

ORIGINAL

Sustainable Application of Bioles to Improve Agronomic Performance, Productivity, and Soil Health in Cacao (*Theobroma cacao* L.) Propagated Sexually and Asexually

Aplicación sostenible de bioles para mejorar el desempeño agronómico, la productividad y la salud del suelo en cacao (*Theobroma cacao* L.) propagado sexual y asexualmente

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ABSTRACT

Introduction: the research was conducted at the experimental farm “La Represa” of the Technical State University of Quevedo with the aim of evaluating the sustainable application of bioles as a strategy to improve agronomic performance, productivity, and soil health in cacao (*Theobroma cacao* L.) propagated sexually and asexually. The study addressed the need for sustainable alternatives to chemical fertilizers and for reducing the accumulation of heavy metals in agricultural systems.

Method: a randomized complete block design with split plots was implemented, comprising 21 treatments (seven bioles and one control) with three replications. Physiological, phenological, sanitary, productive, and soil variables were evaluated. Data were analyzed using Tukey’s test ($p \leq 0,05$).

Results: bioles produced significant differences in most variables. Treatment T14 (potassium-based biol in grafted cacao) achieved the highest yield with 2790,67 kg/ha/year and 30,17 healthy pods per plant. For vegetative traits, T15 recorded the largest stem diameter (12,05 cm) and vigor, while T12 notably reduced the incidence of witch’s broom (*Moniliophthora perniciosa*). Soil analyses confirmed increases in macro- and micronutrients, as well as marked reductions in cadmium and lead concentrations.

Conclusions: the sustainable application of bioles improved agronomic performance, enhanced productivity, and strengthened soil health in cacao propagated both sexually and asexually. Among the treatments, potassium-based biol proved to be the most effective, establishing itself as a viable agroecological alternative for sustainable cacao production systems in Ecuador.

Keywords: Liquid Biofertilizers; Agricultural Sustainability; Cacao Productivity; Sexual and Asexual Propagation; Soil Health.

RESUMEN

Introducción: la investigación se realizó en la finca experimental “La Represa” de la Universidad Técnica Estatal de Quevedo con el fin de evaluar la aplicación sostenible de bioles como estrategia para mejorar el desempeño agronómico, la productividad y la salud del suelo en cacao (*Theobroma cacao* L.) propagado sexual y asexualmente.

sexual y asexualmente. El estudio respondió a la necesidad de alternativas sostenibles que sustituyan a los fertilizantes químicos y mitiguen la acumulación de metales pesados en los sistemas agrícolas.

Método: se implementó un diseño de bloques completamente al azar con parcelas divididas, que incluyó 21 tratamientos (siete bioles y un testigo) y tres repeticiones. Se evaluaron variables fisiológicas, fenológicas, sanitarias, productivas y de suelo. Los datos se analizaron mediante la prueba de Tukey ($p \leq 0,05$).

Resultados: los bioles generaron diferencias significativas en la mayoría de variables. El tratamiento T14 (biol potásico en cacao injertado) presentó el mayor rendimiento con 2790,67 kg/ha/año y 30,17 frutos sanos por planta. En variables vegetativas, T15 alcanzó el mayor diámetro de tallo (12,05 cm) y vigor, mientras que T12 redujo la incidencia de escoba de bruja (*Moniliophthora perniciosa*). Los análisis de suelo confirmaron incrementos en macro y micronutrientes, así como disminuciones notorias en cadmio y plomo.

Conclusiones: la aplicación sostenible de bioles mejoró el desempeño agronómico, aumentó la productividad y fortaleció la salud del suelo en cacao propagado sexual y asexualmente. Entre los tratamientos evaluados, el biol potásico destacó como la opción más eficaz, consolidándose como una alternativa agroecológica viable para sistemas cacaoteros sostenibles en Ecuador.

Palabras clave: Biofertilizantes Líquidos; Sostenibilidad Agrícola; Productividad del Cacao; Propagación Sexual y Asexual; Salud del Suelo.

INTRODUCTION

The prolonged and intensive use of synthetic fertilizers in agricultural systems has had negative impacts on soil health, production costs, and environmental sustainability, particularly in tropical countries dependent on permanent crops.⁽¹⁾ This problem has driven the development of alternatives based on bio-inputs, including bioles, liquid biofertilizers obtained by anaerobic fermentation of animal manure and plant biomass, rich in assimilable nutrients, bioactive compounds, and beneficial microorganisms.⁽²⁾ Their application is associated with improvements in root, leaf, and reproductive growth, as well as reducing dependence on high-cost chemical inputs.⁽³⁾

In this context, cocoa (*Theobroma cacao* L.) is a strategic crop in Latin America. It originated in the northwestern Amazon region, where the current territories of Ecuador, Peru, and Colombia are the primary areas of domestication and genetic diversification.⁽⁴⁾ Ecuador, in particular, is the world's leading producer of fine aroma cocoa, with more than 500 000 hectares under cultivation and an approximate 65 % share of this specialized market.⁽⁵⁾ More than 600 000 families depend directly on its production, reinforcing its economic, social, and cultural importance.⁽⁶⁾

Despite this relevance, the national average productivity (419 kg/ha/year) remains below the genetic potential of the cultivated materials.⁽⁷⁾ Among the limiting factors are: soil degradation, poor sustainable fertilization, fungal diseases (*Moniliophthora roreri* and *Crinipellis perniciosa*), aging plantations, and limited technical assistance for small producers.⁽⁸⁾ During the "golden age" (1860-1920), Ecuador led global exports; however, monoculture, phytosanitary crises, and a lack of clonal renewal led to a sustained decline in production.⁽⁹⁾ The introduction of improved materials, such as the CCN-51 clone, allowed competitiveness to be regained by combining yield, tolerance, and agronomic stability.⁽¹⁰⁾

A critical aspect that has been little addressed is the method of crop propagation. Sexual propagation by seed, which is predominant on 70 % of farms, generates heterogeneous populations with low phenotypic uniformity and variable responses to fertilization.⁽¹¹⁾ In contrast, asexual techniques (grafting, layering, cuttings) allow the replication of superior genotypes with better performance and resistance to biotic and abiotic stress.⁽¹²⁾ However, there is a research gap regarding how the application of bioles interacts with different propagation methods in the establishment and initial development of cocoa, especially in smallholder farm conditions. Current studies focus on annual crops, specific soils, or homogeneous genetic materials, without considering physiological differences between plants of sexual and clonal origin.

Given this scientific limitation, the present study aimed to evaluate the effect of biol on the initial growth of cacao (*Theobroma cacao* L.), considering different propagation methods. The hypothesis is that the application of biol significantly improves the crop's agronomic performance, with different responses depending on the type of propagation used, thereby allowing the generation of sustainable technical recommendations adapted to Ecuadorian production systems.

METHOD

Location and environmental conditions

The research was carried out at the "La Represa" experimental farm of the Technical State University of Quevedo, located at kilometer 7,5 of the Quevedo-San Carlos road, Fanta district, Los Ríos province, Ecuador.

Its geographical location corresponds to 1°03'18" south latitude and 79°25'24" west longitude, at an altitude of 73 m above sea level.

The site belongs to the Tropical Rainforest (bh-T) life zone according to Holdridge's classification, with an average annual rainfall of 2200-2600 mm, an average temperature of 24-27 °C, and relative humidity above 80 %. The predominant soils are of alluvial origin, with a loamy-clay texture, slightly acidic pH (5,5-6,5), and good organic matter content.

Plant material and establishment

The trial was conducted with established *Theobroma cacao* L. plants that were 36 months old at the start of the study (January 2024). Transplanting to the final field was carried out in January 2021, using three propagation methods:

Grafting and twig: CCN-51 variety (Trinitario).

Propagation by seed: nacional and ETT-103 (Forastero) materials.

