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# Potential of chemical insecticides on fourth instar caterpillars of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

# Potencial de inseticidas químicos sobre lagartas de quarto instar de *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae)

**GAZOLI, Leonardo(1); DUARTE, Rogério Teixeira(2)**

(1) 0000-0003-1020-4528; University of Araraquara (*Universidade de Araraquara*). Araraquara, São Paulo (SP), Brazil. Email: leogazoli@hotmail.com.
(2) 0000-0002-4908-6349; University of Araraquara (*Universidade de Araraquara*). Araraquara, São Paulo (SP), Brazil. Email: rogerio.tduarte@yahoo.com.br.



## ABSTRACT

*Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is considered an important pest of Brazilian agriculture, whose management is oriented mainly towards the control of immatures. The objective of this research was to analyze the effect of chemical insecticides on fourth-instar caterpillars of *H. armigera*. The research was conducted under laboratory conditions, corresponding to the control treatments, zeta-cypermethrin (37.5 mL / 500 L); lambda-cyhalothrin (75 mL/ 150 L); chlorpyrifos (1.0 L / 300 L); bifenthrin + carbosulfan (1.0 L / 350 L); and beta-cyfluthrin (60 mL / 250 L). The bioassays were conducted in plastic containers containing artificial diet. After application of the treatments on the food substrate, and subsequent drying, two caterpillars were inserted per container, and the set of four containers was considered a repetition, totaling four replicates per treatment, with mortality evaluations after 24, 48, 96 and 144 hours of the applications. The sublethal effect was analyzed by evaluating the average weight of caterpillars and pupae (g), and emergence of adults (%). The active ingredients chlorpyrifos and bifenthrin + carbosulfan caused 100% mortality of fourth-instar caterpillars of *H. armigera* from 48 hours of the applications. After 96 hours of phytosanitary application, lambda-cyhalothrin was responsible for causing mortality above 80%. Regarding the sublethal effect, better results were observed in the lambda-cyhalothrin and beta-cyfluthrin treatments, influencing the average weight of the caterpillars and the emergence of adults. The active ingredients chlorpyrifos, bifenthrin + carbosulfate and lamba-cyhalothrin were presented as potential insecticides in the management of fourth-instar caterpillars of *H. armigera*.

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***Palavras-Chave*:**
controle químico, lepidópteros, imaturos, manejo

## RESUMO

*Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) é considerada uma importante praga da agricultura brasileira, cujo manejo é orientado principalmente frente ao controle de imaturos. O objetivo da pesquisa foi analisar o efeito de inseticidas químicos sobre lagartas de quarto ínstar de *H. armigera*. A pesquisa foi conduzida em condições laboratoriais, correspondendo aos tratamentos controle, zeta-cipermetrina (37,5 mL /500 L); lambda-cialotrina (75 mL/ 150 L); clorpirifós (1,0 L / 300 L); bifentrina + carbosulfano (1,0 L / 350 L); e beta-ciflutrina (60 mL / 250 L). Os bioensaios foram conduzidos em recipientes plásticos contendo dieta artificial. Após aplicação dos tratamentos sobre o substrato alimentar, e posterior secagem, foram inseridas duas lagartas por recipiente, sendo o conjunto de quatro recipientes considerado uma repetição, totalizando quatro repetições por tratamento, com avaliações de mortalidade após 24, 48, 96 e 144 horas das aplicações. O efeito subletal foi analisado por intermédio da avaliação do peso médio das lagartas e pupas (g), e emergência de adultos (%). Os ingredientes ativos clorpirifós e bifentrina + carbosulfano ocasionaram 100% de mortalidade de lagartas de quarto ínstar de *H. armigera* a partir de 48 horas das aplicações. Após 96 horas da aplicação fitossanitária, lambda-cialotrina foi responsável por ocasionar mortalidade acima de 80%. Em relação ao efeito subletal, foi observada melhores resultados nos tratamentos lambda-cialotrina e beta-ciflutrina, influenciando no peso médio das lagartas e na emergência de adultos. Os ingredientes ativos clorpirifós, bifentrina + carbosulfato e lamba-cialotrina apresentaram-se como potenciais inseticidas no manejo de lagartas de quarto instar de *H. armigera*.

