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Evaluation of Insecticides Targeting Control of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)

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Abstract

Insecticides from different chemical groups were tested by laboratory bioassay to verify the percentage mortality of *Helicoverpa armigera* (Hubner 1808) (Lepidoptera: Noctuidae). The experiment was conducted in the Crop Science laboratory—Prof. CinobelinaElvas Campus—UFPI, Bom Jesus, PI, from January to June, 2013. The populations utilized came from the University's own insect breeding laboratories. Third instar larvae of *H. armigera* were used to conduct the bioassay. The experimental design was fully randomized, with 13 treatments and four replications. Five larvae were used per replication, with 12 insecticides from 9 different chemical groups and a control. Each treatment consisted of three doses. The methods of application used were topical contact and ingestion in artificial diet. According to the results the percentage mortality of *H. armigera* larvae varied among the treatments. The results demonstrated that chlorpyrifos and spinosad were effective against third instar *H. armigera* larvae both on contact and by ingestion. Flubendiamide, acephate, methomyl, *Bacillus thuringiensis*, dimethoate, chlorantraniliprole and fipronil had good responses to control of *H. armigera*.

Keywords

Bollworm, Bioassay, Chemical Control, Insecticides, Soybeans

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1. Introduction

In Brazil agriculture is a driving force for broad economic growth. According to a survey by the National Supply Company, 184.3 million tons of grain was produced in the 2012/2013 harvest [1]. Production can be limited by a variety of factors, including fertilizers, water and crop genetic potential, as well as organisms that feed on plants.

Among the pests, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) represents a challenge for agricultural production around the world. It feeds on diverse economically important crops, among them more than 60 species of cultivated and non-cultivated plants belonging to more than 47 families, including soybeans, cotton, sorghum, corn, sunflower, peanuts, beans, tomatoes and peppers [2].

H. armigera was recently identified attacking cotton and soybean crops in twelve Brazilian states, with the highest incidence in Bahia. The Brazilian states that have already been affected by this pest are: Mato Grosso, Mato Grosso do Sul, Minas Gerais, Bahia, Pará, Goiás, Paraná, São Paulo, Maranhão and Piauí. In March 2013 a phytosanitary emergency was declared in Bahia, the state that has most suffered from this pest, with damage calculated at more than US$ 420 million [3].

The success of this pest can be derived from its life history characteristics (polyphagia, high mobility, high fecundity and facultative diapause), that permit it to survive in adverse environments and to adapt to the most abrupt seasonal changes [4]. Management strategies have been proposed for *H. armigera*, such as use of biological control; pest monitoring; reduction of the seeding window for corn, soybeans and cotton; and adoption of refuge areas of conventional plants near transgenic cultivars. However, chemical control is still the form of control most used by Brazilian producers. Considering the lack of action levels for controlling *H. armigera* under Brazilian conditions, chemical insecticides should be used on an emergency basis respecting control levels in the international literature. The recommended dosages of the insecticides should be observed, avoiding super- and sub-dosages, since the effectiveness of control can be reduced, as well as contributing to selection for populations resistant to the insecticides applied. Multiple applications of an average dosage are generally more effective than a single application in overdose. Rotation of insecticides with different modes of action is also recommended to avoid selection for resistant populations [5].

The synthetic insecticides currently used for control purposes in countries that suffer from damage caused by *H. armigera* are: indoxacarb, methoxyfenozide, emamectin benzoate, novaluron, chlorfenapyr, imidacloprid, fluvalinate, endosulfan, spinosad, abamectin, deltamethrin, cypermethrin, lambda-cyhalothrin, carbaryl, methomyl, profenofos, thiodicarb and chlorpyrifos [6].

In Brazil until recently *H. armigera* was considered an A1 quarantine pest. The first cases of this pest were registered in the 2012/2013 season; therefore, there were no specific insecticides registered to combat this type of pest on cotton and soybeans and the effectiveness of these products is still little known. Thus this work proposes to test different chemical groups by laboratory bioassay and verify the percentage mortality in *H. armigera* larvae.

