

Use of natural insecticides and biocontrol agents in the control of fall armyworm (*Spodoptera frugiperda* J. E. Smith) of maize

Uso de insecticidas naturales y biocontroladores en el control de gusano cogollero del maíz (*Spodoptera frugiperda* J. E. Smith)

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Abstract: With the aim of controlling the fall armyworm (*Spodoptera frugiperda* J. E. Smith) by applying natural insecticides and biocontrol agents, an experimental plot was set up in a completely randomised block design (CRBD) with 4 treatments, 4 replicates and 16 experimental units at the Los Laureles site in the parish of Julcuy, Manabí. There were four treatments (T1: control (no application), T2: *Bacillus thuringiensis* (Bt), 150 mL/20 L, T3: *Beauveria bassiana* (Bb), and T4: Neem (*Azadirachta indica*), 25 mL/20 L). The variables studied were: percentage incidence (Pinc), percentage severity (Psev), plant height (ADP), number of internodes (NDEN), stem width (ADT), leaf area (AF), number of rows per ear (NHPM), number of grains per ear (NDGPM), size per ear (TDM), ear width (ADM), weight of 100 grains (P100G) and ear weight (PDM). The results determined that Bb and Bt were the best treatments with the lowest Psev. Bb was the best treatment for NHPM with 15.12 rows and NDGPM with 549.02 grains, compared to the control with 14.24 rows and 496.53 grains. There was a high, positive and significant correlation ($P < 0.05$) for NDEN with ADP, NFGPM with NDEN and NHPM, ADM with TDM and PDM with P100G.

Keywords: yield, severity percentage, incidence percentage, grain weight.

Resumen: Con el objetivo de controlar el gusano cogollero del maíz (*Spodoptera frugiperda* J. E. Smith) aplicando insecticidas naturales y biocontroladores, fue implementada una parcela experimental en un diseño de bloques completamente aleatorios (DBCA) con 4 tratamientos, 4 repeticiones y 16 unidades experimentales en el Recinto Los Laureles de la parroquia Julcuy, Manabí. Los tratamientos fueron cuatro (T1: testigo (sin aplicación), T2: *Bacillus thuringiensis* (Bt), 150 mL/20 L, T3: *Beauveria bassiana* (Bb), y T4: Neem (*Azadirachta indica*), 25 mL/20 L). Las variables estudiadas

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fueron: porcentaje de incidencia (Pinc), porcentaje de severidad (Psev), altura de planta (ADP), número de entrenudos (NDEN), ancho de tallo (ADT), área foliar (AF), número de hileras por mazorca (NHPM), número de granos por mazorca (NDGPM), tamaño por mazorca (TDM), ancho de mazorca (ADM), peso de 100 granos (P100G) y peso de mazorca (PDM). Los resultados determinaron que Bb y Bt fueron los mejores tratamientos con un menor Psev. Bb fue el mejor tratamiento para NHPM con 15,12 líneas y NDGPM con 549,02 granos, respecto del testigo con 14,24 hileras y 496,53 granos. Hubo una correlación alta, positiva y significativa ($P < 0,05$) para el NDEN con la ADP, el NFGPM con el NDEN y el NHPM, el ADM con el TDM y el PDM con P100G.

Palabras clave: rendimiento, porcentaje de severidad, porcentaje de incidencia, peso de grano.

Introduction

The fall armyworm (FAW) (*Spodoptera frugiperda* J. E. Smith) is the most important pest worldwide, causing considerable losses in maize yield. The FAW was first reported in 1797 as an endemic devouring pest of the subtropical and tropical regions of America (Abbas et al., 2022). This insect belongs to the Noctuidae family, order *Lepidoptera*, and was first reported on the African continent (Goergen et al., 2016). The BOL is a devastating pest that damages 186 plant species belonging to 42 families. Poaceae, Fabaceae, Solanaceae, Asteraceae, Rosaceae, Chenopodiaceae, Brassicaceae, and Cyperaceae are the most affected (Abbas et al., 2022). It results in a yield loss of approximately 58% in maize [Kumar et al., 2022, Kenis et al., 2022]. GC is known to voraciously feed on more than 350 plant species, especially maize, rice and sorghum, causing significant agricultural losses worldwide (Kenis et al., 2022, Montezano et al., 2018, Wang et al., 2022). GC is the most important pest affecting maize cultivation in Ecuador and in various producing countries in the region (González-Maldonado et al., 2015, Yasem de Romero y Romero, 2013). The losses it causes are substantial, reducing yields by 0.8 t/ha of dry maize, which is equivalent to 40% of production.