The plants were in the early stages of production, with flowering, fruit formation, and expression of health symptoms characteristic of the crop. A planting frame of 3 m × 3 m ($\approx 1,111$ plants/ha) was used under regulated shade.

Each experimental unit consisted of 10 useful plants, with two additional plants as a border, totaling 630 plants distributed across 63 experimental units (21 treatments × 3 replicates). Cultural practices (manual weeding, sanitary pruning, irrigation, and supplementa) were applied uniformly to avoid bias in the response to treatments.

Research Design

As shown in table 1, the research was conducted under a randomized complete block design with a split-plot arrangement to simultaneously evaluate the effects of the propagation method and the application of bioles on *Theobroma cacao* L. plants in the early productive stage. The factor corresponding to the main plot consisted of three propagation methods: clonal twigs of the CCN-51 variety, grafting of the same variety, and sexual propagation using seeds from the Nacional and ETT-103 materials. Seven bioles of different nutritional and microbiological composition were evaluated in the subplots, including a traditional artisanal control and six commercial formulations (Albio Root, Alico-Cal SC, Albio-Potasio, Albiobacth, Najoga Plus, and Bioru de minerales).

The factorial combination of the two factors yielded 21 treatments, each repeated three times, for a total of 63 experimental units. Each unit consisted of ten useful plants, with two additional plants serving as a border to reduce lateral interference. The distribution of treatments within each block was carried out by random assignment, respecting the hierarchical structure between main plots and subplots.

Statistical analysis was performed using ANOVA for split-plot designs, accounting for the main effects of the factors, their interaction, and the block as a random factor. When significant differences were detected, the means were compared using Tukey's test at the $p \leq 0,05$ significance level. Data processing was performed using InfoStat software (professional version, National University of Córdoba, Argentina), which ensured the proper treatment of errors associated with each level of the experimental design.

Table 1. Table title		
Source of variation	Formula	Degrees of freedom
Large plot	a.r-1	8
Methods(A)	a-1	2
Block	r-1	2
Error (a)	(a-1)(r-1)	4
Bioles (B)	(b-1)	6
Inte.AxB	(a.-1)(b-1)	12
Error (b)	a (r-1) (b-1)	36
Total	a.b.r-1	62

Description of treatments

The experiment included a total of twenty-one treatments, resulting from the factorial combination of the three propagation methods evaluated and the seven types of bioles applied. Each treatment was coded according to the type of biol and the corresponding propagation method, allowing comparison using a multifactorial

approach, as shown in table 2.

The bioles used included a handmade control and six commercial formulations with different microbiological, mineral, and organic bases. The handmade biol was prepared from rabbit excrement and fermented legumes, with a pH of 6,6 and organic matter, nitrogen, phosphorus, and potassium contents in line with its traditional composition. The commercial bioles were formulated to act on different physiological and edaphic processes: Albio Root, composed of humic, fulvic, and ulmic acids; Alico-Cal SC, designed as a calcium amendment to correct acidity and salinity; Albio-Potassium, aimed at sugar synthesis and structural resistance; and Najoga Plus, providing macronutrients, sulfur, and amino acids. Likewise, microbiological formulations such as Albiobacth, with consortia of *Azospirillum brasilense*, *Azotobacter chroococcum*, *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, *Bradyrhizobium japonicum*, and Bioru minerals, derived from ruminal material enriched with volcanic ash, rock flour, and efficient microorganisms such as *Bacillus subtilis*, were used.

Each biol was applied to the three forms of propagation evaluated: clonal twig of the CCN-51 variety, graft of the same variety, and sexual propagation using seeds from the Nacional and ETT-103 materials. This arrangement generated twenty-one experimental combinations, differentiated by the code b□a□, where “b” identifies the type of biol and “a” the propagation method. These combinations were distributed in the field with experimental uniformity and analyzed under the same statistical scheme.

The integration of these treatments enabled contrasting the differential effects of biofertilizers on plants with different physiological origins, thereby facilitating the detection of potential interactions between the type of propagation and the composition of the biol. This approach enabled evaluation of the crop’s productive, physiological, and health responses under homogeneous management conditions, using a robust methodological framework.

Table 2. Description of treatments: propagation by cuttings and grafting was carried out with the CCN-51 (Trinitario) variety, while propagation by seed corresponded to the ETT-103 (Forastero) and Nacional varieties

No. Treatments	Code	Details of the type of biol and propagation method
1	b0a0 Control 1	Artisanal control biol with twig
2	b0a1 Control 2	Artisanal control with graft
3	b0a2 Control 3	Artisanal control with seedling
4	b1a0	Biol albio root with twig
5	b1a1	Biol albio root with graft
6	b1a2	Biol albio root with seedling
7	b2a0	Biol Alico- cal Sc with twig
8	b2a1	Biol Alico- cal Sc with graft
9	b2a2	Biol Alico- cal Sc with seed
10	b3a0	Biol Albio- potassium with twig
11	b3a1	Biol Albio - potassium with graft
12	b3a2	Biol Albio - potassium with seed
13	b4a0	Biol Albiobacth with twig
14	b4a1	Biol Albiobacth with graft
15	b4a2	Biol Albiobacth with seed
16	b5a0	Biol Najoga Plus with twig
17	b5a1	Biol najoga plus with graft
18	b5a2	Biol najoga plus with seed
19	b6a0	Biol bioru mineral fertilizer with twigs
20	b6a1	Biol bioru of minerals with grafting
21	b6a2	Mineral bioru with seed

Experiment management

The trial was conducted under homogeneous field conditions to ensure that the observed differences in the variables were attributable solely to the evaluated factors. The bioles were applied over 120 days (January-April 2024), every two weeks, for a total of 8 applications. The dose used was 200 mL per plant, distributed as 100 mL via foliar application and 100 mL as a collar drench, applied with a 20 L Stihl motor pump equipped with

a calibrated conical nozzle (0,45 L·min⁻¹). The applications were conducted in the morning (7:00-9:30 a.m.), and the equipment was washed after each use to prevent cross-contamination.

The application schedule was as follows: January 5 and 20, February 4 and 19, March 5 and 20, and April 4 and 19, 2024. During this period, no synthetic fertilizers or systemic fungicides were applied to evaluate the interaction between propagation type and bioles. Weed control was performed manually every 30 days, while sanitary pruning was carried out preventively. Supplemental irrigation was applied only when weekly precipitation was less than 20 mm, maintaining a depth of 25-30 mm per event.

Measurements were taken on five central plants in each experimental unit, using standardized protocols and calibrated equipment. Plant height was measured with a metal stadiometer from the stem collar to the functional apex. Diameter was recorded with a digital caliper (0,01 mm accuracy), 30 cm above the ground, with two orthogonal readings averaged. Chlorophyll content was determined using an SPAD-502 meter, taking three fully expanded leaves per plant and three readings per leaf, and then averaging the individual values. Bud sprouting and flowering were quantified by direct counting on flower cushions and vegetative axes, and the data were expressed as percentages.

For health variables, the incidence of *Moniliophthora roreri* and *Moniliophthora perniciosa* was expressed as a percentage of affected fruits or shoots relative to the total observed per plant. Physiological wilting (cherelle wilt) was calculated as the proportion of aborted fruits relative to set fruits in each evaluation cycle. For production variables, the number of healthy fruits was counted every two weeks during physiological harvest (5-6 months of development), and the fresh weight of almonds was determined with an analytical balance (0,01 g) on representative cobs from each plant.

Finally, soil analyses were performed before starting the applications and at the end of the trial to determine changes in pH, organic matter, nitrogen, phosphorus, and potassium. This comprehensive management enabled accurate evaluation of the effects of treatments on the physiological, sanitary, and productive performance of cocoa under controlled field conditions.

RESULTS

Physiological Variables

Bud sprouting

Figure 1 shows the initial and final values of bud sprouting in the different treatments. In the evaluation conducted on day 0, no significant differences were detected among treatments ($p > 0,05$), confirming homogeneous conditions before bioles application.

