

Joint Toxic Action of Chlorantraniliprole with Certain Insecticides against Cotton Leafworm Larvae

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ABSTRACT

Toxicity of the anthranilic diamid insecticide chlorantraniliprole, the macrocyclic lactone insecticide emamectin benzoate, the neonicotinoid insecticide imidacloprid and the bioinsecticide Agree[®] (*Bacillus thuringiensis* subsp. *aizawai* GC-91) against the 2nd, 3rd and 4th larval instars of *Spodoptera littoralis* (Boisd.) were studied. The joint toxic action of chlorantraniliprole with each of the other three insecticides was also investigated against the 4th instar larvae of *S. littoralis*. Emamectin benzoate against 2nd, 3rd and 4th larval instars (LC₅₀ = 0.0004, 0.0020 and 0.0048 ppm, respectively) was more toxic than chlorantraniliprole (LC₅₀ = 0.17, 0.51 and 1.5 ppm, respectively), followed by imidacloprid (LC₅₀ = 1.19, 43.65 and 1085.69 ppm, respectively) and *Bacillus thuringiensis* (LC₅₀ = 754.65, 1205.67 and 2279.91 ppm, respectively) after 96 hrs of treatment by dipping leaf disc technique. Chlorantraniliprole/imidacloprid mixtures resulted in antagonistic effect more than the chlorantraniliprole/emamectin benzoate mixtures and chlorantraniliprole/*Bacillus thuringiensis* mixtures at different concentration levels. The chlorantraniliprole at LC₅₀/other tested insecticides at LC₅₀ mixtures revealed antagonistic effects higher than the mixtures of chlorantraniliprole at LC₂₅/other tested insecticides at LC₂₅. The mixture of chlorantraniliprole at LC_{12.5}/*Bacillus thuringiensis* at LC_{12.5} resulted in an additive effect. Therefore, mixtures of chlorantraniliprole with these tested insecticides can't be used for cotton leafworm control.

Key words: chlorantraniliprole, emamectin benzoate, imidacloprid, *Bacillus thuringiensis*, cotton leafworm.

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.); is one of the most destructive agricultural lepidopterous pests. It can attack numerous economically important crops all the year round. On cotton, the pest may cause considerable damage by feeding on the leaves, fruiting points, flower buds and, occasionally, also on bolls. The chemical control of *S. littoralis* has been extensively reported in relation especially to cotton in Egypt (Issa *et al.*, 1984a & b and Abo-El-Ghar *et al.*, 1986). The frequent use of insecticides against agricultural pests usually leads to the development of resistance in the target pests as to contaminate the environment. Although the importance of the insecticide use for agriculture to prevent insect associated losses cannot be

overlooked, there is a greater need to develop alternative or additional techniques, which would allow a rational use of pesticides and provides adequate crop protection for sustainable food, feed and fiber production.

A number of insecticides with different modes of action, (chlorantraniliprole, emamectin benzoate, imidacloprid and bioinsecticide; *Bacillus thuringiensis* var. *aizawai*) were chosen for this study.

Chlorantraniliprole is a new anthranilic diamid insecticide, which effectively controls pest insects belonging to Lepidoptera, Coleoptera, Diptera and Hemiptera, and has been shown to be effective against insects that have developed resistance to older classes of chemistry (Bentley *et al.*, 2010). Anthranilic diamides selectively bind to ryanodine receptors in insect muscles resulting in an uncontrolled release of calcium from internal stores in the sarcoplasmic reticulum (Lahm *et al.*, 2005 and Cordova *et al.* 2006), causing impaired regulation of muscle contraction leading to feeding cessation, lethargy, paralysis, and death of target organisms. Anthranilic diamides have very low vertebrate toxicity due to a >500-fold differential selectivity toward insect over mammalian ryanodine receptors (Cordova *et al.* 2006).

Emamectin benzoate is a novel semi-synthetic derivative of natural product abamectin in Avermectin family. Avermectins including emamectin benzoate have been shown to be effective against broad spectrum of arthropod pests (Putter *et al.*, 1981). This materials act by interfering with the action of gamma aminobutyric acid (GABA) (Fritz *et al.*, 1979). It blocks post-synaptic potentials of neuromuscular junction, leading to paralysis. Imidacloprid (a chloronicotinyl insecticide) is widely used to control sucking pests (Elbert *et al.*, 1991). The entomopathogenic bacteria, *Bacillus thuringiensis* represents a good example for nonchemical control of insect pests. This bacterium, proved to be a highly successful for controlling some agricultural insect pests (Armengol *et al.*, 2007).

