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(Original Article)

Toxicity of Certain Insecticides Against Different Stages of Cotton leafworm, *Spodoptera littoralis* (Boisd.)

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Abstract

The current study investigated the toxicity of five insecticides, indoxacarb, thiamethoxam+chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron against the 2nd and the 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* using leaf-dip bioassay under laboratory conditions. Based on the LC_{50s} values of the tested insecticides for the 2nd instar larvae of *S. littoralis*, the most toxic insecticide was indoxacarb (0.009, 0.006 and 0.001 ppm), followed by thiamethoxam + chlorantraniliprole (0.016, 0.01 and 0.009 ppm fold), chlorantraniliprole (0.21, 0.12 and 0.052 ppm fold), fipronil (3.79, 2.81 and 0.661 ppm fold) and lufenuron (5.19, 3.21 and 0.916 ppm fold) after 24, 48 and 72 hrs. post exposure, respectively. The toxicity index and relative potency values showed indoxacarb was more toxic for the 2nd instar larvae than thiamethoxam + chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron by (1.78, 23.33, 421.11 and 576.67), (1.67, 20.00, 468.33 and 535.0) and (9.0, 52.0, 661.0 and 916.0) fold, respectively. The 4th instar larvae showed high susceptibility to indoxacarb compared thiamethoxam + chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron. The LC_{50s} values of the tested insecticides revealed that indoxacarb was more effective than other insecticides (0.83, 0.61 and 0.32 ppm), followed by thiamethoxam + chlorantraniliprole (7.11, 5.23 and 1.11 ppm), fipronil (10.11, 9.13 and 3.26 ppm) and lufenuron (16.38, 14.39 and 4.11 ppm) after 24, 48 and 72 hrs. exposure, respectively. Therefore, our study recommended using indoxacarb, thiamethoxam + chlorantraniliprole and chlorantraniliprole in controlling CLW because of their mode of action are different and promising for using these insecticides in Integrated Pest Management programs.

Keywords: *Insecticides, Toxicity, leaf-dip bioassay, Spodoptera littoralis*

Introduction

The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is considered one of key insect that reason incredible harm to co). Thelants and various other field crops, including cotton and vegetables, leading to severe damage in Egypt (Al-Shannaf *et al*., 2006; Dahi *et al*., 2009; Saleh *et al*., 2018; Suarez-Lopez *et al*., 2022). The instar larvae of this insect pest can feed on approximately ninety economically important plant species belonging to 40 families. Synthetic chemical insecticides with a low degree of selectivity (broad spectrum) remain the main method

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for controlling this species. However, the continuous and exclusive application of these compounds has led to the development of populations resistant to many active substances (Kranthi *et al*., 2002; Hawkins *et al*., 2019; Suarez-Lopez *et al*., 2022). Various strategies are being explored to develop control options that minimize dependence on broad-spectrum synthetic insecticides. These include the creation of selective insecticides and the incorporation of biological control agents within integrated pest management (IPM) programs. Recently, the use of innovative insecticides like oxadiazines (indoxacarb), neonicotinoids (thiamethoxam), diamides (chlorantraniliprole), phenylpyrazoles (fipronil), and insect growth regulators (IGRs) such as benzoylphenyl urea (lufenuron) has become increasingly common. These insecticides are more selective in targeting only arthropods, yet their application raises concerns about the potential emergence of resistant populations (Osorio *et al*., 2008; Mosallanejad and Smagghe, 2009) and can affect natural enemies (Suarez-Lopez *et al*., 2020; Suarez-Lopez *et al*., 2022).

Indoxacarb represents a new class of insecticides, oxidiazines, with its stomach and contact action. It blocks the movement of sodium ions into the nervous system, resulting in paralysis and death of the pest (Wing *et al*., 2000; Wise *et al*., 2006). Indoxacarb has consistently been reported as highly effective against lepidopteran pests, particularly noctuids (Ahmad *et al*., 2003; Cook *et al*., 2004). Indoxacarb was a remarkably potent compound for controlling the cotton leafworm, *S. littoralis* and the beet armyworm, *S. exigua* (Moadeli *et al*., 2014). Diamide insecticides, including chlorantraniliprole, represent one of the most promising new classes of insecticides, offering exceptional effectiveness alongside minimal risk to mammals (Lahm *et al*., 2005; Bentley *et al*., 2010) and it is mode of action (Groups 28, IRAC). Anthranilic diamides interact with ryanodine receptors in insect muscle cells, triggering an uncontrolled release of calcium from the sarcoplasmic reticulum's internal stores. This process results in feeding inhibition, paralysis, and ultimately the death of the targeted insects (Hussein and Eldesouky, 2019). Chlorantraniliprole has an insecticidal effect on a wide range of lepidopteran pests (Hannig *et al.,* 2009; Lahm *et al*., 2005) besides other orders including Coleoptera, and Diptera (Lanka *et al.,* 2013; Sattelle *et al.,* 2008). This insecticide proves highly effective in managing lepidopterous insects, particularly those resistant to older insecticide classes. Its insect growth regulators (IGRs) work by hindering development, disrupting the regular functioning of the endocrine system, and interfering with reproduction or metamorphosis in the target pests (Kai *et al*., 2009).

Fipronil is pyrazole insecticide commercially used since 1993 (Tingle *et al*., 2003). Fipronil operates through a unique mode of action, distinct from the conventional biochemical pathways utilized by classical insecticides such as pyrethroids, organophosphates, and carbamates, to which many insects have developed resistance (Aajoud *et al*., 2003). Fipronil affects the GABA-gated ion channels, disrupting normal nerve signal transmission by targeting the GABA-regulated chloride channels. This interference impedes the flow of chloride ions, and at high enough doses, it leads to excessive neural excitation, severe paralysis, and ultimately, insect death (Gant *et al*., 1998; Cole *et al.,* 1993; Aajoud *et al*., 2003; IRAC, 2025). In addition, fipronil demonstrates a selective toxicity toward insects by having a tighter binding affinity toward the GABAregulated chloride channels of insects than the mammalian GABA receptors (Hainzl and Casida, 1996). Lufenuron, a chitin synthesis inhibitor, affects the development of lepidopteran larvae and leads to the production of infertile eggs. Insects exposed to this compound develop normally up to the moulting stage but are unable to complete the process due to disrupted synthesis of new cuticle (Tunaz and Uygun, 2004; El-Sheikh, 2015).

Therefore, the aim of this study was to assess the toxicity of five insecticides from different new groups: oxadiazines (indoxacarb), neonicotinoids- diamides (thiamethoxam + chlorantraniliprole), phenylpyrazoles (fipronil) and benzoylphenyl urea (lufenuron) against the 2nd and the 4th instar larvae of the cotton leafworm (laboratory strain) under laboratory conditions.

Materials and Methods

1. Test insect strain

The strain of the laboratory cotton leaf worm, *Spodoptera littoralis*, utilized in this study has been sustained in the Plant Protection Laboratory at the Faculty of Agriculture, Assiut University, Egypt, for over fifteen years without any exposure to insecticides. The insects are reared on castor leaves following the method outlined by Eldefrawi *et al.* (1964), under controlled conditions of 27 ± 2°C temperature, 65 ± 5% relative humidity, with a photoperiod of 16:8 hrs (light:dark).

2. Insecticides

Tested insecticides active ingredient, trade names, percentage of active ingredients, formulation types, chemical group, and (IRAC) mode of action classifications are listed in Table 1 and their structures are illustrated in Figure 1.

Table 1. Descriptions of the insecticides used against different stages of cotton leafworm, *S. littoralis* under laboratory conditions.