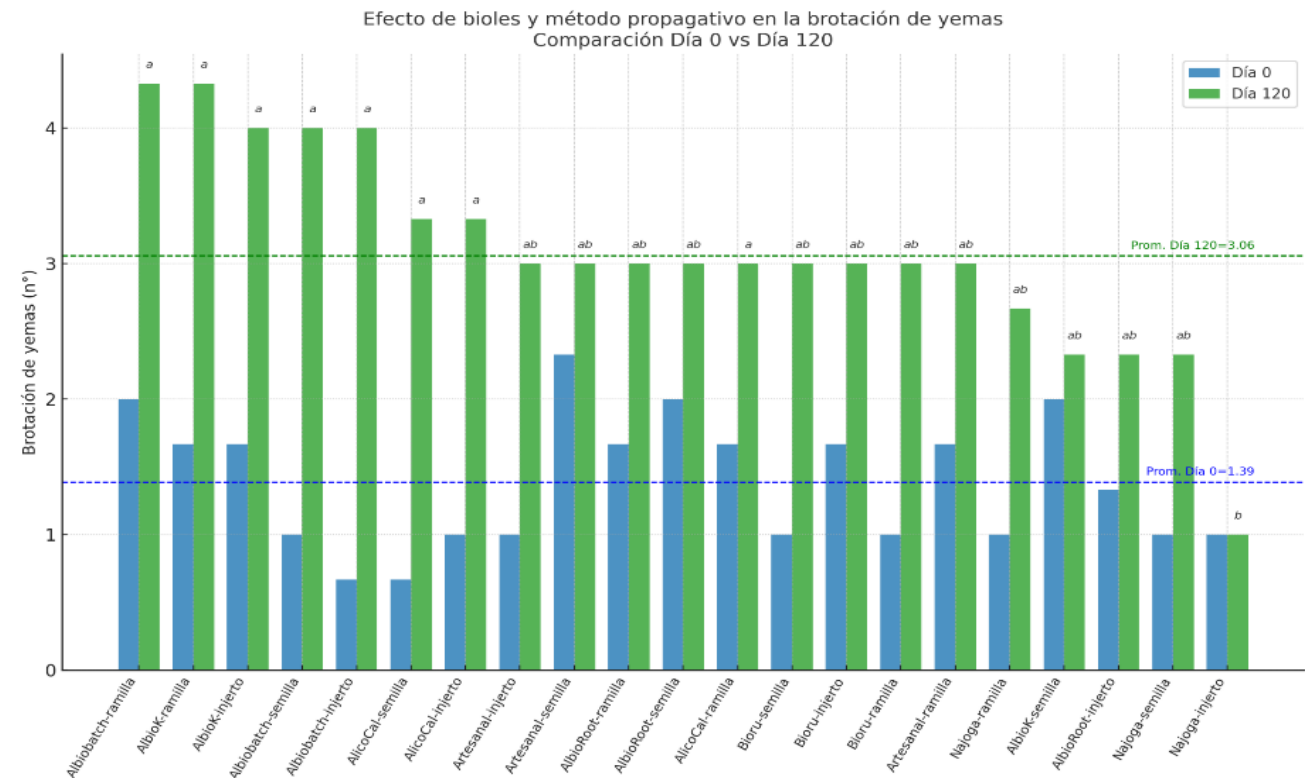


Figure 1. Effect of the application of bioles on propagation methods, physiological variable bud sprouting on day 0 without application and day 120 after application

Note: *Scale 1 = 0 % Absence 2 = 25 % Low 3 = 50 % Light 4 = 75 % Moderate 5 = 100 % Abundant

At 120 days, the analysis of variance showed highly significant differences between treatments ($p \leq 0,05$). Treatment T13 (Biol Albiobacth applied to plants propagated by cuttings) had the highest average, with 4,33 buds per plant. In contrast, the lowest value was recorded in treatment T17 (applied to grafted plants), with an average of 1,00 buds. The overall mean value at the end of the trial was 3,06 buds, compared to 1,39 at the beginning. The coefficient of variation reached 22,65 %, reflecting moderate variability between treatments.

Flowering

Figure 2 shows the initial and final flowering values for the different treatments evaluated. In the day 0 measurement, the analysis of variance revealed no significant differences among treatments ($p > 0,05$), confirming the initial uniformity of the experimental units. After 120 days of biocide application, highly significant differences were observed between treatments ($p \leq 0,05$). Treatment T14 (Albiobacth applied to grafted plants) recorded the highest average, with 5,0 flowers per plant. The overall average at the end of the trial was 2,94 flowers, compared with the initial average of 1,00 flowers recorded before the applications. The coefficient of variation was 24,12 %, indicating moderate variability between treatments.

The lowest values were recorded in treatments T21, T2, T4, T5, T19, and T17, which averaged 2,00 flowers per plant. These values were lower than those for the treatments with the highest responses on day 120, although they exceeded the initial values.

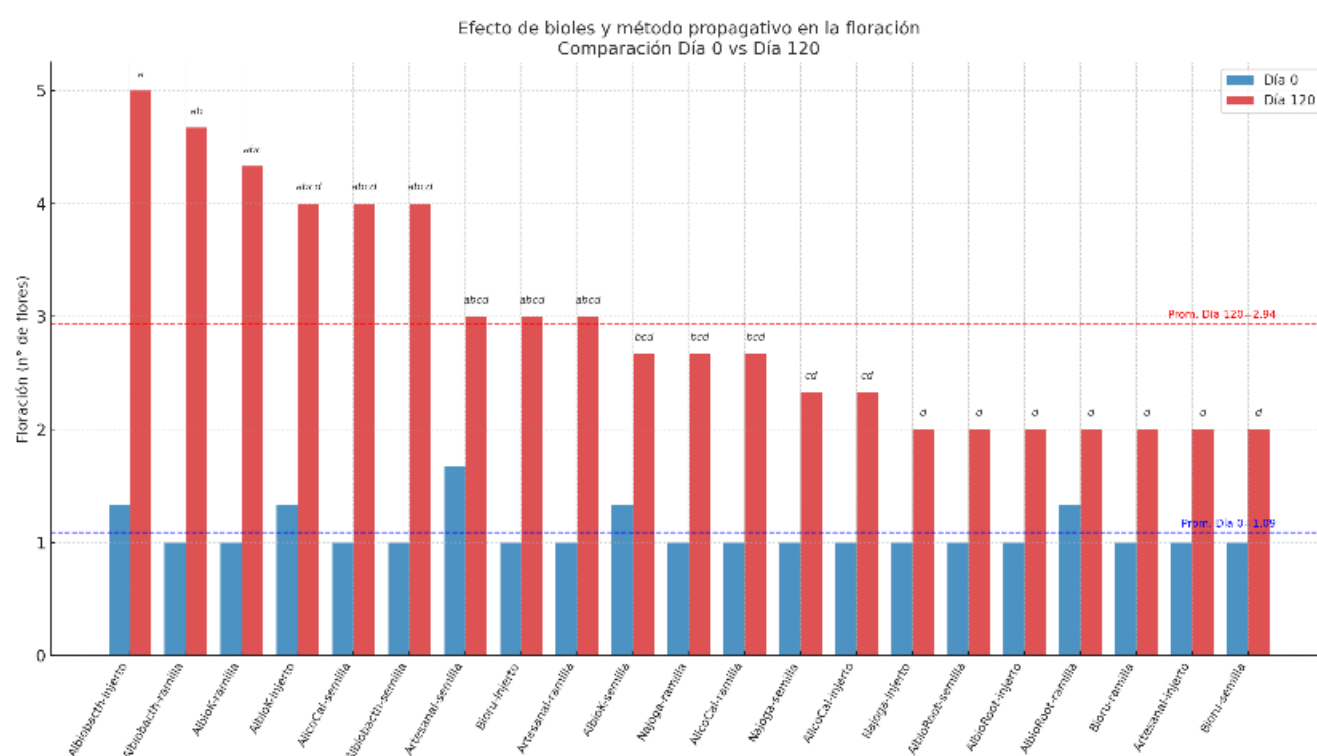


Figure 2. Analysis of the effect of bioles on propagation methods in the physiological variable flowering on day 0 without application and day 120 with application

Note: *Scale 1 = 0 % Absence 2 = 25 % Little 3 = 50 % Light 4 = 75 % Moderate 5 = 100 % Abundant

Fruiting

Figure 3 shows the fruiting values recorded at the start of the trial (day 0) and 120 days after the application of bioles. In the initial evaluation, the analysis of variance showed no significant differences among treatments ($p > 0,05$), indicating homogeneous conditions before the intervention.