Resistance to pesticides is probably the biggest challenge facing pesticides research today. Consequently, insecticides from different chemical groups with different mode of action and also some of their combination should be tested against *S. littoralis* to

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help developing a sound control program in the future (Ghoneim, 2002).

The combination of such bioactive agent with insecticides was investigated as attempt to increase their efficiency on *Spodoptera* and reduce the amounts of insecticides release in the environment which is appreciable from the environmental safety point of view (Aly and El-dahan, 1987).

The objective of the present study was to assess the toxicity of chlorantraniliprole, emamectin benzoate, imidacloprid and *Bacillus thuringiensis* against the 2nd, 3rd and 4th larval instars of *S. littoralis*. In addition, this work was carried out to study the compatibility of mixing chlorantraniliprole with these insecticides.

MATERIALS AND METHODS

Insects: Larvae of *S. littoralis* were obtained from the Plant Protection Research Institute, Cairo, Egypt, and reared on castor oil leaves under lab conditions (27 ± 2 °C, RH 65 %) for several years, according to Eldefrawi *et al.*, (1964). The second instar larvae (1.5 ± 0.1 mg per larva) and the third instar (16.95 ± 0.3 mg per larva) and the fourth instar (46.6 ± 0.4 mg per larva) were used in the bioassay.

Insecticides: chlorantraniliprole [95.3% technical product] was provided by DuPont Crop Protection, emamectin benzoate [96.2% technical product] by M/s. Crystal Phosphate Ltd., NewDelhi, and Imidacloprid [95% technical product] by Changlong Chemical Industrial Group (Changzhou, Jiangsu, China). Agree[®] 50% WG (*Bacillus thuringiensis* subsp. aizawi strain GC-91) commercial formulation was obtained from Plant Protection Research Institute, Agricultural Research Centre, Cairo, Egypt.

Toxicity of insecticides against *S. littoralis* larval instars: Toxicity of the fore-mentioned insecticides against the 2nd, 3rd and 4th larval instars of *S. littoralis* was evaluated. Castor oil leaves were cut into discs (2 cm²) (Eldefrawi *et al.*, 1964; Mansour *et al.*, 1966). Each disc was dipped in a series of the insecticides concentrations containing 0.01% triton X100 and 1% dimethyl formamide (DMF) for Chlorantraniliprole or 1% acetone for Emamectin benzoate and imidacloprid or without solvents for agree[®]. Dipping was performed for 10sec., 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, 10 larvae in each cup, starved larvae for 2-4 hrs, were placed. Each concentration was replicated three times. Mortality percentages were recorded after 24, 48, 72 and 96 hrs of treatment for chlorantraniliprole,

emamectin benzoate and imidacloprid and for 7 days for the bioinsecticide agree[®], percent mortality was corrected according to Abbott equation (Abbott, 1925) and subjected to probit analysis (Finney, 1971). From which the corresponding concentration probit lines (LC-p lines) were estimated in addition to determine values of 12.5, 25 and 50% mortalities, slope values of tested compounds were also estimated.

Joint toxic action of chlorantraniliprole with insecticides against the 4th instar of *S. littoralis* larvae: Joint toxic action of chlorantraniliprole with the insecticides (emamectin benzoate, imidacloprid and agree[®]) against the 4th instar larvae of *S. littoralis* was investigated. Chlorantraniliprole was mixed with these insecticides at different concentrations. In addition, LC₂₅ of chlorantraniliprole was mixed with the LC₂₅ of the other insecticides. Also, LC_{12.5} of chlorantraniliprole was mixed with LC_{12.5} of the insecticides. Three control groups were subjected to calculate the expected mortalities. Co-toxicity factors (Mansour *et al.*, 1966) were calculated as follows:

Co-toxicity factor =

$$\frac{\text{observed \% mortality} - \text{expected \% mortality}}{\text{expected \% mortality}} \times 100$$

This factor was used to categorize the results into three categories as follow: Co-toxicity factors ≥ +20 meant potentiation; co-toxicity factors < - 20 meant antagonism; and co-toxicity factors between -20 and +20 meant additive effect.

