

Effect of bioproducts on the development of coffee seedlings in the nursery

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Abstract

Currently, problems are reported in the quality of coffee (*Coffea arabica* L.) seedlings obtained in nurseries, a determining factor in the achievement of future highly productive plantations. A trial was carried out in 2020 at the CCS Juan Ramón Chávez in the municipality of Remedios, province of Villa Clara, with the aim of evaluating the effect of the bioproducts Enerplant, Nitrofix and Viusid agro on the quality of coffee seedlings in the nursery. For this, a completely randomized experimental design was established with five treatments that included the three bioproducts, an absolute control and a control where the provisions of the technical standards for the crop were applied, with three repetitions. The bioproducts were applied prior to the sowing of the seeds and when the seedlings reached the third pair of leaves. The percentages of germination at 50, 60 and 70 days after sowing were determined and the height, stem diameter, number of leaf pairs, leaf area and total dry mass of the seedlings were evaluated at seven months. The results show that the treatment with the bioproducts accelerates the germination of seeds and favorably stimulates the growth of coffee seedlings in the nursery, with increases between 35 and 48% in the leaf area with respect to the control without application, the best results are achieved when applying Nitrofix. The bioproducts applied contribute to raising the quality of the seedlings that are taken to the new coffee plantations.

Keywords: *Coffea arabica* L., biostimulants, growth, production of seedlings, seedlings.

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Introduction

Coffee ranks second after oil in terms of international trade figures, generating annual revenues of more than USD 15 billion for exporting countries and providing jobs for more than 20 million people in the world; it holds a primordial place in the world beverage market and constitutes an essential element in the daily life of the different populations in most of the planet, where it is enjoyed as a complement to the activities of daily life (Canet and Soto, 2016).

Coffee belongs to the Rubiaceae family, within this the species that stands out the most is *Coffea arabica* L., which represents approximately 80% of world production and in Cuba, it is the species of greatest economic importance and is recognized as the main crop in the mountainous regions of the Macizo Guamuaya (López, 2016). Increasing the levels of coffee production is an economic necessity and its work in areas outside the mountains is today an important project for the promotion of this species and the local development of many municipalities of the country.

According to Sánchez *et al.* (2018), the fundamental premise to achieve highly productive coffee plantations is the obtaining of healthy and vigorous seedlings, which must be preceded by a correct selection of the seed. Due to the intensive work in this phase of the crop, it is necessary to investigate new alternatives that involve reducing the use of fertilizers and reducing the volumes of organic matter in the substrates, contributing to the reduction of production costs without affecting the quality of coffee seedlings (Díaz *et al.*, 2016).

Biological products can help solve this problem since their advantages reach both manufacturers and agricultural producers and consumers. In Cuba there are many biostimulants and biofertilizers that allow plants to overcome environmental stress situations and favor growth and development, as well as yield, which allows reducing the use of chemical substances (Bustamante *et al.*, 2018).

Among these products are Viusid agro[®], Enerplant[®] and Nitrofix[®], which despite having demonstrated biostimulant effect on the agronomic behavior of several crops, no conclusive research has been developed regarding their effectiveness in the growth and development of coffee seedlings in the nursery phase.

Materials and methods

The experimental work was carried out on the La Magda farm belonging to a producer associated with the CCS Juan Ramón Chávez in the Municipality of Remedios, province of Villa Clara. The soil that predominates on the farm is red Ferralitic according to Hernández *et al.* (2015). A substrate was formed with the soil characteristic of the place and totally decomposed organic matter (filter cake), in a ratio of three parts of soil to one of organic matter (3:1 volume: volume). The containers were filled according to the established technical standards and black polyethylene bags of 14 cm in diameter by 22 cm in height, with a capacity of approximately 1 kg of substrate, were used.

The bags were arranged grouped in raised beds properly identified according to the treatments and distributed according to the experimental design used. For sowing, certified coffee seeds of the Island 5-3 variety were used, two seeds were sown per bag, leaving only one seedling when they reached the butterfly stage. To evaluate the effect of bioproducts on seed germination and the growth and development of coffee seedlings, a completely randomized experimental design was established with five treatments, including an absolute control and a control where the provisions of the technical standards for the crop were applied, with three repetitions.