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## Introduction

*Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is one of the most impactful and significant pests in agriculture in Asia, Europe, Africa and Australia, causing economic damage, but also environmental and social cost (Tay et al., 2013). In Brazil, this species has been considered a quarantine pest A1 since 1999 (Oliveira et al., 2003), with initial reports of its occurrence from the 2011/2012 harvest, erroneously identified as Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae) (Thomazoni et al., 2013).

This pest has a polyphagous aspect, causing damage to different crops of economic importance, mainly soybeans, corn, beans and cotton (Czepak et al., 2013a; Thomazoni et al., 2013). Immatures can cause injury in the vegetative and reproductive phases of agricultural crops, but have a preference for shoots, inflorescences, fruits and pods (Reed, 1965; Wang; Li, 1984). Another extremely important characteristic in the characterization of *H. armigera* is related to its high capacity for dispersion, adaptation and multivoltinism (Pedgley, 1985; Fitt, 1989), which favors its rapid dissemination and establishment in different Brazilian agricultural regions.

Among the control strategies of *H. armigera*, the chemical method has been the main form of population reduction of this pest in the field, which has propitiated the evolution of the resistance of this species to different active ingredients of insecticides, such as fipronil, chlorfenapir, spinosad and indoxacarb (Ahmad et al. 2003; Wu 2007). In this perspective, there are different mechanisms of resistance of *H. armigera* to chemical insecticides (Ahmad, 2007), but studies show that the early stages of development of immature *H. armigera* are the most susceptible to the use of chemical control (Ahmad et al., 2001).

However, under field conditions, only a single instar of a given phytophagous species is not necessarily found, and thus, it´s necessary to conduct research that analyzes the efficiency of chemical molecules of insecticides from the fourth instar, at which time it has been evidenced greater difficulty of management (Kuss et al., 2016). The objective of this research was to analyze the effect of chemical insecticides on fourth-instar caterpillars of *H. armigera*.

## Material and Methods

The research was carried out at the Laboratory of Agricultural Entomology (*Laboratório de Entomologia Agrícola*) (LEA-UNIARA), Department of Management and Technology Sciences, University of Araraquara (*Universidade de Araraquara* - UNIARA). A laboratory population of *H. armigera* was used to conduct the bioassays. For the bioassays, fourth-instar caterpillars were used, packed in rectangular plastic containers 7 cm long by 5 cm wide and 3 cm high, containing approximately 10 $cm^3$ of artificial diet suitable for breeding *H. armigera*.

The experiment consisted of five treatments, one of which was a control, consisting of the application of distilled water, and treatments based on zeta-cypermethrin (Fury® 400EC - FMC Química do Brasil Ltda.; recommended dosage of 37.5 mL/ha with syrup volume of 500 L/ha); lambda-cyhalothrin (Karate Zeon® 50CS - Syngenta Proteção de Cultivos Ltda.; recommended dosage of 75 mL/ha with syrup volume of 150 L/ha); chlorpyrifos (Klorpan® 480EC - Sumitomo Chemical; recommended dosage of 1.0 L/ha with syrup volume of 300 L/ha); bifenthrin + carbosulfan (Talisman® - FMC Química do Brasil Ltda.; recommended dosage of 1.0 L/ha with syrup volume of 350 L/ha); and beta-cyfluthrin (Turbo® - Bayer S.A.; recommended dosage of 60 mL/ha with syrup volume of 250 L/ha) (Agrofit, 2018).