RESULTS AND DISCUSSION

Toxicity of chlorantraniliprole and certain insecticides against the 2nd, 3rd and 4th *S. littoralis* larval instars: Regression lines were established for the tested insecticides on the three larval instars. Toxicity of the anthranilic diamide insecticide chlorantraniliprole, the macrolactones insecticide emamectin benzoate, the neonicotinoide insecticide, imidacloprid and the bio-insecticide Agree[®] (*Bacillus thuringiensis* subsp. aizawi strain GC-91) against the larval instars were recorded. Table (1), showed that the toxicity of tested insecticides decreased with the advancement of larval instar. Emamectin benzoate was the most effective against *Spodoptera* 2nd, 3rd and 4th larval instars followed by chlorantraniliprole then imidacloprid and agree; respectively. LC₅₀ values at after 96 hrs of treatment were 0.0004, 0.17, 1.19 and 754.65 ppm for 2nd instar, 0.0020, 0.51, 43.65 and 1205.67 ppm for 3rd instar, and 0.0048, 1.53, 1087.69 and 2279.91 ppm for 4th instar larvae; respectively.

Joint toxic action of chlorantraniliprole with insecticides against the 4th *Spodoptera* larval instar:

To determine the effect of applying mixtures of chlorantraniliprole (LC₅₀, LC₂₅ and LC_{12.5}) with the tested insecticides (LC₅₀, LC₂₅ and LC_{12.5}), 100%, 50% and 25% mortality was expected results, when the mixtures were used. Since the average weights of the 4th instar larvae used in each test varied, thus, the expected mortality for the concentrations applied in every test varied accordingly. So, the expected mortality was calculated for each insecticide in the mixture in every test by treating larvae by each one alone. Therefore, the expected mortality for the mixture of two insecticides was the sum of the mortalities of each concentration used in the mixture. The joint toxic action of chlorantraniliprole with the tested insecticides at different concentrations is shown in Tables (2 and 3). It is clear that, all mixtures of chlorantraniliprole (at LC₅₀) with the tested insecticides (at LC₅₀) resulted in antagonistic effect with co-toxicity factors (CTFs) ranged between -20 to -53.33 at all exposure times (Table 3). The highest antagonistic effect was observed, after 96 hrs of treatment, when chlorantraniliprole was mixed with imidacloprid at different concentration levels. CTFs values were -46.88, -53.33 and -41.38 when chlorantraniliprole (LC₅₀) was mixed with emamectin benzoate (LC₅₀), imidacloprid (LC₅₀) or agree[®] (LC₅₀), respectively, while CTFs were -46.15, -50.00 and -50.00 when Chlorantraniliprole (LC₂₅) was mixed with the same compounds (LC₂₅), respectively. And the lowest CTFs, -33.33, -42.86 and -20.00 were obtained when Chlorantraniliprole (LC_{12.5}) was mixed with the same compounds (LC_{12.5}), respectively after 96 hrs of treatment.

DISCUSSION

This study evaluated the acute toxicities of chlorantraniliprole, emamectin benzoate, imidacloprid and Agree[®]. The joint toxic action chlorantraniliprole in mixtures with these insecticides against *Spodoptera* larval instars were also studied.

Chlorantraniliprole (Rynaxpyr and coragen[®]) is a recently developed compound by Dupont belonging to a new class of relatively selected insecticides (anthranilic diamides) featuring a novel mode of action. This insecticide is being developed worldwide in a broad range of crops to control a range of pests belonging to the order Lepidoptera and some Cleoptera, Diptera and Isoptera species. This insecticide possesses a new mode of action, high biological activity, relatively low mammalian toxicity and selectivity to non-target arthropods. Chlorantraniliprole is primarily formulated as a 20 % w/v (200 g/l) suspension concentrate (Coragen[®]) showing good tank-stability and compatibility with conventional crop protection products. Unless otherwise specified, the results reported in this work refer to the coragen formulation.

Trends in pest management in the last four decades emphasis on methods on controlling insect pest a part from conventional insecticides had stimulated much research on the use of the mimics of the natural products and similar. Chlorantraniliprole (new anthranilic diamide derivative), imidacloprid (is a neanicotinoid, agonist of nAChR), and emamectin benzoate (a derivative of abamectin, chloride channel activator) were used and evaluated against *Spodoptera* larvae, the major lepidopterous cotton leaf worm infesting more than 150 crop in Egypt.