2. Materials and Methods

2.1. Insects

The populations used came from the University's own insect breeding laboratory, where the insects were maintained on an artificial diet adapted [7]. Newborn larvae (<24 h of age) were isolated and transferred to 100 mL plastic containers with lids containing artificial diet until reaching pupal phase. The adults were transferred to 40 cm × 30 cm PVC cages containing sheets of bond paper for oviposition. They were fed a honey-based solution (10%) and maintained in the laboratory (25˚C ± 2˚C, 60% ± 10% RH, 14:10 L.D). Eggs were collected and stored in plastic bags until eclosion of the larvae.

2.2. Pesticides

The products and dosages were selected based on their use in other countries for control of *H. armigera*, or in Brazil for control of *Heliothis virescens* (Fabricius 1781), a pest belonging to the same family. The average dose was used as the standard dose, this being the recommended one, along with a lower dose 20% less than the average dose, and a dose 20% above the average dose, this being the higher dose (**Table 1**).

Table 1. Active ingredients and chemical groups used for bioassay and their respective doses.

Active ingredient	Chemical group	Conc. a.i.	Doses in L/ha^{-1}		
			Dose 1	Dose 2	Dose 3
Chlorantraniliprole	Diamide	200	0.08	0.10	0.12
Flubendiamide	Diamide	480	0.08	0.10	0.12
Abamectine	Avermectin	18	0.80	1.00	1.20
Acephate	Organophosphate	750	1.20	1.50	1.80
Bacillus thuringiensis	*Bacillus thuringiensis*	33.6	0.56	0.70	0.84
Chlorpyrifos	Organophosphate	480	1.20	1.50	1.80
Diflubenzuron	Benzoylurea	480	0.12	0.15	0.18
Dimethoate	Organophosphate	400	1.20	1.50	1.80
Fipronil	Phenylpyrazole	200	0.20	0.24	0.28
Lambda-cyhalothrin	Pyrethroid/pyrethrin	250	0.12	0.15	0.18
Methomyl	Carbamate	215	1.20	1.50	1.80
Spinosad	Spinosyn	400	0.06	0.08	0.10
Control	-	-	0.00	0.00	0.00

a.i.: active ingredient.

2.3. Contact Bioassay

This experiment was conducted in the Crop Science laboratory—Prof. CinobelinaElvas Campus—UFPI, Bom Jesus, PI, from January to June 2013. For evaluation by contact the products were diluted in 100 mL of distilled water, of which 10 µl was applied with a micropipette to the dorsal thorax of each larva. Distilled water was used as the control treatment. The experimental design was fully randomized in a product x dose factorial scheme, using eight insecticide treatments with three doses each and four replications and a control. The insecticides used for contact testing were: abamectin, acephate, chlorantraniliprole, chlorpyrifos, flubendiamide, lambda-cyhalothrin, methomyl and spinosad (concentrations and doses **Table 1**). After treatment, the larvae were maintained under standard breeding conditions (temperature 25˚C ± 1˚C, relative humidity 60% ± 2%) and the percentage mortality was evaluated five days after application.

2.4. Ingestion Bioassay

Concentrations of insecticides were incorporated in 250 g of artificial diet (concentrations and doses **Table 1**), of which 13 g was weighed and distributed in 100 mL plastic pots. Each larva was individually transferred to a plastic pot containing the treated diet. The control consisted of artificial diet without addition of insecticide. The experimental design was fully randomized, in a product x dose factorial scheme, using 12 insecticide treatments with three doses each and four replications and a control. The insecticides used for ingestion testing were abamectin, acephate, *Bacillus thuringiensis*, chlorantraniliprole, chlorpyrifos, diflubenzuron, dimethoate, fipronil, flubendiamide, lambda-cyhalothrin, methomyl and spinosad. After treatment, the larvae were maintained under standard breeding conditions (temperature 25˚C ± 1˚C, relative humidity 60% ± 1%); the percentage mortality was evaluated five days after application of the insecticide in the artificial diet. The larvae were considered dead if they were desiccated or there was general darkening of the cuticle or they were not capable of moving in a coordinated way when disturbed with the tip of a forceps.

2.5. Data Analysis

Analysis of variance was performed by F-test on the two methods of application and followed by median test by the SNK method at 5% probability when a significant difference was observed. Statistical analyses were performed using the SAS® program [8].