After the preparation of the control and the insecticide suspensions, they were individually allocated in a magnetic stirrer (Model MAG-01H, Marte Científica, São Paulo, SP, Brazil), aiming at homogenization for two minutes. In each plastic container, 200 µL of the insecticidal solution related to each treatment was applied superficially to the diet, with the aid of an automatic pipette [Model P100 (10-100 µL), Kasvi - São José dos Pinhais, PR, Brazil]. After evaporation of excess moisture, two fourth-instar caterpillars were inserted per container, and the set of four containers was considered a repetition, totaling four replicates per treatment. The control treatment followed the same methodology described above, only with the replacement of the insecticide solution by distilled water.

After the assembly of the bioassays, the containers were packed in the experimentation room of the LEA-UNIARA, and the mortality evaluations were measured after 24, 48, 96 and 144 hours of the treatments. Total mortality was corrected for mortality observed in the control (Abbott, 1925). The sublethal effect of treatments based on chemical insecticides on the surviving caterpillars of *H. armigera* was analyzed by the biological parameters mean weight of the caterpillars (g) after the last evaluation of mortality (144 hours) and mean weight of pupae (g), conducted by means of an analytical balance (Model AUY220, Shimadzu do Brasil - Barueri, SP, Brazil), in addition to the emergence of adults (%).

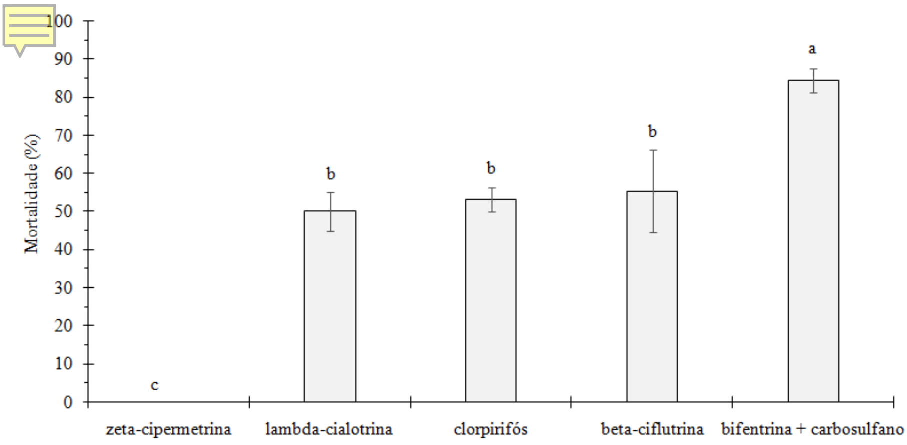
The experiment was conducted in a completely randomized design, with the data being subjected to analysis of variance and the means compared by Tukey's test ($P < 0.05$), through the statistical program Sisvar 5.6 (Ferreira, 2014).

## Results and Discussion

In the first 24 hours after offering the treated diet, mortality of fourth-instar caterpillars of *H. armigera* was observed between 0.0 and 84.38%, with significant results among the insecticides analyzed ($F = 28.590$; gl = 4.15; $P < 0.05$), and highlight the use of the active ingredient bifenthrin + carbosulfan, whose mortality was above 80% (Figure 1).

**Figure 1.**

*Mean cumulative mortality (%) of fourth-instar caterpillars Helicoverpa armigera after 24 hours of treatment application. Values followed by the same letter do not differ significantly from each other by Tukey's test (P < 0.05). The error bar corresponds to the standard error (EP).*