Historically, Ecuador has managed 250,000 hectares of maize. In previous years, 214,000 hectares were reported as planted; however, the area is trending downward, with an estimated 50% located in the province of Los Ríos, 40% in Manabí, and the rest in Guayas. Ninety per cent of maize planting takes place in winter. In the summer season,

16,000 hectares were planted with a lower than normal average yield of 1.82 tonnes per hectare (Drouet Candell, 2018, Llanos et al., 2020).

Large-scale farming creates conditions conducive to the pest's reproduction and spread. The corn rootworm is one of the most aggressive insects, acting as a soil worm, cutworm or armyworm and as a corn earworm, which is its most characteristic habit in corn (Santos et al., 2014). This pest is of tropical origin and attacks late plantings on the coasts and in warm irrigated regions more severely. Less infested are the cornfields of the highlands, where the earworm attack decreases when the rains begin or when the plants reach a metre in height. To reduce the harmful effects of the earworm, chemical insecticides are used, which are sprayed or dusted on the crop (). The effects of control have been low, because these measures have been taken after the critical moment of the pest and the most appropriate phenological stage of the crop, or after the damage is irreversible. Attempts have even been made to reduce the damage when the crop has practically reached a size that makes it impossible for machines to enter the field (Drouet Candell, 2018).

Biological control strategies are more appropriate for farmers who do not have the financial capacity to purchase chemical insecticides and expensive seeds (Abate et al., 2000). Microbial and natural insecticide formulations are available on the market, made from pathogens, natural enemies of arthropods, and organic substances, which are more cost-effective in agricultural systems (Angulo-Escalante et al., 2004, Arias et al., 2009, Pilkington et al., 2010, Morales, 2015, Kenis et al., 2022). Recently, the production costs of microbial formulations have been significantly reduced because they are mainly mass-produced in liquid media (Mahmoud, 2016, Angel-Ríos et al., 2021, Kenis et al., 2022). The repetitive use of synthetic pesticides in the field can be harmful to humans and the environment, has increased the cost of inputs, and can also lead to resistance and resurgence (Ullah et al., 2019, 2020a, 2020b, Qu et al., 2020). Fall armyworm larvae burrow deep into corn leaves and cobs, resulting in control failures. However, it feeds on plants at night, dawn, and dusk (Day et al., 2017, Avila-martínez et al., 2023).

The simplest way to control insect pest outbreaks is to formulate a self-propelling, self-perpetuating system for the restoration or stimulation of self-sustaining biological control tactics. Biological control agents, such as predators, parasitoids, and pathogens, were supplied to maintain close synchronisation with the community of other organisms (Sánchez et al., 2019, Valverde et al., 2020). *Trichogramma* and *Telenomus* are the most active biological control agents, parasitising the eggs of GC

and other key pests (Desneux et al., 2010, Zang et al., 2021, Barrera, 2023). Parasitoids are closely associated with one of the stages of the pest and have a higher level of specificity (Gowda et al., 2021). Predators are rarely linked to a specific insect pest. They feed on prey with a lower trophic level of specificity than other animals. For example, earwigs, ladybirds, podisus and orius feed on different life stages of GC. Entomopathogens include bacteria, fungi, viruses, protozoa and nematodes, which are the main cause of disease in insects. The GC was infected by several entomopathogens, including *Bacillus thuringiensis* (Bt), *Metarhizium anisopliae*, *Beauveria bassiana* (Bb), and multiple nucleopolyhedroviruses of *Spodoptera frugiperda* (Portela-Dussán et al., 2013, Drouet, 2018, García et al., 2018, Assefa & Ayalew, 2019, Litwin et al., 2020, 2022).