3. Results

3.1. Mortality Bioassay Contact

According to the results the percentage mortality of third instar larvae of *H. armigera* varied among the treatments. In the contact bioassay (**Table 2**) chlorpyrifos and spinosad presented the greatest percentage mortality in the three doses tested, ranging from 94.7% to 100%, respectively. They were followed by flubendiamide and acephate that obtained percentage mortalities of 74.2% and 68.4%, respectively, for the lower dose and remained constant for the average and higher doses tested. The other treatments presented mortality below 50%.

3.2. Mortality Bioassay Ingestion

In the ingestion bioassay (**Table 3**) chlorpyrifos, spinosad, methomyl, flubendiamide, acephate, dimethoate and fipronil presented 100% mortality at the lower dose tested, followed by chlorantraniliprole, lambda-cyhalothrin

Table 2. Average percentage mortality of *Helicoverpa armigera* five days after contact exposure to different insecticides at three different doses.

Treatment	% mortality		
	Dose 1	Dose 2	Dose 3
Abamectin	0.0 ± 0.00 Ca	0.0 ± 0.00 Ba	5.3 ± 6.25 Ca
Acephate	68.4 ± 13.77 Aa	79.5 ± 4.73 Aa	84.8 ± 11.81ABa
Chlorantraniliprole	0.0 ± 0.00 Ca	5.3 ± 5.00 Ba	5.3 ± 5.00 Ca
Chlorpyrifos	100 ± 0.00 Aa	100 ± 0.00 Aa	100 ± 0.00 Aa
Flubendiamide	74.2 ± 9.57 Aa	74.2 ± 12.58 Aa	79.5 ± 8.21 ABa
Lambda-cyhalothrin	10.5 ± 5.77 BCa	5.3 ± 5.00 Ba	10.5 ± 5.77 Ca
Methomyl	21.2 ± 8.26 Ba	26.5 ± 15.00 Ba	47.7 ± 4.79 Ba
Spinosad	100 ± 0.00 Aa	94.7 ± 5.00 Aa	100 ± 0.00 Aa
Control	0.0 ± 0.00 Ca	0.0 ± 0.00 Ba	0.0 ± 0.00 Ca

Means followed by the same letters, capitals in the column and small letters in the line, do not differ by SNK test ($P > 0.05$, $F = 29.89$, $CV = 27.03$ [SAS Institute, 2002]).

Table 3. Average percentage mortality of *Helicoverpa armigera* fed for 5 days with artificial diet containing different insecticides at three different doses.

Treatment	% mortality		
	Dose 1	Dose 2	Dose 3
Abamectin	26.3 ± 4.73 Bb	5.3 ± 5.00 BCc	89.4 ± 10.00 Aa
Acephate	100 ± 0.00 Aa	100 ± 0.00 Aa	100 ± 0.00 Aa
Bacillus thuringiensis	74.2 ± 5.00 Aa	74.2 ± 11.09 Aa	100 ± 0.00 Aa
Chlorantraniliprole	95.4 ± 5.00 Aa	100 ± 0.00 Aa	79.5 ± 1.25Ab
Chlorpyrifos	100 ± 0.00 Aa	100 ± 0.00 Aa	100 ± 0.00 Aa
Diflubenzuron	21.2 ± 8.16 Ba	15.9 ± 9.87 Ba	21.2 ± 8.26 Ca
Dimethoate	100 ± 0.00 Aa	100 ± 0.00 Aa	100 ± 0.00 Aa
Fipronil	100 ± 0.00 Aa	100 ± 0.00 Aa	94.7 ± 5.00 Aa
Flubendiamide	100 ± 0.00 Aa	100 ± 0.00 Aa	100 ± 0.00 Aa
Lambda-cyhalothrin	74.2 ± 15.00 Aa	53.0 ± 17.08 Aa	47.7 ± 11.97 Ba
Methomyl	100 ± 0.00 Aa	100 ± 0.00 Aa	100 ± 0.00 Aa
Spinosad	100 ± 0.00 Aa	100 ± 0.00Aa	100 ± 0.00 Aa
Control	0.0 ± 0.00 Ca	0.0 ± 0.00 Ca	0.0 ± 0.00 Da

Means followed by the same letters, capitals in the column and small letters in the line, do not differ by SNK test ($P > 0.05$, $F = 37.20$, $CV = 12.40$ [SAS Institute, 2002]).

and *Bacillus thuringiensis* with 94.5%, 74.2% and 74.2% mortality, respectively. There was not a significant difference among these treatments. At this same dose abamectin and diflubenzuron had mortality below 30%.