Active ingredient (a.i.)	Trade name % (a.i.)	Chemical group	(IRAC) Mode of action classifications
Indoxacarb	Avaunt 15% EC	Oxadiazines	(22A) Voltage-dependent sodium channel blockers
Thiamethoxam + chlorantraniliprole	Voliam flexi 40% WG	Neonicotinoids + Diamides	(4A) Nicotinic acetylcholine receptor (nAChR) competitive modulators -(28)
Chlorantraniliprole	Coragen 20% SC	Diamides	(28) Ryanodine receptor modulators
Fipronil	Coach 20% SC	Phenylpyrazoles	(2B) GABA-gated chloride channel blockers
Lufenuron	Reguron 5% EC	Benzoylphenyl urea	(15) Inhibitors of chitin biosynthesis affecting CHS1

* EC: Emulsifiable concentrate, WG: Wettable granules, SC: Suspension concentrate,

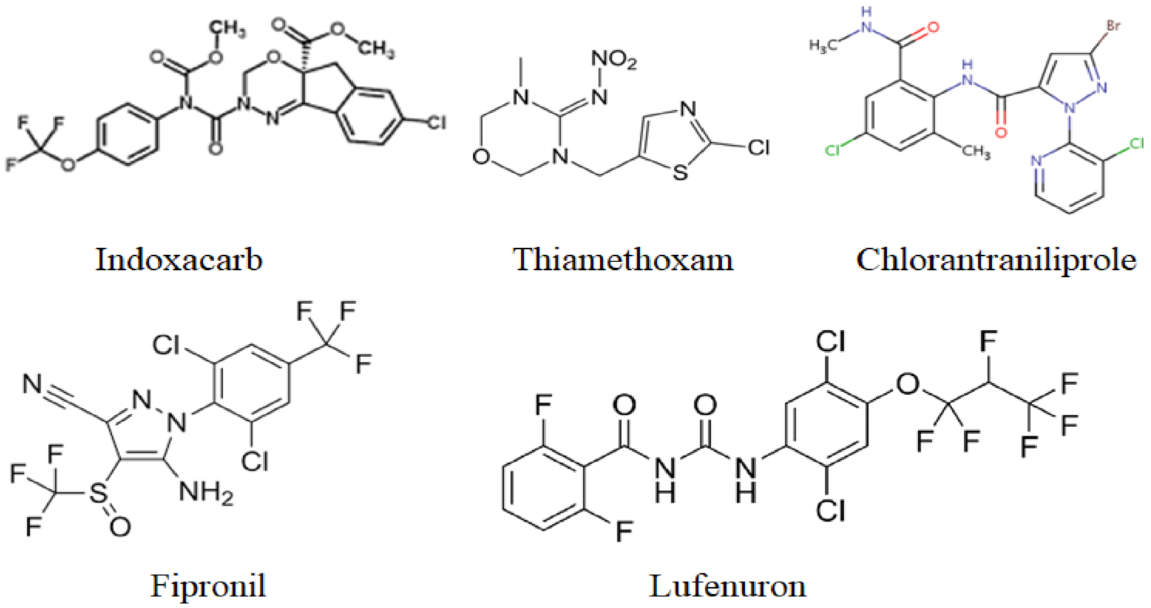

Fig. 1. Structure of selected insecticides, oxadiazines (indoxacarb), neonicotinoids (thiamethoxam), diamides (chlorantraniliprole), phenylpyrazoles (fipronil) and benzoylphenyl urea (lufenuron) used against different stages of cotton leafworm, *S. littoralis* under laboratory conditions.

3. Laboratory bioassay

The insecticidal properties of the specified compounds were evaluated against the 2nd and 4th instars of the cotton leafworm using leaf dipp bioassay method (O'Brien *et al.,* 1992). This study presents the laboratory test results aimed at determining the concentrations of these chemical compounds required to achieve a 50% mortality rate (LC_{50}) for the 2nd and 4th instars of the cotton leafworm, incorporating modifications in toxicity testing procedures. Six concentrations of an aqueous solution of each insecticide, supplemented with 0.05% Triton X-100 as a surfactant, were prepared. Subsequently, six sequentially diluted concentrations, ranging from 0.001 to 1000 ppm, were freshly prepared before application.

2nd and 4th instar larvae of the cotton leafworm were utilized. Serial concentrations of indoxacarb, thiamethoxam + chlorantraniliprole, chlorantraniliprole, fipronil, and lufenuron were prepared using TritonX-100 (0.05%) as a detergent and tap water as the solvent. Portions of castor bean leaves were immersed for 10 seconds in each test concentration and then placed in Petri dishes lined with filter paper for half an hour, allowing the treated leaves to air-dry. The selected larvae were first allowed to feed on these treated leaves for 24 hours and then on untreated fresh leaves for subsequent 24-hour and 48-hour intervals. Larval mortality was assessed 24, 48, and 72 hours after treatment. Control larvae were fed exclusively on treated leaves only with tape water plus detergent. Larvae were considered dead if no movement was observed upon gentle probing with a small brush. The toxicity experiment for each tested insecticide was performed twicely, and the results were adjusted using Abbott's formula (1925).

4. Statistical analysis

The median lethal concentrations (LC_{50} and LC_{90}) along with slope values of the insecticides were calculated through probit regression analysis using SPSS software (Version 25.0 for Windows, SPSS Inc., Chicago, USA). The results were expressed in parts per million (mg/L or ppm) as per Finney's (1971) methodology and were presented with their corresponding 95% confidence limits (CLs). As a result, LC_{50} values were utilized to calculate the toxicity index (Sun, 1950), which served to compare the relative effectiveness of the insecticides tested. Significant differences in lethal concentrations were determined when the corresponding 95% confidence limits did not overlap. All figures and statistical analyses were performed using GraphPad Prism 5 software (San Diego, CA).

Results

1. Toxicity of certain insecticides on the 2nd instar larvae of cotton leafworm, *S. littoralis*

The susceptibility of the 2nd instar larvae of *S. littoralis* to toxic effects of indoxacarb, thiamethoxam + chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron is shown in Table 2, 3 and 4 and Fig. 2 A, B and C. There are direct correlations between insecticide and period of exposure (24, 48 and 72 hrs), as the toxicity increases with an increasing period of exposure. Comparison between the LC_{50s} of the tested insecticides for the 2nd instar larvae of *S. littoralis* showed that the most toxic insecticide was indoxacarb (0.009, 0.006 and 0.001ppm), followed by thiamethoxam + chlorantraniliprole (0.016, 0.01 and 0.009 ppm), chlorantraniliprole (0.21, 0.12 and 0.052 ppm), fipronil (3.79, 2.81 and 0.661 ppm and lufenuron (5.19, 3.21 and 0.916 ppm) after 24, 48 and 72 hrs. exposure, respectively. Y value for each line estimated by probit regression was equal to zero when LC_{50} (x) was converted to log base 10. The slope values of the regression lines of the tested insecticides (Table 2, 3 and 4) and (Fig. 2 and 3 A, B and C) in ascending order are chlorantraniliprole (0.98, 1.11 and 1.41), lufenuron (1.09, 1.15 and 1.30), indoxacarb (1.20, 1.36 and 1.55), fipronil (1.52, 1.65 and 1.79) and thiamethoxam + chlorantraniliprole (1.78, 1.81 and 1.88) after 24, 48 and 72 hrs. exposure, respectively, which indicates that the response of the insect population to thiamethoxam + chlorantraniliprole is higher than the other tested insecticides and more homogenous.

Comparison between the LC_{90s} of the tested insecticides for the 2nd instar larvae of *S. littoralis* (Table 2, 3 and 4) and (Fig. 2 and 3 A, B and C) showed that the highest effective one is indoxacarb (0.056, 0.035 and 0.023 ppm), followed by thiamethoxam + chlorantraniliprole (0.53, 0.36 and 0.77 ppm), chlorantraniliprole (5.08, 0.83 and 0.98 ppm), fipronil (17.22, 13.29 and 7.11 ppm) and lufenuron (38.14, 19.36 and 8.95 ppm) after 24, 48 and 72 hrs. exposure, respectively.

