# Toxicity of Chlorantraniliprole and Lufenuron Against the Cotton Leafworm Larvae in Relation to Their Effects on AST, ALT and ALP Enzymes Activity

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**ABSTRACT:** The toxicity of chlorantraniliprole (an anthranilic diamide) and lufenuron (an insect growth inhibitor) against the 2nd and 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.) was investigated. The *in vivo* effects of chlorantraniliprole and lufenuron on the activity of each of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatases (ALP) activities of the *S. littoralis* 4th larval instar were also assessed. The bioassay of the 2nd instar larvae revealed that chlorantraniliprole ($LC_{50}$ = 0.009, 0.005 and 0.003 mg/l) was approximately as 234.3, 206.2 and 226.3 fold more toxic than lufenuron ($LC_{50}$ = 2.019, 1.031 and 0.679 mg/l) after three different exposure periods (48, 72 and 96 hrs, respectively). Concerning the 4th instar larvae, chlorantraniliprole ($LC_{50}$ = 6.645, 0.028 and 0.006 mg/l) was approximately as 2.01, 105.36 and 226.3 fold more toxic than lufenuron ($LC_{50}$ = 13.37, 2.95 and 1.87 mg/l) after 48, 72 and 96 hrs of exposure, respectively. In vivo effect of chlorantraniliprole and lufenuron enhanced the activity of AST and ALT and that enhancement was concentration and time of exposure dependent. Both chlorantraniliprole and lufenuron inhibited ALP activity; which was also concentration and time of exposure dependent. Based on the obtained results, chlorantraniliprole and lufenuron would be involved within the current foliar insecticide applications used for controlling *S. littoralis* in cotton fields.

**Keywords:** *Spodoptera littoralis*, chlorantraniliprole, lufenuron.

## INTRODUCTION

The cotton leafworm (CLW), *Spodoptera littoralis* (Boisd.) is one of the most destructive agriculture lepidopterous insect-pests. In Egypt, it can attack numerous economically important crops throughout the year (**Hatem *et al.*, 2009**). The chemical control of *S. littoralis* has been extensively reported especially in relation to cotton in Egypt (**Abo-El-Ghar *et al.*, 1986**). Extensive use of insecticides, multiple generations of CLW per annum and the availability of host crops, all over the year have contributed to the development of resistance in this insect-pest to many insecticide groups (**Abo Elghar *et al.*, 2005; Abou-Taleb, 2010**). Therefore, searching for an effective alternatives and/or pest control strategies is needed to avoid the increase of the selection pressure of the insect population to insecticides and provide adequate crop protection for sustainable food, feed and crops of fiber production. This need is met in part by the insecticide chlorantraniliprole, which has a novel mechanism of action and can be applied as an alternative insecticide for managing CLW (**Bentley *et al.*, 2010**).

Chlorantraniliprole is discovered by DuPont; it is also known as rynaxypyr (**Bentley *et al.*, 2010**). Studies have shown that chlorantraniliprole has exceptional insecticidal activity on a range of lepidopteran pests and many other orders, such as Coleoptera, Diptera, Isoptera and Hemiptera (**Sattelle *et al.*, 2008**; **Lahm *et al.*,**

**2009**). Chlorantraniliprole activates the unregulated release of internal calcium stores leading to $Ca^{2+}$ depletion, feeding cessation, lethargy, muscle paralysis and finally insect death (**Lahm *et al.*, 2005**). It blocks the feeding of lepidopteran larvae rapidly; the feeding cessation time being equivalent to that of nerve agents. Accordingly, it was ranked as the fastest-acting insecticide for lepidopteran control (**Hannig *et al.*, 2009**). In addition, the low ecotoxicology to non-target organisms such as birds, fish, mammals, earthworms and many other arthropods (**Lahm *et al.*, 2007**; **Larson *et al.*, 2012**) and no cross-resistance with other older classes of chemistry (**Cao *et al.*, 2010**; **Sial *et al.*, 2010**; **Wang *et al.*, 2010**) make it an excellent pest management tool.