After 120 days, highly significant differences were observed between treatments ($p \leq 0,05$). Treatment T14 (biological product applied to grafted plants) recorded the highest average, with 5,0 fruits per plant. The overall average at the end of the study was 2,90 fruits, compared to the initial value of 1,42 fruits. The coefficient of variation was 29,10 %, indicating moderate dispersion among the treatments.

The lowest values on day 120 were recorded in treatments T19, T20, and T21, with an average of 1,0 fruit per plant.

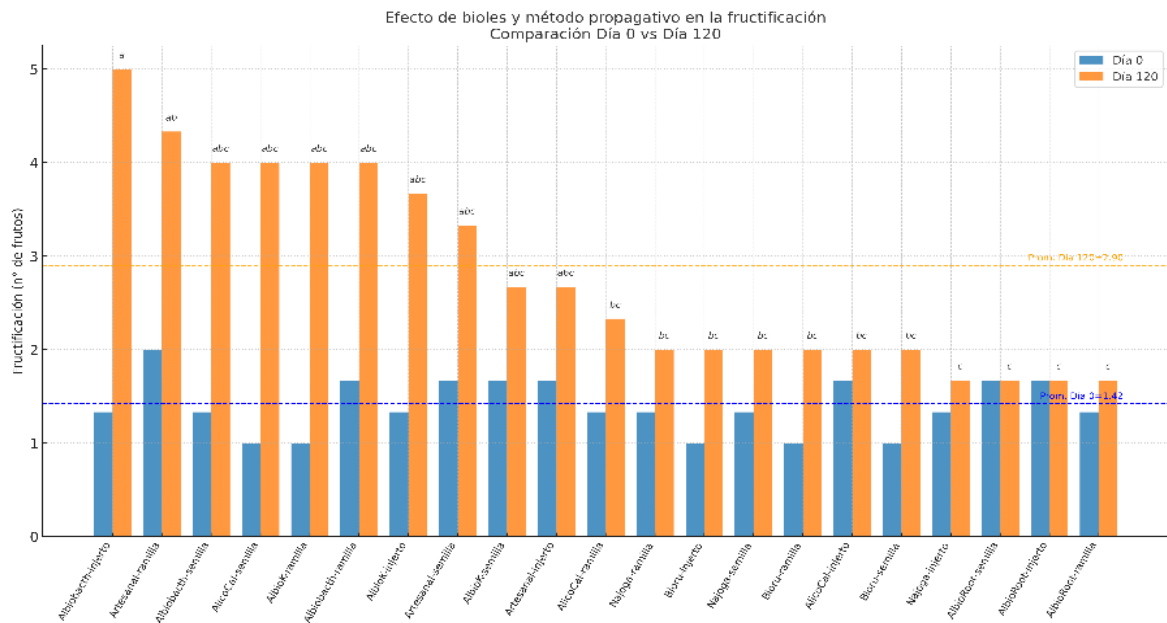


Figure 3. Analysis of the effect of biofertilizers on propagation methods in the physiological variable of fruiting on day 0 without application and day 120 with application
Note: *Scale 1 = 0 % Absence 2 = 25 % Little 3 = 50 % Light 4 = 75 % Moderate 5 = 100 % Abundant

Phenological variables
Plant height

Figure 4 shows the plant height values evaluated at the start of the trial (day 0) and 120 days after the application of the biofertilizers. In the initial measurement, significant differences were observed between the treatments ($p \leq 0,05$), with an overall average of 2,73 m.

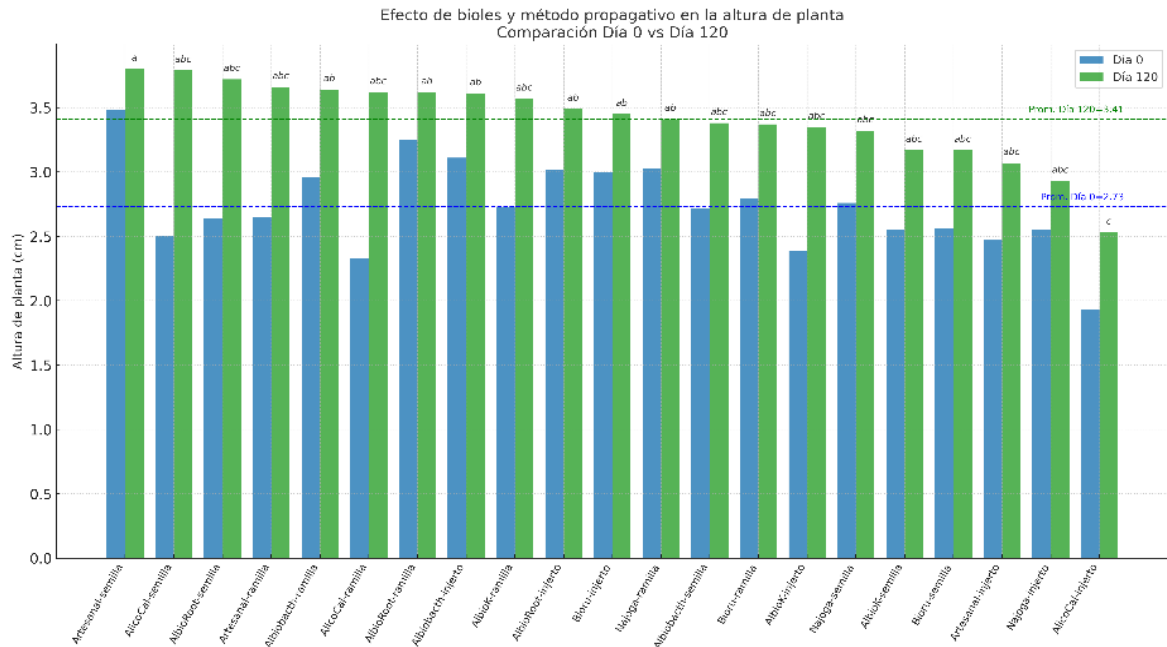


Figure 4. Analysis of the effect of biofertilizers on propagation methods on the phenological variable plant height on day 0 without application and day 120 with application

At the end of the 120 days, the analysis of variance showed no statistically significant differences between treatments ($p \geq 0,05$). However, an increase in average values was recorded, reaching an overall average of 3,41 m. The coefficient of variation was 12,66 %, which shows low variability between treatments. Treatment T3 (artisan biol applied to seed-propagated plants) recorded the highest value at the end of the period, increasing from 3,48 m to 3,80 m. The lowest value was observed in the treatment T8 (Allico-Cal applied to seed-propagated plants), with a final height of 3,41 m.

Stem diameter

Figure 5 shows the stem diameter values recorded at the start of the experiment (day 0) and 120 days after the application of the bioles. In the initial evaluation, significant differences were observed between treatments ($p \leq 0,05$), with an overall average of 7,81 mm. At the end of the evaluation period, significant differences were also observed ($p \leq 0,05$), with the overall average increasing to 8,38 mm. The coefficient of variation was 17,79 %.

