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AGRONOMIA

EFFICIENCY OF *Bacillus thuringiensis* IN CONTROLLING THE CORN FALL ARMYWORM IN LAB CONDITIONS

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ABSTRACT: The region of Triângulo Mineiro, in the state of Minas Gerais, has one of the highest corn productivity in Brazil. However, such productivity is significantly affected by the damage of the fall armyworm, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae), one of the most important pests in corn fields. Due to the necessity of a well-established control, the purpose of this study was to evaluate the efficacy of some insecticides commonly used by growers in this region, both biological and chemical. Under controlled laboratory conditions, we assessed mortality of second-instar *S. frugiperda* larvae on artificial diet treated with insecticides using manufacturer-recommended dosages. Three chemical insecticides, one bioinsecticide and one control (distilled water + Tween® 0.5 %) were tested. The larval mortality was evaluated 12, 24, 48, 72, 96 and 120 hours after the treatment of single application. It was possible to conclude that the bioinsecticide had the same efficiency as all the insecticides tested, with all treatments causing around 100% mortality of *S. frugiperda* larvae. The corrected mortality (CM) was calculated though the Schneider-Orelli formula and, it was possible to conclude that all the insecticides tested were efficient controlling S. frugiperda larvae, with calculated CM higher than 92 %, with no difference between the insecticide treatments. These results show that the products used by the corn growers from the region of Triângulo Mineiro, in the state of Minas Gerais, are efficient in controlling *S. frugiperda* under controlled conditions. Moreover, the results of Bt-bioinsecticide show that it is as efficient as the chemical insecticides, and can be used for controlling this pest in corn fields after regulation.

Keywords: *Insecticide. Integrated pest management. Maize.*

EFICIÊNCIA DE *Bacillus thuringiensis* NO CONTROLE DE LAGARTA DO CARTUCHO DO MILHO EM CONDIÇÕES DE LABORATÓRIO

RESUMO: A região do Triângulo Mineiro possui uma das maiores produtividades de milho do Brasil. Tal produtividade, no entanto, é afetada significativamente pelos danos causados pela lagarta-do-cartucho, *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae). Devido à necessidade de o controle da praga ser bem executado, objetivou-se avaliar alguns inseticidas químicos e um biológico, comumente utilizados pelos produtores do Triângulo Mineiro, em relação à eficiência no controle de lagartas *S. frugiperda*. As lagartas foram cedidas pela empresa Vitae Rural, situada em Uberaba-MG. Utilizou-se um delineamento inteiramente casualizado, no qual foram transferidos para cada recipiente plástico (100 mL) uma unidade de 1 cm^3 de dieta artificial previamente imersa na solução de cada tratamento e uma lagarta de segundo ínstar de *S. frugiperda*, sendo avaliadas cinco repetições de dez indivíduos por tratamento. Foram testados três inseticidas químicos e um bioinseticida, seguindo as dosagens recomendadas pelos fabricantes, e um controle (água destilada + Tween® 0,5 %). Avaliou-se a mortalidade das lagartas 12, 24, 48, 72, 96 e 120 horas após o tratamento de aplicação única. A eficiência de controle (EC) foi calculada pela fórmula de Schneider-Orelli e concluiu-se que o bioinseticida foi tão eficiente no controle de lagartas de *S. frugiperda* quanto os demais inseticidas testados, com todos tratamentos apresentando EC calculada superior a 94%, sem diferença significativa entre eles. Além disso, os resultados obtidos com o bioinseticida demonstram que esse é tão eficiente quanto os inseticidas químicos e pode ser usado para controle dessa praga em milho após a regulação de seu uso.

Palavras-chave: *Inseticida. Manejo integrado de pragas. Milho*.

