



Effect of the Application Timing of VIUSID Agro[®] on the Growth of *Coffea arabica* L. Seedlings

Bustamante-González, Carlos A.^{1*} ; Vázquez-Osorio, Yudmila¹; Álvarez-Morales, Roxana¹ 

¹ Instituto de Investigaciones Agro-Forestales. Unidad de Ciencia y Tecnología de Base Tercer Frente. Cruce de los Baños, Tercer Frente, Santiago de Cuba. Cuba. C.P. 92700.

* Correspondence: marlonalejandros2012@gmail.com; sanvegetal3@tercerfrente.inaf.co.cu

ABSTRACT

Objective: VIUSID Agro[®] is a biostimulant that contains amino acids, vitamins and minerals. In Cuba, its beneficial effects have been primarily demonstrated in vegetables, sugarcane and tobacco. However, there is limited information regarding its use in coffee. Therefore, this study was conducted to evaluate the effect of application timing on the growth of coffee seedlings.

Design/methodology/approach: The experiments were conducted at nursery of the Agro-Forest Research Institute in Tercer Frente, from December 2019 to July 2020 and from October 2020 to June 2021, under saran mesh shade. Using a completely randomized design, four treatments were evaluated: without VIUSID Agro[®] (Control); monthly applications from the second to the fifth leaf pairs; application on the second, fourth and sixth leaf pairs; and applications on the third and fifth leaf pairs. Height, stem diameter, dry mass, leaf area, seedlings quality index, as well as agronomic efficiency were assessed. Data were analyzed using a one-way analysis of variance, and Tukey test was applied to compare the means.

Results: The coffee seedlings responded positively and significantly to the biostimulant. The applications enhanced the morphological variables and improved the application efficiency.

Findings/conclusions: Foliar applications of VIUSID Agro[®] on the third and fifth pair of leaves were the most efficient, reducing the production costs of coffee plants.

Keywords: biostimulant, coffee seedlings, growth, efficiency, promoter.

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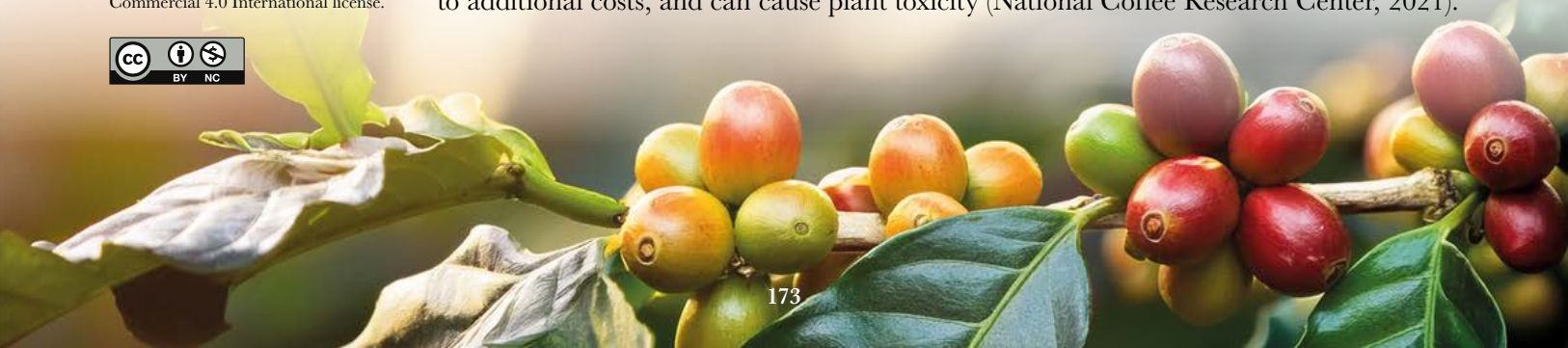
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INTRODUCTION

It is well known that splitting fertilizer applications in crops is a strategy to increase their efficiency (Mattson and van Iersel, 2011). Fertilizers can be applied either through the soil or via foliar application. The effectiveness of foliar application depends on several factors, including the fertilizer source, the application method, the timing (Salamanca and González-Osorio, 2020), and the nutrients applied (Fageria *et al.*, 2009).

In Colombia, it is recommended to apply phosphate fertilizer to the soil at the time of transplanting seedlings, with additional applications one month and three months after transplanting (Sadeghian and Ospina, 2021). On the other hand, it has been suggested that foliar applications do not affect the dry matter of seedlings (Sadeghian, 2022), may lead to additional costs, and can cause plant toxicity (National Coffee Research Center, 2021).



Furthermore, foliar fertilization applied at different doses, timings, and fertilizer sources did not significantly improve coffee cherry production or the physical quality of the beans (Salamanca and González-Osorio, 2020).