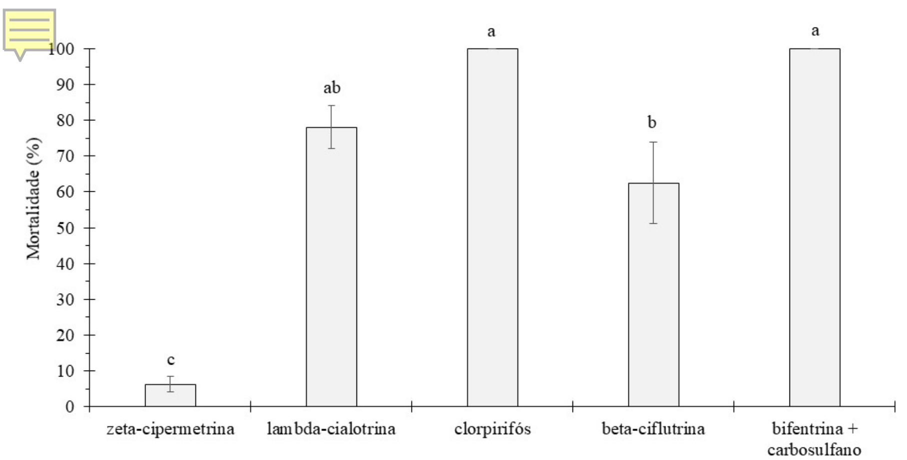



Mortality (%), zeta-cypermethrin, lambda-cyhalothrin, chlorpyrifos, beta-cyfluthrin, bifenthrin + carbosulfan

It was observed that after 48 hours of the application of the treatments, the mean accumulated mortality was between 6.25 and 100%, with total control of fourth-instar caterpillars in the treatments related to the application of the active ingredients chlorpyrifos and bifenthrin + carbosulfan, differing significantly from the treatments based on beta-cyfluthrin (62.5 ± 11.41%) and zeta-cypermethrin (6,25 ± 2,15%) (F = 36,476; gl = 4, 15; $P$ < 0,05) (Figure 2).

**Figure 2.**

*Mean cumulative mortality (%) of fourth-instar caterpillars Helicoverpa armigera after 48 hours of treatment application. Values followed by the same letter do not differ significantly from each other by Tukey's test (P < 0.05). The error bar corresponds to the standard error (EP).*

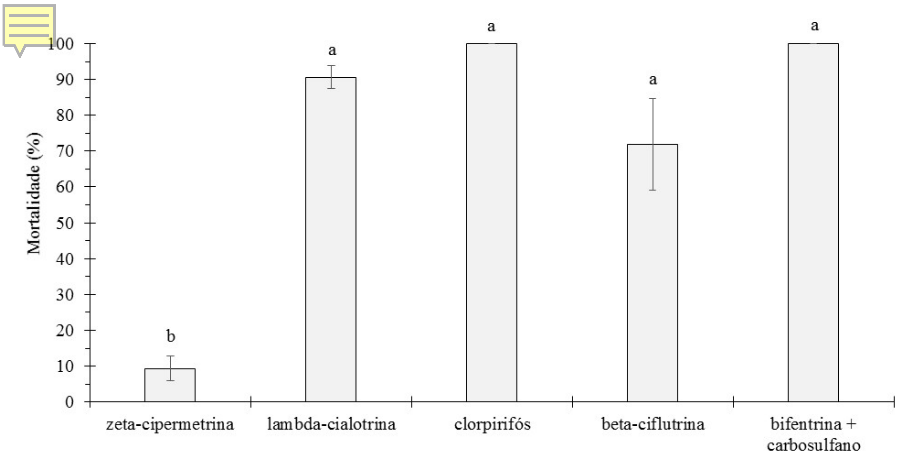



Mortality (%), zeta-cypermethrin, lambda-cyhalothrin, chlorpyrifos, beta-cyfluthrin, bifenthrin + carbosulfan

After 96 hours of insecticide application it was observed, in most treatments, the mean accumulated mortality of fourth-instar caterpillars of *H. armigera* above 70%, with a statistical difference of these in comparison with the active ingredient zeta-cypermethrin, whose mortality was of 9,38 ± 3,41% (F = 27,537; gl = 4, 15; *P* < 0,05) (Figure 3).