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INTRODUCTION

Corn is a multifunctional crop grown worldwide, with a variety of possible uses, such as human and animal feeding, bioenergy, biodegradable plastic, among others. The largest producer areas are the United States of America, China, Brazil and European Union, being Brazil the third largest producer in the year of 2016 (FEDERAÇÃO DAS INDÚSTRIAS DO ESTADO DE SÃO PAULO, 2016).

In Brazil, corn is the second most grown grain, in an area of approximately 15.69 million hectares in the 2015/2016 growing season, with an average productivity of 5.39 ton of grains per hectare (COMPANHIA NACIONAL DE ABASTECIMENTO, 2016). In the State of Minas Gerais, where this study was carried out, the region known as Triângulo Mineiro features high productivities of this crop, due to the high-level of technology applied.

Although technology constantly provides higher yields, one of the main factors reducing such productivity is the infestation of insect-pests, especially the fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), considered to be the main pest in the corn fields in Brazil (FARIAS et al., 2014). It is generally distributed in all growing regions of the country, due to its polyphagous feeding behavior and the favorable weather (CRUZ, 1995).

Larvae vary from light tan to black with three light yellow stripes down the back. There is a wider dark stripe and a wavy yellow-red blotched stripe on each side. Larvae have four pairs of fleshy abdominal prolegs in addition to the pair at the end of the body (BESSIN, 2017). First, instar larvae feed on their own egg's shell and have positive phototropism. After this first feeding, they remain resting for two to ten hours before foraging for other sources of food. *S. frugiperda* larvae may be cannibalistic, and this type of behavior favors the natural regulation of populations (CRUZ, 1995). In the fields, the larvae feed on new plant tissues, leaves and inflorescence, going through six ecdysis before reaching the pupa stage (GALLO et al., 2002). Although the species is polyphagous, it is specifically well adapted to feed on grasses, and it is able to cope with maize chemical defenses (GLAUSER et al., 2011). Among the host plants it feeds on, we can include cotton, corn and soybean (CAPINERA, 2008), in addition to using many alternative hosts for maintaining itself in the agricultural ecosystems (BARROS et al., 2010).

In corn fields, the damages may occur from the seedling stage until tasseling and kernelling stages. Young larvae initially consume leaf tissue from one side, leaving the opposite epidermal layer intact. By the second or third instar, larvae begin to make holes in leaves, and eat from the edge of the leaves inward (CAPINERA, 2014). Larger larvae are usually found deep in the whorl often below a "plug" of yellowish brown frass (BESSIN, 2017).

In late infestation, the larvae may be found in the ear destroying silk and grains (ÁVILA et al., 1997). This direct damage to the ears may be even more important than leaf damage. Besides, the damage to the developing grains may facilitate the infestation of secondary pests and microorganisms that produce mycotoxins, resulting in a potential loss in grains productivity and quality (WILLIAMS et al., 2005).

Chemical control is an efficient and viable tool to manage *S. frugiperda* in corn fields. According to Raga (1997), the use of pesticides reduces the number of plants damaged by this pest, resulting in increased grains productivity. However, synthetic pesticides, besides being toxic to the environment, do not show efficiency when applied incorrectly (ROEL et al., 2000). Furthermore, in several regions of the world, insecticide overuse has resulted in the development of populations highly resistant to certain chemicals (YU et al., 2003; AHMAD; ARIF, 2010). Also, in some cases, the strategies are inconsistent and unsatisfactory because the larvae tend to move to the whorl, where they are protected from insecticide applications (BESSIN, 2017).

Some alternatives to the use of chemical pesticides are biopesticides and transgenic plants, which are based on microorganisms and are beneficial for agriculture and less harmful for public health (GUPTA; DIKSHIT, 2010). Among the group of bacteria used for biocontrol means, *Bacillus thuringiensis* has been successfully used for many years as both a bioinsecticide and a source of genes for transgenic plants (FANG et al., 2009; RAYMOND et al., 2010).

In this context, the Integrated Pest Management has been proposed, associating the knowledge of both the environment and the species population dynamics, using appropriate methods to manage the pest. Such methods should be the most compatible as possible with other techniques to control pests, in addition to being capable to maintain the pest infestation lower than the economic threshold.

