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New Alternative to Control *Stenoma impressella* (Lepidoptera: Elachistidae) Using *Bacillus thuringiensis* Commercial Formulations in Oil Palm Crops

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Abstract: Using chemical insecticides in IPM is possible and could be sustainable. To find a sustainable alternative to control *S. impressella*, we assessed the biological activities of five commercial formulations of *Bacillus thuringiensis*. First, these formulations were evaluated under laboratory conditions. No differences were observed between the commercial formulations Bt_A_1, BT_K_2, and Bt_K_3. Then, the three formulations were compared in further experiments. This bioassay was performed under field conditions in palms naturally infested by *S. impressella*, and differences in larval mortality rates were observed between commercial formulations. The mortality rates caused by Bt_A_1 and BT_K_3 did not significantly differ. The third step evaluated different doses of Bt_A_1 and BT_K_3 formulations (250, 500, 750, and 1000 g/Ha) under field conditions. Seven days after spraying, differences were only observed between Bt_A_1 and BT_K_3 and the control. Finally, these two formulations were evaluated under field conditions. The mortality rates caused by Bt_A_1 and BT_K_3 were 77.2% and 85.3%, respectively. These findings show that commercial formulations of *B. thuringiensis* subsp. aizawai (Bt_A_1) and *B. thuringiensis* var. *aizawai* (BT_K_3) exhibit high biological activities against *S. impressella* larvae and can be included in the integrated management of *S. impressella*.

Keywords: *Stenoma cecropia*; biological control; hybrid palm; oil palm; *Elaeis guineensis*; Colombia

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1. Introduction

Stenoma impressella Busck 1914 (=*Stenoma cecropia* Meyrick 1916; Lepidoptera: Elachistidae) is an important polyphagous pest of different crops in Latin America. This defoliator has been reported in crops such as apple, coffee, eucalyptus, guava, and oil palm [1,2]. In Colombia, *S. impressella* is a frequent pest in oil palm plantations [3]. It is commonly controlled with chemically synthesized insecticides, affecting the environment and the people who work and live near the oil palm plantations [4,5].

The more frequently used active ingredients used to control *S. impressella* are Teflubenzuron [4,6], Chlorantraniliprole, Flubendiamide, and others. According to evaluations under field conditions made by Cenipalma (Colombian Oil Palm Research Center), *S. impressella* larvae mortality can reach up to 89% 7 days after the spraying chemical insecticides.

Stenoma impressella is present in two of the four oil palm Colombian regions (34% of the national growing oil palm area), central and southwest oil palm regions [6,7]. In the central oil palm region, *S. impressella* is present in approx. 90 000 Ha. The cost of controlling *S. impressella* in oil palm plantations is between USD 30 and USD 55/Ha/sprays, and in some cases six sprays per Ha are necessary (depending on the supplies and the sprayer equipment selected and used, the cost can change).

Stenoma impressella has a lot of natural enemies, including egg parasitoids [8], larval and pupal parasitoids, and predators [9–11] that can be affected by the use of conventional

insecticides. Furthermore, other natural enemies of *S. impressella* are the fungi *Cordyceps cateniannulata* (Z. Q. Liang) Kepler, B. Shrestha, and Spatafora (Hypocreales: Cordycipitaceae) or *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), virus of the *Alphabaculovirus* genus, and the bacteria *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae) [4,5,7,9,12–15].

In sustainable crop production, the use of *B. thuringiensis* formulations is one of the most important alternatives for pest control in integrated pest management [16–20]. Notably, these *B. thuringiensis* formulations are composed of a mixture of different insecticidal proteins (Cry, Cyt, and vegetative insecticidal proteins), and there are specific proteins affecting different insect pests (i.e., Coleoptera, Diptera, Hymenoptera, and Lepidoptera) [21–24].

In Colombia, the biological activities of commercial formulations of *B. thuringiensis* and specific proteins from *B. thuringiensis* have been evaluated to control Lepidoptera and Coleoptera [25–27]. However, information about the activity of commercial formulations of *B. thuringiensis* against *S. impressella* does not exist, and the grower cannot compare and choose the best formulation against this specific target. Therefore, the present study evaluated the biological activities of five commercial formulations of *B. thuringiensis* against *S. impressella* larvae.