Based on the relative potency, indoxacarb was more toxic for the 2nd instar larvae than thiamethoxam + chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron by (1.78, 23.33, 421.11 and 576.67), (1.67, 20.00, 468.33 and 535.0) and (9, 52.0, 661.0 and 916.0) fold, respectively. These results indicated that indoxacarb, thiamethoxam + chlorantraniliprole and chlorantraniliprole were the most toxic insecticides against the 2nd instar larvae of *S. littoralis*, followed by fipronil and lufenuron (Fig. 4 A, B and C).

Table 2. Toxicity of certain insecticides on the 2nd instar larvae of cotton leafworm, *S. littoralis* after 24 hrs exposure.

Insecticides	Slope± SE	LC_{50} (ppm)	95% Confidence Limits (CL)		Toxicity Index (1)	Relative Potency(2)	LC_{90} (ppm)	95% Confidence Limits (CL)		χ^2(df)
			Lower	Upper				Lower	Upper	
Indoxacarb	1.20 ±0.19	0.009a	0.006	0.012	100	1	0.056a	0.051	0.061	2.55(5)
Thiamethoxam-chlorantraniliprole	1.78 ±0.22	0.016b	0.011	0.21	56.25	1.78	0.53b	0.46	0.59	2.17(5)
Chlorantraniliprole	0.98 ±0.18	0.21c	0.17	0.28	4.28	23.33	5.08c	4.71	5.88	4.22(5)
Fipronil	1.52±0.21	3.79d	3.61	3.91	0.24	421.11	17.22d	16.96	18.57	1.83(5)
Lufenuron	1.09±0.20	5.19e	3.16	7.89	0.17	576.67	38.14e	32.44	43.11	4.68(5)

(1): Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) × 100]. (2): Relative Potency = LC_{50} for least toxic tested compound/LC_{50} of the tested compound. LC_{50} and LC_{90} values having different letters are significantly different (95% CL did not overlap).

Table 3. Toxicity of certain insecticides on the 2nd instar larvae of cotton leafworm, *S. littoralis* after 48 hrs exposure.

Insecticides	Slope± SE	LC_{50} (ppm)	95% Confidence Limits (CL)		Toxicity Index(1)	Relative Potency(2)	LC_{90} (ppm)	95% Confidence Limits (CL)		χ^2(df)
			Lower	Upper				Lower	Upper	
Indoxacarb	1.36±0.19	0.006a	0.003	0.009	100	1	0.035a	0.031	0.039	2.31(5)
Thiamethoxam-chlorantraniliprole	1.81±0.21	0.01b	0.008	0.07	60.00	1.67	0.36b	0.28	0.43	1.70(5)
chlorantraniliprole	1.11 ±0.18	0.12c	0.08	0.16	5.00	20.00	0.83c	0.79	0.96	3.11(5)
Fipronil	1.65±0.20	2.81d	2.73	2.81	0.21	468.33	13.29d	12.61	14.93	1.70(5)
Lufenuron	1.15 ±0.21	3.21e	2.91	3.56	0.19	535.00	19.36e	16.23	23.18	4.50(5)

(1): Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) × 100]. (2): Relative Potency = LC_{50} for least toxic tested compound/LC_{50} of the tested compound. LC_{50} and LC_{90} values having different letters are significantly different (95% CL did not overlap).

Table 4. Toxicity of certain insecticides on the 2nd instar larvae of cotton leafworm, *S. littoralis* after 72 hrs exposure.

Insecticides	Slope± SE	LC_{50} (ppm)	95% Confidence Limits (CL)		Toxicity Index(1)	Relative Potency(2)	LC_{90} (ppm)	95% Confidence Limits (CL)		χ^2(df)
			Lower	Upper				Lower	Upper	
Indoxacarb	1.55±0.21	0.001a	0.0007	0.005	100	1	0.023a	0.017	0.026	1.92(5)
Thiamethoxam-chlorantraniliprole	1.88±0.24	0.009b	0.006	0.013	11.11	9.00	0.77b	0.71	0.82	1.55(5)
Chlorantraniliprole	1.41 ±0.22	0.052c	0.048	0.068	1.92	52.00	0. 98c	0. 91	1.04	2.23(5)
Fipronil	1.79±0.20	0.661d	0.61	0.73	0.15	661.00	7.11d	6.98	7.45	1.37(5)
Lufenuron	1.30±0.19	0.916e	0.78	0.99	0.11	916.00	8.95e	8.18	9.33	3.61(5)

(1): Toxicity index = [(LC_{50} of the most toxic tested compound/LC_{50} of the tested compound) × 100]. (2): Relative Potency = LC_{50} for least toxic tested compound/LC_{50} of the tested compound. LC_{50} and LC_{90} values having different letters are significantly different (95% CL did not overlap).