This study was designed to gather information that might improve the management of *S. frugiperda* in corn fields, using chemical insecticides and a bioinsecticide that have different modes of action. This bioinsecticide is recommended for controlling *S. frugiperda* in alfalfa, sugarcane, rice and pasture, but not for corn, according to the product leaflet. Therefore, the interest in comparing the efficacy of this bioinsecticide with chemical insecticide commonly used in corn fields is raising. Hence, the objective of this study was to evaluate the mortality of *S. frugiperda* under laboratory conditions, caused by the most commonly used insecticides in the region of Triângulo Mineiro, Brazil, and compare those insecticides with a Bt-bioinsecticide.

MATERIAL AND METHODS

The assays were carried out in July 2015, in the Laboratory of Entomology at Instituto Federal do Triângulo Mineiro – Uberaba, Minas Gerais, Brazil, whose geographic coordinates are 19° 39' S and 47° 57' W; with 780 m altitude. Trials were performed under controlled laboratory conditions (25 ± 2 ºC, 70 ± 10

% RH, and a photoperiod of 12:12h L:D).

A completely randomized design was used, with five treatments and five replications consisting of ten second instar *S. frugiperda* larvae, for a total of 50 individuals per treatment. Each larva was maintained in one individual 100 mL plastic container with perforated cover, and a group of ten containers was considered one replication. Each larva was maintained with a 1 cm3 cube of artificial diet previously immersed for 5 min in the solutions of the respective treatments. The ingredients used to make the artificial diet were proposed by Nalin (1991) (Table 1).

Table 1. Composition of artificial diet for rearing Spodoptera frugiperda under laboratory conditions (NALIN, 1991).

Components	Quantity
Bean (variety "Carioquinha")	165.00 g
Wheat germ	79.20 g
Brewer yeast	50.50 g
Methyl 4-hydroxybenzoate (Nipagin)	3.15 g
Ascorbic Acid	5.10 g
Sorbic Acid	1.65 g
Formaldehyde 10%	12.50 ml
Agar	25.50 g
Water	1195.00 ml

The treatments were three chemical insecticides recommended for S. frugiperda in corn, one *B. thuringiensis* based biological insecticide not currently recommended for *S. frugiperda* in corn, and a water control (Table 2).

Table 2. Insecticide and bioinsecticide dosages used as treatments to control Spodoptera frugiperda.

Active ingredient	Chemical group[1]	Recommended dosage	Tested dosage (Active ingredient)
Bacillus thuringiensis	*Bacillus thuringiensis* and the insecticidal proteins they produce	33.60 g L^{-1}	12.50 g 500 ml^{-1}
Chlorpyrifos	Organophosphates	0.40 – 0.60 L ha^{-1}	0.12 ml 500 ml^{-1}
Lufenuron	Benzoylurea	0.30 L ha^{-1}	0.75 ml 500 ml^{-1}
Lambda-cyhalothrin	Pyrethroids	0.15 L ha^{-1}	0.37 ml 500 ml^{-1}

[1]Chemical group according to IRAC classification (INSECTICIDE RESISTANCE ACTION COMMITTEE, 2018).

The chemical insecticides and bioinsecticide were diluted in a solution of distilled water + Tween® (0.5%), following the quantity of active ingredient recommended by the manufacturer, as shown in Table 1. The cubes used for control treatment were immersed in a solution containing only distilled water + Tween® (0.5%). After immersion, the cubes were put on plastic trays, and dried for 30 min under laboratory conditions. Once dried, each cube was put in a plastic container with one larva. Larval mortality was evaluated 12, 24, 48, 72, 96 and 120 hours after treatment (HAT).