2. Materials and Methods

2.1. Evaluation of Bacillus thuringiensis Formulations

We selected five commercial formulations registered with the Colombian Agricultural Institute (referred to as ICA, for its acronym in Spanish) (Table 1). The first step of the screening process comprised the evaluation of these formulations under laboratory conditions. The bioassay was performed at the Entomology Laboratory in the Experimental Field La Vizcaína of Cenipalma, Colombia, at a temperature of 26.8 ± 0.4 °C and relative humidity of 73.7 ± 9.2%.

Table 1. *Bacillus thuringiensis* commercial formulations were evaluated to control *Stenoma impressella* larvae under laboratory conditions (i.e., 26.8 ± 0.4 °C and 73.7 ± 9.2% relative humidity).

Commercial Name	Code Formulation	Composition	Dose (g/Ha)	Manufacturer's Headquarters
Bacillus Agrogen WP	Bt_K_1	*Bacillus thuringiensis* var. *kurstaki*	1000	Yáser S.A.S., Cali, Colombia
BT-Biox WP	Bt_K_2	*Bacillus thuringiensis* var. *kurstaki*	500	Semillas Valle S.A., Yumbo, Colombia
Bassar WP	Bb_Bt_1	*Beauveria bassiana* and *Bacillus thuringiensis*	1000	Natural Control, Antioquia, Colombia
Xentari WDG	Bt_A_1	*Bacillus thuringiensis* var. *Aizawai*	500	Valent BioSciences, Libertyville, IL
Dipel WP	Bt_K_3	*Bacillus thuringiensis* var. *kurstaki*	500	Bayer AG, Leverkusen, Germany

Dilutions of 2.5 g or 5.0 g of each formulation were used per liter of water, according to the required dose (i.e., 500 g/Ha or 1000 g/Ha, respectively; Table 1). Furthermore, each dilution was prepared in an emulsifying oil (i.e., unsaturated carboxylic acid), using 3 mL of oil per 1000 mL of water. The water that was used had a pH below 7. Moreover, a manual sprinkler was calibrated to spray 0.5 mL of the dilution per leaflet.

The bioassay was conducted in a complete randomized design, with five repetitions. Each observational unit consisted of a larva of *S. impressella*, in instar III–IV, which is the optimal stage in which to control *S. impressella*, placed on a leaflet inside an acetate tube. The tube was 28 cm long and 4 cm in diameter, with cotton plugs on the extremities. Ten observation units per treatment formed the experimental unit. The control was not sprayed. The mortality of *S. impressella* larvae was the response variable and was evaluated for nine days.

2.2. Formulation Comparison

The second step in the screening process was the comparison of the formulations that caused the highest mortality under laboratory conditions. The bioassay was performed in oil palms naturally infested with *S. impressella* under field conditions (26.2 ± 3.4 °C; 84.6 ± 27.1% relative humidity). The palms that were selected had leaves with a minimum of 15 *S. impressella* larvae, in instar III–IV. One leaf formed an observational unit.

All formulations were evaluated at a dose of 500 g/Ha. The dilution was prepared using 2.5 g of formulation and 3 mL of an emulsifying oil (unsaturated carboxylic acid) per liter of water. The water that was used had a pH below 7. The dilutions were sprayed with a manual backpack sprayer (Clasica Royal Cóndor, Soacha, Colombia) that had a 20 L capacity and hydraulic pressure and was fitted with a cone-shaped nozzle (RC 350B101X). The sprayer was calibrated for spraying 145 mL per leaf, which resulted in good coverage of the oil palm leaves with the spray.

The bioassay was conducted in a complete randomized design, with five repetitions. The control was absolute. The experimental unit was formed by two observational units (i.e., two infested leaves). The mortality of *S. impressella* larvae was the response variable. The mortality was evaluated seven days after spraying the formulations.

2.3. Dose Evaluation

The third step in the screening process was to determine the optimal doses of the formulations selected in the previous bioassays, which were the formulations that caused high *S. impressella* larval mortality. The dose evaluation was performed for each formulation in independent bioassays and under field conditions. The weather conditions were the same as those recorded in the previous evaluation.

The palms selected to perform the bioassays had leaves with a minimum of 15 *S. impressella* larvae in instar III–IV, and one leaf formed an observational unit. The evaluated doses were 200, 250, 500, and 1000 g/Ha. To prepare the solutions of each formulation, we used 1.0, 1.25, 2.5, and 5.0 g of each formulation per liter of water (pH < 7) and the same emulsifying oil as that used in the previous bioassays. The water volume used as a reference was 200 L/Ha.

