VARIATION OVER TIME OF BIOINSECTICIDES EFFECT ON S*podoptera frugiperda* INCIDENCE IN CORN (*Zea mays*) IN DRY SEASON IN MOCACHE, LOS RÍOS, ECUADOR †

[VARIACIÓN EN EL TIEMPO DEL EFECTO DE BIOINSECTICIDAS SOBRE LA INCIDENCIA DE *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) EN MAÍZ (*Zea mays*) EN LA TEMPORADA SECA EN MOCACHE, LOS RÍOS, ECUADOR]

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SUMMARY

Background. Corn cultivation – one of the main crops in the world – is important in Ecuador for human and animal nutrition, but one of the main limitations in production is the damage caused by the insect pest *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). **Objective**. Therefore, the objective of this research is to evaluate the effectiveness over time of three bio-insecticides on pest incidence in maize hybrid INIAP H601 in Mocache, Los Ríos, Ecuador in the dry season. **Methodology**. A completely randomized design was used with five treatments and four replicates: (1) Natural neem (*Azadirachta indica*) leaf extract in doses of 1.88 L; (2) Bassigeos (*Beauveria bassiana*) in doses of 5 L; (3) Neem + *B. bassiana* mix in doses of 7 L; (4) Chemical bait in doses of 80 kg.ha^{-1}; and (5) Control group without application, processed by a linear model of repeated measures. **Results**. A significant variation was observed in the treatments due to the influence of time. **Implications**. Corn agricultural production has a long tradition and importance in Ecuador, especially in the Province of Los Ríos where productions are affected by *S. frugiperda* whose major control is by using polluting chemical products. **Conclusions**. Because the effect of the applied bio-insecticide treatments changes overtime, pest incidence is modified through a linear model of repeated measures. The lowest incidence percentages are achieved with the use of *B. bassiana* (Bb), *A. indica* (neem) and Bb + Neem mix after day 15, whereas the chemical control group achieves it throughout the evaluated time interval.
Key words: Corn; neem; *Beauberia bassiana*; *Spodoptera frugiperda*; incidence.

RESUMEN

Antecedentes. El maíz constituye uno de los principales cultivos en el mundo y en el Ecuador es importante para la nutrición humana y animal, pero una de las principales limitaciones productivas es el daño causado por la plaga de insectos *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). **Objetivo**. El objetivo de esta investigación es evaluar el efecto de tres bioinsecticidas sobre la incidencia de este insecto en el híbrido INIAP H601, en la época seca en Mocache, Los Ríos, Ecuador, a través del tiempo. **Metodología**. Se utilizó un diseño completamente al azar, con cinco tratamientos y cuatro repeticiones. Los tratamientos fueron (1) extracto natural de hoja de neem (*Azadirachta indica*) en dosis de 1.88 L; (2) Bassigeos (*Beauveria bassiana*) en dosis de 5 L; (3) Mezcla de Neem + *B. bassiana* en dosis de 7 L; (4) Cebo químico en dosis de 80 kg.ha^{-1}; y (5) un grupo control sin aplicación, procesado por un modelo lineal de medidas repetidas. **Resultados**. Existe una variación significativa en los tratamientos por influencia del tiempo. **Implicaciones**. La producción agrícola de maíz tiene una larga tradición e importancia en el Ecuador, especialmente en la provincia de Los Ríos, cuyas producciones se ven afectadas por el ataque de *S. frugiperda*, cuyo control mayoritario es con el uso de productos químicos contaminantes. **Conclusiones**. La incidencia de la plaga por efecto de los tratamientos aplicados para su control se modifica con el tiempo a través de un modelo lineal de medidas repetidas. Los porcentajes de incidencia más bajos se logran con el uso de *B.*

† Submitted September 2, 2021 – Accepted September 6, 2022. http://doi.org/10.56369/tsaes.3958

ISSN: 1870-0462.

bassiana (Bb), Neem y Bb + Neem mezclado después de 15 días, mientras que el control químico lo logra en todo el intervalo de tiempo evaluado.
Palabras claves: maíz; neem; *Beauberia bassiana*; *Spodoptera frugiperda*; incidencia.

INTRODUCTION

Corn (*Zea mays*) in Ecuador – one of the most important agricultural products of the national economy – is the main raw material to produce concentrated (balanced) feeds for animal industry, especially commercial poultry, which is one of the most dynamic activities in the agricultural sector. According to the Ecuadorian Institute of Statistics and Censuses (INEC, 2013), 338 130 hectares (ha) of hard corn are planted in the country, of which 133 876 ha are located in the Province of Los Ríos, 70 007 ha in the Province of Manabí, 49 903 ha in the Province of Guayas province and the rest in the Province of Loja. Around 90% of corn sowing takes place in the rainy season (INIAP, 2014).

As in all agricultural crops, corn is affected by the incidence of pests, which directly or indirectly cause production losses (Lima *et al*., 2017). However, of all the pests found in corn, the main one is the fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) due to the level of damage it causes in the crop when it reaches 20.0 % of infestation and can cause reductions up to 60.0 % (Valarezo *et al*., 2011). The fall armyworm is a polyphagous pest that appears in tropical and subtropical areas causing considerable damage not only in corn but also in other crops with a wide distribution in the American continent (Valencia *et al*., 2014 and Barrera *et al*., 2017). Populations of *S. frugiperda* were correlated with the reduced abundance of resident communities of stem borers and maize parasitoids in Kenya production fields (Mawuko *et al*., 2021).