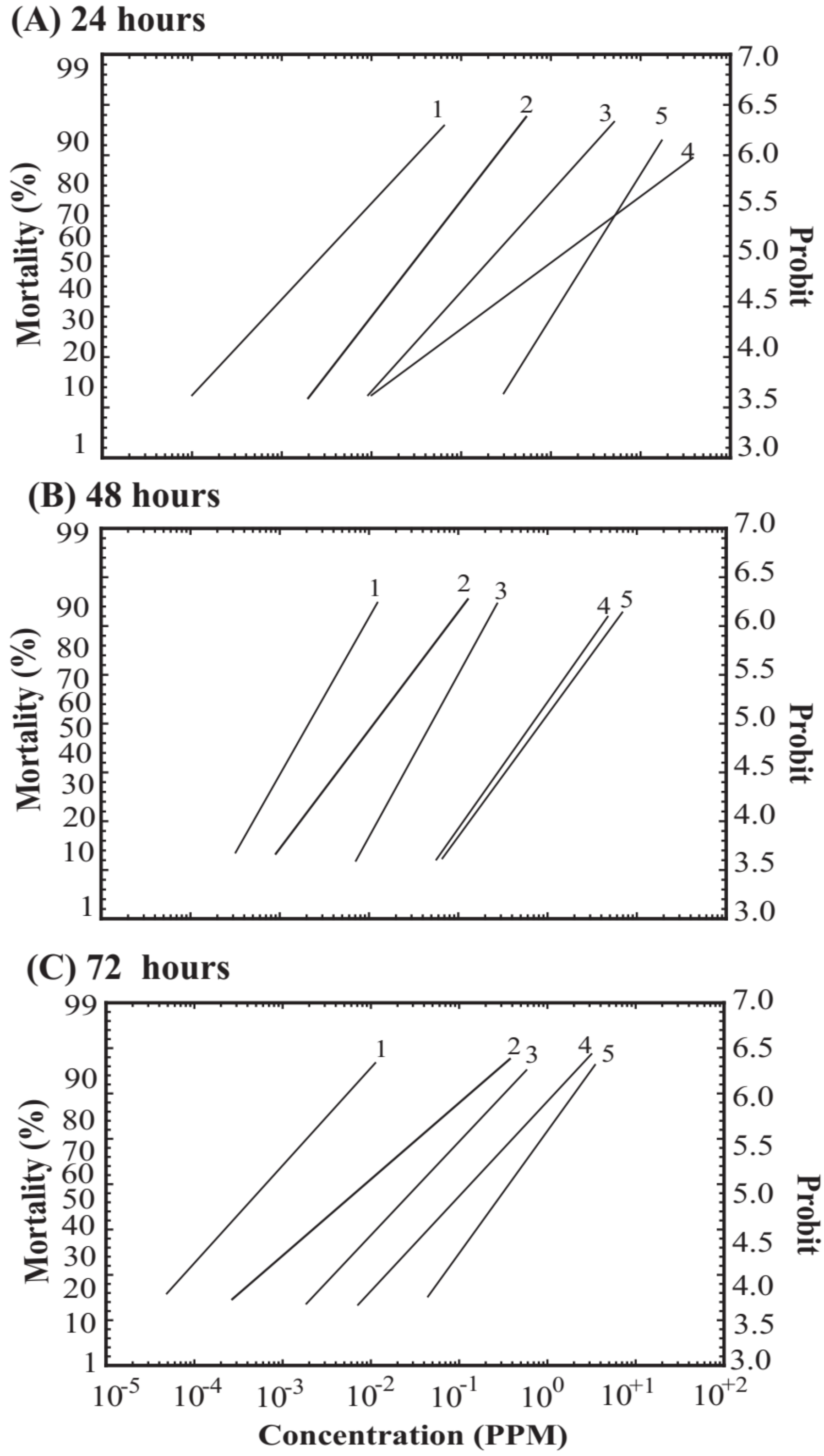

Fig. 2. Toxicity lines of certain insecticides on the 2nd instar larvae of cotton leafworm, *S. littoralis* after 24h (A), 48h (B) and 72 h. (C) exposure. 1: Indoxacarb, 2: Thiamethoxam-chlorantraniliprole, 3: chlorantraniliprole, 4: Fipronil and 5: Lufenuron.

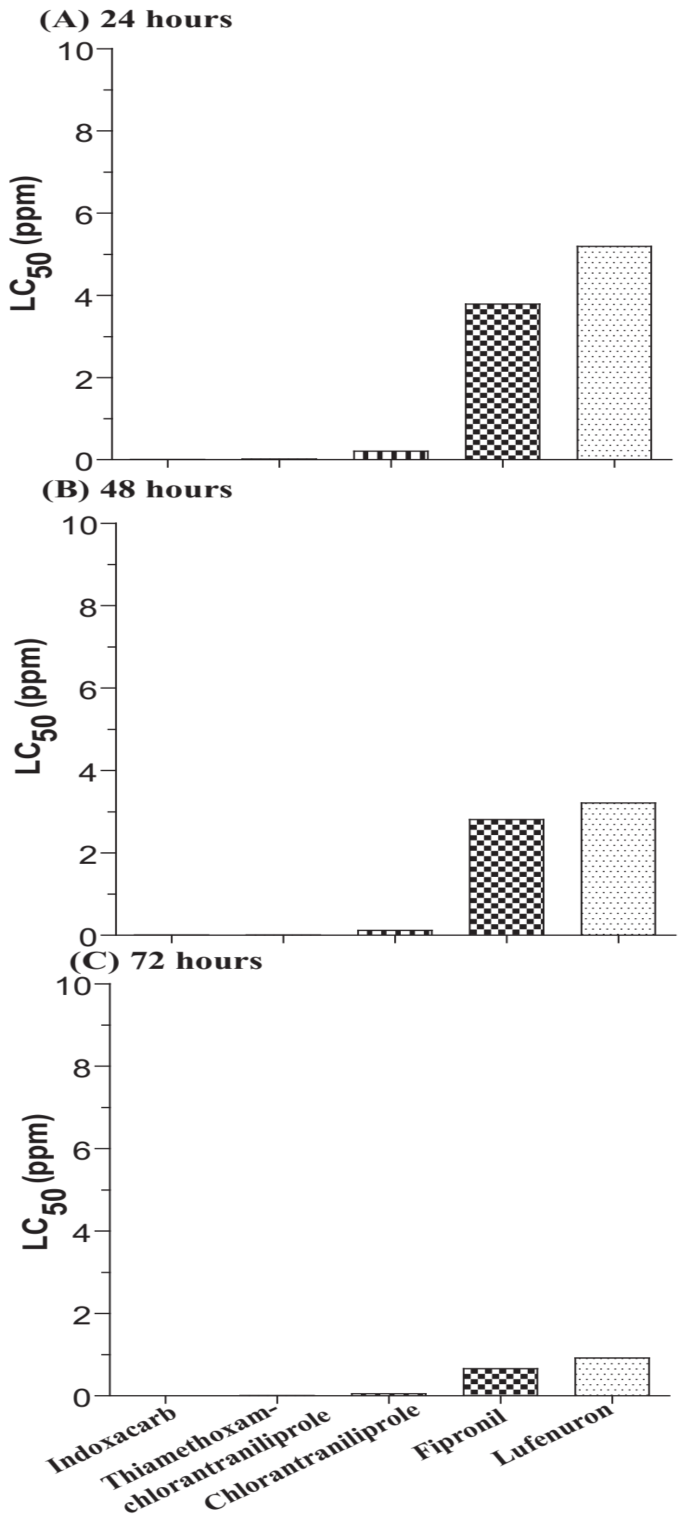

Fig. 3. LC_{50} (ppm) of certain insecticides on the 2nd instar larvae of cotton leafworm, *S. littoralis* after 24h (A), 48h (B) and 72h (C) exposure.

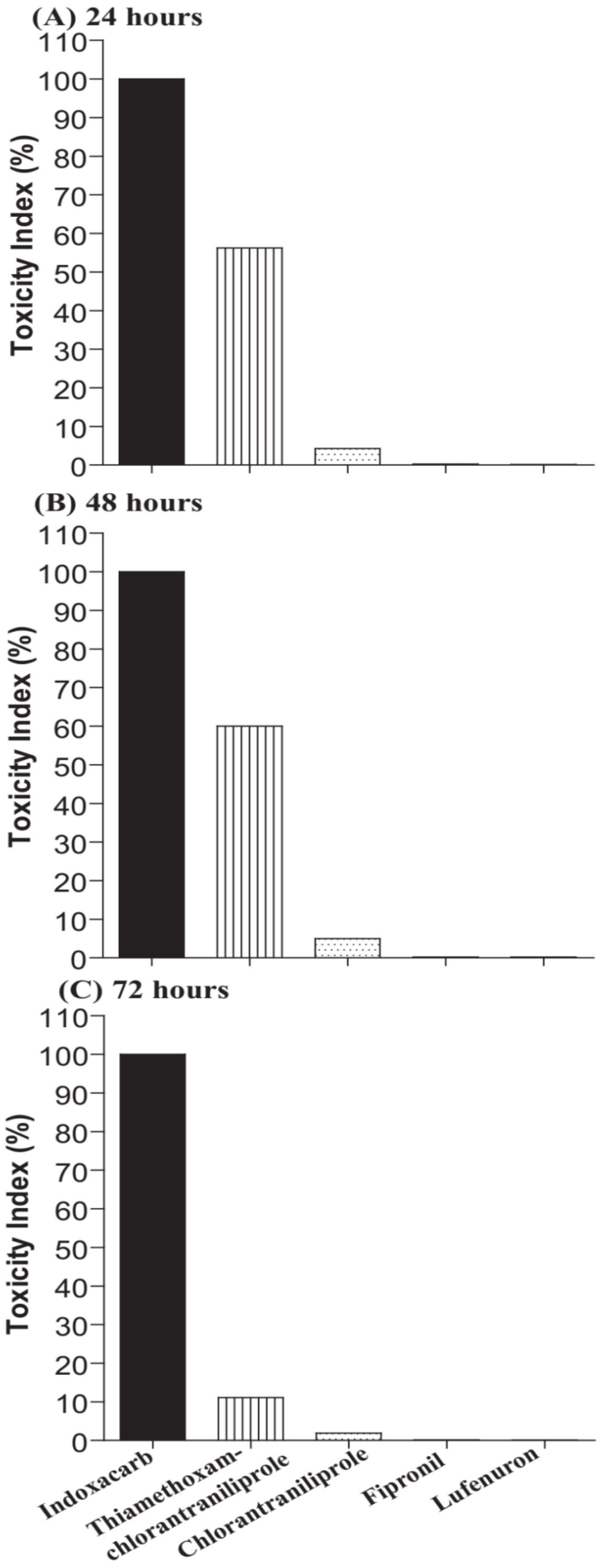

Fig. 4. Toxicity index of certain insecticides on the 2nd instar larvae of cotton leafworm, *S. littoralis* after 24h (A), 48h (B) and 72h (C) exposure.