Each repetition had 50 plants; they constituted the experimental units. The treatments used are listed below: Treatment 1. No application of biostimulants and NPK (Control). Treatment 2. Application of NPK to the substrate (technical standards, according to MINAG, 2013). Treatment 3. Application of Enerplant (concentration 0.4 ml L⁻¹). Treatment 4. Application of Nitrofix (concentration 200 ml L⁻¹). Treatment 5. Application of Viusid agro (concentration 0.5 ml L⁻¹). Prior to sowing, the seeds were immersed for three hours in dilutions of the bioproducts in water, at the concentrations defined in the treatments, in the case of the control treatment and technical standards, the imbibition was only in water.

Two foliar applications of the bioproducts were made, when the seedlings reached the second and fourth pair of leaves, with the same concentration of the bioproducts specified in the treatments. The foliar application was carried out with a Matabi backpack of 16 L capacity. The agrotechnical activities for the production of seedlings were carried out according to the Technical Instructions for Arabica Coffee (MINAG, 2013).

The percentages of seed germination in the nursery were determined at 50, 60 and 70 days after sowing. For this, daily observations were made to all the bags sown, counting as the moment of emergence of the seedlings. Seven months after sowing, the following were evaluated: plant height, stem diameter, leaf area and total dry mass, in addition, an efficiency index for the leaf area was calculated.

In all cases, the growth variables were evaluated as follows: Plant height (cm): it was performed with a graduated ruler from the root neck of the plant to the apex. Stem diameter (mm): it was performed with a vernier graduated in millimeters at the base of the stem of the plant. Leaf area (cm²): it was estimated with the use of the method proposed by Soto (1980), from the linear dimensions of the leaf: formula: LA= length x width x 0.64. Total dry mass: after proper washing to remove all the substrate from the roots, the plants were placed in an oven for drying at a temperature of 65 °C, until constant mass was achieved.

Efficiency index (EI); (%). It was used to determine the effect of the application of biostimulants, based on the formula proposed by Siqueira and Franco (1988), cited by Sánchez (2001). In this case, it was applied to the variables leaf area, taking the plants obtained in the control without inoculation as a reference control.
$$EI = \frac{\text{Leaf area of the treatment with application} - \text{Leaf area of the control}}{\text{Leaf area of the control}} \times 100.$$

In all cases the results achieved were subjected to an analysis of variance processed in the statistical package Statgraphics 5.0, applying the Tukey test (for $p < 0.05$) as a comparative criterion between the treatments in cases where significant differences were found, with a previous transformation of the data, in the analysis of germination percentages, by the arcsine of the square root of the proportion, according to the expression $\arcsin\sqrt{\%}$.

Results and discussion

Effect of bioproducts on germination percentages

The results of the evaluation show that, in the germination of coffee seeds in the first two samplings (50 and 60 DAS), the bioproducts had a significant effect, with statistical differences in relation to the control treatment without application and in the one where the technical standards were applied, the latter does not differ from the treatment with Enerplant at 50 DAS. In the last sampling (70 DAS), no statistical differences were found between all treatments, but the tendency to improve this indicator continues in those where biological products were applied.

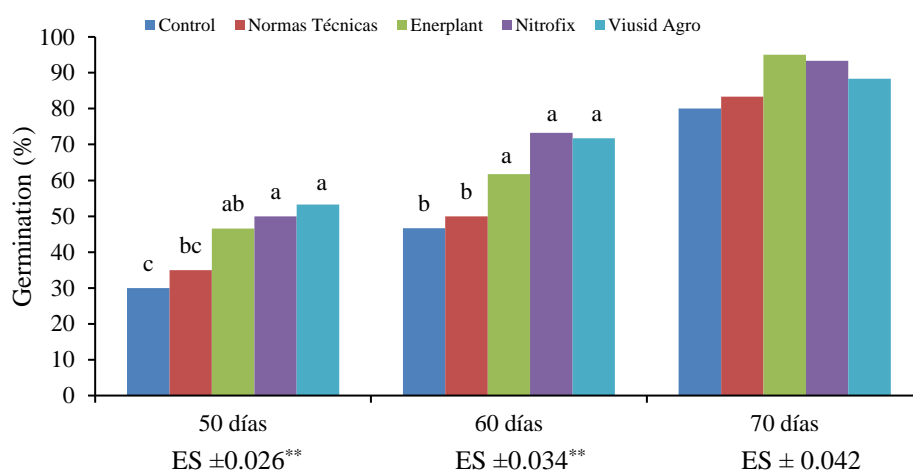


Figure 1. Percentage of germination of coffee seeds.