The presence of *S. frugiperda* in corn crop has been a limiting factor in low yields, since no adequate management has been provided, and if the conditions are favorable, the crop can be lost in its entirety. The main method for its control is the use of chemical pesticides, but these substances have a negative impact on human health and on beneficial insects (Hernández *et al*., 2018, 2019). Furthermore, the uncontrolled application of synthetic pesticides, on the one hand increases costs (Guedes *et al*., 2016) and on the other hand creates a type of resistance that insect pests develop to these products (Motta and Murcia, 2011, Ramírez *et al*., 2018 and Cerna *et al*., 2022). Furthermore, synthetic pesticides cause effects on all the surrounding living organisms where they are applied due to their negative effects on wildlife, in general, but especially on birds and pollinating insects or those that exert the function of natural biological insect pest control.

In water quality, these synthetic pesticides, in turn, cause damage to aquatic species and those that feed on them, in addition to the contamination that occurs in soil and, consequently, the silent contamination of humans with their consequent social cost (Pimentel and Lehman, 1993). Currently, estimates have considered that more than 250,000 species of higher (vascular) plants on Earth have the possibility of providing an inexhaustible source of bioactive compounds with potential pharmaceutical, agrochemical and biopesticide uses (Draz *et al*., 2019, Pérez *et al*., 2019 and Yparraguirre *et al*., 2021). In this sense, the purpose of this research is to evaluate the effectiveness over time of three bio-insecticides on pest incidence in maize hybrid INIAP H601 in Mocache, Los Ríos, Ecuador in the dry season.

MATERIALS AND METHODS

This research study was performed in UTEQ (Universidad Técnica Estatal de Quevedo) facilities, Campus "La María", located at Km 7 via Quevedo - El Empalme between 01º 06' 18” S latitude and 79º 27' 24” W longitude at an altitude of 75 m a.s.l.

The area has a humid tropical climate with an average annual temperature of 24.4 ^{0}C, a relative humidity of 75.0 %, annual heliophany of 894 h, average annual rainfall of 2252.2 mm, which is divided into two seasons, one rainy with 97.0 % and the other one dry with 3.0 % remaining. Topography is slightly regular, clay loamy soil type, and pH of 5.7.

For sowing corn, the hybrid INIAP H601 from the Instituto Nacional de Investigaciones Agropecuarias was used, which has tolerance to biotic factors (pests, diseases), as well as abiotic factors (such as drought), 55 days to flowering and a vegetative cycle of 120 days. Its yield of 7 400 kg.ha^{-1} in flat terrain conditions and with irrigation (INIAP, 2004). A completely randomized experimental design with five treatments and four replicates was applied with the characteristics shown in Table 1.

Experimental treatments and descriptions

The experimental treatments were the following: **T_1:** Natural neem (*Azadirachta indica*) leaf extract. Dose 1.88 L; **T_2**: Commercial product Bassigeos (*Beauveria bassiana*). Dose 5 L; **T_3**: Neem+ *B.*

bassiana) Mix. Dose 7 L; **T4**: Chemical Bait (Sand + Lorsban). Dose 80 kg.ha^{-1}; **T5**: No application.

Table 1. Characteristics used in this research study to evaluate three bio-insecticides for the control of *Spodoptera frugiperda* in corn (*Zea mays*) in the area of Mocache, Los Ríos, Ecuador in the dry season.

Experimental surface	456 m^2 (16 m x 28.5 m)
Plot area	19.2 m^2 (3.20 m x 6 m)
Useful plot area	9.6 m^2 (1.60 m x 6 m)
Number of plots	20
Number of rows	4
Number of useful rows per plot	2
Row spacing	0.80 m
Distance between plants	0.20m
Plants per row	30
Plants per plot	120

Observations were made of the presence of fresh foliar damage on the new leaf or bud in 10 randomly selected corn plants in each of the two central rows of each treatment (20 plants per treatment); averages of fresh foliar damage presence threshold were obtained of the pest *S. frugiperda* from 25.0 to 30.0 %, for which the following treatments were applied.

Treatment 1 (T_1). Natural Neem Leaf Extract

Treatment 1 consisted of elaborating neem leaf natural extract with the methodology described by Osuma (2005), using 800 g of leaves in 8 L of water. The extract preparation was placed in an oven at 40.0 ºC in a water bath for 15 min using the water with the leaves previously washed; then, the leaves and water were separated. Once this procedure was performed, half of the water obtained was taken and the reserved leaves were used again and placed in the oven in a water bath for 15 minutes; after that, the leaves were separated again obtaining 2 L of neem leaf extract; the dose for the application was of 1.88 L in a 200-L tank of water.ha^{-1}.

Treatment 2 (T_2). Commercial product Bassigeos (*Beauveria bassiana*)

For Treatment 2, the commercial product Bassigeos based on *Beauveria bassiana* was used in doses of 5 L in a 200-L tank of water.ha^{-1}.

Treatment 3 (T_3). Mix (Neem + *Beauveria bassiana*)

Treatment 3 consisted of mixing the doses previously established for neem leaf natural extract and the commercial product Bassigeos (*Beauveria bassiana*) in a 200-L tank of ha^{-1}.

Treatment 4 (T_4). Chemical Bait (Sand + Lorsban)

The methodology proposed (Páliz and Mendoza, 1985) was used for the preparation of Lorsban chemical bait plus sand in relation to 300 cc (Lorsban) + 80 kg (riviersand).ha^{-1}. For bait preparation, the chemical product was diluted in 1 L of water, and then a CP3 pump was used to sprinkle it on the sand until it was uniformly moistened. The bait was applied directly to the plant buds affected by *S. frugiperda*.