The excessive application of agrochemicals, coupled with monoculture in certain agricultural areas of the country, means that it is common to see cases where the recommended commercial doses of certain products for pest control have to be doubled to increase their efficiency, thus increasing their impact on the environment (Vélez et al, 2021). This is why there is growing interest in using organic products, in this case biological and natural, to control pests without affecting crop production (Drouet Candell, 2018, Villarreal et al., 2018). Therefore, the objective of this research was to control the fall armyworm (*Spodoptera frugiperda*) by applying natural insecticides and biocontrol agents.

Methodology

Location

The research was conducted on the farm of producer Galo Holguín in Los Laureles, Julcuy parish, Manabí. The parish is bordered to the north by Jipijapa canton, to the south by Pedro Pablo Gómez parish, to the east by América parish, and to the west by Puerto López canton. Los Laureles is located at 1° 25' 44" south latitude and 80° 35' 16" west longitude, at an altitude of 329.22 metres above sea level, with an average temperature of 24^o, RH of 81% and an average annual rainfall of 250 mm [Julcuy Parish Land Use Plan (PDOT, 2023)].

Factors under study

The factors under study were natural insecticides and biocontrol agents.

Treatments

The treatments applied were as follows (Table 1):

Table 1 . Four treatments were applied in the trial, as shown below

Treatments	Natural insecticide and biocontrol agents	Dose
T1	Control	No application
T2	<i>Bacillus thuringiensis (Bt)</i>	150 mL/20 L of water
T3	<i>Beauveria bassiana (Bb)</i>	150 mL/20 L of water
T4	Neem (<i>Azadirachta indica</i>)	25 mL/20 L of water

Experiment management

The land was prepared one week before sowing, stubble was removed, minimum tillage was applied, and then the plots were marked out. Sowing was carried out at a density of 0.80 m between rows and 0.20 m between plants, placing one seed per site. There were 16 experimental units, covering an area of 285 m². Weeds were controlled with the pre-emergent herbicides Glyphosate (2 L/ha), Terbutryn (500 cc/ha), and 2,4-D (2,4-dichlorophenoxyacetic acid (amine) (500 mL/ha). Post-emergence manual weeding was carried out 35 days after sowing (dds). Fertilisation was carried out according to the nutritional requirements of the maize crop. Pest control was carried out using the treatments proposed in the research, for which a 20 L Jacto brand backpack sprayer equipped with a solid cone nozzle was used. 150 mL/20 L was applied for the biocontrol agents and 25 mL/20 L for the natural insecticide. Harvesting was carried out at 130 days after sowing.

Experimental Design

The study plot was implemented in a completely randomised block experimental design, with 4 replicates and 4 treatments (Gabriel *et al.*, 2022). In 4 m long furrows, totalling 16 experimental units, covering an area of 285 m².

The following variables were evaluated: *Percentage of incidence (%) (Pinc)*. Using the scale suggested by Agrofy News (2018), which

consists of evaluating the percentage of affected leaves out of the total number of leaves evaluated in each experimental unit, multiplied by 100. *Percentage of severity (%) (Psev)*. The percentage incidence of damage caused by *Spodoptera frugiperda* was determined at the phenological stages of maize cultivation (development stage, flowering stage, and reproduction stage) at 20, 30, and 45 days after sowing. Data were collected in each useful plot, for which an evaluation scale was developed according to the Davis scale (Table 2 and Figure 1).

Table 2. Scale of percentage of damage caused by *Spodoptera frugiperda* in maize.

Scale	Percentage of damage	Characteristics of the damage
1	0–20	Healthy
2	21–40	Mild
3	41 – 60	Moderate
4	61 – 80	Strong
5	81 – 100	Severe



1. Davis scale for damage assessment (Agrofy News, 2018)

1: No damage or pinhole-like lesions caused by first-stage larvae. It is more common to find a greater number of hatched larvae eggs during this stage.

2-4: This is the optimal time for control, when window-like lesions are observed, small circular lesions 1 to 1.5 mm in diameter, scraped without holes. These lesions are caused by second and third instar larvae

5-6: Holes of different sizes, visible damage to the bud with signs of a small amount of soft faeces.

7-8-9: Obvious destruction of the bud to varying degrees with sawdust-like faecal plugs that completely prevent control (Agrofy News, 2018).