2. Toxicity of selected insecticides on the 4th instar larvae of cotton leafworm, *S. littoralis*

Similar to the 2nd instar, the 4th instar larvae showed high susceptibility to indoxacarb compared thiamethoxam + chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron in both acute and chronic toxicity (Table 5, 6 and 7). Based on the LC_{50s} values of the tested insecticides for the 4th instar larvae of *CLW* showed that indoxacarb was more effective than other insecticides (0.83, 0.61 and 0.32 ppm), followed by thiamethoxam + chlorantraniliprole (7.11, 5.23 and 2.18 ppm), chlorantraniliprole (8.76, 6.89 and 1.17 ppm), fipronil (10.11, 9.13 and 2.19 ppm and lufenuron (16.38, 14.39 and 4.11 ppm) after 24, 48 and 72 hrs. exposure, respectively. The slopes of the regression lines of the tested insecticides (Table 5, 6 and 7) and (Fig. 5 A, B and C) in ascending order are indoxacarb (0.78, 0.85 and 1.10), lufenuron (0.0.95, 1.10 and 1.30), chlorantraniliprole (0.98, 1.20 and 1.40), thiamethoxam + chlorantraniliprole (1.20, 1.35 and 1.55) and fipronil (1.25, 1.40 and 1.65) after 24, 48 and 72 hrs. exposure, respectively, which indicates that the response of the insect population to fipronil is higher than the other tested insecticides and more homogenous. The slope of the insecticide's toxicity lines is higher in chronic toxicity (72 hrs.) than that in acute toxicity (24 hrs).

The LC_{90s} of the tested insecticides for the 4th instar larvae of *S. littoralis* (Table 5, 6 and 7) and (Fig. 5) showed that the highest effective one is indoxacarb (6.12, 3.18 and 6.11 ppm), followed by thiamethoxam + chlorantraniliprole (46.41, 27.22 and 11.84 ppm), chlorantraniliprole (64.13, 38.14 and 12.77 ppm), fipronil (78.11, 46.53 and 13.10 ppm) and lufenuron (229.32, 201.46 and 31.16 ppm) after 24, 48 and 72 hrs. exposure, respectively.

As a results of the toxicity index and relative potency, indoxacarb was more toxic than thiamethoxam + chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron by (8.56, 10.55, 12.18 and 19.73), (8.57, 11.29, 14.96 and 23.59) and (3.66, 9.31, 10.18 and 12.84) fold, respectively. These results indicated that indoxacarb, thiamethoxam + chlorantraniliprole and chlorantraniliprole were the most toxic insecticides against the 4th instar larvae of *S. littoralis*, followed by fipronil and lufenuron (Table 5, 6 and 7) and (Fig. 7 A, B and C).

Table 5. Toxicity of certain insecticides on the 4th instar larvae of cotton leafworm, *S. littoralis* after 24 hrs exposure.

Insecticides	Slope± SE	LC_{50} (ppm)	95% Confidence Limits (CL)		Toxicity Index(1)	Relative Potency(2)	LC_{90} (ppm)	95% Confidence Limits (CL)		χ^2(df)
			Lower	Upper				Lower	Upper	
Indoxacarb	0.78 ±0.19	0.83a	0.76	0.91	100	1	6.12a	5.96	6.43	5.11(5)
Thiamethoxam-chlorantraniliprole	1.20±0.19	7.11b	6.98	7.35	11.67	8.56	46.41b	42.33	49.56	2.61(5)
chlorantraniliprole	0.95 ±0.18	8.76c	8.61	8.88	9.47	10.55	64.13c	61.18	68.01	2.31(5)
Fipronil	1.25 ±0.19	10.11d	9.78	11.36	8.21	12.18	78.11d	76.31	80.14	3.14(5)
Lufenuron	0.95 ±0.19	16.38e	14.23	18.81	5.07	19.73	229.32e	219.11	236.17	4.19(5)

(1): Toxicity index = [(LC_{50} of the most toxic tested insecticide/LC_{50} of the tested insecticide) × 100]. (2): Relative Potency = LC_{50} for least toxic tested insecticide/LC_{50} of the tested insecticide. LC_{50} and LC_{90} values having different letters are significantly different (95% CL did not overlap).

Table 6. Toxicity of certain insecticides on the 4th instar larvae of cotton leafworm, *S. littoralis* after 48 hrs exposure.

Insecticides	Slope± SE	LC_{50} (ppm)	95% Confidence Limits (CL)		Toxicity Index(1)	Relative Potency(2)	LC_{90} (ppm)	95% Confidence Limits (CL)		χ^2(df)
			Lower	Upper				Lower	Upper	
Indoxacarb	0.85 ±0.18	0.61a	0.52	0.78	100	1	3.18a	2.98	3.31	3.46(5)
Thiamethoxam-chlorantraniliprole	1.35 ±0.20	5.23b	5.16	5.28	11.66	8.57	27.22b	26.19	29.41	1.81(5)
chlorantraniliprole	1.20 ±0.19	6.89c	6.61	6.96	8.85	11.29	38.14c	36.98	39.71	2.19(5)
Fipronil	1.40 ±0.22	9.13d	9.07	9.25	6.68	14.96	46.53d	44.11	49.16	1.55(5)
Lufenuron	1.10 ±0.20	14.39e	11.86	18.23	4.24	23.59	201.46e	189.31	221.14	3.16(5)

(1): Toxicity index = [(LC_{50} of the most toxic tested insecticide/LC_{50} of the tested insecticide) × 100]. (2): Relative Potency = LC_{50} for least toxic tested insecticide/LC_{50} of the tested insecticide. LC_{50} and LC_{90} values having different letters are significantly different (95% CL did not overlap).

Table 7. Toxicity of certain insecticides on the 4th instar larvae of cotton leafworm, *S. littoralis* after 72 hrs exposure.

Insecticides	Slope± SE	LC_{50} (ppm)	95% Confidence Limits (CL)		Toxicity Index(1)	Relative Potency(2)	LC_{90} (ppm)	95% Confidence Limits (CL)		χ^2(df)
			Lower	Upper				Lower	Upper	
Indoxacarb	1.10 ±0.19	0.32a	0.27	0.39	100	1	6.11a	5.98	6.33	1.61 (5)
Thiamethoxam-chlorantraniliprole	1.55 ±0.22	1.17b	1.11	1.26	27.35	3.66	11.84b	10.13	12.86	2.11(5)
chlorantraniliprole	1.40 ±0.20	2.18c	1.96	2.48	10.74	9.31	12.77c	10.65	14.91	1.98(5)
Fipronil	1.65 ±0.22	3.26d	2.99	3.44	14.16	10.18	13.10d	12.98	13.25	1.73(5)
Lufenuron	1.30 ±0.20	4.11e	3.98	5.18	10.29	12.84	31.16e	28.19	35.43	2.28(5)

(1): Toxicity index = [(LC_{50} of the most toxic tested insecticide/LC_{50} of the tested insecticide) × 100]. (2): Relative Potency = LC_{50} for least toxic tested insecticide/LC_{50} of the tested insecticide. LC_{50} and LC_{90} values having different letters are significantly different (95% CL did not overlap).

