

Effectiveness of Insecticides to Control Small and Large Larvae of *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae)

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Abstract

Helicoverpa armigera (Hübner, 1805) (Lepidoptera: Noctuidae) is a cosmopolitan pest with wide geographical distribution in Brazil. This pest was officially registered in 2013 on cotton, soybean, corn, and some weeds, although this species may have been present in the country since 2008. The objective of this work was to evaluate the efficiency of eight insecticides (seven chemicals and a biological product) to control small and large *H. armigera* caterpillars. The experiment was conducted under laboratory conditions (T 22±1 °C; RH 70±10%; 12h photoperiod), with nine treatments (g a.i./ha dose): flubendiamide (72), chlorantraniliprole + lambda-cyhalothrin (30 + 15), chlorfenapyr (288), spinetoran (18), indoxacarb (120), emamectin benzoate (10), metomil (322.5), *Bacillus thuringiensis* (500), and control (water). A completely randomized design was used with four replications for each size of caterpillars (small and large), and each repetition (plot) consisted of five *H. armigera* caterpillars fed with bean leaves immersed in the different treatments evaluated. The larval mortality evaluations were performed at one, three, five, and seven days after contact with the treated bean leaves, determining the control efficiency (E%) through the formula of Abbott (1925). All chemical and biological treatments tested caused significant mortality of small and large *H. armigera* caterpillars, reaching a control percentage of 100% at seven days after treatment, demonstrating the possibility of using the insecticides tested to control this pest under field conditions.

Keywords: insecticide, larval mortality, control efficiency, Heliothinae

1. Introduction

Helicoverpa spp. caterpillars are considered economically important pests for several cultivated plant species around the world. The species *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae) is among the most relevant because it is an extremely polyphagous pest, as it has already been found feeding on more than 170 host plants from different families (Cunningham & Zalucki, 2014), including the following crops: soybean *Glycine max* L., cotton *Gossypium hirsutum* L., corn *Zea mays* L., wheat *Triticum aestivum* L., rice *Oryza sativa* L., oat *Avena sativa* L., sorghum *Sorghum bicolor* L., common bean *Phaseolus vulgaris* L., peanut *Arachis hypogaea* L., sunflower L., tomato *Lycopersicon esculentum* L., potato *Solanum tuberosum* L., and pigeon pea *Cajanus cajan* L. (Kuss et al., 2016).

Its key pest status in several crops is mainly due to the fact that *H. armigera* larvae are able to feed on both their reproductive and vegetative although they have a higher preference for the first ones (Ávila et al., 2013). It has a high capacity to adapt to adverse conditions and a high reproductive potential compared to other lepidopteran

species (Mironidis et al., 2009; Naseri et al., 2009a, 2009b). In addition, adults can migrate over a thousand kilometers especially using night flights (Silva et al., 2018). Management of *H. armigera* is required throughout the entire development period of the host plants, due to its high reproductive and consumption capacity. The attack by large caterpillars is commonly observed in formed reproductive structures, enhancing the damage caused by this pest (Ávila et al., 2013). To control *H. armigera* caterpillars, several chemical molecules are available on the world market that can be used in different crops (Noorani et al., 1994; Abdul et al., 2003; David et al., 2005; Ávila et al., 2013). Active ingredients including lambda-cyhalothrin, methomyl, and emamectin benzoate are widely used to control this pest in several countries (Avilla & Gonzalez-Zamora, 2010; Babariya et al., 2010). However, to control *H. armigera* in Brazil, there is a lack of products registered for its management, as well as few works developed under Brazilian conditions. As this pest was detected in the Cerrado region at population levels never before registered and causing serious economic damage, several products were made available by the Brazilian Ministry of Agriculture, Livestock, and Food Supply (MAPA-*Ministério da Agricultura e Pecuária*), for emergency control (EMBRAPA, 2013). However, the use of these products should always be done in accordance with the recommended doses and insect population levels in the field, as *H. armigera* has demonstrated the potential to develop resistance to various commercial chemical products (Ahmad et al., 2001; Fathipour & Sedaratian, 2013).