It is critically important to establish the susceptibility of insects to newly developed insecticides before their widespread use. Therefore, the aim of this work was to assess the insecticidal activity of chlorantraniliprole compared to lufenuron against the 2nd and 4th larval instars (laboratory strain) of the cotton leafworm. The *in vivo* effects of chlorantraniliprole and lufenuron on certain enzymes activities [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatases (ALP)] of the 4th instar larvae of the cotton leafworm (laboratory strain) were also investigated.

## MATERIALS AND METHODS

### Experimental insect:

A laboratory strain of the cotton leafworm, *Spodoptera littoralis* larvae used for testing the evaluated insecticides was reared under the laboratory conditions of 25±2 ºC and 65±5 % RH according to **Eldefrawi *et al.* (1964)**.

### Tested insecticides:

Chlorantraniliprole (Coragen® 20% SC) was provided by DuPont Agricultural Chemicals Ltd.). Lufenuron (Match®5% EC) was supplied by Syngenta.

### Bioassay studies:

Toxicity of the formulated chlorantraniliprole (Coragen® 20% SC) and lufenuron (Match®5% EC) against 2nd and 4th instar larvae of *S. littoralis* laboratory strains was evaluated. Homogenous pieces of castor oil leaves were dipped in a series of chlorantraniliprole or lufenuron concentrations for 10 sec., held vertically to allow excess solution to drip off and dried at room temperature. Treated pieces of castor oil leaf were transferred to a plastic cups, and an appropriate and fixed number (10 larvae per cup) of starved larvae were added. Each concentration was replicated four times. Mortality percentages were recorded after 24, 48, 72, 96 hrs of treatment. Mortality counts were recorded and corrected according to Abbott equation (**Abbott, 1925**) and subjected to probit analysis (**Finney, 1971**).

**Biochemical studies**

***In vivo* effect of chlorantraniliprole and lufenuron on AST and ALT activities of 4th instar larvae of *S. littoralis* (Lab. strain)**

**Tissue preparation:** Starved 4th instar larvae of *S. littoralis* laboratory strain were fed on castor oil leaves dipped in chlorantraniliprole and lufenuron solution at $^1/_{10}$ $LC_{50}$, ½ $LC_{50}$ and $LC_{50}$. Larvae fed on untreated castor oil leaves were used as control. Suitable numbers of total larvae of each treatment were collected after 96 hrs post-treatment. These larvae were homogenized (1:10 w/v) in glass distilled water (pH=7) using glass homogenizer. The homogenate was centrifuged at 5000 rpm for 30 min at 4ºC using IEC-CRU 5000 cooling centrifuge. The supernatant was used for the estimation of AST (Aspartate aminotransferase), and ALT (Alanine aminotransferase) activities.

**Enzyme assay:** Activity of both enzymes AST and ALT was measured according to the method of **Reitman and Frankel (1957)**, using Diamond Diagnostic kit (Diamond Co. Egypt). In this method, 100 µl of enzyme source was added to 500 µl of 100 mM phosphate buffer of pH=7.2 containing 80 mM L-aspartate as a substrate for AST or 80 mM D-L-alanine as a substrate for ALT, and 4 mM ά-ketoglutarate. This mixture was incubated for 30 min at 37˚C. After that, 500 µl of developing color reagent (4 mM 2, 4-dinitrophenylhydrazine) was added and the solution was incubated for 20 min at room temperature. Lastly, 5 ml of 0.4 N NaOH was added then mixed and left at room temperature for five min. An assay mixture without enzyme source was used as the blank and the absorption was measured at the wave length of 546 nm using spectrophotometer (Milton Roy Spectronic 601). AST and ALT specific activities were determined as IU/mg protein/hr and calculated as a percentage of control.

***In vivo* effect of *S. littoralis* (Lab. strain) 4th instar larvae ALP activity by chlorantraniliprole and lufenuron**

**Tissue preparation:** Starved laboratory strain 4th instar larvae of *S. littoralis* were fed on castor oil leaves dipped in chlorantraniliprole and lufenuron solution at $^1/_{10}$ $LC_{50}$, ½ $LC_{50}$ and $LC_{50}$ plus the untreated larvae which have been used as control. Midguts were collected, after 96 hrs post-treatment, excised, repeatedly washed with ice-cold saline solution (0.9% NaCl) to remove foodstuff. These midguts were homogenized (1:10 w/v) in 100 mM phosphate buffer (pH= 9.8), using glass homogenizer. The homogenate was centrifuged at 5000 rpm for 30 min at 4ºC using IEC-CRU 5000 cooling centrifuge. The supernatant was used for ALP (alkaline phosphatases) activity estimation.