Treatment 5 (T_5)

No App. In this treatment, no type of control was performed to allow *S. frugiperda* to develop freely.

Agricultural practices in the experiment

To prepare the agricultural practices for the experiment, the following were performed: (1) Soil preparation was carried out following the methodology for growing corn in Ecuador;

(2) Hierba luisa (*Aloysia citriodora*) from the family Verbenaceae was sown as a living barrier to protect the crop from agents external to the investigation;

(3) Sowing was carried out manually by depositing 2 seeds of INIAP H-601 hybrid corn per site according to the sowing distance under study of 0.80 x 0.20 m;

(4) Weed control was performed manually with a weeding device during the crop pre-emergence and emergence stages; subsequent manual weeding was performed every 15 days with a machete;

(5) Plant thinning was done 12 days after sowing, leaving the most vigorous plants found per site;

(6) For the control of the fall armyworm (*S. frugiperda*), the study treatments were applied. For disease control, no other product had to be applied since very low thresholds were observed;

(7) Sprinkler irrigation was applied twice a week before flowering and four times a week when the crop was in the flowering stage;

(8) Fertilization was applied 12 days after sowing, and compost was applied in doses of 1 kg.m^{-2}. The same compost dose was again applied after 30 days to incorporate nutrients into the soil;

(9) Harvest was performed manually when the grains reached the physiological maturity of each experimental plot. The ears were collected and later manually shelled.

Variables evaluated

Incidence percentage of *Spodoptera frugiperda* larvae

The fall armyworm incidence percentage was determined, making the readings of the two central rows of each experimental unit at random at 3, 6, 9, 12, 15, 18, 21 and 28 days after the product application in 20 plants (100.0 %). Applications were made to the foliage in the morning before 09:00 am or in the afternoon at 16:00 pm.

The incidence percentage value was obtained by applying the methodology proposed (González *et al*., 1995), which is based on the observation of fresh damage presence in the plants caused by the fall armyworm, through the following formula:

Incidence (%) = (Number of plants affected with fresh damage / Number of plants evaluated) x 100

Statistical analyses

The values of the pest incidence percentages were transformed by the equation of the arc sine of the square root of the value of each percentage over 100, to comply with the normal distribution of the data (Shapiro-Wilk) and the homogeneity of the variances univariate (Levene), for the significant difference between the means, the Bonferroni test was used to control the error rate that is referred to the probability of committing type I errors, in order to adjust the significance (Sig.) or critical level and the confidence intervals. In the case of normal distribution and multivariate homoscedasticity, the Mardia (1970) and Box's M tests were applied, respectively (Rencher, 2002).

The statistical analyses of the results were performed through a linear model of repeated measures, in which the time factor with its eight-time intervals in days was considered as intra-subject variable, and the five treatments were considered as inter-subject factor for pest control through a complete factorial model.

Mauchly's sphericity test (Mauchly, 1940) was determined to verify variance similarity assumption of the eight-time factors or sphericity intervals of the variance-covariance matrix (Winer *et al*. 1991). In addition, the test of the intra-subject effects of the time factors and interaction time by treatments was carried out through the Assumed Sphericity, Greenhouse-Geisser, Huynh-Feldt and Lower Bond.

Multivariate analysis of variance was applied to demonstrate the influence of measurements over time in relation to treatments as a whole using Pillai's Trace, Wilks' Lambda, Hotelling's Trace and Roy's Largest Root statistical tests. The within-subjects test and multivariate approach were performed to test the null hypothesis that the means of the five treatments for the control of the pest incidence did not vary over time. In addition, the intra-subject contrast test was used to determine the best relationship established between the time factor and treatments. For the tests of the intra-subject effects, the multivariate approach and intra-subject contrast test, the value of significance, partial Eta squared or effect size index, non-centrality parameter and power were determined observed.

Figures represented the profile graph of the estimated marginal means of the time factor, treatment factor and interaction time by treatment, where the global mean observed and the error bars for the confidence interval of measurement in each figure are represented at 95.0 of confidence. All statistical processing was performed using the statistical package SPSS, version 26 (IBM, 2019) and PAST software 4.07 (Hammer *et al*., 2001) for the normal distribution and multivariate homoscedasticity.

RESULTS AND DISCUSSION

For Mauchly's sphericity test (Table 2) in the linear models of repeated measures, as is the case, the assumption that the variances of the eight time intervals are similar must be fulfilled, which implies that the variance-covariance matrix is spherical (Winer *et al*., 1991) and contrasted through the sphericity test of Mauchly. Since the significance value is greater than 0.05 for these results, the assumption of sphericity of the data matrix of the experiment is fulfilled. Therefore, the variances of the time intervals are not different.

Table 2. Mauchly's test of sphericity to check the assumption of variance similarity of the eight time intervals for the control of *Spodoptera frugiperda* in corn (*Zea mays*) in the area of Mocache, Los Ríos, Ecuador in the dry season. Sig., is the significance (p).