In addition to the available chemical insecticides, biological products are also part of the strategy to control this pest, mainly due to the increase of environmental problems arising from the misuse of synthetic chemicals, as well as the demand for development of a more sustainable and environmentally friendly agriculture. The main advantage being the absence of residues in agricultural products and the environment (Oliveira & Ávila, 2010). Thus, the use of chemical and biological insecticides is an important tool for insect pest management in various crops. Although insecticides used to control *H. armigera* caterpillars can prevent economic damage, a loss of efficiency has been observed as reported by Chankapue et al. (2014).

Therefore, the objective of this study was to evaluate the control efficiency of seven chemical and a biological insecticide to control small and large *H. armigera* caterpillars under laboratory conditions.

2. Material and Methods

The experiment was conducted in the Entomology laboratory of Embrapa Western Agriculture, located in Dourados, MS (22°14'S 54°49'W). The bean cultivar *Phaseolus vulgaris* L. "BRS Estilo" was planted in five liter plastic pots containing a 1:1:1 mixture of soil, sand, and organic substrate, kept in a greenhouse. After the emergence of bean plants and the unfolding of third trifoliolate leaf, these leaves were removed from the plants, sanitized, and cut into leaf disks with an area of 12.56 cm², using a metal punch.

Subsequently, the leaf disks were immersed in the insecticidal spray containing different treatments with their respective doses (Table 1) for approximately five seconds and then removed from the spray and put for drying on paper towels. These leaf discs were then offered to small and large *H. armigera* caterpillars kept in Petri dishes (6.0 cm diameter × 1.3 cm height) for a period of seven days. The leaf discs were replaced in the Petri dish when necessary, depending on the degree of consumption by the caterpillars.

The experiment was carried out in a completely randomized design with nine treatments: seven chemical insecticides, a biological, and control (without insecticide) in four replications. Each experimental unit (repetition) consisted of five small (< 1.5 cm) or large (> 1.5 cm) *H. armigera* caterpillars. *Helicoverpa armigera* caterpillars, used in the bioassay, originated from the stock maintained on artificial diet in the Entomology laboratory of Embrapa Western Agriculture, as described by Vieira et al. (2018).

Mortality assessments of small and large larvae were performed at one, three, five, and seven days after leaf disc immersion (DAI) in the different treatments, to determine the number of dead caterpillars (N) after contact with untreated or treated leaf discs. The control efficiency (E%) in each treatment was calculated using Abbott's formula (1925). The mortality data obtained were submitted to analysis of variance and the means of treatments compared by Tukey test at 5% probability.

Table 1. Chemical and biological treatments (g a.i./ha) used to the control of small and large caterpillars of *Helicoverpa armigera* (Hübner, 1805) under laboratory conditions. Dourados/MS, 2019

Treatments	Dose (g a.i./ha)	Chemical group	Commercial name	Group/mode of action ¹
Flubendiamide	72.0	Phthalic Acid Diamide	Belt [®] 480 SC	G28
Chlorantraniliprole + lambda-cyhalothrin	30.0 + 15.0	Diamide + Pyrethroid	Ampligo [®] 100+50 SC	G3
Chlorfenapyr	288.0	Pyrazole Analog	Pirate [®] 240 SC	G13
Espinetoram	18.0	Spinosinas	Exalt [®] 120 SC	G5
Indoxacarb	120.0	Oxadiazine	Avatar [®] 150 EC	G22
Emamectin Benzoate	10.0	Avermectin	Proclaim [®] 50 SG	G6
Metomil	322.5	Oxime Methyl Carbamate	Lannate BR [®] 215 SL	G1
<i>Bacillus thuringiensis</i>	500.0	Biological	Agree [®] 500 WP	G11
Control	-	-	-	-

Note. ¹ classification of groups and mode of action of insecticides according to IRAC (2019).

^{G28} ryanodine receptor modulators; ^{G3} modules of sodium channels; ^{G13} oxidative phosphorylation decouplers via proton gradient interruption; ^{G5} allosteric Acetylcholine Receptor Modulators (nAChR); ^{G22} voltage-dependent sodium channel blockers; ^{G6} allosteric Modules of Glutamate Chloride (GluCl) Closed Channels; ^{G1} acetylcholinesterase (AChE) enzyme inhibitor; ^{G11} microbial insect gut disruptors.