**Figure 3.**

*Mean cumulative mortality (%) of fourth-instar caterpillars Helicoverpa armigera after 96 hours of treatment application. Values followed by the same letter do not differ significantly from each other by Tukey's test (P < 0.05). The error bar corresponds to the standard error (EP).*

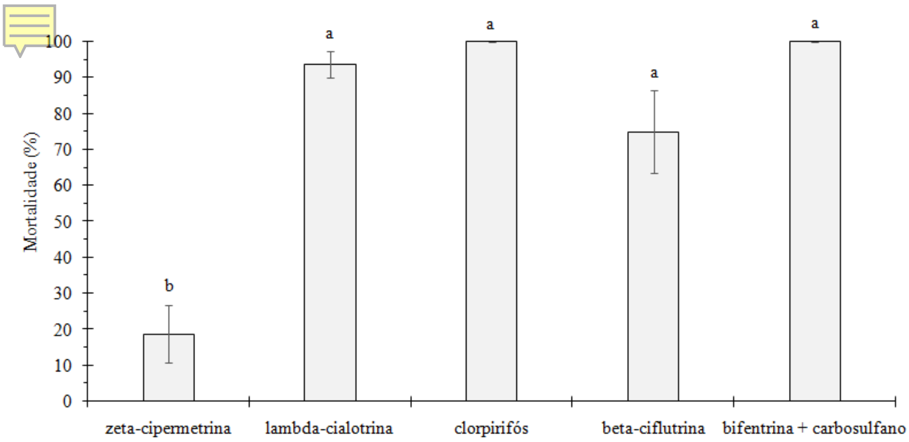



Mortality (%), zeta-cypermethrin, lambda-cyhalothrin, chlorpyrifos, beta-cyfluthrin, bifenthrin + carbosulfan

For the last evaluation, characterized 144 hours after the beginning of the experiment, the mean accumulated mortality was above 75% in most treatments, differing statistically only from the treatment based on the active ingredient zeta-cypermethrin, whose mortality was 18,75 ± 8,07% (F = 28,406; gl = 4, 15; *P* < 0,05) (Figure 4).

**Figure 4.**

*Mean cumulative mortality (%) of fourth-instar caterpillars Helicoverpa armigera after 144 hours of treatment application. Values followed by the same letter do not differ significantly from each other by Tukey's test (P < 0.05). The error bar corresponds to the standard error (EP)*




Mortality (%), zeta-cypermethrin, lambda-cyhalothrin, chlorpyrifos, beta-cyfluthrin, bifenthrin + carbosulfan

Regarding the management of inmature fourth-instar *H. armigera* with insecticides, Kuss et al. (2016) observed high mortality from three days of exposure of immatures to soybean leaves harvested after 24 hours of phytosanitary applications, especially for the chemical molecules flubendiamide, chlorantraniliprole, chlorpyrifos and indoxacarb, responsible for causing 100% mortality of immatures of the said species. This study corroborates what was observed in the present study for the active ingredient chlorpyrifos, given that, from 48 hours after the application of the treatments, total mortality of immatures of fourth instar of *H. armigera* was observed (Figure 1).

An important factor to be considered in the research conducted by Kuss et al. (2016), is consistent with the moment by which the immature fourth star of *H. armigera* comes into contact with the food substrate treated with insecticide, evidencing that, after 72 hours of spraying on the soybean foliage, with subsequent introduction of the caterpillars in this food environment, no satisfactory control was observed in any of the treatments marked in the chemical control, in order to characterize the moment of application an important factor within the management of this pest.