Each bioassay was conducted in a complete randomized design with six repetitions. The solutions were sprayed with the same equipment and volume per leaf as in the previous bioassays (see Section 2.2). The control was absolute. Two observational units per palm formed the experimental unit. The mortality of *S. impressella* larvae, which was evaluated seven days after spraying the formulations, was the response variable.

2.4. Sprayed Commercial Formulations under Field Conditions

The final step in the screening process was the spraying of the two chosen formulations under commercial oil palm plantation conditions. The formulations were sprayed at the dose selected in the previous bioassays (Section 2.3).

The sprays were made in two plots of 6 and 10 ha, respectively, where 9-year-old Coarí × La Me commercial hybrids (*E. oleifera* (Kunth) Cortés × *E. guineensis*), naturally infested with *S. impressella*, were planted at a density of 115 palms/Ha. Before spraying each selected formulation, each plot was sequentially sampled. Sampling was performed by selecting one palm for every eight palms and every eight lines of palms (8 × 8). Then, one leaf at the foliar level 17 (middle-third of the palm) was selected in each sampled palm, and the number of *S. impressella* larvae on that leaf was quantified.

The formulations were then sprayed with an electrostatic nebulizer with a tube (Martignani, S. Agata sul Santerno, Italy), calibrated for spraying 200 L/Ha (i.e., 1.7 L/palm). The dilutions were measured according to each area, using the selected dose and the same emulsifying oil as that used in previous bioassays (3 mL per liter). Seven days after spraying, sequential sampling was repeated. The sample sizes were 11 and 17 palms per plot (plots with 690 and 1150 palms, respectively).

2.5. Statistical Analysis

The mortality of *S. impressella* larvae was corrected in all bioassays according to Schneider-Orelli's formula [28]. In each bioassay, the homogeneity of variance and normality distribution of the data were verified, and then the data were analyzed with an analysis of variance, with treatment means separated by Tukey's honestly significant differences, using the SAS 9.4 software. The data analysis of the evaluation under field conditions was performed by determining the 95% confidence intervals ($\alpha = 0.05$).

3. Results

3.1. Evaluation of Bacillus thuringiensis Formulations

All the formulations were pathogenic to *S. impressella* larvae under laboratory conditions. The Bt_K_3 formulation caused the highest mortality, without any variability during the bioassay. Furthermore, differences were found between Bt_A_1 and Bt_K_2 formulations, and Bb_Bt_1, and the Bt_K_1 formulation ($F = 47.11$; $df = 4, 20$; $p < 0.0001$). However, the comparison of means revealed no significant differences between the Bt_A_1 and Bt_K_2 formulations (Table 2).

Table 2. Mean mortality of *Stenoma impressella* larvae achieved with five commercial formulations of *Bacillus thuringiensis* evaluated under laboratory conditions (i.e., 26.8 ± 0.4 °C and 73.7 ± 9.2% relative humidity), over nine days.

Code Formulation	Composition	Dose (g/Ha)	Mortality (%)	Standard Error	Corrected Mortality (%)
Bt_K_3	*Bacillus thuringiensis* var. *kurstaki*	500	100	-	100.0
Bt_A_1	*Bacillus thuringiensis var. aizawai*	500	94 a*	2.4	93.9
Bt_K_2	*Bacillus thuringiensis* var. *kurstaki*	500	84 a	6.8	83.7
Bb_Bt_1	*Beauveria bassiana* and *Bacillus thuringiensis*	1000	52 b	7.3	51.0
Bt_K_1	*Bacillus thuringiensis* var. *kurstaki*	1000	22 c	7.3	20.4
Control	-	-	2 c	2.0	-

Corrected mortality according to Schneider-Orelli's formula (28). Bt_K3 treatment was not included in the data analysis because no variability was observed, * different letters in the same column indicate significant differences (Tukey, $p = 0.05$).

The Bt_K_3, Bt_A_1, and Bt_K_2 formulations were selected to continue the screening process because they caused the highest *S. impressella* larval mortality rates.

3.2. Formulation Comparison

The Bt_A_1 and Bt_K_3 formulations caused the highest mortality in *S. impressella* larvae seven days after spraying, and the mortality rates of these formulations significantly differed from those of the Bt_K_2 formulation and the control, respectively ($F = 69.05$; $df = 3, 16$; $p < 0.0001$; Figure 1). Notably, the mortality of *S. impressella* larvae was the highest under field conditions (i.e., 88% mortality), and the larval cadavers turned dark and flaccid, which are typical characteristics of larvae affected by *B. thuringiensis*.