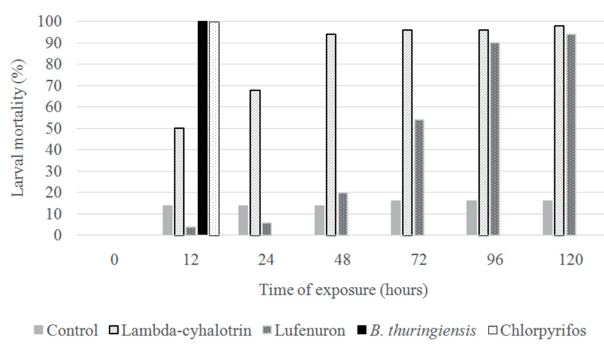
The data were initially checked for the variance analysis assumptions. The Shapiro-Wilk (1965) test was run to verify the residue's normality, and the Bartlett (SOKAL; ROHLF, 1969) test was run to verify the homogeneity of variance. Since these assumptions were not met, a non-parametric analysis was run, through the Kruskal-Wallis (THEODORSSON-NORHEIM, 1986) test (p<0.05). The Bonferroni post-hoc test was used to compare the median values of mortality (p<0.05). After the statistical analysis, the mortality data was transformed using the Schneider-Orelli formula (PÜNTENER, 1981).

RESULTS AND DISCUSSION

The larval mortality caused by the tested insecticides is related to their mode of action, absorption, transportation and excretion by the insect, type of formulation and residual effect. These factors may lead to an immediate toxic action or negatively influence the insect's development. The quantity of active ingredient deposited over the diet cubes is also determinant for the insecticide efficacy.

The larval mortality was observed six times, being the two first observations in a 12-hour interval, and the other ones in a 24-hour interval. Even though no statistical analysis was used to compare the mortality in the different intervals, a graphical representation of the mortality dynamic is shown in Figure 1.

Figure 1. Distribution of accumulated mortality of second instar Spodoptera frugiperda larvae over time, comparing control, chemical insecticides and bioinsecticide.



The Kruskal-Wallis test showed significant differences in the treatments order (p-value = 0.0011 and $|^{2}_{calculated}$ = 18.26). According to Bonferroni test, the median values of *B. thuringiensis*, chlorpyrifos, lambda-cyhalothrin and lufenuron are statistically equal and greater than the control median. Furthermore, it ispossible to observe small variation in the results' distribution, especially in the treatments *B. thuringiensis*, chlorpyrifos and lambda-cyhalothrin (Table 3).

Table 3. Average and median mortality of second instar *Spodoptera frugiperda* larvae caused by different insecticides.

Treatment	Median Mortality (%)	Corrected Mortality[1] (%)
B. thuringiensis	100 ± 0 a*	100.00
Chlorpyrifos	100 ± 0 a	100.00
Lambda-cyhalothrin	100 ± 4,0 a	95.24
Lufenuron	100 ± 4.0 a	92.60
Control	10 ± 8.12 b	-

*Medians followed by the same letters do not differ by Bonferroni post-hoc test (p<0.05).

C.V. = 12.19%

[1]Corrected mortality calculated with the Schneider-Orelli formula (PÜNTENER, 1981).

Chlorpyrifos caused 100% corrected mortality in second instar *S. frugiperda* larvae, 12 hour after treatment (HAT) (Figure 1). This insecticide is an organophosphate and acts as an acetylcholinesterase inhibitor. Neurotoxic insecticides, such as chlorpyrifos, usually cause a rapid mortality in early instar insects. Cessa; Melo, Lima Júnior. (2013) observed that neurotoxic insecticides used in single or combined application with other insecticides, had relatively high efficiency in controlling *S. frugiperda* up to 48 HAT, under laboratory conditions. However, such efficiency wasn't observed by Wangen, Pereira Júnior, Santana. (2015), who tested this insecticide under field conditions.