Within subject effect	Mauchly´s W	Sig.
Time	0.056	0.132

The significance of the F-statistic value in the within-subject effects tests for the Assumed Sphericity and the remaining three Greenhouse-Geisser, Huynh-Feldt and Lower Limit tests (Table 3), both time factor independently and time per treatment interaction were significant for a critical level of p = 0.05 and coincide with the results obtained with the multivariate statistics tests (Table 4). Therefore, the hypothesis that the means of the treatment are equal in time and in time interaction per treatment is rejected. It can be assumed that the incidence evaluation through time (days) shows general variations and with effect on the treatments. These results show that time per treatment interaction is significant or the differences that occur between the different treatments are modified over time.

In the intra-subject contrast tests (Table 5), the time factor that refers to the polynomial contrasts for the type of relationship established between the time factor and treatments, seven orthogonal contrasts appear because they are the number of factor levels minus the unit. In this sense, the table shows seven contrasts. However, the linear and quadratic contrasts are the ones that showed the lowest critical value of significance and less than p = 0.05. Nevertheless, between these two contrasts, the linear contrast is the one with the best fit because it shows the highest value of partial Eta squared or correlation ratio with a value of 0.918 very close to 1. This result indicates that the time factor explains a proportion of 0.918 or that 91.8 % of that proportion is with respect to the variance in the linear model. Thus, the size or proportion of the effect of time is very high in that model; additionally, the linear contrast reached the highest values of the parameter without centrality, and the maximum power was observed.

The partial Eta squared is an effect size index that quantifies the magnitude of the difference between two means (Wilkinson, 1999) and the proportion of the variance of the dependent variable. In this case, the time variable is made up of the different time intervals in the days that evaluated, which is explained by the independent or predictive variables for this research in the treatments used. The effect size or Eta values are between 0 and 1, whose values above 0.14 for repeated measures, linear models are considered to have a large effect size (Cárdenas and Arancibia, 2014). A large effect was found in all the statistical analyses performed in this article.

Table 3. Tests withing-subjects effects to check whether the means of the treatments vary over time for the control of *Spodoptera frugiperda* in corn (*Zea mays*) in the area of Mocache, Los Ríos, Ecuador in the dry season. Sig., is the significance (p).

Source		Sig.	Partial Eta Squared	Non-centrality Parameter	Observed Power
Time	Sphericity Assumed	0.000	0.748	310.900	1.000
	Greenhouse-Geisser	0.000	0.748	184.613	1.000
	Huynh-Feldt	0.000	0.748	310.900	1.000
	Lower-bound	0.000	0.748	44.414	1.000
Time x Treatment	Sphericity Assumed	0.000	0.814	460.255	1.000
	Greenhouse-Geisser	0.000	0.814	273.299	1.000
	Huynh-Feldt	0.000	0.814	460.255	1.000
	Lower-bound	0.000	0.814	65.751	1.000

Table 4. Statistical tests of multivariate analysis of variance withing-subjects effects to check if the means of the treatments vary over time from a multivariate approach for the control of *Spodoptera frugiperda* in corn (*Zea mays*) in the area of Mocache, Los Ríos, Ecuador in the dry season. Sig., is the significance (p).

Effect		Sig.	Partial Eta Squared	Non-centrality Parameter	Observed Power
Time	Pillai's Trace	0.000	0.969	281.384	1.000
	Wilks' Lambda	0.000	0.969	281.384	1.000
	Hotelling's Trace	0.000	0.969	281.384	1.000
	Roy's Largest Root	0.000	0.969	281.384	1.000
Time x Treatment	Pillai's Trace	0.032	0.516	51.188	0.956
	Wilks' Lambda	0.001	0.693	76.384	0.992
	Hotelling's Trace	0.000	0.862	187.695	1.000
	Roy's Largest Root	0.000	0.958	271.513	1.000

Table 5. Test of the polynomic type intra-subject contrasts of the relationship between the time factor with respect to the treatments for the control of *Spodoptera frugiperda* in corn (*Zea mays*) in the area of Mocache, Los Ríos, Ecuador in the dry season. Sig., is the significance (p).

Source	Time	Sig.	Partial Eta Squared	Non-centrality Parameter	Observed Power
Time	Linear	0.000	0.918	168.305	1.000
	Quadratic	0.000	0.615	23.947	0.995
	Cubic	0.540	0.026	0.394	0.091
	Order 4	0.186	0.114	1.921	0.255
	Order 5	0.882	0.002	0.023	0.052
	Order 6	0.706	0.010	0.148	0.065
	Order 7	0.479	0.034	0.528	0.105
Time x Treatment	Linear	0.000	0.935	216.256	1.000
	Quadratic	0.004	0.619	24.404	0.940
	Cubic	0.001	0.677	31.489	0.981
	Order 4	0.040	0.467	13.153	0.699
	Order 5	0.205	0.311	6.756	0.396
	Order 6	0.013	0.551	18.442	0.851
	Order 7	0.553	0.173	3.135	0.195

The non-centrality parameter is considered as the degree to which a null hypothesis is false. Central distributions, such as Fisher's or F test used in this analysis, are a type of non-central distribution that differs from central distributions by a non-centrality parameter. This non-centrality parameter represents the degree to which the test statistics mean deviates from it when the null hypothesis is true, which is determined by multiplying the degrees of freedom by the value of F of the analysis. When the non-centrality parameter is further from zero, the probability of rejecting the null hypothesis is very low (Castro, 2013). One of the uses of this parameter is in the determination of confidence limits for the effect on sizes, since they are linear functions of the non-centrality parameters.

The non-centrality for the intra-subject test effectors (Table 3) reached the highest values in the time factor in the Assumed Sphericity and Hynh-Feldt tests with a value of 310.90; while for time x treatment interaction, both tests reached again the highest values, even above the time factor as an independent one with a value of 460.255.