The results indicate that the application of bioproducts through the imbibition of coffee seeds, prior to sowing, accelerated the germination process, counted from the emergence of small seedlings. These results demonstrate that seeds treated with biostimulants can achieve a higher germination speed and percentage, which may be related to the fact that this product contains levels of tryptophan and other amino acids (Santana *et al.*, 2016).

Seed germination is a key process for reproduction (World Coffee Research, 2021). Coffee seeds germinate slowly, the first manifestation of this process is the presence of the radicle, which appears between 25 and 30 days of sowing, the emergence of seedlings can occur in a period from 50 to 90 days (Cortés *et al.*, 2010) and sometimes it can be advanced when making applications of growth-stimulating substances (Díaz *et al.*, 2016) or delayed if environmental conditions are not conducive, mainly linked to low temperatures (Cortés *et al.*, 2010).

Several studies have been developed related to the use of biological products aimed at improving the germination rates of coffee seeds in nurseries. Díaz *et al.* (2016) reported a favorable effect of the biostimulant FitoMas E on the germination of seeds of this crop. These authors argue that the biostimulant used in the study stimulates and invigorates virtually any type of crop from germination to fruiting, increases and accelerates the germination of seeds, whether botanical or agamic, and stimulates the development of roots, stems and leaves.

González *et al.* (2015) managed to stimulate the germination of coffee (*Coffea arabica* L. cultivar ‘Caturra rojo’) seeds with the application of 200 ml L⁻¹ of Bioenraiz[®], and Ferrás *et al.* (2020) concluded that the viability and mean germination time of coffee seeds tended to improve when they were soaked in the bioproduct, based on efficient native microorganisms, Ihplus[®] at 6%. Both authors mention that these bioproducts improve the vitality and viability of the seeds due to their hormonal effect similar to that of gibberellic acid, hence the influence that could exist in the decrease of the average time of germination of coffee seeds.

There is no reference to the effect of bioproducts used in the research on the germination of coffee seeds; nevertheless, there are several reports of their influence on the germination of seeds of other crops and rooting of those that propagate by the agamic route (Biotec International, 2000; Catalysis, 2014; MINAG, 2020).

Gómez *et al.* (2019) report that the inoculation of Nitrofix[®] causes a stimulating response on the root growth of sugarcane varieties evaluated in their research paper, while Peña *et al.* (2015) determined the effect of Viusid agro on the germination of beans (*P. vulgaris*) and growth of seedlings under *in vitro* conditions. They concluded that the immersion of the bean seed for three hours in a solution of Viusid agro at 0.02% favors germination speed and seedling development.

The bioproducts used in the research showed positive effects on the growth and development of coffee seedlings in the nursery (Table 1). In general, in all the variables evaluated, the seedlings treated with the bioproducts exceeded those of the control treatment and showed a behavior also superior to those of the treatment where the technical standards were applied, although without statistical differences in the variable stem diameter.

Table 1. Influence of treatments on the growth of coffee seedlings

Treatments	Height (cm)	Diameter (cm)	Pairs of leaves
Control	18.53 c	0.38 b	6.65 c
Technical standards	23.42 b	0.41 ab	6.75 bc
Enerplant	25.52 ab	0.42 ab	6.67 bc
Nitrofix	28.67 a	0.49 a	7.5 a
Viusid agro	28.14 a	0.45 ab	7.16 ab
ES ±	0.848 **	0.011*	0.086**
CV (%)	18.81	20.21	9.7

*= means with different letters in the same column differ significantly ($p \leq 0.05$).

Statistically, there were no highly significant differences between the treatments where the bioproducts were applied, except for the variable pairs of leaves, where differences were found between the treatments with application of Nitrofix and Enerplant. In general, the best results were obtained in the treatment where Nitrofix was applied, with significant differences, for all the variables under study, with the control treatment, and in the variables plant height and pair of leaves with respect to the treatment where the technical standards were applied.