The effect of biostimulants and biofertilizers has been positive in coffee seedlings (Silva *et al.*, 2020; Canseco Martínez *et al.*, 2020; Ferrás *et al.*, 2021) as well as in coffee crops under production (Chacón-Villalobos *et al.*, 2021), with yield increases of up to 74% following two or three foliar applications. The application of *Azolla filiculoides* as a biofertilizer every 15 days for five months improved the physico-chemical properties of the soil, as well as coffee bean production and quality in Ecuador (Vásquez and Espinosa-Palacios, 2023). In India, soil and foliar applications of humic acid increased coffee yield, quality, and profitability (Kishor *et al.*, 2020).

In Cuba, VIUSID Agro[®] is registered as one of the formulations used as a plant growth stimulant. In *Coffea arabica* under production, doses of 2.0 and 4.0 mL of VIUSID Agro[®] dissolved in 5 L of water increased the number of fruits per plant, the equatorial and longitudinal diameters of the fruits, yield per plant, and yield per hectare of coffee (Maldonado, 2016). However, when applied to coffee in Ecuador, it was concluded that higher doses and application frequencies must be explored to achieve conclusive results (Cargua Chávez *et al.*, 2022). Recent information on the use of VIUSID Agro[®] in producing coffee seedlings is available (Bustamante *et al.*, 2023); however, alternatives must be sought to improve its efficiency. Therefore, this study was conducted to evaluate the effect of application timing on the growth of coffee seedlings.

MATERIALS AND METHODS

Study Site

The experiment was conducted at the nursery of the Agroforestry Research Institute (UCTB Tercer Frente) (20° 08' 11.06" N, 76° 16' 22.27" W), located in the Consejo Popular Cruce de Los Baños, Tercer Frente municipality, during two periods: the first from December 2019 to July 2020 and the second from October 2020 to June 2021.

During the experimental period, the average temperature for both years was approximately 24 °C. The total rainfall accumulation was 626 mm in 2020 and 1221 mm in 2021.

Treatments

The following treatments were studied:

- No application (control).
- Monthly application from the second to the fifth leaf pair.
- Application on the second, fourth, and sixth leaf pairs.
- Application on the third and fifth leaf pairs.

Each treatment consisted of 28 plants, of which 10 were evaluated per treatment. Black polypropylene bags with dimensions of 12.5 cm in width and 25 cm in length were used.

Two seeds of *Coffea arabica* L., variety “Isla 6-14”, were planted in each polyethylene bag, which were filled with a substrate of Pardo soil / filter cake in a 3:1 (volume/volume) ratio.

The shading used was a saran mesh, which provided 60% shade to the plants.

Application of VIUSID Agro[®]

VIUSID Agro[®] was applied at a concentration of 0.6 mL L⁻¹ using a 16-liter Matabi backpack sprayer, during the early morning hours, when the dew had already evaporated, but there was still no strong sunlight.

Recorded Variables

Plant height (cm): Measured from the base of the stem to the apical meristem using a graduated ruler.

Stem diameter (mm): Measured with a Dijite digital caliper, 1 cm above the base of the stem.

Leaf area (cm²): Estimated by measuring the linear dimensions of the leaves (Soto, 1980) using the formula: $AF = \text{length} \times \text{width} \times 0.64$

Dry mass (g): The plants were separated by organs (leaves, stems, and roots), washed with water, blotted with paper, and then dried in a forced-air oven at 70 °C until a constant weight was reached.

Quality index based on the dry mass values, height, and stem diameter.

Efficiency index (E.I.): This was calculated for the variables evaluated in the experiment using the following formula:

$$E.I.\% = \left(\frac{\text{Value of the variable in the treatment} - \text{Value of the control}}{\text{Value of the control}} \right) \times 100$$

Statistical Analysis

The analysis of variance was performed using a completely randomized design according to the linear model of fixed effects. The data were processed using the Statistica software for Windows. The normality of the data was tested using the Kolmogorov-Smirnov test, and the homogeneity of variance was assessed using Levene's test. Tukey's test ($p \leq 0.05$) was used to determine the differences between the treatments.

RESULTS AND DISCUSSION

The coffee seedlings responded positively in both experimental years to the application of the biostimulant, with an increase in the evaluated variables, regardless of the application timing. In 2020, the monthly application of the biostimulant significantly increased all the evaluated variables, with the maximum values for each treatment showing a 7% increase in height and total dry mass, a 23% increase in the quality index, and a 12% increase in the leaf area of the coffee seedlings compared to the control (Table 1).

Table 1. Effect of the moment of application of the biostimulant on morphological variables of coffee seedlings. 2020.

Moments	Height, cm	Stem diameter, mm	Dry mass, g	Quality index	Leaf area, cm ²
Control	19.45 b	2.92 ab	3.30 b	0.30 b	383.06 b
Monthly	20.84 a	3.21 a	3.54 a	0.37 a	431.51 a
2 nd , 4 th y 6 th pair of leaves	20.01 b	2.89 b	3.35 b	0.31 b	386.40 b
3 rd , and 6 th pair of leaves	20.93 a	3.17 ab	3.38 b	0.32 ab	409.16 ab
E. E., \bar{x}	0.323*	0.054*	0.08*	0.01*	12.46*

* Means with different letters indicate significant differences (Tukey, $p \leq 0.05$).