The highest value on day 120 corresponded to treatment T15 (Albiobacth applied to seed-propagated plants), which went from 11,17 mm to 12,05 mm. In contrast, the lowest values were recorded in treatments T5 (Albio Root with grafting) and T14 (Albiobacth with grafting), with final averages close to 5,24 mm and values below the average for all treatments.

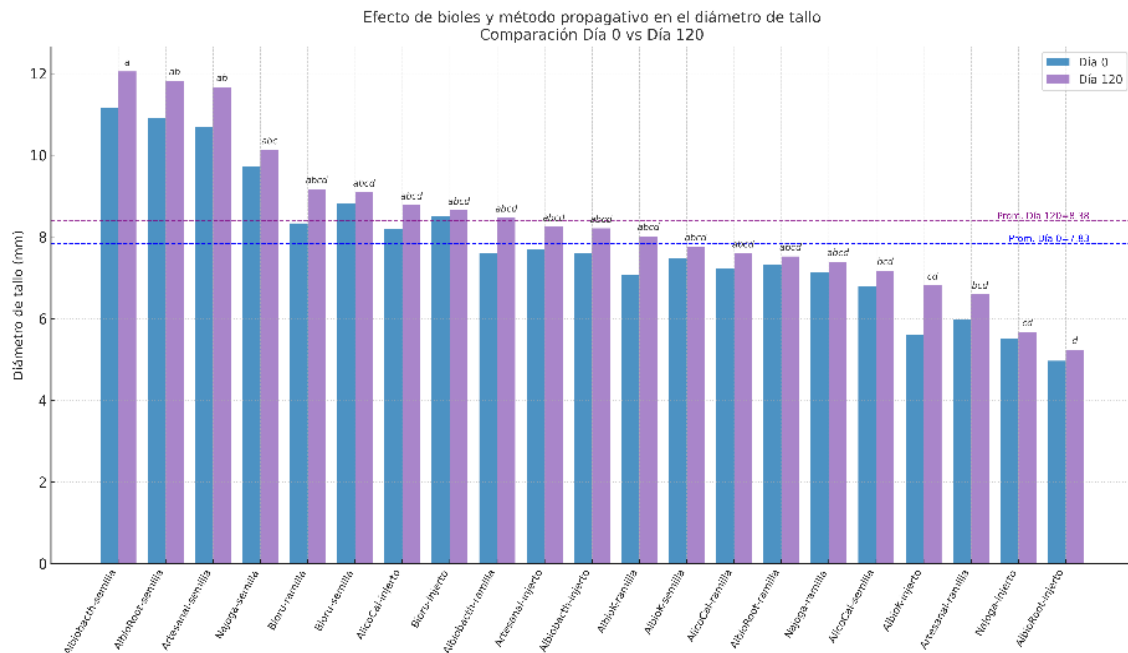


Figure 5. Analysis of the effect of bioles on propagation methods on the phenological variable stem diameter on day 0 without application and day 120 with application

Vigor

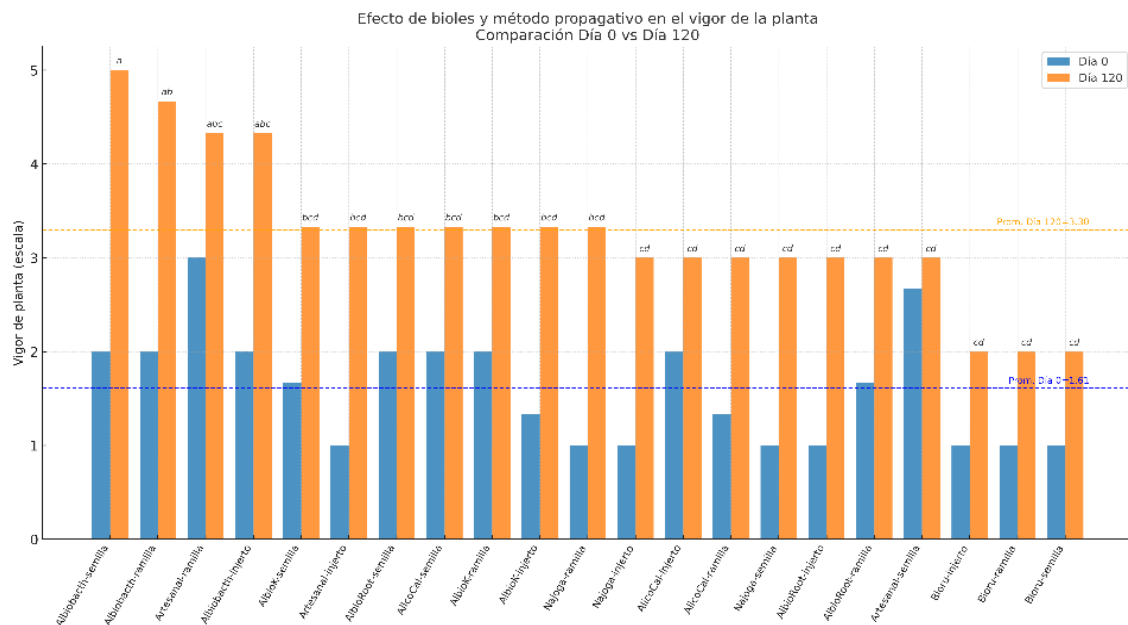


Figure 6. Analysis of the effect of bioles on propagation methods on the phenological variable vigor of the cacao plant on day 0 without application and day 120 with application

Figure 6 shows the plant vigor values measured at the start of the trial (day 0) and 120 days after bioles application. In the initial evaluation, no significant differences were recorded between treatments ($p > 0,05$), with an overall average of 1,61 on the scale used.

At 120 days, highly significant differences were observed ($p \leq 0,05$). The highest value corresponded to treatment T15 (Albiobacth applied to seed-propagated plants), with an average of 5,0 on the evaluation scale. In contrast, the lowest values were found in treatments T19, T20, and T21, with final averages close to 2,0. The overall average for this period was 3,30, an increase from the initial value. The coefficient of variation was 13,58 %, indicating homogeneity in the treatments' responses.

Leaf color

Figure 7 shows the results of the leaf color variable before the application of bioles (day 0) and 120 days later. In the initial evaluation, no significant differences were detected between treatments ($p \geq 0,05$), with an overall average of 2,11 on the scale used.

At the end of the experimental period, statistically significant differences were observed ($p \leq 0,05$), with an overall average of 2,75 and a coefficient of variation of 11,55 %, indicating a relatively uniform response between treatments. The highest value corresponded to treatment T15 (Albiobacth applied to seed-propagated plants), with an average of 3,67 on the color scale. In contrast, treatments T1, T2, and T18 recorded the lowest values, with averages close to 2,00.