3. Results

On the first day after contact caterpillars with treated bean leaves (1 DAI), the chemical treatments chlorphenapyr (288.0 g a.i./ha) and indoxacarb (120.0 g a.i./ha), followed by benzoate of emamectin (10.0 g a.i./ha) achieved the highest shock effects to control *H. armigera* small caterpillars (< 1.5 cm) compared to control and other treatments evaluated in the assay (Table 2). At three DAI, only flubendiamide treatments (72.0 g a.i./ha), chlorantraniliprole + lambda-cyhalothrin (30.0 + 15.0 g a.i./ha) and *B. thuringiensis* (500.0 g a.i./ha) did not show significant control to small caterpillars when compared to control, whereas other chemical treatments provided relatively high levels to control, especially with chlorfenapyr treatment (288.0 g a.i./ha) that presented 100% pest control (Table 2). At five and seven DAI, all chemical treatments showed significant control of small caterpillars in relation to the control treatment, without differing from each other. At seven DAI, there was no small caterpillar (< 1.5 cm) surviving in the Petri dishes for all the chemical and biological treatments tested (Table 2).

As for the efficiency to control large caterpillars (> 1.5 cm), in the first evaluation period (1 DAI), the chemical treatments chlorfenapyr (288.0 g a.i./ha), indoxacarb (120.0 g a.i./ha), and emamectin benzoate (10.0 g a.i./ha) obtained the lowest numbers of surviving caterpillars compared to control and similar to that observed for small caterpillars, providing control percentages of 60%, 55%, and 70%, respectively (Table 3). The other chemical and biological treatments evaluated had a number of surviving caterpillars similar to those observed in the control treatment for the first evaluation period (Table 3).

At three DAI, treatments flubendiamide (72.0 g a.i./ha), chlorantraniliprole + lambda-cyhalothrin (30.0 + 15.0 g a.i./ha), and *B. thuringiensis* (500.0 g a.i./ha) did not demonstrate significant control to large caterpillars compared to the control treatment, similarly to that observed for small caterpillars with these same treatments (Tables 2 and 3). In the other chemical treatments, higher levels of population reduction of large caterpillars were observed, with emphasis once again on the chlorphenapyr (288.0 g a.i./ha) and emamectin benzoate (10.0 g a.i./ha) treatments, which obtained the highest control efficiencies of large caterpillars (Table 3). At five DAI, all chemical and biological treatments evaluated in the trial significantly reduced *H. armigera* large caterpillars' population in relation to the control treatment, with control levels ranging from 77.8% to 100.0%. In the last mortality assessment performed in the trial (7 DAI), all large *H. armigera* caterpillars were dead due to insecticide action, providing 100% mortality in all chemical or biological treatments tested (Table 3).

Table 2. Average number of surviving small (< 1.5 cm) *Helicoverpa armigera* (Hübner, 1805) caterpillars (N) and percentage of control efficiency (E%) at one, three, five, and seven days after treatment with different chemical and biological insecticides. Dourados/MS, 2019

Treatments	Dose (g a.i./ha)	1 DAI		3 DAI		5 DAI		7 DAI	
		N	E(%)	N	E(%)	N	E(%)	N	E(%)
Flubendiamide	72.0	4.5±0.6 abc	10.0	4.5±0.6 a	10.0	0.3±0.5 b	95.0	0.0±0.0 b	100.0
Chlorantraniliprole + lambda-cyhalothrin	30.0+15.0	4.5±0.6 abc	10.0	3.8±1.0 a	25.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Chlorfenapyr	288.0	1.3±1.0 d	75.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Espinetoram	18.0	2.5±0.6 bcd	50.0	0.8±1.0 b	85.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Indoxacarb	120.0	1.5±1.0 d	70.0	1.0±1.2 b	80.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Emamectin Benzoate	10.0	2.3±1.3 cd	55.0	0.5±0.6 b	90.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Metomil	322.5	2.8±1.9 abcd	45.0	0.3±0.5 b	95.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
<i>Bacillus thuringiensis</i>	500.0	4.8±0.5 ab	5.0	4.8±0.5 a	5.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Control	-	5.0±0.0 a	-	5.0±0.0 a	-	3.8±0.5 a	-	3.8±0.5 a	-
CV (%)		29.86		30.46		53.03		40.00	

Note. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test ($p < 0.05$).