**Enzyme assay:** Activity of ALP was determined according to the method of **Dgkc (1972)**, using Diamond Diagnostic kit (Diamond Co. Egypt). In this method, 20 µl of the enzyme source was added to 1000 µl of 0.9 M diethanolamine buffer (pH 9.8) containing 0.6 mM magnesium ions and 1 mM p-nitrophenyl phosphate, then mixed in the cuvette, incubated for 30 seconds in the spectrophotometer (Milton Roy Spectronic 601), using a stopwatch simultaneously and the reading was done again after exactly 1, 2 and 3 minutes at 405 nm. ALP specific activity was determined as IU/mg protein/hr and calculated as a percent age of control.

**Statistical analysis:** Data was subjected to analysis of variance (ANOVA) (**CoStat Statistical Software, 1990**). The standard deviation (SD) of four replications was calculated. Means were compared with each other using Student-Newman Keuls (SNK) test (LSD at $P < 0.05$).
Insecticides relative potency was calculated as the following equation:

$$\text{Relative potency} = \frac{Lc_{50} \text{ of the least effective insecticide}}{Lc_{50} \text{ of the other insecticide}}$$

## RESULTS:

### Toxicity of chlorantraniliprole and lufenuron against the 2nd and 4th larval instars of *S. littoralis*

Toxicity of chlorantraniliprole and lufenuron against the 2nd and 4th larval instars of *S. littoralis* by leaves dipping technique (mixing the insecticide with food) after different exposure times is shown in Tables 1 and 2. Regarding the 2nd instar larvae, chlorantraniliprole ($LC_{50}$ = 0.009, 0.005 and 0.003 mg/l) was approximately 234.33, 206.20 and 226.33 fold more toxic than lufenuron ($LC_{50}$ = 2.019, 1.031 and 0.679 mg/l) after 48, 72 and 96 hrs post-treatment, respectively (Table 1).

**Table (1): Toxicity of chlorantraniliprole and lufenuron against 2nd instar larvae of *S. littoralis* after different exposure times**

| Insecticide | Exposure time (hrs) | $LC_{50}$ (mg/l) (95% CL) (Lower-Upper) | $LC_{25}$ (mg/l) (95% CL) (Lower-Upper) | $LC_{10}$ (mg/l) (95% CL) (Lower-Upper) | Slope ± SE | Relative potency at $LC_{50}$ level |
|---|---|---|---|---|---|---|
| Chlorantraniliprole | 48 | 0.009 (0.008 – 0.013) | 0.004 (0.003 - 0.005) | 0.002 (0.001 - 0.003) | 1.84 ± 0.27 | 234.33 |
| | 72 | 0.005 (0.005 - 0.006) | 0.002 (0.002 - 0.003) | 0.001 (0.001 - 0.002) | 2.03 ± 0.18 | 206.20 |
| | 96 | 0.003 (0.003 - 0.004) | 0.002 (0.001 - 0.002) | 0.001 (0.0005 - 0.009) | 1.92 ± 0.19 | 226.33 |
| Lufenuron | 48 | 2.109 (1.806 – 2.471) | 0.886 (0.682 - 1.073) | 0.406 (0.274 - 0.540) | 1.79 ± 0.17 | 1.00 |
| | 72 | 1.031 (0.859 – 1.24) | 0.373 (0.277 - 0.469) | 0.149 (0.093 - 0.21) | 1.53 ± 0.15 | 1.00 |
| | 96 | 0.679 (0.599-0.809) | 0.255 (0.183-0.327) | 0.106 (0.064-0.152) | 1.59 ± 0.147 | 1.00 |