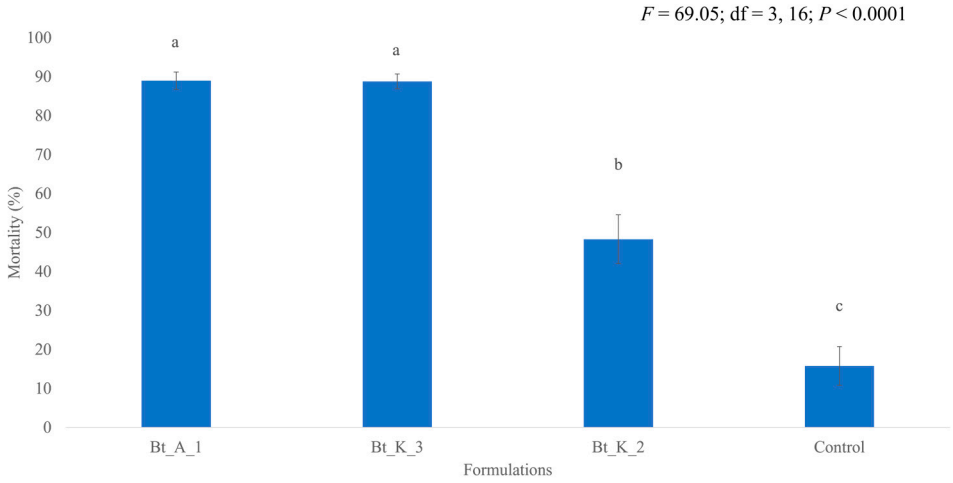

Figure 1. Mean (±standard error) mortality *of Stenoma impressella* larvae achieved with three commercial formulations of *Bacillus thuringiensis*, each at a dose of 500 g/Ha, seven days after spraying under field conditions (i.e., 26.2 ± 3.4 °C, 84.6 ± 27.1% relative humidity, and 137 mm rainfall during the bioassay). Different letters indicate significant differences (Tukey, $p = 0.05$).

3.3. Dose Evaluation

After spraying different doses of the Bt_K_3 and Bt_A_1 commercial formulation, significant differences were found between the doses and the control ($F = 69.05$; $df = 3, 16$; $p < 0.0001$). Notably, the mortality of *S. impressella* larvae sprayed with the formulations varied between 51.9% and 96.7% (Table 3). Moreover, no differences were observed among the doses in either of the two bioassays. The effects of the lowest doses, namely 250 g/Ha and 500 g/Ha, did not differ from those of the higher doses. However, the use of low doses of *B. thuringiensis* is not recommended because of an increased risk of resistance. Therefore, the 500 g/Ha dose was selected for the final Bt_K_3 and Bt_A_1 evaluation.

Table 3. Mean mortality of *Stenoma impressella* larvae obtained with two commercial formulations of *Bacillus thuringiensis* at different doses under field conditions, seven days after spraying.

Code Formulation	Composition	Dose (g/Ha)	Mortality (%)	Standard Error	Corrected Mortality (%)
Bioassay 1 (27.8 ± 4.2 °C and 83.9 ± 19.7% RH)					
Bt_K_3	*Bacillus thuringiensis* var. *kurstaki*	250	51.9 a*	8.6	43.3
		500	56.2 a	4.0	48.3
		750	70.8 a	9.0	65.6
		1000	69.8 a	8.3	64.4
Control	-	-	15.2 b	7.9	-
Bioassay 2 (30.5 ± 6.3 °C and 80.2 ± 22.7% RH)					
Bt_A_1	*Bacillus thuringiensis* var. *aizawai*	250	93.4 a	1.1	92.8
		500	96.7 a	1.2	96.4
		750	93.3 a	2.6	92.7
		1000	96.3 a	1.9	96.0
Control	-	-	8.3 b	3.8	-

Corrected mortality according to Schneider-Orelli's formula (28). RH: relative humidity, * different letters in the same column indicate significant differences (Tukey, $p = 0.05$).