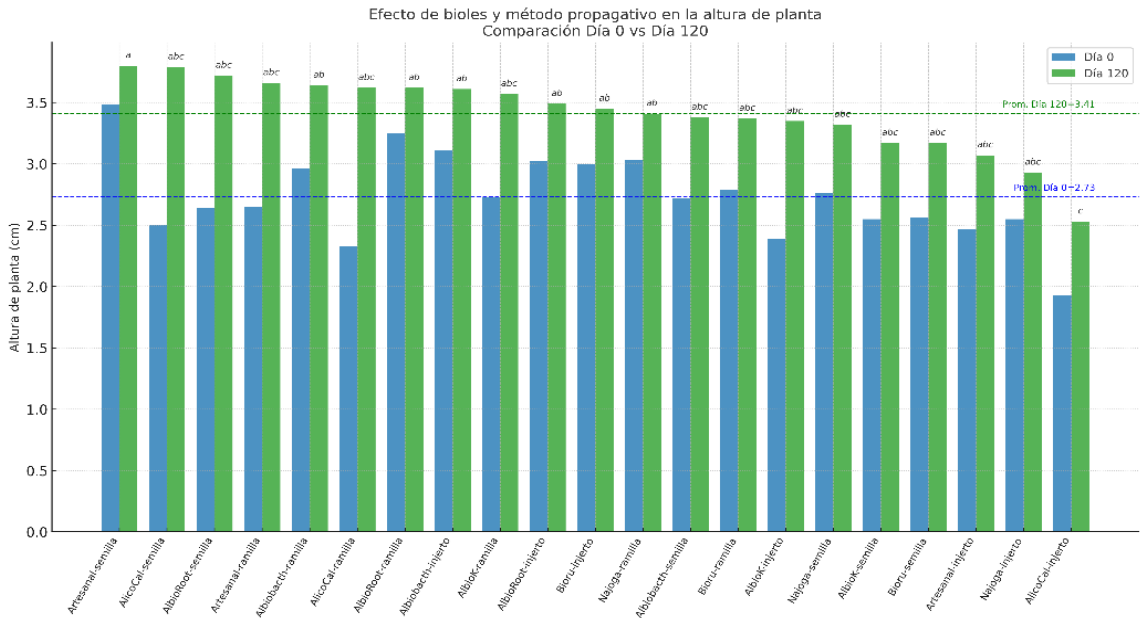


Figure 7. Analysis of the effect of bioles on propagation methods on the phenological variable leaf color on day 0 without application and day 120 with application

Health variable

Incidence of witch's broom

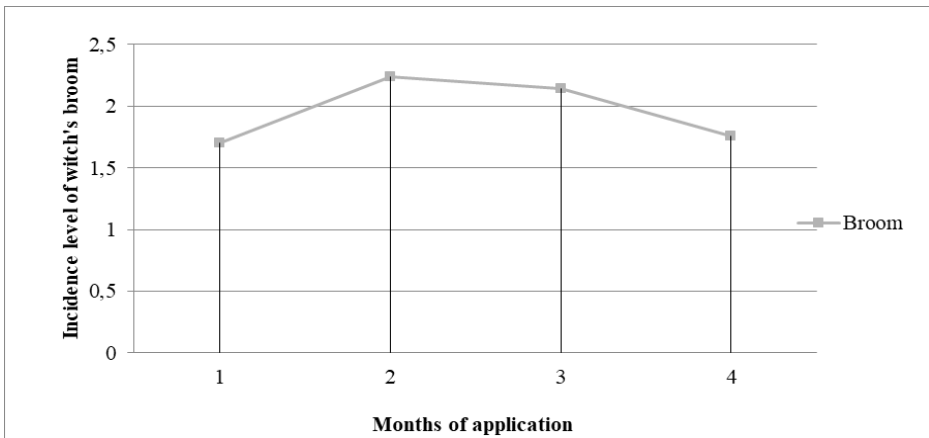


Figure 8. Incidence of witches' broom in cocoa during the four months of potassium biol application

Figure 8 shows the results of witch's broom incidence during the four months of evaluation. The analysis of variance performed on day 120 did not reveal any statistically significant differences between treatments ($p \geq 0,05$).

The overall average value for this variable was 1,76, with a coefficient of variation of 43,49 %. The highest value was recorded in treatment T21 (Bioru biofertilizer with minerals), with 2,67, while the lowest was observed in treatment T12 (Albio-Potassium biofertilizer applied to seed-propagated plants), with an average of 1,00.

Cherelles wilt

Table 3 shows the results of the incidence of cherelles wilt in cacao plants during the 120 days of evaluation. The analysis of variance showed no statistically significant differences between treatments ($p \geq 0,05$). The overall average value obtained on day 120 was 1,10, lower than that recorded in the initial evaluation (1,17 on day 30). The coefficient of variation in this period was 23,95 %, indicating moderate data dispersion.

Among the treatments, the highest value was observed in T1 (artisan biol applied to grafted plants) at 1,67. At the same time, most of the other therapies remained at 1,00, which coincides with the absence or minimal incidence reported on the different evaluation dates.

Cherelle Wilt (Chw)			Days			
No.	Code	Description of treatments	30	60	90	120
1	b0 a0	Artisanal control with twig	2,33 to	2,00 a	1,00 b	1,67 a
2	b0 to1	Artisanal control with graft	2,00 a b	1,00 b	1,00 b	1,00 a
3	b0 to 2	Artisanal control with seed	1,00 c	1,00 b	1,00 b	1,67 a
4	b1 a0	Biol albio root with twig	1,33 b c	1,00 b	1,00 b	1,00 a
5	b1 a1	Biol albio root with graft	1,00 c	1,00 b	1,00 b	1,00 a
6	b1 a2	Biol albio root with seed	1,00 c	1,00 b	1,00 b	1,00 a
7	b2a0	Biol Alico- cal Sc with twig	1,00 c	1,00 b	1,33 a b	1,00 a
8	b2 a1	Biol Alico- cal Sc with graft	1,00 c	1,00 b	1,00 b	1,00 a
9	b2 a2	Biol Alico- cal Sc with seed	1,00 c	1,00 b	1,00 b	1,00 a
10	b3 a0	Biol Albio- potassium with twig	1,00 c	2,00 a	1,67 a b	1,33 a
11	b3a1	Biol Albio - potassium with graft	1,00 c	2,00 a	1,33 a b	1,33 a
12	b3 a2	Biol Albio - potassium with seed	1,00 c	1,00 b	1,00 b	1,00 a
13	b4 a0	Biol Albiobachth with twig	1,00 c	1,00 b	1,00 b	1,00 a
14	b4a1	Biol Albiobachth with graft	1,00 c	1,00 b	1,00 b	1,00 a
15	b4 a2	Biol Albiobachth with seed	1,00 c	1,00 b	1,00 b	1,00 a
16	b5a0	Biol najoga plus with twig	1,00 c	1,00 b	1,00 b	1,00 a
17	b5 a1	Biol najoga plus with graft	1,00 c	1,00 b	1,00 b	1,00 a
18	b5a2	Biol Najoga Plus with seed	1,00 c	1,00 b	1,33 a b	1,00 a
19	b6a0	Biol bioru mineral with twig	1,00 c	1,00 b	1,00 b	1,00 a
20	b6a1	Biol bioru of minerals with grafting	1,33 b c	1,00 b	1,00 b	1,00 a
21	b6a2	Biol bioru minerals with seed	1,67 a b	1,00 b	2,00 a	1,00 a
Average			1,17	1,14	1,13	1,10
C.V (%)			21,89	19,09	23,27	23,95

Number of diseased ears

Table 4 shows the evolution of the number of diseased ears at the four evaluation times (30, 60, 90, and 120 days). The analysis of variance indicated statistically significant differences between treatments on day 120 ($p \leq 0,05$). The overall average value in this last evaluation was 0,43, lower than the initial average recorded at 30 days (0,79). The treatment that recorded the highest value on day 120 was T10 (Albio-Potassium biofertilizer in plants propagated by cuttings), with an average of 1,33 affected ears. In contrast, most treatments recorded values of 1,00 or less during this same period. The coefficient of variation was 79,61 %, indicating high dispersion among treatments. The general trend shows a progressive reduction in the number of affected ears in most treatments as the experimental cycle progressed.