**Table (2): Toxicity of chlorantraniliprole and lufenuron against 4th instar larvae of *S. littoralis* after different exposure times**

| Insecticide | Exposure time (hrs) | LC50 (mg/l) (95% CL) (Lower-Upper) | LC25 (mg/l) (95% CL) (Lower-Upper) | LC10 (mg/l) (95% CL) (Lower-Upper) | Slope ± SE | Relative potency at LC50 level |
|---|---|---|---|---|---|---|
| Chlorantraniliprole | 48 | 6.645 (1.27 – 177.8) | 0.025 (0.008 - 0.07) | 0.0002 (0.0001 - 0.00025) | 0.28 ± 0.03 | 2.01 |
| | 72 | 0.028 (0.02 - 0.04) | 0.005 (0.003 - 0.007) | 0.0009 (0.0004 - 0.002) | 0.87± 0.08 | 105.36 |
| | 96 | 0.006 (0.005 - 0.008) | 0.001 (0.001 - 0.002) | 0.0003 (0.0001 - 0.0006) | 0.99 ± 0.1 | 311.67 |
| Lufenuron | 48 | 13.37 (10.73 – 21.78) | 6.90 (5.92 – 7.96) | 3.80 (2.36 – 4.72) | 2.35± 0.50 | 1.00 |
| | 72 | 2.95 (2.55 – 3.39) | 1.35 (1.04 – 1.64) | 0.667 (0.442 - 0.889) | 1.98 ± 0.19 | 1.00 |
| | 96 | 1.87 (1.62 – 2.12) | 0.991 (0.741- 1.20) | 0.559 (0.535-0.747) | 2.45 ± 0.303 | 1.00 |

Concerning the treated 4th instar larvae, chlorantraniliprole ($LC_{50}$ = 6.645, 0.028 and 0.006 mg/l) was approximately 2.01, 105.36 and 311.67 times more toxic than lufenuron ($LC_{50}$ = 13.37, 2.95 and 1.87 mg/l) after 48, 72 and 96 hrs post-treatment, respectively (Table 2).

### *In vivo* effect of chlorantraniliprole and lufenuron on AST activity of *S. littoralis* 4th instar larvae

The *in vivo* effects of chlorantraniliprole and lufenuron on *S. littoralis* AST activity after different exposure times are presented in Tables 3 and 4. It is clear that, the activity of AST is increased with the increase of chlorantraniliprole and lufenuron concentrations and as will as the increase of the exposure time. The AST activity reached to its highest levels after 96 hrs of exposure, where it was 190.1% as compared to control at the concentration of 0.006 mg/l ($Lc_{50}$) of chlorantraniliprole (Table 3). In the case of lufenuron, AST reached to the highest activity (316.2% as compared to control) after 96 hrs at 1.870 mg/l ($Lc_{50}$) (Table 4).

### *In vivo* effect of chlorantraniliprole and lufenuron on ALT activity of *S. littoralis* 4th instar larvae

*In vivo* effects of chlorantraniliprole and lufenuron on ALT activity of *S. littoralis* 4th instar larvae after different exposure times are presented in Tables 5 and 6. It is clear that the exposure of CLW 4th instar larvae to different tested concentrations of chlorantraniliprole or lufenuron after different exposure times resulted in the increase of the ALT activity. When the 4th instar *S. littoralis* larvae were exposed to chlorantraniliprole at 0.006 mg/l for an exposure period of 96hrs, ALT activity was 173.4% as a percentage of control (Table 5). The activity of ALT was 221.1% as that of control (100%) when CLW 4th instar larvae were exposed to lufenuron at concentrations of 1.870 mg/l (Table 6).

### *In vivo* inhibition of alkaline phosphatase (ALP) activity of *S. littoralis* 4th instar larvae by chlorantraniliprole and lufenuron at different exposure times

*In vivo* inhibition of ALP activity within treated *S. littoralis* 4th instar larvae by chlorantraniliprole after different exposure times was investigated (Table 7). When *S. littoralis* larvae were treated with chlorantraniliprole at concentrations of 0.0006, 0.003 and 0.006 mg/l, ALP activity was 92.7, 81.8, and 68.2% as a percentage of control (100%), respectively after 48 hrs of exposure. ALP activity after 72 hrs of exposure was 78.1, 61.6, and 50.5% of that of control at the same concentrations, respectively. After 96 hrs of exposure, the ALP activity was 68.2, 59.5 and 47.3% of that of control at the same concentrations, respectively. The i*n vivo* inhibition of ALP activity of *S. littoralis* 4th instar larvae treated with lufenuron was also investigated after different exposure times (Table 8).