These results may be due, among other elements, to the fact that the chemical composition and concentration of nutrient solutions determine the nutrition of seedlings, with contributions of nitrogen and potassium, which are the nutrients required in greater quantity in this first cycle. The results of the application of Nitrofix in the growth of coffee seedlings are in correspondence with what was stated by Gómez *et al.* (2019), who point out that *Azospirillum*, bacteria that make up the bioproduct Nitrofix, is the most studied genus of free-living rhizobacteria, not only for their ability to fix biological nitrogen, but also for their mechanism of production of indole acetic acid (IAA) auxins, which can modify the phytohormone content of plants, leading to the stimulation of their growth, in this regard it is recognized that Nitrofix can fix nitrogen from the air, stimulate plant growth and systemic plant resistance (MINAG, 2020).

On the other hand, according to Falcón *et al.* (2015), the oligosaccharides that make up the bioproduct Enerplant are organic substances capable of inducing not only the production of ethylene, but also of activating certain physiological and biochemical mechanisms, especially enzymatic and hormonal, this is because these substances regulate the events related to the absorption and translocation of nutrients, which reinforces the resistance of the plant to biotic and abiotic factors, altering their phytohormonal levels.

Peña *et al.* (2015) state that Viusid agro[®] increases its effectiveness in crops without altering their properties and therefore, invigorates and stimulates plant development and increases yields, they report that this growth promoter has been evaluated in different crops with positive results. Different studies developed from the use of bioproducts coincide in giving these products great importance as they are able to influence different physiological processes that occur in the plant and stimulate the growth and development of plants (Quintero *et al.*, 2018).

Several authors recognize the beneficial effect of biostimulants on plant physiology. Terry *et al.* (2017) point out that bioproducts exert various beneficial effects on plants, such as the induction of defensive mechanisms and the stimulation of plant growth, in addition to having the advantage of not being harmful to plants or the environment. For their part, Aguilar *et al.* (2016) state that the benefits of these products generate vigorous growth of roots, foliage, flowering and fruiting, which allows plants greater resistance against pests and diseases and their rapid recovery after harvest.

In this regard, Valverde *et al.* (2020) ensure that these biological products are capable of improving the efficiency, absorption and assimilation of nutrients, stimulate and invigorate plants from germination to fruiting, also provide greater tolerance to biotic, abiotic stress and improve some of their agronomic characteristics. This author found a significant physiological response of coffee seedlings to biostimulants because these are compounds of biogenic stimulants, metabolic enhancers, positive growth regulators and plant strengtheners.

In coffee crops, some authors have developed research related to the development of coffee seedlings in nurseries treated with bioproducts. Álvarez and Damião (2018) obtained increases in the variables plant height, number of pairs of leaves and stem diameter of coffee seedlings when treated with a biofertilizer based on efficient microorganisms in the nursery phase.

The effect of this biofertilizer on the growth and development of coffee seedlings in nurseries has been studied by several researchers (Sánchez, 2001; Hernández *et al.*, 2013; Barroso *et al.*, 2015; Sánchez *et al.*, 2018), who report a positive effect on the indicators of quality of the seedlings of this crop.

The leaf area index and the total dry mass of the coffee seedlings of the treatments with bioproducts were higher with respect to the control treatment and the technical standards (Table 2).

Table 2. Influence of treatments on the leaf area and dry mass of coffee seedlings.

Treatments	Leaf area (cm ²)	Total dry mass (g)
Control	377.82 c	2.53 c
Technical standards	488.54 b	3.93 b
Enerplant	509.66 ab	4.29 ab
Nitrofix	559.72 a	4.61 a
Viusid agro	531.19 ab	4.20 ab
ES ±	10.6**	0.125**
CV (%)	16.64	18.29

* = means with different letters in the same column differ significantly ($p \leq 0.05$).

In both variables, the treatment with Nitrofix keeps the best results, with significant differences with respect to the control treatment and technical standards and without differences in relation to the treatments with Viusid agro and Enerplant. The leaf area of coffee seedlings, according to Soto (1980), is the morphological indicator that most clearly expresses the growth of the coffee tree in the nursery stage, while dry matter is one of the most measured growth and development variables in coffee plants to analyze the effect of biofertilizers (Ibarra *et al.*, 2014).

As noted above, these results can be determined by the contribution of Nitrofix in the fixation of atmospheric nitrogen, and by its participation in various transformations in the nitrogen cycle; as well as by the ability of the genus *Azospirillum* to produce phytohormones such as 3-indole acetic acid (IAA), a substance that participates in the development of the root system and causes an increase in the intake of minerals and water, which favors the increase of the leaf area and the accumulation of dry matter in coffee seedlings (Hernández *et al.*, 2013).