At the higher doses tested chlorpyrifos, spinosad, methomyl, flubendiamide, acephate, dimethoate, fipronil, *Bacillus thuringiensis* and chlorantraniliprole maintained effectiveness in percentage mortality in comparison with the lower dose tested. Lambda-cyhalothrin had a reduction in percentage mortality at higher doses tested in comparison with the lower dose tested. Abamectin had low percentage mortality from doses 1 and 2, however, the higher dose tested showed itself effective with percentage mortality above 80%.

4. Discussion

The results show a significant difference among treatments and the different dosages can be attributed mainly to the different modes of action of the products. Chlorpyrifos, methomyl, acephate and dimethoate belong to the organophosphate and carbamate chemical groups that possess toxic action inhibiting certain important enzymes of the nervous system, such as cholinesterases (ChE). This can explain the similar results obtained for these insecticides in this study. Comparing the effectiveness of insecticides and demonstrating that the maximum percentage mortality of third instar H. armigera larvae was found with chlorpyrifos and methomyl [9]. Chlorpyrifos was effective against 1st to 3rd instar *H. armigera* larvae [10].

The spinosyns act by inducing allosteric activation of nicotinic acetylcholine receptors, causing death of the insects [11]. Spinosad and chlorpyrifos had the greatest efficacy against third instar cotton bollworm compared to endosulfan and acephate [12] [13]. Compared chemical insecticides in the field and laboratory, spinosad had the greatest toxicity for *H. armigera* after five days of application of insecticides [14].

Abamectin acts by blocking the neurotransmitter gamma aminobutyric acid (GABA), in the neuromuscular junction of the insects [15]. Abamectin showed toxicity only at the highest dose. Abamectin had low effectiveness against the American bollworm, owing to the resistance of the cuticle to penetration, a higher concentration being necessary for effectiveness [16]. Studies of insect toxicity abamectin markedly reduced the fecundity and longevity of adults [17].

Diflubenzuron acts by inhibiting the synthesis of chitin. This insecticide had low effectiveness in control of *H. armigera*. In their study, diflubenzuron incorporated into an artificial diet and applied to cotton disks had no toxic effect on *H. armigera* [18].

Fipronil belongs to the phenylpyrazole insecticide family. Phenylpyrazoles are potent blockers of GABA (gamma aminobutyric acid), a regulator of chloride channels interfering with the central nervous system [19]. Fipronil was effective in the control of *H. armigera*. These authors reported that fipronil was extremely toxic to *H. armigera* and the level of resistance to the same was low. *Bacillus thuringiensis* demonstrated effectiveness based on percentage mortality of *H. armigera* mainly at the highest dose. Insecticidal proteins and found them sufficiently toxic and effective for control of *H. armigera*e *Helicoverpa punctigera* (Wallengren 1860) (Lepidopera: Noctuidae) [20].

Chlorantraniliprole and flubendiamide, diamides that act as modulators of ryanodine receptors, were effective in control of *H. armigera*. In a study of the effectiveness of insecticides for control of *H. armigera*, indicated that chlorantraniliprole can successfully be used against various larval stages of *H. armigera*, but its effectiveness can depend of various factors, such as the origin of the populations and the phases of the larvae the larger the *H. armigera* larvae the lower the mortality [21]. These results could explain the fact that the middle dose had a higher rate of mortality than the other doses. Insecticides with different modes of action can differ in their levels of control, can also provide appropriate control of the pest and can retard the development of resistance to the insecticide.

Chlorpyrifos and spinosad were effective against third instar *H. armigera* larvae in laboratory bioassay both on contact and by ingestion. Flubendiamide, acephate, methomyl, *Bacillus thuringiensis*, dimethoate, chlorantraniliprole and fipronil had a good response to control of *H. armigera* on application by ingestion. In laboratory the biological and environmental factors are controlled, so that we can better understand the behavior of this pest; studies should be conducted under greenhouse and field proven efficacy, so that the selected insecticides may be proposed for use in products rotation.

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