**Table (3): *In vivo* effect of chlorantraniliprole on the AST activity of *S. littoralis* 4th instar larvae after different exposure times**

| Insecticide concentration (mg/l) | Activity after different exposure times (hrs) | | | | | |
|---|---|---|---|---|---|---|
| | 48 | | 72 | | 96 | |
| | S.A* ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD |
| 0.0000 (control) | 670.9$^{c}$ ± 12.9 | 100.0 ± 1.92 | 580.4$^{d}$ ± 16.1 | 100.0 ± 2.77 | 571.7$^{d}$± 11.2 | 100.0 ± 1.96 |
| 0.0006 | 775.2$^{b}$± 22.3 | 115.5 ± 3.32 | 687.7$^{c}$ ± 26.6 | 118.5 ± 4.58 | 608.0$^{c}$± 21.0 | 106.3 ± 3.67 |
| 0.0030 | 803.7$^{b}$ ± 36.5 | 119.8 ± 5.44 | 742.0$^{b}$± 36.2 | 127.8 ± 6.23 | 686.7$^{b}$± 26.3 | 120.1 ± 4.60 |
| 0.0060 | 843.4 $^{a}$± 44.7 | 125.7 ± 6.66 | 823.8 $^{a}$± 41.3 | 141.9 ± 7.11 | 1086.8$^{a}$± 30.9 | 190.1 ± 5.40 |

*S.A = Specific activity (IU / mg protein / hr). Numbers within the same column followed by the same letter(s) are not significantly different according to Student-Newman Keuls (SNK) test ($LSD_{0.05}$).

**Table (4): *In vivo* effect of lufenuron on the AST activity of *S. littoralis* 4th instar larvae after different exposure times**

| Insecticide concentration (mg/l) | Activity after different exposure times (hrs) | | | | | |
|---|---|---|---|---|---|---|
| | 48 | | 72 | | 96 | |
| | S.A* ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD |
| 0.000(control) | 860.9d ± 32.4 | 100.0 ± 3.8 | 490.6c ± 36.3 | 100.0 ± 7.4 | 381.7d ± 11.2 | 100.0 ± 2.9 |
| 0.187 | 885.2c ± 52.5 | 102.8 ± 6.1 | 497.8c ± 6.7 | 101.5 ± 1.4 | 468.0c ± 0.0 | 122.6 ± 0.0 |
| 0.935 | 981.7b ± 26.7 | 114.0 ± 3.1 | 552.1b ± 46.2 | 112.5 ± 9.4 | 596.7b ± 6.3 | 156.3 ± 1.7 |
| 1.870 | 1153.4a ± 34.3 | 134.0 ± 4.0 | 654.9a ± 51.3 | 133.5 ± 10.5 | 1206.9a ± 30.9 | 316.2 ± 8.1 |

*S.A = Specific activity (IU / mg protein / hr). Numbers within the same column followed by the same letter are not significantly different according to Student-Newman Keuls (SNK) test ($LSD_{0.05}$).

**Table (5): *In vivo* effect of chlorantraniliprole on the ALT activity of *S. littoralis* 4th instar larvae after different exposure times**

| Insecticide concentration (mg/l) | Activity after different exposure times (hrs) | | | | | |
|---|---|---|---|---|---|---|
| | 48 | | 72 | | 96 | |
| | S.A* ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD |
| 0.0000(control) | 465.1c ± 27.4 | 100.0 ± 5.9 | 414.5d± 18.7 | 100.0 ± 4.6 | 381.8d± 22.0 | 100.0 ± 5.8 |
| 0.0006 | 592.4b ± 19.8 | 127.4 ± 4.3 | 510.8c± 10.5 | 126.9 ± 2.6 | 451.9c± 15.4 | 118.4 ± 4.0 |
| 0.0030 | 622.5b ± 12.5 | 133.9 ± 2.9 | 600.6b±9.7 | 118.9 ± 1.9 | 520.5b± 9.6 | 136.4 ± 2.5 |
| 0.0060 | 805.1a ± 9.2 | 173.2 ± 2.0 | 746.5a± 3.2 | 147.8 ± 0.6 | 661.7a ± 9.4 | 173.4 ± 2.5 |

*S.A = Specific activity (IU / mg protein / hr). Numbers within the same column followed by the same letter are not significantly different according to Student-Newman Keuls (SNK) test ($LSD_{0.05}$).