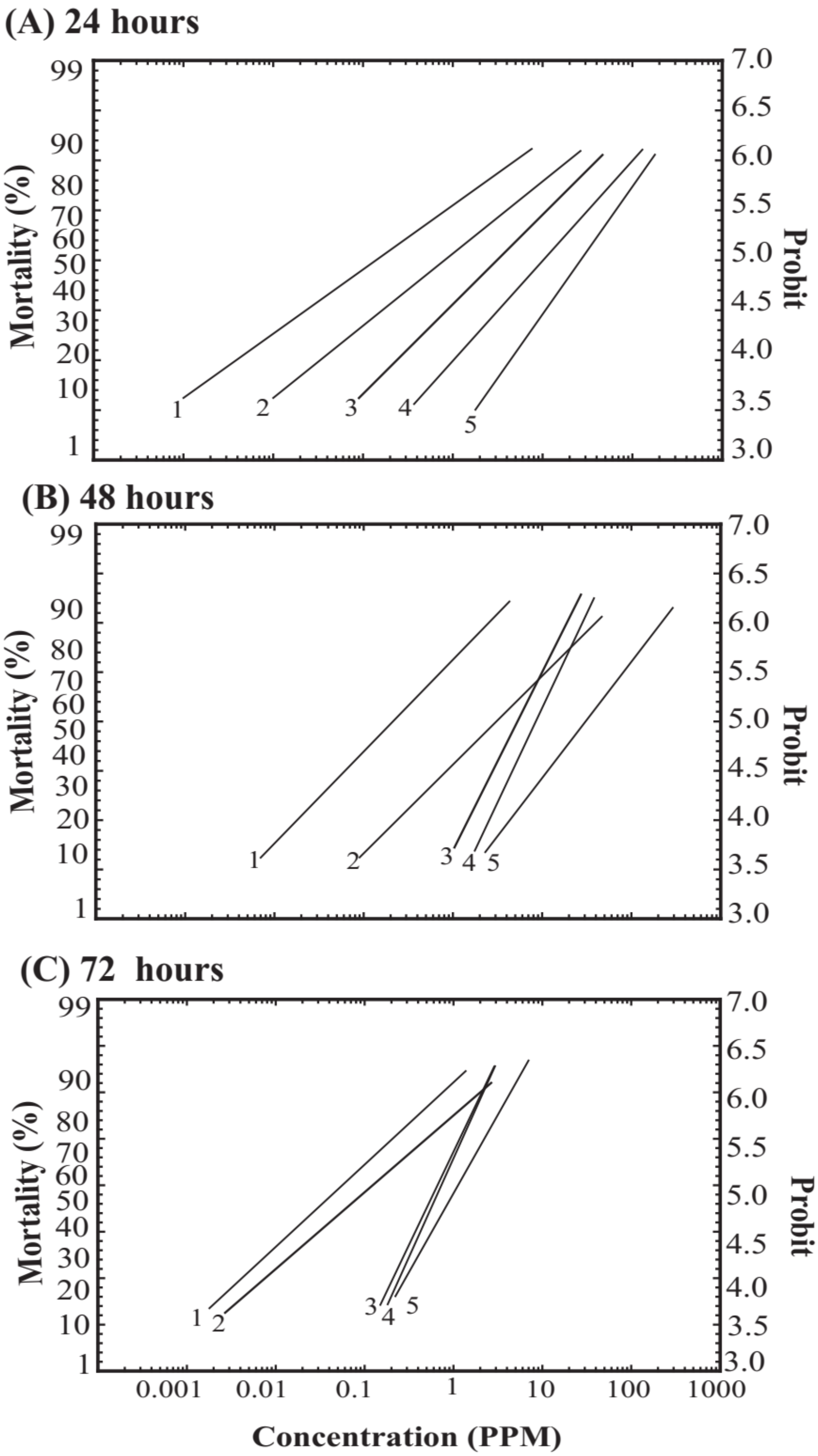

Fig. 5. Toxicity lines of certain insecticides on the 4th instar larvae of cotton leafworm, *S. littoralis* after 24h (A), 48h (B) and 72h (C) exposure. 1: Indoxacarb, 2: Thiamethoxam-chlorantraniliprole, 3: chlorantraniliprole, 4: Fipronil and 5: Lufenuron.

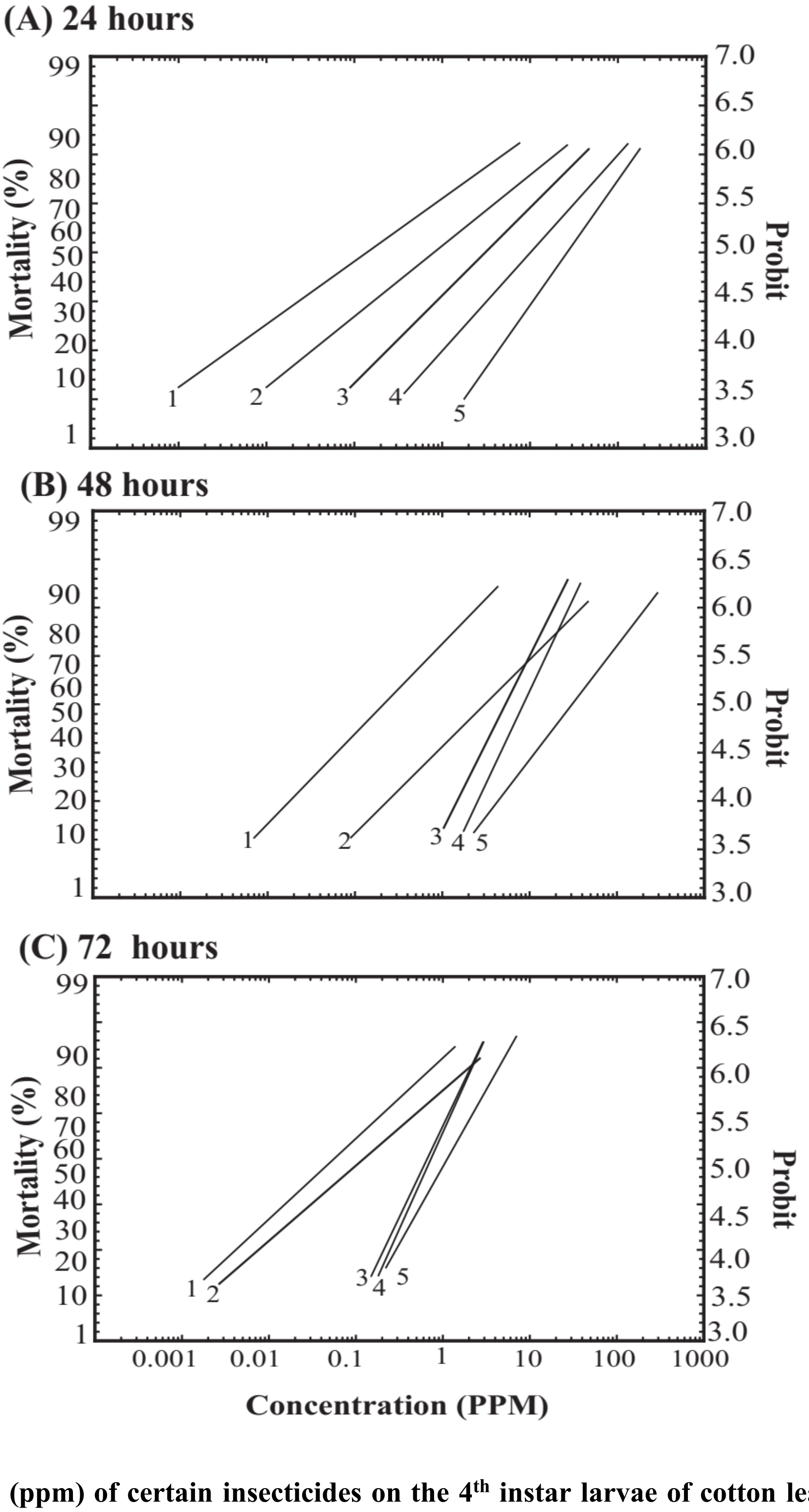

Fig. 6. LC50 (ppm) of certain insecticides on the 4th instar larvae of cotton leafworm, *S. littoralis* after 24h (A), 48h (B) and 72h (C) exposure.