Table four shows the non-centrality parameter value for the time factor and four multivariate tests of 281.384, higher than those reached in time interaction per treatment.

The final non-centrality parameter assessment in tables three and four show that moving away from zero, the probability of accepting the null hypothesis increases or the time factor influences in the results of the treatments applied.

Table five is related with the effect of intra-subject contrast effects. The non-centrality parameter of the time factor and time interaction per treatment was the highest for the linear model with respect to the rest, which confirms the lineal tendency of the time with respect to the treatments.

The statistical power is the probability that an effect of a given size can be distinguished from the intrinsic random variation of the variable (Gent *et al*., 2018 and Vargas, 2021). When its value is 1 (maximum value), it indicates that the statistical test used has a high capacity to reject the null hypothesis. Thus, it is considered an index that validates the experimental results (Bono and Arnau, 1995). When the size effect increases, the statistical power also increases (Cohen, 1992). An acceptable power value is considered when it is 0.8 or 80.0 % (Cohen, 1988), whose study is based on the probability of making the type II error. Thus, this error should be as small as possible, and its complement – the statistical power - should be as high as possible (Lapeña *et al*., 2011).

The power values observed in Table 3 for the tests of intra-subject effects, both for the time factor and time x treatment interaction, reached the maximum value, which corresponds to the value of 1. These results are repeated in Table 4 for the time factor and also in the interaction in the multivariate tests of the Hotelling Trace and in the Roy's Largest Root, and values very close to 1 for the remaining multivariate tests. In Table 5 for the time factor and in the interaction, the maximum observed power that can be reached (value of 1), corresponded to the linear model. To sum up, in all the statistical tests used in this investigation, the power value observed was above 0.80, which validates the experimental results and indicates that the statistical tests used have a high capacity to reject the null hypothesis.

The determination of the effect size index and statistical power is considered demands and requirements of the research process related to the use of statistical tests for processing experimental data for ethical reasons and technical consideration (Grissom and Kim, 2012; Cárdenas and Arancibia, 2014). For the effect size, indices are currently proposed that do not vary depending on the different models (Vandekar *et al*. 2020). Figure 1 shows the profile graph of the estimated marginal means of pest incidence with respect to the time factor from day 3 to 30. A decrease was observed in pest incidence with a marked linear trend through time but in the opposite direction, which corroborates the linear type contrast as the best fit model that was carried out in the intra-subject contrast test. Above the observed global mean, the highest incidence percentages were reached at days three, six, nine and 12, while from days 15 to 30 incidence percentages were below the observed global mean. According to the confidence intervals, the pest incidence percentages between three and six days were significantly higher in relation to those framed between 15 and 30, which denotes the significant influence that the time factor exerts on pest incidence percentage.

The confidence intervals of the treatments in Figure 2 of marginal means denote significant differences between the five treatments. The lowest pest incidence occurred when the treatment with the chemical product was applied, which significantly exceeded the rest. The treatments without significant differences between them and below the value of the global average of pest incidence percentage observed, they corresponded to those with products of biological, origin such as *B. basiana*, Neem and the combination of both. A much higher marginal mean was achieved by the treatment without product (control) than the global estimated mean.

For all the days in which pest incidence was determined (Figure 3), the treatment with the chemical product maintained the lowest values or carried out a better pest control and maintained the incidence below 5.0 % and below the value of the global average observed. The rest of the treatments at three, six and nine days did not differ statistically from each other but showed incidence values above the global mean value. From day 12, a clear bifurcation of the treatments was observed, in one direction the treatment without product or control showed a sudden increase in pest incidence with percentages above 30.0 % and in the other direction, the treatments of *B. basiana*, Neem and the *B.* basiana-Neem combination were grouped. For this group of biological products as of day 12, a reduction in pest incidence was observed below 15.0 % of the global average, and their effects were almost equal in pest incidence reduction compared to the chemical treatment without significant differences between them. The control treatment is the only treatment that does not respond in a linear and inverse way through the days evaluated, so it is assumed that it is the treatment responsible for others types of models such as the quadratic and the polynomials of order four and six to show significance ($p < 0.05$), but it is emphasized that the best fit is the linear model.

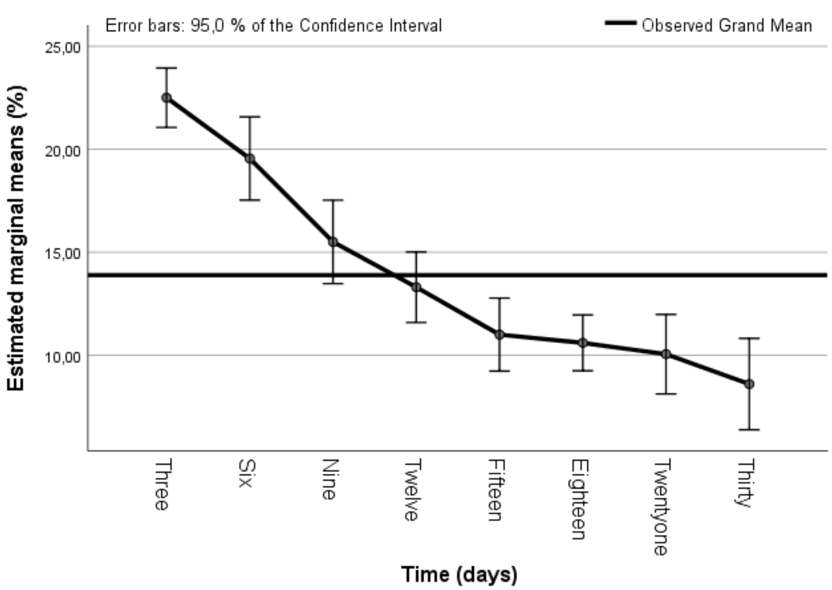

Figure 1. Profile graph of the estimated marginal means of *Spodoptera frugiperda* pest incidence in corn (*Zea mays*) with respect to the time factor in the area of Mocache, Los Ríos, Ecuador in the dry season. Note: Confidence interval error bars that do not overlap between days indicate no significant differences for $p < 0.05$.