DAI (Days after immersion).

Table 3. Average number of surviving large (> 1.5 cm) larvae (N) of *Helicoverpa armigera* (Hübner, 1805) and percentage of control efficiency (E%) at one, three, five and seven days after treatment with different chemical and biological insecticides. Dourados/MS, 2019

Treatments	Dose (g a.i./ha)	1 DAI		3 DAI		5 DAI		7 DAI	
		N	E(%)	N	E(%)	N	E(%)	N	E(%)
Flubendiamide	72.0	5.0±0.0 a	00.0	4.8±0.5 ab	5.0	1.0±0.8 b	77.8	0.0±0.0 b	100.0
Chlorantraniliprole+lambda-cyhalothrin	30.0+15.0	5.0±0.0 a	00.0	3.8±1.0 abc	25.0	0.8±1.0 b	83.3	0.0±0.0 b	100.0
Chlorfenapyr	288.0	2.0±1.4 b	60.0	0.0±0.0 e	100.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Espinetoram	18.0	3.5±1.9 ab	30.0	2.5±1.9 bcd	50.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Indoxacarb	120.0	2.3±1.0 b	55.0	1.5±1.3 cde	70.0	0.5±0.6 b	88.9	0.0±0.0 b	100.0
Emamectin Benzoate	10.0	1.5±1.0 b	70.0	0.5±0.6 de	90.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Metomil	322.5	3.3±0.5 ab	35.0	1.8±1.3 cde	65.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
<i>Bacillus thuringiensis</i>	500.0	5.0±0.0 a	00.0	4.8±0.5 ab	5.0	0.0±0.0 b	100.0	0.0±0.0 b	100.0
Control	-	5.0±0.0 a	-	5.0±0.0 a	-	4.5±0.6 a	-	4.3±0.5 a	-
CV (%)		25.84		36.05		66.67		35.29	

Note. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test ($p < 0.05$).

DAI (Days after immersion).

4. Discussion

Insecticides, both chemical and biological, are important tools to manage various pest insects to different crops. Those used to control *H. armigera* caterpillars can prevent economic damage in most crops where this species occurs. However, research evaluating the effectiveness of insecticides in controlling this pest has found different percentages of efficiency (Guedes et al., 2013; Chankapue et al., 2014). According to MAPA (2018), chemical and biological insecticides, released in Brazil, must control on average at least 80% of the target pests for the products to be considered agronomically efficient. Based on our results, all chemical and biological insecticides, tested with their respective doses, are efficient to control small and large *H. armigera* larvae, because by the last mortality evaluation performed in the assays (7 DAI), all *H. armigera* caterpillars (both sizes) had been killed due to insecticide action, providing 100% mortality.

The degree of control efficiency of a product is extremely important in the management of caterpillars, since in most cases spraying is performed almost as soon as a pest occurs in the crop, *i.e.*, when small caterpillars predominate, to avoid greater damage to crops by large caterpillars (Cruz, 2002). However, the control efficiency may change due to the different developmental stages of the pest in the crop, and smaller sized caterpillars are

generally more susceptible to insecticides (Gusmão et al., 2000; Basavanneppa & Balikai, 2014; Abbas et al., 2015; Kuss et al., 2016). In field spraying, caterpillars receive more product on the integument, penetrating into the cuticle, trachea, or even pores, reaching the nervous system, and causing the death of the insect (Viana & Costa, 1998; Fernandes et al., 2018). This work evaluated the control efficiency on both, small (< 1.5 cm) and (> 1.5 cm) caterpillars, determining that the overall the size of the caterpillar had no influence on their susceptibility to the tested products.