Plant height (cm) (ADP). Measured with a flexometer at 55 dds on 10 plants chosen at random from each useful plot (Anchundia & Tumbaco, 2021). *Number of internodes (NDEN)*. The nodes of each plant were counted from 55 dds. *Stem width (cm) (ADT)*. A vernier was used at a height of 3 cm from the soil surface. *Leaf area (cm²) (AF)*. The length of the leaf was measured from the tip of the blade to the ligule, and the width of the leaf was measured at the centre of the blade. These data were multiplied by a constant factor of 0.73. *Number of rows per ear (NHPM)*. The NDPM was counted. *Number of grains per ear (NDGPM)*. The NDGPM was counted. *Ear size (cm) (TDM)*. A tape measure was used. *Ear width (cm) (ADM)*. A tape measure was used. *Weight of 100 grains (g) (P100G)*. One hundred seeds were weighed with a gram scale. *Ear weight (g) (PDM)*. The harvested ears were weighed on a scale.

Statistical analysis

Based on the defined model, analysis of variance (ANOVA) was performed to test hypotheses about fixed effects, as well as comparisons of treatment means using Tukey's test ($P < 0.05$). The ANOVA was also used to estimate the variance components for random effects. Likewise, the correlation between all variables was performed to determine the degree of association between them using Pearson's coefficient. All analyses were performed using Infostat software (2020).

Normality and homogeneity of variance analysis

Normality was analysed using the Shapiro-Wilks test at 5%, and homogeneity of variance was analysed using Levene's test at 5% (Gabriel *et al.*, 2022). The data for the variables evaluated were not significant for either test, indicating normality and hom of the data, which allowed the ANOVA and comparison of means to continue.

Results

analysis of severity and incidence

Table 3 shows the ANOVA for Psev and Pinc and morpho-agronomic traits in maize cultivation. There were significant differences ($P < 0.05$) for the treatments in Psev and highly significant differences ($P < 0.01$) for ADP, NDEN, ADT, and AF. The coefficients of variation (CV) ranged from 3.97% to 17.60%.

Table 3. ANVA y medias para variables morfo-agronómicas.

ANOVA

FV	gl	Mean squares					
		Psev	Pinc	ADP	NDEN	ADT	AF
Tra	3	27.59 *	304.92 ns	3176.28 **	2.43 **	0.64 ns	2.03 **
Rep	3	8.41	74.25 ns	1117.96 **	0.74 ns	2.38 ns	2.11 **
Error	9	4.03	191.58	131.78	0.23	1.74	0.15
Total	15						
CV		4.84	17.60	4.57	3.57	7.43	3.97

Comparison of means

Tra	n	Pseudo	Pinc	ADP	NDEN	ADT	AF
<i>Bb</i>	4	38.87 to	80.50 to	271.49 to	13.98 to	18.01 to	10.05 to
<i>Bt</i>	4	40.01 to	86.00 to	268.01 to	13.81 to	17.97 to	10.34 to
NEEM	4	42.18 ab	66.00 a	210.32 b	12.25 b	17.81 a	8.73 b
Control	4	44.83 b	82.00 a	255.25 a	13.43	17.15	9.97 a
DSH		4.43	30.55	25.34	1.05	2.91	0.85

Psev: percentage of severity, Pinc: percentage of incidence, ADP: plant height, NDEN: number of internodes, ADT: stem width (mm), AF: leaf area (cm^2), *Bb*: *Beauveria bassiana*, *Bt*: *Bacillus thuringiensis*, Neem: neem oil. Means with the same letter are not significantly different ($P < 0.05$). HSD: Honest Significant Difference.

Analysis of the treatment means using Tukey's multiple range test ($P < 0.05$) (Table 3) showed that *Bb* (38.87%) and *Bt* (40.01%) were the best treatments, with a lower Psev, which is not significantly different from the control, which showed a Psev of 44.83%. With regard to ADP, the NDEN and AF were less favourable with 12.25 nodes, 17.81 mm and 8.73 cm^2 respectively. Variables such as Pinc and ADT were not significant.

Table 4 shows the ANOVA of the productive variables, where the NHPM and NDGPM in corn plants were significantly different ($P<0.05$). The TDM, ADM, P100G grains and PDM were not significant. The CVs ranged from 2.63% to 5.54%.