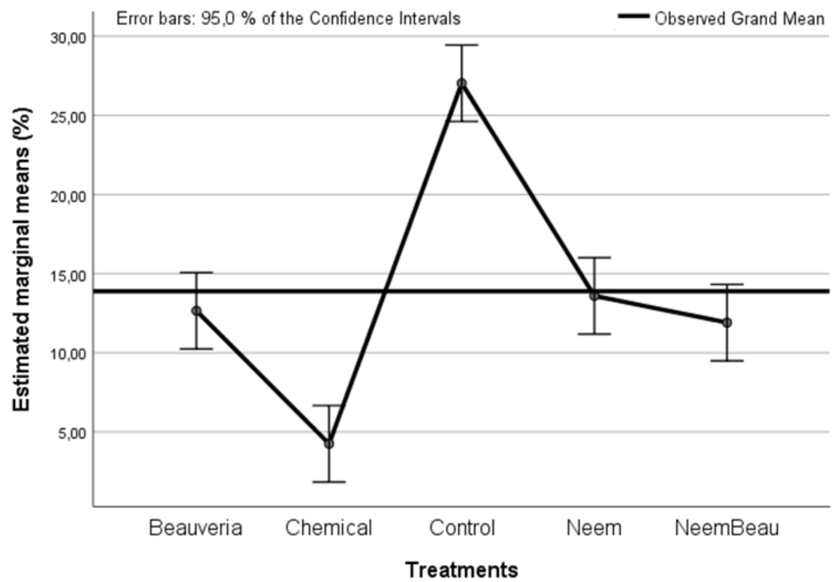

Figure 2. Profile graph of the estimated marginal means of the pest *Spodoptera frugiperda* in corn (*Zea mays*) incidence with respect to the treatments in the area of Mocache, Los Ríos, Ecuador in the dry season. Note: Confidence interval error bars that do not overlap between treatments indicate no significant differences at $p < 0.05$. NeemBeau, is the treatment Neem + Beauveria.

The control treatment is the only treatment that does not respond in a linear and inverse way through the days evaluated, so it is assumed that it is the treatment responsible for other types of models such as the quadratic and the polynomials of order four and six with significance ($p < 0.05$), in the treatment's interaction in relation to the days, but it is emphasized that the best fit is the linear model.

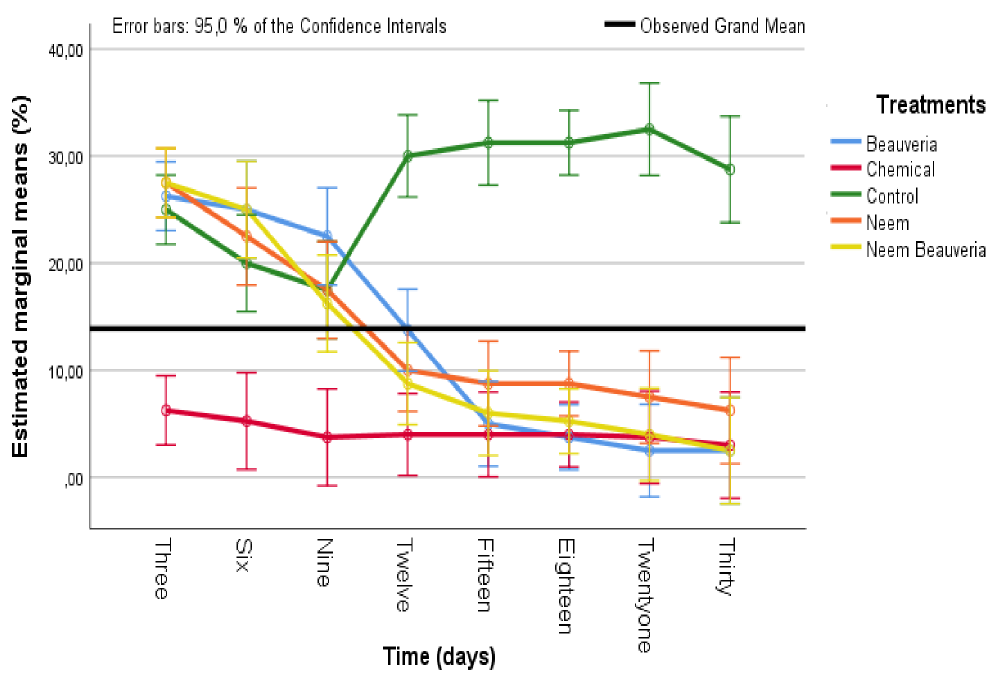

Figure 3. Profile graph of the estimated marginal means of each of the treatments to control *Spodoptera frugiperda* incidence in corn (*Zea mays*) on each of the days of the time factor in the area of Mocache, Los Ríos, Ecuador in the dry season. Note: Confidence interval error bars that do not overlap between treatments across eight time intervals indicate no significant differences at $p < 0.05$.