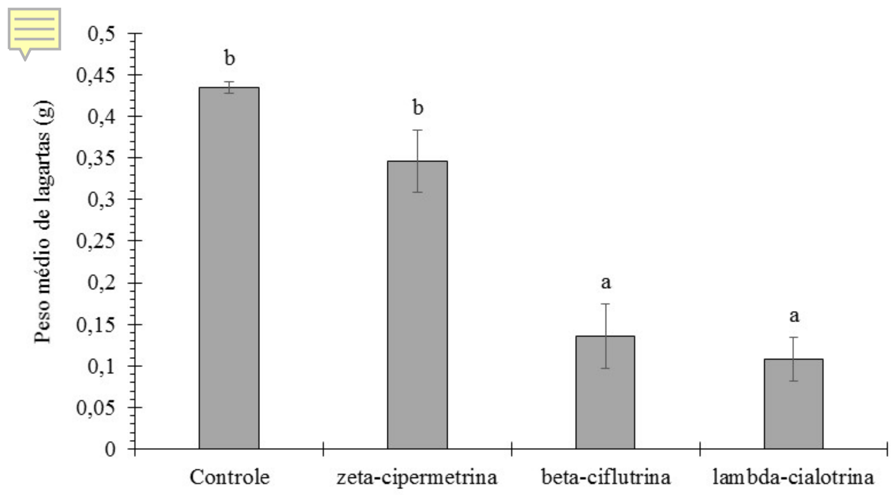
Another factor of interference in the sensitivity of immatures of *H. armigera* to chemical insecticides concerns the texture of their integument, represented by leathery characteristics, with differences in relation to other species of Helionthinae presents in Brazil, which may be closely related to the capacity of tolerance to contact insecticides, especially in relation to pyrethroids, organosphosphorates and carbamates (Ávila et al., 2013; Czepak et al., 2013b). In these research, it was evident lower efficiency of the pyrethroid group in relation to the others, especially zeta-cypermethrin, which may be related in terms of the insect integument, thus hindering the contact action of the chemical molecule in relation to its site of action.

The genetic issue of the species itself may also have been a relevant factor in relation to the efficiency of the insecticides used in the present research in relation to the management of *H. armigera*, especially in the case of the chemical group of pyrethroids, given that, according to Durigan et al. (2017), the populations of *H. armigera* in Brazil have low susceptibility to this chemical group due to the cytochrome P450 gene CYP 337B3, present in most populations of this species, related to Brazil, which can also better explain the results obtained regarding the use of the active ingredient zeta cypermethrin, whose mortality was much lower compared to other treatments.

Regarding the sublethal effect of the surviving caterpillars of *H. armigera*, a significant reduction was observed in relation to the weight of the immatures related to the treatments with the active ingredients beta-cyfluthrin and lamba-cyhalothrin, when compared with the other treatments, being represented with an average weight of 0,1358 ± 0,039 g and 0,1079 ± 0,027 g, respectively (F = 35,596; gl = 3, 9; $P$ < 0,05) (Figure 5).

**Figure 5.**

*Average weight (g) of surviving caterpillars of Helicoverpa armigera submitted to treatments. Values followed by the same letter do not differ significantly from each other by Tukey's test (P < 0.05). The error bar corresponds to the standard error (EP).*

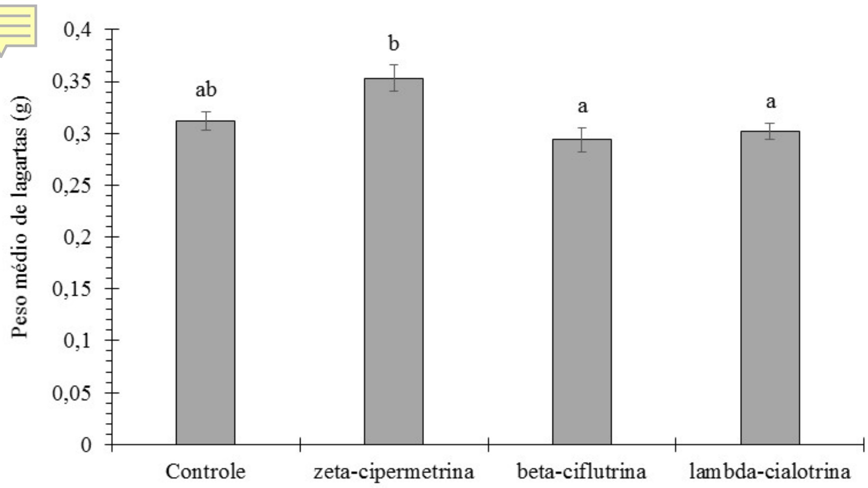



Average weight of caterpillars (g), Control, zeta-cypermethrin, beta-cyfluthrin, lambda-cyhalothrin