Lambda-cyhalotrin caused 95.24% corrected mortality 48 HAT (Fig. 1). This insecticide belongs to the pyrethroids group, which are sodium channel modulators, and the toxic effects of which are mediated through preventing the closure of the voltage-gated sodium channels in the axonal membranes. Due to causing an immediate paralysis and a rapid mortality, pyrethroids are known as "knock down" insecticides. Such rapid mortality observed in this treatment may also be related to the low molecular weight (391.30), which facilitates the ingredient's penetration in the insect body. The corrected mortality observed was higher than the efficiency of lambda-cyhalotrin + chlorantranilipole (63%) in the field obtained by Wangen, Pereira Júnior, Santana. (2015).

Lufenuron was highly effective, but not immediately. The mortality was lower before 96 HAT, reaching a total corrected mortality of 94% only 120 HAT (Fig. 1), similar to results found in the literature (BUSATO et al., 2006; CRUZ et al., 2010). Lufenuron has been largely used in crop fields for a long time, being the insecticide with higher efficiency in the field (GRÜTZMACHER et al., 2000). Being an insect growth regulator, with mode of action of inhibitors of chitin biosynthesis type 0, lufenuron had a high efficacy but demanded a longer period to reach it. Furthermore, lufenuron has a heavier molecular weight (505.20) compared to the other insecticides, and substances with such characteristic have a lower penetration rate in the insect cuticle (STOCK; HOLLOWAY, 1993). Busato et al. (2006) also observed that chitin synthesis inhibitors act slower than neurotoxic insecticides, causing disturbances in insect physiology.

The *B. thuringiensis* based bioinsecticide caused 100% corrected mortality in the first 12 HAT (Fig. 1). Such product is not registered to be used for controlling *S. frugiperda* in corn fields. However, the results obtained in the present study agree with other studies showing *B. thuringiensis* strains causing high mortality in *S. frugiperda* larvae, such as Silva-Werneck et al. (2000). This entomopathogenic bacterium acts as a disruptor of the mesenterum, usually leading to death by septicemia and has proven to be effective against pests of different orders such as Lepidoptera, Coleoptera, and Diptera (MAGALHÃES et al., 2015). Considering only the Spodoptera genus, several papers have determined the specificity of individual *B. thuringiensis* toxins against this pest and identified toxins with the highest potential to be used as bioinsecticides or for the development of transgenic crops (HERRERO et al., 2016).

Bioinsecticides cause less environmental impacts compared to chemical insecticides. Selective Bt-bioinsecticides boost the natural biological control in the agricultural ecosystems by allowing the conservation of beneficial organisms, including parasitoids and predators and reducing chemical insecticides applications (DE BORTOLI et al., 2017).

Recently, this pest has caused economic damage to maize hybrids in several regions of Brazil and high resistance levels to certain insecticides have been confirmed (FARIAS et al. 2014; MONNERAT et al., 2015). In this scenario, Bt-bioinsecticides can be used in the Bt-corn resistant *S. frugiperda* management. As shown by Jakka, Knight, Jurat-Fuentes. (2014), *S. frugiperda* strains resistant to Bt-corn showed high susceptibility to the same Bt-bioinsecticide used in this study.

All the insecticides tested caused a corrected mortality higher than 90%, and similar results were verified by Busato et al. (2006) and Gonçalves et al. (2016), who tested the efficiency of many insecticides in controlling *S. frugiperda* larvae, among them lufenuron, lambda-cyhalotrin and chlorpyrifos. Thus, the lower efficiency observed in the fields may be related to the spray operation. Factors such as wind speed, droplet size, equipment pressure, tractor speed, insect exposure and weather conditions are determining for the control success.

CONCLUSION

Based on the results, it is possible to conclude that the preference by the corn growers in Triângulo Mineiro for the insecticides tested is confirmed by their high efficiency under laboratory conditions. Furthermore, even though the commercial product based on B. thuringiensis tested is not registered for managing *S. frugiperda* in corn fields, such insecticide can be potentially registered for this means in the future, based on the results verified in this study.

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