Three days after germination, significant differences were found between the five treatments for the pest incidence percentage. The highest incidence was found in the treatments with neem, *B. bassiana*, neem + *B. bassiana* mix and in the control treatment where no product was applied. This last one, significantly exceeded the treatment with chemical bait, indicating that three days after germination, the treatment with chemical products achieved the greatest control over the pest with the lowest incidence values in a proportion four times higher than the rest of the treatments. This same trend was maintained when the applications of the five treatments were made at six and nine days after germination but with the trend from day three to nine of a gradual decrease in pest incidence percentage in the treatments with neem, *B. bassiana* and the mixture of both.

From day 12 after the seeds germinated, significant differences were maintained, when the highest incidence continued in the control treatment and significantly exceed the rest. However, from that date on the order of merit of the remaining treatments started to change, especially those made from biological products neem, *B. bassiana* and the mixture of both. In these biological treatments the incidence percentages began a reduction three times lower than the values found on the third day after germination. No significant differences between these three treatments were observed although still with a higher incidence than the treatment in which a chemical product was applied.

The control treatment was maintained with the highest incidence percentages among all the treatments with 30.0 %. The incidence percentages from day 12 after germination and until day 30, the significant differences between all treatments were maintained. The highest incidence continued in the treatment with no application or control treatment, with the singularity that the treatments made with biological products, such as neem, *B. bassiana* and the mixture of both did not show significant differences with the treatment with chemical bait, indicating that from day 15 after germination and up to day 30, the biological treatments decreased pest incidence percentage at the same level as the treatment with chemicals. The results suggest that for the locality under study – at the time it was performed and with the characteristics of the hybrid maize used – if an attack of *S. frugiperda* occurs within the first nine days after sowing and could affect crop yields, chemical products should be applied and *B. bassiana* and neem tree extract mix applied as a biopesticide from day nine on. In the case the pest occurs before nine days in a mild way, eliminate the chemical product and only make applications with the aforementioned biopesticide mixt, with the consequent reduction in costs and protection of the environment.

The superiority of the chemical bait treatment that contains Lorsban, whose active ingredient is chlorpyrifos, is an insecticide that has an activity by contact, ingestion and inhalation, which is why its mechanism of action is almost immediately (Albuja *et al*., 2016). González *et al.* (2015) highlighted that Lorsban can control 100.0 % of the pest, which indicates that this product is highly toxic to the pest insect.

González *et al.* (2015) evaluated different biological products for *S. frugiperda* control, among them *B. bassiana* and neem oil, and found that the most effective product was neem oil with pest mortality higher than 86.0%, followed by *B. bassiana* with 71.0%. They also considered that the combination of neem-based products with those based on microorganisms is also possible to increase effectiveness, whereas Lorsban, as a chemical control, caused 100.0% mortality, which indicates its high toxicity for both the pest insect and natural beneficial insects and pollinators. On the other hand, Agarwaal *et al.* (2006) recommend the combination of biorational products and bioinsecticides that increase pest insect mortality, attributed to a synergy due to their joint mode of action. The chemical insecticide Lorsban (Chlorpyrifos ethyl), is an organophosphate chemical compound, whose action is by contact, ingestion and inhalation, and is considered a moderately toxic product for humans with neurological effects, developmental disorders and the appearance of autoimmunity in exposures excessive (Cao *et al*., 2011 cited by González *et al*., 2015).

With respect to the neem tree extract – a substance known as azadirachtin – is a tetranorditerpenoid ($C_{35}H_{44}O_{16}$), a compound that inhibits the oviposition and metamorphosis of *S. frugiperda* (Adel and Sehnal, 2000). On the biopreparations based on the neem tree, the formulations based on crushed seeds achieved the best results with a duration of crop protection that lasted up to seven days in corn cultivation under field conditions (Cortez *et al*., 2011). Capataz *et al*. (2007) evaluated the antifeedant effect (AE) of the cell suspension extracts of *A. indica* on *S. frugiperda* under laboratory conditions. These authors concluded that the intra and extracellular extracts of these suspensions had a high antifeedant effect on *S. frugiperda* larvae, reaching 100 % AE for the intracellular extracts produced at 15 ºC and darkness and constituting an indicator of cell suspension potential as a biotechnological tool for product production with antifeedant effect on lepidoptera. For future research, the previous authors suggest analyzing the robustness of the extract antifeedant effect through non-choice bioassays and its effect on growth inhibition in *S. frugiperda* larvae as well as the minimum lethal dose LC50 to ensure the commercial potential of *A. indica* suspension cells as generators of metabolites.

The mode of action of the neem tree extract is not immediate, since it occurs two or three days after being applied when the insects leave or stop feeding and die. A first effect is a repulsive effect: the insects refuse to consume the treated plants and abandon them. The main effect occurs when the pest ingests the treated plant: they die or suffer physiological and behavioral disorders, lethal at last (3 to 15 days after treatment), such as blocking the metamorphosis of larvae and nymphs, sterilization of the adults or inhibition of eating and chewing processes. Neem behaves as a growth regulator, acts on insects as a juvenile hormone, and azadirachtin, the main active substance ingested by larvae, prevents molting. The insect remains in the larval stage and dies. This second effect is very important in the control of harmful insects because azadirachtin penetrates the plant and waits for the insects to suck the sap (Gimeno, 2009). González *et al*. (2015) found that neem oil caused a repellent effect on the pest, inhibited feeding, caused molting, malformations (known as pronotum) and limited growth and development in the larval phase.