**Table (6): *In vivo* effect of lufenuron on the ALT activity of *S. littoralis* 4th instar larvae after different exposure times**

| Insecticide concentration (mg/l) | Activity after different exposure times (hr) | | | | | |
|---|---|---|---|---|---|---|
| | 48 | | 72 | | 96 | |
| | S.A* ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD |
| 0.000 (control) | 555.7c± 33.2 | 100.0 ± 6.0 | 518.0d± 38.7 | 100.0 ± 7.5 | 431.5d± 32.3 | 100.0 ± 7.5 |
| 0.187 | 765.1b ± 29.8 | 137.7 ± 5.4 | 602.1c± 30.5 | 116.2 ± 5.9 | 593.2c± 25.6 | 137.5 ± 5.9 |
| 0.935 | 799.5b± 22.5 | 143.9 ± 4.0 | 747.1b±29.7 | 144.2 ± 5.7 | 671.6b± 19.9 | 155.6 ± 4.6 |
| 1.870 | 878.2a± 19.2 | 158.0 ± 3.5 | 899.5a± 13.2 | 173.6 ± 2.5 | 953.9a ± 11.1 | 221.1 ± 2.6 |

*S.A = Specific activity (IU / mg protein / hr). Numbers within the same column followed by the same letter are not significantly different according to Student-Newman Keuls (SNK) test ($LSD_{0.05}$).

**Table (7): *In vivo* effect of chlorantraniliprole on the ALP activity of *S. littoralis* 4th instar larvae after different exposure times**

| Insecticide concentration (mg/l) | Activity after different exposure times (hr) | | | | | |
|---|---|---|---|---|---|---|
| | 48 | | 72 | | 96 | |
| | S.A ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD |
| 0.0000 (control) | 943.9a± 22.9 | 100.0 ± 2.4 | 880.4 a ± 26.1 | 100.0 ± 3.0 | 817.7a± 11.2 | 100.0 ± 1.4 |
| 0.0006 | 875.2b± 42.3 | 92.7 ± 4.5 | 687.7b ± 6.6 | 78.1 ± 0.7 | 558.0b± 0.0 | 68.2 ± 0.0 |
| 0.0030 | 771.7c± 16.5 | 81.8 ± 1.7 | 542.0 c± 36.2 | 61.6 ± 4.1 | 486.7c± 6.3 | 59.5 ± 0.8 |
| 0.0060 | 643.4d± 24.7 | 68.2 ± 2.6 | 444.8 d± 41.3 | 50.5 ± 4.7 | 386.8d± 30.9 | 47.3 ± 3.8 |

*S.A = Specific activity (IU / mg protein / hr). Numbers within the same column followed by the same letter (s) are not significantly different according to Student-Newman Keuls (SNK) test ($LSD_{0.05}$).

**Table (8): *In vivo* effect of lufenuron on the ALP activity of *S. littoralis* 4th instar larvae after different exposure times.**

| Insecticide concentration ( mg/l ) | Activity after different exposure times (hr) | | | | | |
|---|---|---|---|---|---|---|
| | 48 | | 72 | | 96 | |
| | S.A* ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD | S.A ± SD | Activity (%) ± SD |
| 0.000 (control) | 1172.4a ± 8.7 | 100.0 ± 0.7 | 1048.8a± 16.6 | 100.0 ± 1.6 | 1086.6a ± 1.2 | 100.0 ± 0.1 |
| 0.187 | 672.0b± 26.5 | 77.03 ± 3.0 | 681.7b± 5.6 | 65.0 ± 0.5 | 644.0b± 2.0 | 59.3 ± 0.02 |
| 0.935 | 605.7c± 16.9 | 69.43 ± 1.9 | 382.1c ± 26.5 | 36.4 ± 2.5 | 388.7c± 5.3 | 35.8 ± 0.49 |
| 1.870 | 477.7d± 14.2 | 54.76 ± 1.6 | 106.4d± 31.3 | 10.1 ± 3.0 | 165.2d± 19.9 | 15.2 ± 1.83 |

*S.A = Specific activity (IU / mg protein / hr). Numbers within the same column followed by the same letter are not significantly different according to Student-Newman Keuls (SNK) test ($LSD_{0.05}$).