Table 2. Observed mortality of chlorantraniliprole, emamectine benzoate, imidacloprid and *Bacillus thuringiensis* against 4th instar *Spodoptera* larvae at different times after treatment

Insecticide	Concentration levels ¹	Concentration(ppm)	Observed (%) mortality		
			After 48 hr.	After 72 hr.	After 96 hr.
Chlorantraniliprole	LC ₅₀	1.5	3.33	23.33	53.33
	LC ₂₅	0.3	3.33	6.67	23.33
	LC _{12.5}	0.1	0.00	3.33	10.00
Emamectin benzoate	LC ₅₀	0.005	13.33	30.00	53.33
	LC ₂₅	0.0015	0.00	3.33	20.00
	LC _{12.5}	0.0006	0.00	3.33	10.00
Imidacloprid	LC ₅₀	1100	16.67	33.33	46.67
	LC ₂₅	100	6.67	6.67	23.33
	LC _{12.5}	20	0.00	3.33	13.33
<i>Bacillus thuringiensis</i>	LC ₅₀	2300	0.00	6.67	43.33
	LC ₂₅	600	0.00	3.33	23.33
	LC _{12.5}	200	0.00	0.00	6.67

¹Concentration level of each insecticide was calculated from its corresponding LC-p lines at 4th day after treatment.

Different larval instars were subjected to the pesticidal toxic evaluations. These were referred specifically in the experiment and the corresponding results.

Bacillus thuringiensis is one of the few microbes that have been used successfully against certain agricultural insects pest species. Many strains are identified and has been used either specifically or commonly against several insect pests for practical control. Each *Bt* strain usually carries several toxins genes that determine the range of insects affected. Although most of the isolated *Bt* strains specific against Lepidoptera, some strains are specified only to Diptera or to Coleoptera, and a few are lethal to both Lepidoptera and Diptera insects. Many of the *Bt* strains that affect Lepidoptera have quite different efficacies on different insects. Liquid and powder formulations of this bacterium hold a small but growing share of the pest – control – agent market. Appropriate formulations and the mode of field application, would consider being the future challenge in the practical application of *Bt* in insect control. In spite of the fact that this approach is quite different from *Bt* genetically modified crops, but it is considered the safer and cheaper way.

Though field studies are necessary for quantifying insecticide performance at the farm level, laboratory bioassays are useful for explaining insecticide activity in the field (Hutchison, 1993). In the present study, toxicity of the anthranilic diamide insecticide was more toxic than imidacloprid and agree[®], and less toxic than emamectin benzoate against the 2nd, 3rd and 4th larval instars of *S. littoralis*, at 96 hrs after treatment. These results were compatible with the results obtained by Hanafy and El-Sayed (2013) who found that emamectin benzoate has a good efficacy in the control of *T. absoluta* and *H. armigera* than chlorantraniliprole.

Barrania (2013) stated that chlorantraniliprole seems to be the most powerful than thiamethoxam (neonicotinoide insecticide) and novaluron (benzoylphenyl urea insecticide) formulations at 1, 1/2 & 1/4 field recommended rates against 4th instar *Spodoptera* larvae.

In the present study, the chlorantraniliprole / tested insecticide mixtures with the three insecticides resulted in antagonistic effects, except the chlorantraniliprole (LC_{12.5}) / *Bacillus thuringiensis* (LC_{12.5}) mixture resulted in additive effect. It could be concluded that all tested combinations didn't have positive effect. In contrast it gave negative effects under laboratory condition. So, unfavorable to mix chlorantraniliprole with tested insecticides.

These negative effects may due to the antifeedant effect of chlorantraniliprole, which causes feeding cessation, lethargy, muscle paralysis and ultimately death by activating the ryanodine receptor (Lahm et. al., 2005, Cordova et. al. 2006 and Cao et. al., 2010). Barrania (2013) presented that field doses and sublethal doses of chlorantraniliprole appears antifeedant, growth inhibitory and toxic effects against the cotton leafworm *S. littoralis*.

The development of strategies for the rational use of insecticides within the framework of insect pest management requires a great deal of research. There is tendency with insect pest management research to emphasize the alternative non insecticide methods of control rather than concentrate on insecticides. There is at present a great need for independent work to identify reduced dosage levels that provide adequate control. Finally we can conclude that, it is not preferred to mix chlorantraniliprole with these tested insecticides for controlling *S. littoralis*, which can lead to reduce the efficacy of insecticides.

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