For agricultural productions, the neem tree, in addition to having insecticidal and antifungal properties, has hormonal, nematicidal and antiviral effects, mainly on the leaves and fruits, either in the form of extracts or in the form of oil. The cake that remains after the oil extraction process is a biofertilizer that increases agricultural yields. For soil, the leaves and fruits are easily biodegradable and nourish soil and the macro and micronutrient crops. They also help to eliminate the bacteria responsible for soil denitrification, which added to their antifeedant properties, reduce the insect pests that inhabit the soil, also functioning as a natural soil conditioner (Lokanadhan *et al*., 2012). Its medicinal properties are notorious (Islas *et al*., 2020). Zeesbaan *et al.* (2018), considered that botanical pesticides may play a significant role in insect/mite pest management as substitutes of toxic and hazardous synthetic chemicals. Particularly neem (*A. indica*) and eucalyptus (*E. camaldulensis*) could be effective biorational options against mealybugs and other homopterous pests and should be incorporated in future pest management programs. The latter effect in time on the control of pest incidence of biological insecticides with respect to chemicals may be due to the fact that bio-insecticides need more time to exert their mode of action against pests (Celis *et al*., 2008; Carrillo and Blanco, 2009 and Cayetano *et al*., 2017). In addition, the stability of plant derivatives can be affected by environmental factors.

The lowest incidence achieved with the application of the two treatments with the presence of *B. bassiana* + neem mix. This result is attributed to the fact that both bioinsecticidal treatments contain a living organism (*B. bassiana*) whose spores remain in the culture reactivated, which represents an advantage with long-term residual protection, thus, maintaining its high percentage of efficacy (Crespo *et al*., 2018). When González *et al.* (2015) applied *B. bassiana* for the control of *S. frugiperda*, they found fungus presence in the larvae in certain percentages inside and forming mycosis in the cuticle. The pathogenesis that *B. bassiana* causes in the insect begins with the union of conidia to the host cuticle, which germinate and penetrate to invade the hemocele. The host insect dies due to hemolymph depletion caused by the fungus invasion, as well as toxemia caused by toxic fungal metabolite production. Bassianolide and beauvericin are part of the various secondary metabolites produced by this fungus to which toxic activities against insects have been attributed (Molnár *et al*., 2010 and García *et al*., 2020).

Asare (2020), in the adaptation strategies of small farmers for the fall armyworm (*Spodoptera frugiperda*) management in rural areas of Ghana, concluded that farmers used conventional and unconventional methods to control the pest incidence with demographic and gender differences.

CONCLUSION

Corn producers in the Mocache region and in the Ecuadorian coastal area agree that the pest incidence attack is increasing. This phenomenon is generated by the current climate situation and even by the deficient information on management and phytosanitary control. In addition, accessing to the information, requiring technician assistance or training are difficult to have viable alternatives to achieve production free of toxic products as a challenge of the new proposals accessible to the farmer for sustainable agriculture.

The treatments showed a differentiated response to the pest incidence in the course of the days in which the applications were performed because the lowest incidence was reached with the application of the chemical compound at the first nine days. However, from that date on, the biopesticides began to show the effect on pest incidence reduction, mainly with *Beauveria bassiana* application and *Beauveria bassiana* mixture with the neem tree leaf extract.

These results constitute a sustainable alternative in the control of *Spodoptera frugiperda* for corn producers in Mocache, Los Ríos, Ecuador.

Acknowledgments
The authors are grateful to UTEQ (Universidad Técnica Estatal de de Quevedo), especially Rector Eduardo Díaz Ocampo, PhD for his unrestricted support to research development. To UTEQ facilities of Campus "La María", located at Km 7 via Quevedo - El Empalme. To Eng. Leonardo Matute, M.Sc., Dean of Agricultural Sciences; to Diana Fischer for English Edition.

Funding. Financing for the execution of this research was provided by the first author of this article Eng. Ramiro Gaibor Fernández, M.Sc.

Conflict of interest. The authors declare that the research was carried out in the absence of commercial or financial relationships that could be interpreted as a possible conflict of interest. The products applied were purchased through farmers in the area, and other products were purchased to seek strategies to control the pests in corn cultivation in balance with nature and human life.

Compliance with ethical standards. The authors declare that the entire execution of the investigation was in compliance with the norms and regulations in force in Ecuador for pest management and control, protection of the personnel who carry out the applications, and biodiversity. Technical Manual of Procedures for the Registration and Control of Biological Control Agents, Plant Extracts. Mineral Preparations, Semiochemicals and Related Products for Agricultural Use. Approved on 07-17-2019 by Resolution 0143 of the Phytosanitary and Zoosanitary Regulation and Control Agency of the Republic of Ecuador. https://www.agrocalidad.gob.ec/wp-content/uploads/2020/05/ccc2.pdf

Data availability. The data is available with the corresponding author (sfrodriguez1964@gmail.com)

Author contribution statement (CRediT). R. Gaibor-Fernández – Conceptualization, Investigation, Methodology., **S. Rodríguez-Rodríguez** – Formal analysis, Writting original draft., **C.M. Guevara-Avilés** – Investigation., **J.J. Reyes-Pérez** – Writting original draft., **P. Plaza-Zambrano** – Methodology.

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