When *S. littoralis* larvae were treated with lufenuron at concentrations of 0.187, 0.935, and 1.870 mg/l, the ALP activity was 77.03, 69.43 and 54.76% of that of control, respectively, after 48 hrs of exposure. ALP activity after 72 hrs of exposure was 65.0, 36.4, and 10.1% (as percentages of control), at the same concentrations, respectively. After 96 hrs of exposure, ALP activity was 59.3, 35.8, and 15.2 % of that of control at the same concentrations, respectively. From these data it could be concluded that the inhibition of ALP by chlorantraniliprole and lufenuron is concentration and time dependent.

## DISCUSSION

Many insect pests present an ongoing battle between the grower's ability to control the pest and the pest's ability to resist the available control methods. The discovery of new, novel insect control agents for use against insect pests has served as a focal point for insecticide research for more than five decades, since the insects were actually conquered for the first time about 55 years ago. Because there are several problems resulted from the intensive use of synthetic insecticides, trends in pest management now are emphasis on methods of controlling insect pests apart from it. Chlorantraniliprole is an example for searching about new compounds with high activity in insect control and low ecotoxicity.

In the present study, experiments were conducted to evaluate the susceptibility of *S. littoralis* to chlorantraniliprole and lufenuron. The carried out bioassay enhances the understanding of differential toxicity of these compounds

against the insect. From the data obtained in this study, it is obvious that chlorantraniliprole exert high toxicity against the 2nd and 4th larval instars of *S. littoralis*. The toxicity is increased as the concentration rates and exposure time increased while decreased as the insect instars increased. **Temple *et al.* (2009)** stated that Rynaxypyr® (chlorantraniliprole) demonstrated very good activity at relatively low rates against all three tested major caterpillar pests of cotton in their study, including tobacco budworm, bollworm, and fall armyworm. Because of the high insecticidal activity of Rynaxypyr® (chlorantraniliprole), **Lahm *et al.* (2007)** mentioned that Rynaxypyr® (chlorantraniliprole) could be an excellent option for resistance management strategies as an additional class of chemistry and mode of action for management of lepidopteran pests in cotton. Also, they declared that the high degree of mammalian safety, relatively low use rates compared to standard insecticides (pyrethroids, organophosphates, and carbamates), long residual properties, and broad spectrum of activity against lepidopteran pests will make chlorantraniliprole an excellent control option in an overall integrated pest management system.

Results obtained with both AST and ALT enzymes revealed that the exposure of *S. littoralis* 4th instar larvae to different concentrations of chlorantraniliprole and lufenuron at different exposure times resulted in an increase in the activity levels of these enzymes. The determined changes in the AST and ALT activity levels in the 4th instar larvae of *S. littoralis* following different periods of sub-lethal and lethal chlorantraniliprole and lufenuron exposure suggested that *S. littoralis* exhibited adaptive elevation in the activity levels of both the aminotransferase enzymes, thereby probably aiding gluconeogenesis through transamination of glucogenic amino acids to meet the energy demand under chlorantraniliprole and lufenuron toxicity. These data is in accordance with the arrived at results by other authors. **Ramaswamy *et al.* (1999)** reported that the activity levels of AST and ALT enzymes were elevated when the fish *Sarotherodon mossambicus* had been exposed to sub-lethal (3 mg/l) and lethal (25 mg/l) concentrations of the carbamate insecticide carbaryl. **Radwan *et al.* (1992)** reported that the possible mechanism involved in the elevation of AST and ALT levels may be due to the tissue damage, as a result of the increased synthesis and/or the decreased metabolism of both enzymes.