The average pupae weight of *H. armigera* was also lower in the treatments based on the active ingredients beta-cyfluthrin and lambda-cyhalothrin, with 0.2936 ± 0.012 g and 0.3019 ± 0.008 g, respectively, differing statistically from the treatment with the active ingredient zeta-cypermethrin, with average pupa weight of 0,3532 ± 0,013 g (F = 5,941; gl = 3, 9; $P < 0,05$) (Figure 6).

**Figure 6.**

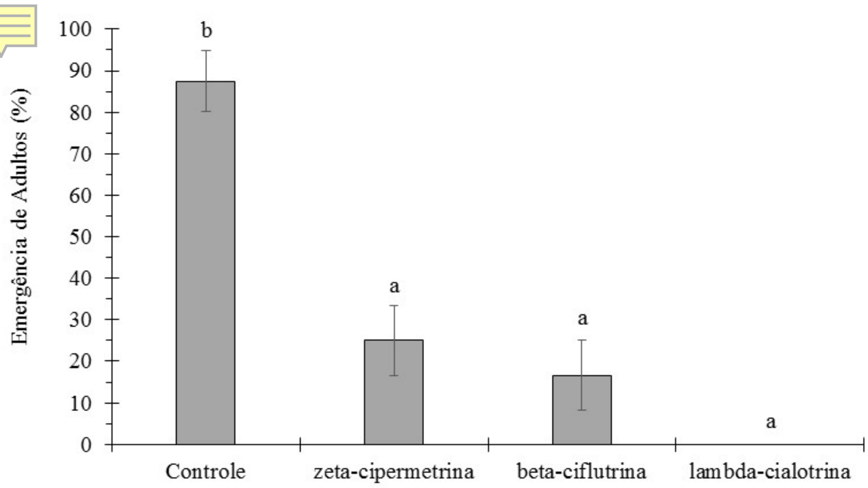
*Average weight of Helicoverpa armigera pupae submitted to treatments. Values followed by the same letter do not differ significantly from each other by Tukey's test (P < 0.05). The error bar corresponds to the standard error (EP).*




In addition, the emergence of adults of *H. armigera* was significantly lower in the treatments based on the insecticides analyzed, with means below 25.0%, when compared to the control, related to 87.5 ± 7.22% of adult emergence (F = 23,589; gl = 3, 9; $P < 0,05$) (Figure 7).

**Figure 7.**

*Mean emergence (%) of adults of Helicoverpa armigera undergoing treatments. Values followed by the same letter do not differ significantly from each other by Tukey's test (P < 0.05). The error bar corresponds to the standard error (EP).*




A very important factor to be considered, is the sub-lethal effect of chemical and biological insecticides on *H. armigera*, in order to intervene greatly in morphological but also physiological aspects of the pest, negatively influencing its development and generation of new offspring, factors evidenced in this research, but also reported by Junior et al. (2009) and Carneiro et al. (2016), thus demonstrating the need for research on the sub-lethal effect of insecticides on agricultural pests, in order to fully understand the action of a certain chemical molecule on the insect pest.

## Conclusions

The best active ingredients for the management of fourth instar caterpillars of *H. armigera* were chlorpyrifos, bifenthrin + carbosulfate and lamba-cyhalothrin.

The active ingredient beta-cyfluthrin caused a sublethal effect on the surviving individuals of *H. armigera*, especially with regard to the average weight of caterpillars and emergence of adults, while the active ingredient zeta-cypermethrin influenced only the emergence of adults of the said species.

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