3.4. Sprayed Commercial Formulations under Field Conditions

The Bt_K_3 and Bt_A_1 formulation sprayed at a dose of 500 g/Ha in commercial plots of oil palm caused larval mortality of more than 77% in *S. impressella* seven days after spraying. After the spraying, the larval populations significantly decreased in both plots (Table 4). The larval cadavers had the typical characteristics of larvae affected by *B. thuringiensis*.

Table 4. Population reduction in *Stenoma impressella* larvae seven days after being spraying by two *Bacillus thuringiensis* commercial formulations in different oil palm plantations.

Code Formulation	Composition	Dose (g/Ha)	No.	Larvae before Spraying (#)	Standard Error	Larvae 7 Days after Spraying (#)	Standard Error	Larvae Reduction (%)
Bt_K_3	*Bacillus thuringiensis* var. *kurstaki*	500	17	7.5	1.6	1.1	0.5	85.3
Bt_A_1	*Bacillus thuringiensis* var. *aizawai*	500	11	15.5	3.9	3.5	1.5	77.2

No: sampled palms (according to the plot area).

4. Discussion

In this study, although Bt_K_3, Bt_A_1, and Bt_K_2 were the best formulations under laboratory conditions, the Bt_K_2 formulation had a low biological activity (less than 50%) against *S. impressella* larvae under field conditions and thus was discarded. As for the Bt_K_3 and Bt_A_1 formulations, they demonstrated high biological activities (>88%) under field conditions. Similar results have been obtained for the activity of *B. thuringiensis* var. *kurstaki* strains against Diptera larvae [29] and that of Bt_K_3 and Bt_A_1 against Lepidoptera defoliators [27,30]. Notably, the results of the present study differ from those of the *S. impressella* controls used in Colombia in the 1980s and the 1990s (i.e., biological activity < 60%) because commercial formulations of *B. thuringiensis* were not used [5].

The quality of *B. thuringiensis* formulations can affect biological activity [5]. The low biological activity of Bb_Bt_1 and Bt_K_1 against *S. impressella* larvae can be explained by the composition of these commercial formulations, which are made with a mixture of insecticidal proteins from *B. thuringiensis* var. *kurstaki* to control other Lepidoptera, and this mixture of proteins showed low biological activity against *S. impressella*. In contrast, the mixture of insecticide proteins used in Bt_K_3 and Bt_A_1 commercial formulations showed high biological activity against *S. impressella* in all bioassays. In addition, all commercial formulations were bought in authorized stores in this study, and their quality was not evaluated. Only the expiry date was checked.

The evaluation of different doses of Bt_K_3 and Bt_A_1 under field conditions revealed that an increase in dose did not increase the mortality of *S. impressella* larvae. Conversely, larval mortality increased with higher doses [29]. Furthermore, in Colombia, low doses have been reported to be inefficient in controlling different pests in oil palm plantations [5]. Therefore, the dose selected to control *S. impressella* larvae in this study was not the lowest. Moreover, applying high doses of *B. thuringiensis* is a strategy to reduce the risk of resistance [31].

The commercial formulation Bt_K_3, when sprayed onto leaves infested with *S. impressella* in commercial oil palm plantations, had high biological activity against this defoliator pest. Notably, similar results were observed with the same formulation to control pests in pomegranate trees [32]. To reduce the risk of resistance, the Bt_K_3 formulation (*B. thuringiensis* var. *kurstaki*) can be rotated with the Bt_A_1 formulation, which contains another variety of *B. thuringiensis* (var. *aizawai*) that also is characterized by its high biological activity against *S. impressella* under field conditions. The use of *B. thuringiensis* to control pests is a sustainable alternative; however, it can cause resistance. Hence, it is crucial to use formulations with protein mixtures [33,34], such as Bt_K_3 and Bt_A_1.

A cost–benefit analysis shows that the commercial formulations of *B. thuringiensis* are a competitive alternative because the cost of *B. thuringiensis* formulations is close to 50% less than the cost of chemical insecticides per Ha. In Colombia, the cost of commercial formulations of *B. thuringiensis* with high biological activity against *S. impressella* are between

USD 13 and USD 18/Ha, and the cost of more frequently used chemical insecticides is between USD 31 and USD 43/Ha.

5. Conclusions

The commercial formulations of *Bacillus thuringiensis* var. *aizawai* (Bt_A_1) and *Bacillus thuringiensis* var. *kurstaki* (Bt_K_3) have high biological activity and, when sprayed at a dose of 500 g/Ha, effectively control *S. impressella* larvae and can be included in the integrated pest management program.

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