Table 4. Health variable number of diseased ears (NDE) on days 30, 60, 90, and 120 after application of the bioles

Number of diseased ears (NDE)			Days			
No.	Code	Description of treatments	30	60	90	120
1	b0 a0	Artisanal control with twig	1,00 a b	1,33 a	1,00 b	0,67 a b
2	b0 to1	Artisanal control with graft	1,00 a b	1,00 a b	1,00 b	0,33 a b
3	b0 to 2	Artisanal control with seed	1,00 a b	1,00 a b	1,00 b	0,00 b
4	b1 to 0	Biol albio root with twig	1,00 a b	1,00 to b	1,00 b	0,00 b
5	b1 a1	Biol albio root with graft	1,00 a b	0,33 b	0,67 b	0,00 b
6	b1 a2	Biol albio root with seed	1,00 a b	0,00 c	0,67 b	0,00 b
7	b2a0	Biol Alico- cal Sc with twig	1,00 a b	0,00 c	1,00 b	0,33 a
8	b2 to a1	Biol Alico- cal Sc with graft	0,67 a b	0,00 c	1,00 b	0,33 a b
9	b2 a2	Biol Alico- cal Sc with seed	0,67 a b	0,00 c	1,00 b	1,00 a b
10	b3 a0	Biol Albio- potassium with twig	0,67 a b	0,00 c	0,00 c	1,33 a
11	b3a1	Biol Albio - potassium with graft	0,00 b	0,00 c	0,00 c	1,00 a b
12	b3 a2	Biol Albio - potassium with seed	1,00 b	0,00 c	0,00 c	1,00 a b
13	b4 a0	Biol Albiobacth with twig	1,00 a b	0,33 b	0,00 c	0,00 b
14	b4a1	Biol Albiobacth with graft	1,00 a b	1,00 a b	0,00 c	0,00 b
15	b4 to 2	Biol Albiobacth with seed	0,67 a b	0,00 c	0,00 c	0,00 b
16	b5a0	Biol najoga plus with twig	0,67 a b	0,00 c	0,00 c	1,00 a b
17	b5 a1	Biol najoga plus with graft	0,67 a b	0,00 c	0,00 c	1,00 a b
18	b5a2	Biol Najoga Plus with seed	1,67 a	0,00 c	0,00 c	0,33 a b
19	b6a0	Biol bioru mineral fertilizer with twigs	0,67 a b	0,00 c	0,00 c	1,00 a b
20	b6a1	Biol bioru minerals with grafting	0,33 b	0,00 c	2,00 a	0,00 b
21	b6a2	Biol bioru minerals with seed	0,00 b	0,00 c	1,000 b	0,00 b
Average			0,79	0,29	0,54	0,43
C.V %)			64,16	78,47	33,01	79,61

Production variable

Number of healthy ears

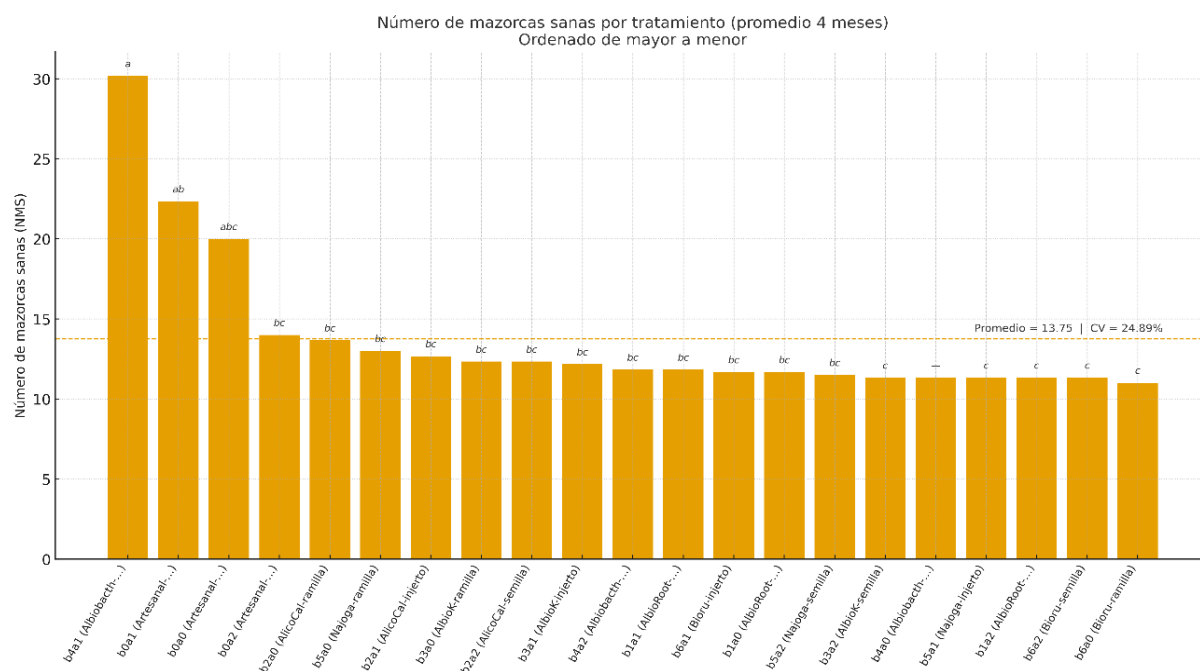
**Figure 9.** Production variable number of healthy ears (NMS), average obtained during the four months of the study

Figure 9 shows the averages obtained for the number of healthy ears per treatment during the four months of evaluation. The analysis of variance showed significant differences between treatments ($p \leq 0,05$), with an overall average of 13,75 fruits per plant and a coefficient of variation of 24,89 %.

The highest value corresponded to treatment T14 (Albiobacth applied to grafted plants), with an average of 30,17 healthy fruits. The second highest value was recorded in T2 (artisan biol with grafting), with 22,33 fruits per plant.

The lowest values were observed in T19 (Bioru mineral biol with twig), with 11,00 fruits, while treatments T6, T12, T13, T17, and T21 recorded values of 11,33 fruits.

Yield (kg/ha/year)

Table 5 shows the estimated yield values based on the fresh weight of healthy ears. The analysis of variance showed significant differences between treatments ($p \leq 0,05$), with an overall average of 1173,00 kg/ha/year and a coefficient of variation of 56,12 %.

The highest value was recorded in treatment T14 (Albiobacth applied to grafted plants), with 2790,67 kg/ha/year. The lowest value corresponded to treatment T21 (Bioru applied to plants from seed), with 580,67 kg/ha/year.

Other treatments with yields above the overall average were T2 (1867,67 kg/ha/year), T13 (1768,67 kg/ha/year), and T7 (1713,00 kg/ha/year). Most of the remaining treatments ranged between 800 and 1300 kg/ha/year.

No.	Code	Description of treatments	Yield (kg/ha/year)	
1	b0 a0	Artisanal Biol control with twigs	1168,33	b
2	b0 a1	Handcrafted witness biol with graft	1867,67	a b
3	b0 a2	Artisanal Biol control with seed	968,33	b
4	b1 a0	Biol albion root with twig	878,67	b
5	b1 a1	Biol Albion root with graft	1056,00	b
6	b1 a2	Biol albion root with seedling	828,00	b
7	b2a0	Biol Allico- cal Sc with twig	1713,00	a b
8	b2a1	Biol Allico- cal Sc with graft	1328,00	b
9	b2a2	Biol Allico- cal Sc with seed	1523,00	b
10	b3a0	Biol Albion- potassium with twig	964,67	b
11	b3a1	Biol Albion - potassium with grafting	984,33	b
12	b3a2	Biol Albion - potassium with seed	947,00	b
13	b4 a0	Biol Albiobacth with twig	1768,67	a b
14	b4a1	Biol Albiobacth with graft	2790,67	a
15	b4a2	Biol Albiobacth with seed	982,33	b
16	b5a0	Biol Najoga Plus with twig	1199,00	b
17	b5 a1	Biol najoga plus with graft	676,00	b
18	b5a2	Biol najoga plus with seed	805,67	b
19	b6a0	Biol bioru mineral with twig	875,67	b
20	b6a1	Biol bioru minerals with graft	728,67	b
21	b6a2	Biol bioru minerals with seed	580,67	b
Average			1173,00	
C.V (%)			56,12	

Analysis of essential nutrients and heavy metals in the soil

Soil nutrients before and after the application of 8 bioles

Table 6 shows the results of the soil analysis carried out before the start of the trial and at the end of the 120 days of bioles application. The pH value increased slightly from 5,5 to 5,6.