Alkaline phosphatases (LPs) are classically described as homodimeric nonspecific metalloenzymes which catalyze phosphomonesterase reactions (**Trowsdale *et al.,* 1990**). Phosphatases have been included in the list of detoxifying enzymes of insecticides; mostly of organophosphorus (**Oppenoorth, 1985**), however, fenvalerate and cypermethrin resistant larvae of *Helicoverpa armigera* showed higher activities of esterases, phosphatases and methyl paraoxon hydrolase compared with susceptible larvae (**Srinivas *et al.,* 2003**). In the present study, chlorantraniliprole and lufenuron inhibited ALP activity. Based on the toxicity of chlorantraniliprole and lufenuron against the laboratory strain and the *in vivo* inhibition of ALP activity, chlorantraniliprole and lufenuron could be usefully incorporated and used for developing a more effective management program for *S. littoralis*.

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## الملخص العربي

# سمية الكلورانترانيبرول واللوفنيرون ضد يرقات دودة ورق القطن وعلاقتها بتأثيرها على نشاط إنزيمات الأسبرتات أمينوترانسفيريز والألانين أمينوترانسفيريز والألكالين فوسفاتيز

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تم دراسة سمية مبيد حشرى من مجموعة الأنثرانيليك ثنائية الأميد (كلورانترانيبرول) ومبيد حشرى من مجموعة مثبطات النمو الحشرية (اللوفنيرون) ضد العمر الثاني والرابع ليرقات دودة ورق القطن. كما تم أيضاً دراسة تأثير كل منهما على نشاط إنزيمات الأسبرتات أمينو ترانسفيريز والألانين أمينو ترانسفيريز والألكالين فوسفاتيز للعمر اليرقي الرابع لدودة ورق القطن. وقد أظهرت نتائج سمية المركبات المختبرة على يرقات العمر الثاني لدودة ورق القطن أن الكلورانترانيبرول (التركيز اللازم منه لقتل ٥٠% من اليرقات المعاملة = ٠,٠٠٩ ، ٠,٠٠٥ ، و ٠,٠٠٣ مجم / لتر) كان أكثر سمية من اللوفنيرون (التركيز اللازم منه لقتل ٥٠% من اليرقات المعاملة = ٢,٠١٩ ، ١,٠٣١ ، ٠,٦٧٩مجم / لتر) حيث كانت سمية الأول تزيد تقريباً ٢٣٤,٣ ، ٢٠٦,٢ ، ٢٢٦,٣ مرة عن سمية الثاني وذلك بعد ٤٨ ، ٧٢ ، ٩٦ ساعة من المعاملة على التوالي. وفيما يتعلق بالعمر اليرقي الرابع أظهرت النتائج أن سمية الكلورانترانيبرول (التركيز المطلوب منه لقتل ٥٠% من اليرقات المعاملة = ٦,٦٤٥ ، ٠,٠٢٨ ، و ٠,٠٠٦ مجم / لتر) وكانت تزيد تقريباً بمعدل ٢,٠١ ، ١٠٥,٣٦ و ٢٢٦,٣ مرة أكثر من سمية اللوفنيرون (التركيز المطلوب لقتل ٥٠% من اليرقات المعاملة =١٣,٣٧ ، ٢,٩٥ ، و١,٨٧ مجم / لتر) بعد ٤٨ ، ٧٢ ، ٩٦ ساعة من المعاملة على التوالي.

وقد أدى كل من الكلورانترانيبرول واللوفنيرون عند التركيزات المختبرة إلى زيادة نشاط انزيمي الأسبرتات أمينوترانسفيريز والألانين أمينوترانسفيريز واعتمدت الزيادة فى النشاط على زيادة التركيز وفترة التعريض. وعلي الجانب الآخر فقد أدى كل من الكلورانترانيبرول واللوفنيرون إلى خفض نشاط إنزيم الألكالين فوسفاتيز، واعتمد هذا التثبيط على التركيز ووقت التعرض للمبيد.

وبالتالى بناءاً على النتائج التى تم التوصل إليها فى هذه الدراسة يمكن التوصية بإستخدام كل من الكلورانترانيبرول واللوفنيرون كمبيدات رش على الأوراق مع المبيدات المستخدمة حالياً فى مكافحة دودة ورق القطن على محصول القطن فى الحقل.