Hernández *et al.* (2013) managed to reduce by 30% the nitrogen fertilization recommended for tomato under the conditions of typical red ferralitic soil, with the inoculation of Nitrofix (*Azospirillum* sp.), they report the greatest economic profit as they obtained an increase in yield with respect to the control with 100% of nitrogen fertilization. In general, the response in the production of leaf area and fresh and dry mass of plants when treated with bioproducts may be related to the changes that occur in the organization and cellular metabolism of plants grown under the influence of these substances or biologically active products (Alarcón *et al.*, 2018).

Viñals *et al.* (2017) report that, with the application of bioactive products, there is a beneficial effect on the nutrition of coffee trees in their nursery stage, reflecting their action on the growth of seedlings, with significant savings in financial resources and reduction of organic material in substrates. The efficiency indices for the indicator leaf area of coffee seedlings (Figure 2) show the increases obtained when applying the treatments with bioproducts and technical standards with respect to the control treatment. The values reached represent between 29 and 48% increase in these treatments when compared with the control.

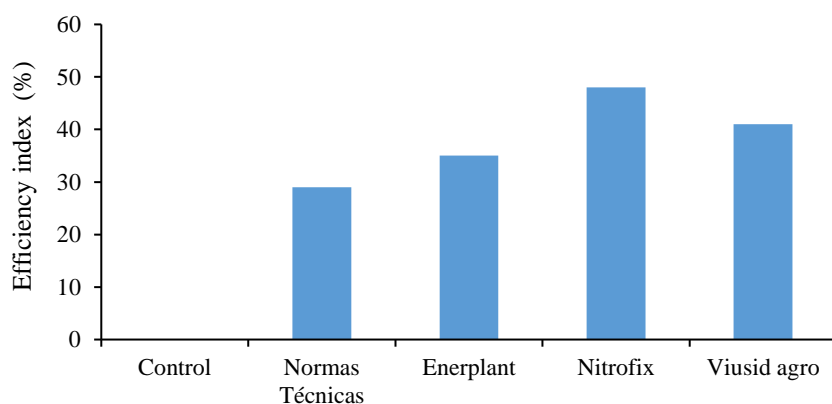


Figure 2. Efficiency indices for the indicator leaf area, of the coffee seedlings of the treatments, with respect to the control.

Specifically, increases of 35, 41 and 48% of leaf area of seedlings treated with Enerplant, Viusid agro and Nitrofix, respectively, were achieved with respect to the control treatment, which demonstrates the positive effect of these bioproducts on this indicator. The largest increase achieved coincides with the treatment where the bioproduct Nitrofix was applied, which is in correspondence with the results shown above, corroborating its positive effect on the growth of coffee seedlings in nurseries.

In this regard, Barroso *et al.* (2015) reported increases in leaf area and total dry mass of 11.03% up to 34.82% and 4.55% up to 12.5%, respectively, of coffee plants treated with FitoMas E with respect to the control without application, they also found increases of 68% when applying the combination of biological alternatives (Mycorrhizae and FitoMas E) together with 75% of mineral fertilization, with respect to the absolute control and 12.59% with respect to the control with 100% of mineral fertilization.

Díaz *et al.* (2021), when applying increasing doses of FitoMas E, in two proportions of organic matter in substrates for the production of coffee seedlings in the nursery, obtained increases between 17.4 and 70.6% with respect to the treatments without application, when evaluating the efficiency index for the indicator leaf area, seven months after sowing the coffee seeds. These authors state that the increase in leaf area in the treatments where bioproducts are applied is the physiological response of the coffee tree when it grows in an environment where there is a greater supply of nutritional elements. For their part, Canseco *et al.* (2020) report positive effects with the incorporation of organic fertilizers and biofertilizers on *Coffea arabica* L., as they generated greater growth and vigor to plants, on the other hand, they improve the structure of the soil by providing nutrients necessary for said crop.

Conclusions

The application of the bioproducts Enerplant, Nitofix and Viusid agro, prior to sowing, accelerates the germination of coffee seeds in nurseries, favorably stimulates the growth of coffee seedlings in the nursery, with the best results in the growth variables evaluated when applying Nitrofix. It was demonstrated that the application of the bioproducts Enerplant, Nitrofix and Viusid agro in coffee nurseries generate increases between 35 and 48% in the leaf area of the treated coffee seedlings, with respect to the control without application.

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