Table 4. ANOVA and means for productive variables.

ANOVA

FV	gl	Mean squares					
		NHPM	NDGPM	TDM	ADM	P100G	PDM
Tra	3	0.66*	2380.22 *	1.35 ns	1.49	0.68 ns	0.47 ns
Rep	3	0.16 ns	581.39 ns	1.13 ns	2.63	11.69 ns	0.63 ns
Error	9	0.15	411.93	0.44	1.48	5.48	0.41
Total	15						
CV		2.63	3.93	3.56	2.71	5.54	3.92

Analysis of means

Tra	n	NHPM	NDGPM	TDM	ADM	P100G	PDM
BB	4	15.12 to	549.02 to	19.33 to	45.66 to	42.68 to	279.03 to
BT	4	14.96 ab	523.04 ab	18.57 to	45.00 a	41.79 a	262.99 a
NEEM	4	14.49 ab	499.55 b	17.95 a	44.23 a	42.12 a	273.22 a
Control	4	14.24 b	496.53 b	18.88 a	44.60 a	42.58 a	249.48 a
DSH		0.85	44.8	1.47	44.60 to	5.17	45.51

NHPM: number of rows of kernels per ear, NDGPM: number of kernels per ear, TDM: ear size (cm), ADM: ear width (cm), P100G: weight of 100 kernels, PDM: ear weight (g). Means with the same letter are not significantly different ($P<0.05$).

Analysis of the treatment means using Tukey's multiple range test ($P<0.05$) (Table 7) showed that *Bb* was the best treatment for NHPM with 15.12 rows and NDGPM with 549.02 grains, compared to the control () with 14.24 rows and 496.53 grains, respectively. The variables TDM, P100G and PDM were not significant, but a trend towards better PDM (279.03 g) was observed with the *Bb* treatment, compared to the control, which had a PDM of 249.48 g.

correlation analysis

Table 5 shows the Pearson correlation analysis, indicating high and significant correlations ($P<0.05$) for NDEN with ADP ($r = 0.92$), NDGPM with NDEN ($r=0.66$) and NHPM ($r=0.87$), ADM with TDM ($r = 0.88$) and PDM with P100G ($r = 0.86$).

%Sev: percentage of severity, %Inc: percentage of incidence, ADP: plant height,

	Psev	%Inc	ADP	NDEN	ADT	AF	NHPM	NDGPM	TDM	ADM	P100G	PDM
Psev	1,00											
Pinc	-0,12	1,00										
ADP	-0,27	0,56	1,00									
NDEN	-0,41	0,54	0,92**	1,00								
ADT	-0,39	0,17	0,33	0,20	1,00							
AF	0,12	0,31	0,68	0,48	0,09	1,00						
NHPM	-0,46	0,40	0,72	0,69	0,53	0,36	1,00					
NDGPM	-0,44	0,25	0,70	0,66 *	0,60	0,22	0,87 *	1,00				
TDM	-0,37	0,05	0,57	0,48	0,68	0,12	0,55	0,83 *	1,00			
ADM	-0,46	-0,10	0,44	0,44	0,64	0,00	0,65	0,80	0,88 *	1,00		
P100G	-0,39	-0,30	0,15	0,14	0,49	-0,19	0,18	0,45	0,77	0,83	1,00	
PDM	-0,38	-0,12	0,49	0,45	0,62	0,06	0,52	0,77	0,95	0,93	0,86 **	1,00

NDEN: number of internodes, ADT: stem width (mm), AF: leaf area (cm²), NHPM: number of rows of grain per ear, NDGPM: number of grains per ear, TDM: ear size (cm), EWB: ear width (cm), P100G: weight of 100 grains, EWG: ear weight (g), *: significant (P<0.05), **: highly significant (P<0.01).

Conclusions

Bb and *Bt* were the best treatments with a lower Psev than the control. *Bb* was the best treatment for NHPM and NDGPM. TDM, P100G and PDM compared to the control. There was a high, positive and significant correlation for NDEN with ADP, NDGPM with NDEN and NHPM, TDM with NDGPM, ADM with TDM, P100G with ADM and PDM with P100G.

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