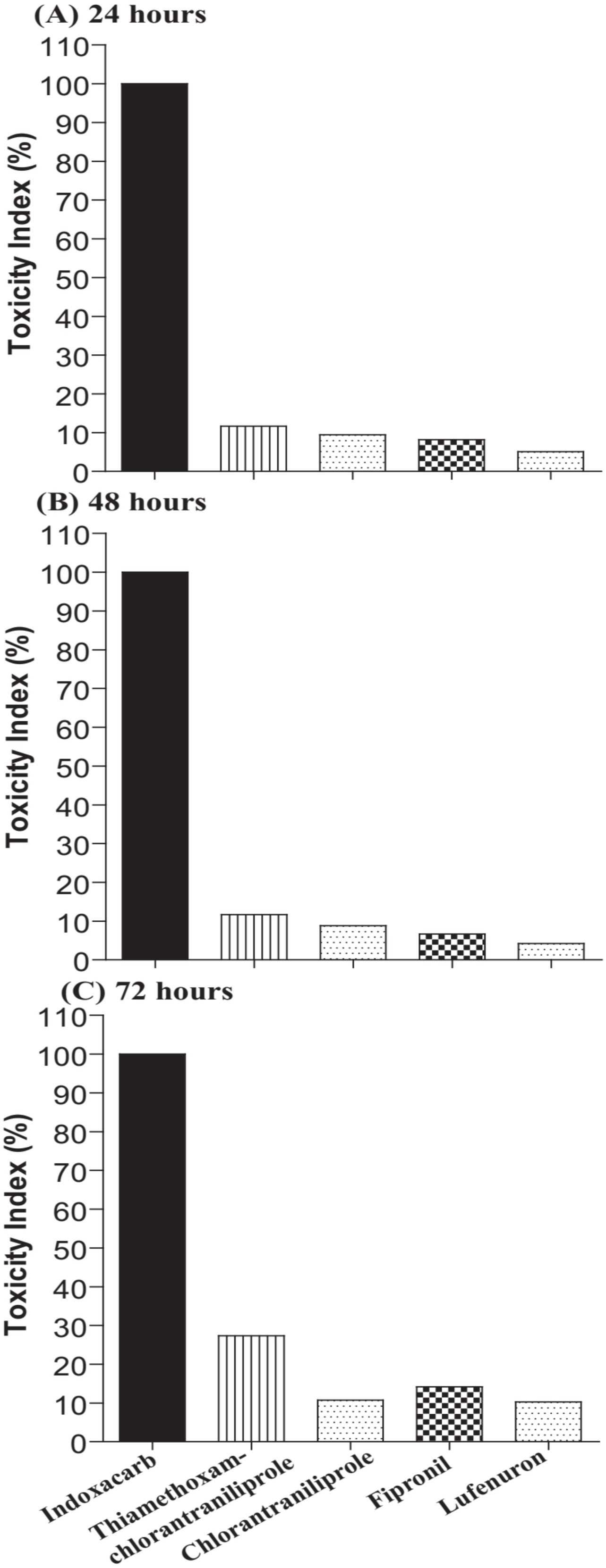

Fig. 7. Toxicity index of certain insecticides on the 4th instar larvae of cotton leafworm, *S. littoralis* after 24h (A), 48h (B) and 72h (C) exposure.

Discussion

In this context, a study was conducted to examine the comparative toxicity of various insecticides, including indoxacarb, thiamethoxam-chlorantraniliprole, chlorantraniliprole, fipronil, and lufenuron, across different groups. The objective was to identify the most effective insecticide targeting the 2nd and 4th instar larvae of CLW. Overall, the 2nd instar larvae of *S. littoralis* exhibited higher sensitivity to the five tested compounds compared to the 4th instar larvae. The LC_{50} and LC_{90} values showed a declining trend with increased duration of insecticide exposure. Indoxacarb, in particular, has proven to be effective against pests from the orders Lepidoptera, Diptera, Thysanoptera, as well as some representative species from Coleoptera and Orthoptera (Wing *et al*., 2000; Gamil *et al*., 2011; Farag *et al*., 2023). Indoxacarb was the most effective insecticide at acute (24 hrs.) and chronic (72 hrs.) levels against both 2nd and 4th larval instars compared with thiamethoxam-chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron. The toxicity of indoxacarb was studied on different insect species and showed high toxic effects in killing a wide variety of lepidopteran pests (Ahmad *et al*., 2003; Cook *et al*., 2004; El-Sheikh, 2015; Farag *et al*., 2023). In other study of the toxic effects of indoxacarb against the 2nd and the 4th instar larvae of *S. littoralis* (Abdu-Allah, 2007; Khaled and Farag, 2015; El-Dewy, 2013; Ismail *et al*., 2018; Moustafa *et al*., 2021; Farag *et al*., 2023). In the present study, indoxacarb showed high toxicity to the 2nd instar larvae of CLW compared with thiamethoxam-chlorantraniliprole, chlorantraniliprole, fipronil and lufenuron with LC_{50} values of (0.009, 0.006 and 0.001ppm) after 24h, 48h and 72hr exposure. These lethal concentrations fall in the range of previous studies on different larval stages of CLW with LC_{50} values ranging between 0.001 to 0.055, and 0.005 to 0.81 mg/L, respectively for the 1st to 6th instars (El-Dewy, 2013; Khaled and Farag, 2015; Ismail *et al*., 2018; Moustafa *et al*., 2021; Farag *et al*., 2023).

Chlorantraniliprole has an insecticidal effect on a wide range of lepidopteran pests besides other orders including Coleoptera, and Diptera (Lahm et al., 2005; Hannig *et al*., 2009; Sattelle *et al*., 2008; Bentley *et al.,* 22010; Lanka *et al*., 2013; Sas *et al*., 2023). Chlorantraniliprole (Coragen®) is a modern insecticide from the relatively recent anthranilic diamides class, classified under Group 28 by IRAC. This innovative category of selective insecticides is particularly effective against lepidopteran pests, including those that have built resistance to older insecticide groups. In the present work, 2nd instar larvae of *S. littoralis* were found to be more susceptible than 4th instar to chlorantraniliprole as evident by the calculated LC_{50} and LC_{90} values. El-Dewy (2013) reported that the LC_{50} value of chloranitraniliprole on the 4th larval instar of *S. littoralis* after 48h was 15.0 mg. Their toxicity increased dramatically after 72h to be 10.03 mg. Sas *et al.* (2023) indicated that the LC_{50} value of laboratory strain for chlorantraniliprole on 4th instar larvae of *S. littoralis* after 72 hrs was 4.09 ppm. While LC_{50} value of field strain after 72 hrs was 13.35ppm. The LC_{50} value of chlorantraniliprole against 4th instar after 48h was 0. 0329 m/l (Abdel Aziz and El-Gabaly, 2021). Research into insecticide mixtures focuses on identifying substances that boost effectiveness while shortening the time needed to achieve results compared to using each agent separately. This approach supports lower application frequencies and reduced dosages of synthetic insecticides without compromising the efficiency of pest control methods. In this study, the combination of thiamethoxam and chlorantraniliprole demonstrated greater toxicity against 2nd and 4th instar larvae of CLW compared to chlorantraniliprole used on its own. This reault agreed with that of Abdel Aziz (2019) who stated the LC_{50} values of Folliam Felixi (thiamethoxam and chlorantraniliprole) against the 2^{nd} and 4^{th} larval instars of *S. littoralis* were 0.076 and 0.233 ppm, respectively.