Among the primary macronutrients, phosphorus went from 21 ppm to 38 ppm, while potassium increased from 0,36 to 0,61 meq/100 mL. Ammoniacal nitrogen remained at similar values (29 ppm at the beginning and 28 ppm at the end).

Among the secondary nutrients, calcium increased from 6 to 10 meq/100 mL, magnesium went from 0,7 to 3,5 meq/100 mL, and sulfur rose from 12 to 17 ppm.

Regarding micronutrients, zinc increased from 3,5 to 5,8 ppm, copper from 6,5 to 13,4 ppm, iron from 279 to 571 ppm, and manganese from 4,8 to 11,4 ppm. Boron was the only element to decrease, from 0,41 to 0,18 ppm.

Table 6. Soil analysis of essential macro- and micronutrients at the beginning and end of applications of ioles in a cocoa orchard on the experimental farm "La Represa"

Soil analysis	pH	ppm		meq/100ml				ppm				
		NH4	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	B
Initial	5,5 Ac	29 M	21 A	0,36 M	6 M	0,7 B	12 M	3,5M	6,5 A	279 A	4,8 B	0,41 B
Final	5,6MeAc	28 M	38 A	0,61 M	10A	3,5A	17 M	5,8 M	13,4 A	571 A	11,4 M	0,18 B

Analysis of organic matter and base interaction before and after the application of bioles

Table 7 shows the results for organic matter content and base ratios before and after bioles application. The percentage of organic matter decreased from 4,4 % to 3,1 %, remaining within the medium range of interpretation.

In terms of base exchange, increases were recorded in the values of Ca/Mg, Mg/K, and the Ca+Mg/K ratio. The sum of bases went from 7,06 meq/100 mL in the initial measurement to 14,11 meq/100 mL at the end of the trial.

Table 7. Results of the analysis of organic matter and base exchange at the beginning and end of the applications in a cocoa orchard on the "La Represa" experimental farm

Soil analysis	OM%	Ca/Mg	Mg/K	Ca+Mg/K	meq/100ml/Σ Bases
Initial	4,4 M	8,5	1,94	18,61	7,06
Final	3,1 M	2,8	5,74	22,13	14,11

Analysis of heavy metals cadmium and lead.

The analysis of heavy metals in the soil, shown in Table 8, showed that the plots without bioles application had higher concentrations than those that received the treatment. In particular, cadmium (Cd) showed a reduction of 23,08 %, from 0,16 mg/kg in the soil without application to 0,13 mg/kg with application. More markedly, lead (Pb) showed a decrease of 98,02 %, falling from 20,00 mg/kg to 10,10 mg/kg after the incorporation of bioles.

Table 8. Results of heavy metal analysis at the beginning and end of applications in a cocoa orchard on the "La Represa" experimental farm

Soil analysis	Cadmium mg/kg	Lead mg/kg
No application	0,16	20,00
With application	0,13	10,10

DISCUSSION

The application of bioles, particularly the T14 treatment (Albiobacth with graft), showed the best performance in physiological, productive, and chemical soil variables, which is consistent with the literature on the role of rhizosphere microorganisms in stimulating growth and productivity in cocoa. Akimien⁽¹³⁾ points out that microbial consortia in liquid biofertilizers are involved in nutrient mineralization, the synthesis of bioactive compounds, and disease reduction. Vásquez⁽¹⁴⁾ indicates that in cocoa cultivation, microorganisms produce amino acids, nucleic acids, sugars, and bioactive metabolites that promote growth and suppress pathogens, which is consistent with the trend observed in the present study.

The treatments with Albiobacth and seed (T15) recorded the highest values for vigor and stem diameter, which can be explained by the presence of Azospirillum, a microorganism reported by Vera⁽²⁾ as a producer of auxins and other phytohormones responsible for floral induction and vegetative growth stimulation. Muñoz⁽¹⁵⁾ describes that the floral dynamics of cocoa depend on hormonal signals and carbohydrate availability, which may be related to the responses observed in the most effective treatments. For his part, Lozano⁽¹⁶⁾ highlights that biotechnological practices, such as the use of bioregulators and microbiota, improve root anchoring, nutrient absorption, and fruit setting, effects reflected in the higher number of flowers and fruits recorded.

In terms of health variables, the reduction in the incidence of *Moniliophthora perniciosa* and the number of diseased ears in treatments with bioles is consistent with the findings of Vera⁽¹⁷⁾, who documented that

antagonistic microorganisms such as *Trichoderma* reduce infection by *M. royeri*. Similarly, Anzules⁽¹⁸⁾ observed that adequate fertilization reduces susceptibility to cherry leaf roll, which supports the lower incidence observed in most treatments in this study. Although not all health variables showed significant differences, the overall trend suggests an indirect effect of bioles on crop health.

Regarding productivity, the highest values for healthy fruits and yield were achieved in T14 (Albiobacth + graft) and T2 (artisanal biol + graft). These results are consistent with those of Rohaman⁽¹⁹⁾ in Indonesia, who observed an increase in the number of ears with biofertilizers, and with Campos⁽²⁰⁾, who documented greater phosphorus availability in soils treated with enriched biofertilizers. The yield obtained in T14 exceeded the average values reported in traditional systems, which shows that the combination of grafting with bioles can enhance metabolic efficiency and fruit filling.

In terms of soil properties, an increase in primary and secondary nutrients was observed after bioles application, in accordance with Campos⁽²⁰⁾ regarding the availability of phosphorus and micronutrients in soils treated with biofertilizers. In addition, the decrease in Cd and Pb in soils treated with bioles coincides with reports by authors who attribute this effect to microbial immobilization processes and changes in soil colloidal structure.⁽²¹⁾

It should be noted that the study has limitations. The trial was relatively short, limiting the evaluation of long-term effects. High coefficients of variation were recorded for some variables, reflecting heterogeneity in crop response. Soil microbiota was not directly quantified, so the biological mechanisms behind the observed changes are inferred from previous literature rather than from our own experimental evidence. Finally, the specific soil and climate conditions of the experimental site limit the extrapolation of the results to other production areas.

In summary, the findings show that integrating bioles into cocoa production systems, especially in combination with grafting, may represent a sustainable and efficient alternative for improving crop growth, productivity, and health, in line with previous literature. However, longer-term research is recommended, including characterization of soil microbiota and validation across different agroecological conditions, to consolidate evidence of its effectiveness.

CONCLUSIONS

The application of bioles in sexually and asexually propagated cocoa (*Theobroma cacao* L.) showed consistent and quantifiable effects on crop performance and soil quality. Treatment T14 (Albiobacth + graft) achieved the highest yield with 2790,67 kg/ha/year and 30,17 healthy fruits per plant, far exceeding the overall average of 1173,00 kg/ha/year. Similarly, treatment T15 (Albiobacth + seed) recorded the largest stem diameter (12,05 mm) and vigor (5,0 on the evaluation scale), confirming the positive action of microbial consortia on vegetative growth.

In terms of health, Albio-Potassium biol reduced the incidence of witch's broom by 62,5 % compared to the control. At the same time, the application of bioles in general decreased the number of diseased ears from 0,79 to 0,43 on average at the end of the trial, demonstrating an indirect effect on pathogen suppression.

In the soil, bioles increased phosphorus from 21 to 38 ppm, potassium from 0,36 to 0,61 meq/100 mL, and magnesium from 0,7 to 3,5 meq/100 mL, and doubled calcium. There was also a 23,08 % reduction in cadmium (from 0,16 to 0,13 mg/kg) and a 49,5 % reduction in lead (from 20,00 to 10,10 mg/kg), highlighting their potential to mitigate heavy metal levels in cocoa systems.

These results confirm that integrating bioles, especially in combination with grafts, is an effective agroecological strategy to improve cocoa productivity, health, and sustainability in Ecuador. However, it is recommended to extend the duration of the studies and evaluate the soil microbiota directly to strengthen the understanding of the biological mechanisms involved.

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