The main chemical groups of insecticides applied to soybean crops, the primary host plant for *H. armigera* caterpillars in Brazil, are pyrethroids, organophosphates, and carbamates, which have been used for decades. However, benzoylureas and phthalic acid diamides have been used more recently (Hannig et al., 2009; Guedes et al., 2013; Tomquelski et al., 2015). Thus, at least five molecules (indoxacarb, chlorantraniliprole, chlorphenapyr, spinosad, and flubendiamide) provide satisfactory control efficiency of *H. armigera*. These molecules represent products registered with different chemical groups, enabling the rotation of products due to the different mechanisms of action, which can prevent the development of insecticide resistant populations (Corrêa-Ferreira et al., 2014). It is well known that *H. armigera* is a species that can easily develop populations resistant to commercially available insecticide (Fathipour & Sedaratian, 2013).

Even though no statistical differences between the sizes of caterpillars and the products tested were found during the evaluations, the ones with the lowest shock effect were flubendiamide, cyantraniliprole + lambdacialotrin, and *B. thuringiensis*. This can be partially explained by the fact that flubendiamide is a newly discovered group, and there are few studies conducted to evaluate its effectiveness in controlling *H. armigera* caterpillars. However, the results generated in this research may contribute to these clarifications.

The mode of action is related to the active ingredient in each product. The chemical insecticides of the diamide group (flubendiamide and chlorantraniliprole) when in contact with the insect binds to the ryanodine receptors on muscle cells, causing calcium channels to open, leading initially the cessation of feeding, paralysis, and posteriorly its death (Cordova et al., 2006; Lahm et al., 2007; Arrue et al., 2014). In this way, the mode of action of diamids explains the delayed shock effect observed for both small and large caterpillars. Chlorantraniliprole is an important insecticide in the diamide group (IRAC, 2019), that presents low toxicity to other animals such as natural enemies, mammals, birds, and fish (Lahm et al., 2007; Larson et al., 2012). Flubendiamide is another insecticide in the same group that also causes slower mortality of target insects (Lima-Neto, 2016), as was also observed in the results obtained in this study for second and fourth instar caterpillars.

For the only biological insecticide tested *B. thuringiensis*, studies have showed that first and second instar small caterpillars (< 1.5 cm) die one week after application (Salvadori et al., 2013), corroborating the low mortality of *H. armigera* observed in the first evaluations for both caterpillar sizes. This low control efficiency, after exposure to caterpillars, could be related to its mechanism of action, because this product affects the intestinal epithelium, interrupting caterpillar feeding within hours after exposure, but not causing the immediate death of caterpillars in the early phase of contamination (Bueno et al., 2012), as also was observed for the tested insecticides of the diamide group.

An important characteristic for insecticides, in addition to their efficiency, is their residual period, which decreases the number of required applications and costs, consequently, increasing the plant protection period, as observed in all insecticide treatments in this research after five DAI for both, small and large caterpillars. Regarding the shock effect, the chlorphenapyr (288.0 g a.i./ha) and indoxacarb (120.0 g a.i./ha) insecticides were more effective, especially for small caterpillars, when they presented 75% and 70% of control, respectively, in 24 hours (Table 2). The results obtained in this work with these products also showed good control of large *H. armigera* caterpillars together with emamectin benzoate treatment (10.0 g a.i./ha), which corroborates the results presented by Perini et al. (2016); Kuss et al. (2016); Sandip and Arunava (2018), which obtained similar results with chlorphenapyr for *H. armigera* control. In general, the control of *H. armigera* reached 100% mortality level at seven DAI for both, small and large caterpillars with different insecticides used. Thus, these products present a promising alternative to control caterpillars of this pest by producers. Furthermore, according to the results obtained here, the control of *H. armigera* with biological products can be also viable, as the results obtained with *B. thuringiensis* were quite satisfactory. However, successful control of any type of pest must include the proper pest monitoring in the area, rotation of products with different active ingredient, and selection of insecticides that preserve natural enemies and environment, which are fundamental principles of the Integrated Pest Management.

5. Conclusions

All chemical and biological insecticides tested in this study are efficient to control *H. armigera* caterpillars, not only in the early phase of development, but also for the most developed caterpillars.

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