Fipronil acts as a noncompetitive antagonist of the GABA receptor, inhibiting GABA and causing hyperexcitation in the insect nervous system (Ffrench-Constant *et al*., 1991). Additionally, recent studies have demonstrated that both fipronil and its sulfone metabolite can block two types of glutamate-gated chloride channels (Zhao and Salgado, 2010). This compound is an effective insecticide for managing CLW, *S. littoralis*, functioning by disrupting gamma-aminobutyric acid (GABA)-gated chloride channels. Lethal effect of fipronil against the 2^{nd} and 4^{th} instar larvae of *S. Littoralis* was investigated using the leaf dipping technique. Results indicated that larvae were more tolerant to fipronil than other insecticides (Tables 2, 3, 4, 5, 6 and 7). However, the LC_{50} values of fipronil as feeding action against the 2^{nd} and 4^{th} instar larvae was only 12.18, 14.96 and 10.18 -fold lower than that of idoxacarb. This suggests that fipronil demonstrated greater efficacy when applied topically compared to the leaf-dipping method. This result is agreement with Barrania (2019) and Sleem *et al.* (2019) who reported that the LC_{50} values of fipronil against 2^{nd} instar of *S. littoralis* larvae after 24 hrs were 0.711 mg L-1 and 0.458 mg L-1 after 48 hrs. The LC_{50} value of fipronil against 4^{th} larval instar was 0.024 µg/m (Swelam *et al*., 2022; Khalifa *et al*., 2023).

Insect growth regulators (IGRs) newly introduced to the market function by targeting and disrupting specific biochemical pathways or processes critical for insect growth and development. This disruption causes improper regulation of hormone-dependent cellular or organ functions, ultimately leading to the insect's death. Among these IGRs, chitin synthesis inhibitors (CSIs), like lufenuron, specifically hinder the process of chitin deposition. IGRs accomplish their effects by interfering with developmental processes, disturbing the endocrine system, and impairing reproduction or metamorphosis in the targeted insects (Kai *et al*., 2009; Saad *et al*., 2015). The results showed that the 4^{th} instar larvae of *S. littoralis* were less susceptible to lufenuron than the 2^{nd} larvae. Moreover, the treatment of lufenuron showed a lower toxicity against 2^{nd} and 4^{th} instars of CLW larvae compared to other tested insecticides. Results obtained with toxicity revealed that the exposure of *S. littoralis* 2^{nd} and 4^{th} instar larvae to different concentrations of lufenuron at different exposure times resulted in an increase in the toxicity levels. This result is agreement with Ismail (2020) who reported that the LC_{50} value of lufenuron against 2^{nd} and 4^{th} larval instar of *S. littoralis* was 3.26 and 49.18 ppm for field strain while the laporatory strain value 2.73 and 5.22ppm, respectively. Osman and Mahmoud (2008) highlighted that the insecticide lufenuron demonstrated strong effectiveness against the 3rd and 5th larval instars of S. littoralis. However, a study by Sabri *et al*., (2016) indicated that lufenuron exhibited low toxicity towards *S. littoralis* larvae.

Conclusion

Thus, it is concluded that, indoxacarb, thiamethoxam + chlorantraniliprole and chlorantraniliprole are the most effective insecticides against 2^{nd} and 4^{th} instar larvae of

S. littoralis, laboratory strain. Although fipronil and lufenuron are the less toxic insecticide on 2nd and 4th instar larvae of *S. littoralis*. The toxicity of mixtures between thiamethoxam and chlorantraniliprole was more effective on the 2nd and 4th instar larvae of CLW than chlorantraniliprole alone. The results showed that the 4th instar larvae of *S. littoralis* were less susceptible to insecticides than the 2nd larvae. Moreover, the treatment of lufenuron showed a lower toxicity against 2nd and 4th instars of CLW larvae compared to other tested insecticides. Results obtained with toxicity revealed that the exposure of *S. littoralis* 2nd and 4th instar larvae to different concentrations of tested insecticides at different exposure times resulted in an increase in the toxicity levels. Therefore, our study recommended using indoxacarb, thiamethoxam + chlorantraniliprole and chlorantraniliprole in controlling *S. littoralis* because of their mode of action are different. These results are promising for using these insecticides in Integrated Pest Management (IPM) programs. Further studies shoud be investigated the potencey of the tested insecticides under field conditions.

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سمية بعض المبيدات الحشرية ضد اطوار مختلفة من دودة ورق القطن *Spodoptera littoralis*

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قسم وقاية النبات، كلية الزراعة، جامعة أسيوط، أسيوط، مصر.

الملخص

هدفت الدراسة الحالية إلى دراسة سمية خمس مبيدات حشرية وهي إندوكساكارب، ثياميثوكسام + كلورانترانيليبرول، كلورانترانيليبرول، فيبرونيل ولوفينورون ضـــد يرقات الطور الثاني والرابع لدودة ورق القطن تحت ظروف المعمل .أظهرت المقارنة بين LC_{50s} للمبيدات الحشـــرية المختبرة ليرقات الطور الثاني أن المبيد الحشـــري الأكثر ســـمية كان إندوكســـاكارب (0.009، 0.006، 0.001 جزء في المليون)، يليــه ثيــاميثوكســــام + كلورانترانيليبرول (0.016 ،0.01، 0.009 جزء في المليون)، كلورانترانيليبرول (0.21، 0.12، 0.052جزء في المليون)، فيبرونيـل (2.81،3.79 ،0.661 جزء في المليون) ولوفينورون (5.19، 0.916،3.21جزء في المليون) بعد التعرض لمدة 24 و48 و72 ســـاعة على التوالي. وبناءًا على مؤشر السمية والفعالية النسبية، كان مبيد الإندوكساكارب أكثر سمية ليرقات الطور الثاني من ثيامثوكســـام + كلورانترانيليبرول، كلورانترانيليبرول، فيبرونيل، ولوفينورون بنســـب (1.78، 23.33، 421.11 ،576.67)، (1.67، 20.00، 468.33، 535.0)، (9، 52.0، 661.0، 916.0) ضـــعفًا، على التوالي .وكما هو الحال في الطور الثاني، أظهرت يرقات الطور الرابع حســـاســـية عالية للإندوكســـاكارب مقارنةً بالثيامثوكســـام + كلورانترانيليبرول، كلورانترانيليبرول، فيبرونيل ولوفينورون في كل من السـمية الحادة والمزمنة .بناءً على قيم التركيز المميت النصــفي للمبيدات الحشــرية المختبرة ليرقات الطور الرابع من دودة ورق القطن، أظهرت أن الإندوكســـاكارب كان أكثر فعالية من المبيدات الحشــرية الأخرى (0.32، 0.61، 0.83 جزء في المليون)، يليه ثيامثوكســام + كلورانترانيليبرول (1.11 .5.23، 7.11 جزء في المليون)، وكلورانترانيليبرول (8.76،6.89،2.18جزء في المليون)، وفيبرونيـل (2.19، 9.13، 10.11جزء في المليون) ولوفينورون (4.11, 14.39، 16.38جزء في المليون) بعد 24، 48 ،72 ساعة من التعرض، على التوالي. لذلك، أوصت دراستنا باستخدام إندوكساكارب وثيامثوكسام + كلورانترانيليبرول وكلورانترانيليبرول في مكافحة دودة ورق القطن لأن طريقة عملها مختلفة وواعدة لاستخدام هذه المبيدات الحشرية في برامج الإدارة المتكاملة للآفات.

الكلمات المفتاحية: المبيدات الحشرية، السمية، التقييم الحيوي بغمر الأوراق، *Spodoptera littoralis*