 2.07 - 3.90 | 0.825 ± 0.071 | - |
| | Field | 12.61 | 9.61 - 16.74 | 1.02 ± 0.09 | 4.5 |
| Methoxyfenozide | Lab. | 6.35 | 5.26 - 7.78 | 1.47 ± 0.14 | - |
| | Field | 46.05 | 37.25 - 57.13 | 1.33 ± 0.12 | 7.3 |
| Chlorfluazuron | Lab. | 3.62 | 2.77 - 4.77 | 1.04 ± 0.09 | - |
| | Field | 12.01 | 9.33 - 15.59 | 1.12 ± 0.10 | 3.3 |

[a] The concentration causing 50% mortality. [b] Slope of the concentration-mortality regression line ± standard error. [c] Resistance ratio equals $LC_{50}$ of field strain / $LC_{50}$ of laboratory strain.

## 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

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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).

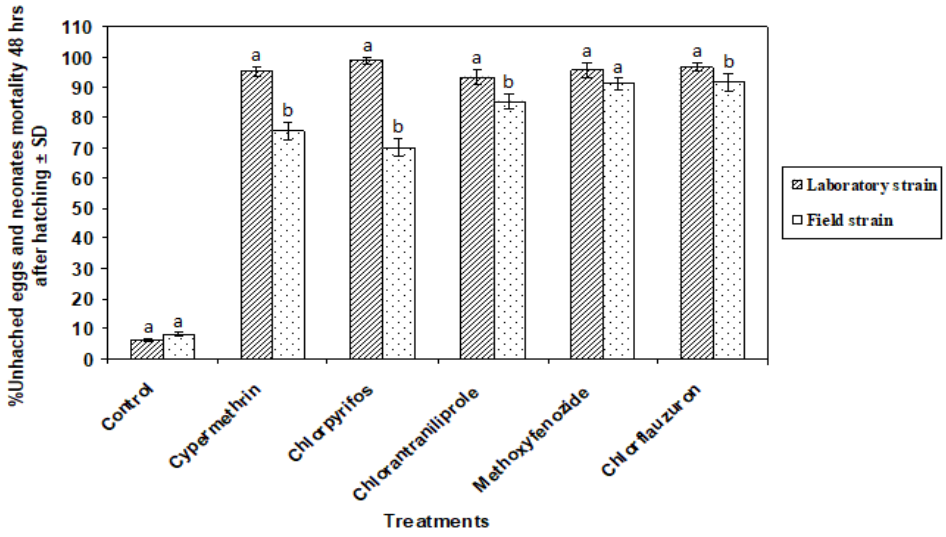


**Figure (1):** Ovicidal and residual toxicity on neonates of some insecticides at field rate against *Spodoptera* laboratory and field strains egg masses. Error bars represent standard deviation (SD) of three replications. Columns within a group with a letter in common are not significantly different according to Student-Newman Keuls (SNK) test (LSD at $P < 0.05$).

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## 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 the laboratory strain (1.53 ΔOD / mg protein / hr) (Table 3).

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

| Larval instar | Specific activity (ΔOD / mg protein / hr) ± SE | | Field / Lab. ratio |
|---|---|---|---|
| | Field strain | Laboratory strain | |
| 2nd | 2.08 a ± 0.08 | 1.04 b ± 0.05 | 2.00 |
| 4th | 3.24 a ± 0.04 | 1.53 b ± 0.07 | 2.12 |

Numbers 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**

| Larval instar | Specific activity (ΔOD / mg protein / min ± SE | | Field / Lab. ratio |
|---|---|---|---|
| | Field strain | Laboratory strain | |
| 2nd | 0.68 a ± 0.01 | 0.18 b ± 0.01 | 3.78 |
| 4th | 0.89 a ± 0.01 | 0.23 b ± 0.01 | 3.87 |

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

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Table (4) shows the activity of esterases in the laboratory and field strains. 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. While esterases activity were 0.18 and 0.23 ΔOD / mg protein / min in the 2nd and 4th larval instars of laboratory strain, it was 0.68 and 0.89 ΔOD / mg protein / min in the 2nd and 4th larval instars of field strain.

## DISCUSSION

Insecticide resistance is a serious worldwide problem, where many different insect species having become 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. **Tong *et al.*, (2013)** mentioned that *Spodoptera sp*. has the ability to develop resistance to wide range of insecticides.

Many studies had reported high resistance levels in CLW against organophosphate, 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

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**(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 activities of 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 complete 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,

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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 between the ovicidal and residual toxicity of cypermethrin, chlorpyrifos, chlorantraniliprole and chlorfluazuron against 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 resistance development to insecticides **(Tikar *et al.*, 2009; Pu *et al.*, 2010)**.

## CONCLUSION

Rotating between insecticides with different mode of action can prevent or delay the development of resistance in this insect to insecticides. In addition, the continuous monitoring of resistance is important for every resistance management program.

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**الملخص العربى**

## مقارنة السمية للعديد من المبيدات على كل من الطور اليرقى والبيض لسلالة معملية وحقلية لحشرة دودة ورق القطن

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تم إجراء تجارب التقييم الحيوى لرصد مستويات مقاومة السلالة الحقلية لدودة ورق القطن لبعض المبيدات مقارنة بالسلالة المعملية (السلالة الحساسة). حيث تم معاملة يرقات العمر الثانى والرابع لكلا السلالتين. وتم معاملة البيض ودراسة تأثير المبيدات المستخدمة على الفقس الحديث. كما تم تقدير نشاط إنزيمات الجلوتاثيون إس ترانسفيريز والإستريزيز لكلا السلالتين. أظهرت النتائج أن العمر اليرقى الثانى أظهر مستويات مختلفة من المقاومة لمركبات السيبرميثرين والكلوربيريفوس والميثوكسفينوزيد بمعدلات مقاومة 12.7 ، 48.0 ، 13.8 على التوالى. بينما أظهر العمر اليرقى الثانى للسلالة الحقلية درجة تحمل 4.6 ، 5.0 لكل من الكلورأنترانيليبرول وال الكلورفلوزيرون على التوالى. أما بالنسبة للعمر اليرقى الرابع للسلالة الحقلية قد أظهر مستويات مقاومة عالية لمبيد السيبرميثرين (120.8) ومستويات مقاومة متوسطة لمبيد الكلوربيريفوس (19.0) كما أظهر درجة تحمل لكلا من الكلورأنترانيليبرول والميثوكسيفينوزيد (7.3) والكلورفلوزيرون (3.3) ومن جهة أخرى عند معاملى كتل البيض بالمعدل الحقلى للمبيدات بالنسبة للسلالة المعملية للسيبرميثرين والكلوربيريفوس والكلورأنترانيليبرول والكلورفلوزيورون كانت نسبة موت الفقس 95.3، 98.9، 93.3، 96.8 % على التوالى. بينما كانت نسبة الموت للسلالة الحقلية 75.6، 70.0، 85.2، 91.6% على الترتيب. أظهرت السلالة الحقلية مستويات نشاط أعلى من السلالة المعملية فى إنزيمات الجلوتاثيون إس ترانسفيريز والإستيرازيز. من ناحية أخرى لم تسجل السلالة الحقلية أو المعملية أى إختلافات جوهرية فى نسبة الموت عند معاملة البيض بمبيد الميثوكسيفينوزيد. من خلال هذه الدراسة يمكن القول أن رصد ظاهرة المقاومة يعتبر خطوة هامة لوضع برامج للحد من ظاهرة المقاومة.