Among the treatments studied, the monthly applications of VIUSID Agro showed a more pronounced and significant effect ($p \leq 0.05$) on the evaluated variables (Table 1). The applications of the biostimulant when the seedlings reached the third and fifth pairs of leaves resulted in an effect statistically similar to that of the monthly applications for height, stem diameter, quality index, and leaf area. In contrast, the applications on the second, fourth, and sixth pairs of leaves showed statistically similar values to the control, except for the height of the seedlings.

In 2021, the highest significant values for height, leaf dry mass, stem dry mass, and total dry mass were found when applying VIUSID Agro[®] to the third and fifth pairs of leaves, which were statistically different from the other treatments (Table 2). This situation represented an increase of 25% for height, 7% for stem diameter, 48% for dry mass, 11% for quality index, and 30% for leaf area.

The application of VIUSID Agro[®] to the second, fourth, and sixth pairs of leaves had a similar effect on stem diameter as the application to the third and fifth pairs of leaves, but both values were statistically higher than the treatment without biostimulant application.

VIUSID Agro[®] increased the quality index of coffee seedlings (Table 2). Statistically similar increases, all higher than the control, were achieved with monthly applications (23%), every two months (19%), and with applications to the third and fifth pairs of leaves (11%).

Table 2. Effect of the moment of application of the biostimulant on morphological variables of coffee seedlings. 2021.

Moments	Height, cm	Stem diameter, mm	Dry mass, g	Quality index	Leaf area, cm ²
Control	21.07 c	3.37 b	2.75 d	0.26 c	402.74 c
Monthly	24.18 b	3.49 b	3.87 b	0.32 a	464.11 b
2 nd , 4 th y 6 th pair of leaves	24.47 b	3.58 a	3.59 c	0.31 ab	504.92 a
3 rd , and 6 th pair of leaves	26.41 a	3.63 a	4.09 a	0.29 b	523.33 a
E. E., \bar{x}	0.33*	0.07*	0.068*	0.007*	7.72*

*Means with different letters indicate significant differences (Tukey, $p \leq 0.05$).

The monthly application of the bioproduct increased the leaf area by 15% compared to the control (Table 2), while applications to the second, fourth, and sixth pairs of leaves resulted in a 25% increase, a value lower than the 30% increase observed when the VIUSID was applied to the third and fifth pairs of leaves. These leaf area increases were lower than those achieved by applying *Azotobacter* monthly after the seedlings reached the third pair of leaves (49%) (Pérez *et al.*, 2002) and those found when applying FitoMas E (between 9% and 56%) after a second application 150 days after seed sowing (Díaz-Medina *et al.*, 2016).

VIUSID Agro[®] is one of the formulations used as a plant growth stimulant (Pérez *et al.*, 2020) and acts as a biostimulant due to its composition of amino acids, vitamins, and minerals (Catalysis, 2016). The amino acids catalyze the synthesis of sugars, starch, and other components of leaves, flowers, and fruits. They contribute to the increase in leaf chlorophyll, thus intensifying the performance of photosynthesis (Maldonado, 2016).

It has been reported that the greatest increase in coffee seedling growth rate and net assimilation rate when applying biofertilizers occurred between 60 and 90 days in the nursery phase. This is related to the fact that during this period, the roots increase their growth and explore a larger volume of soil, thus obtaining greater nutritional resources that promote seedling growth (Cargua-Chávez *et al.*, 2022).

The variation in the response of the seedlings to the timing of bioestimulant application between years could reflect an effect caused by climatic conditions and the duration of the experiment. It was established that this different management of the seedlings resulted in a differentiated response, and that perhaps the key factor to consider when selecting a variant could be the economic analysis.

The efficiency index (E.I.) exhibited different behavior depending on the evaluated variable, the treatment studied, and the year of experimentation. It is known that the ideal balance for the growth of different plant organs is variable, with a certain endogenous concentration potentially promoting the growth of one organ while inhibiting the growth of another (Taiz and Zeiger, 2010). In 2020 (Figure 1), the monthly application was characterized by the highest increase in all evaluated indicators except for height. The application of the bioestimulant at the second, fourth, and sixth leaf pairs showed the lowest efficiency values, while the application at the third and fifth leaf pairs only had a slight superiority in seedling height.

In 2021, the treatment that received the bioproduct at the third and fifth leaf pairs showed the highest I.E. values for all indicators except for the quality index (Figure 2). The treatment with monthly applications was characterized by the lowest efficiency values for height, stem diameter, and leaf area. This situation could be related to the higher level of rainfall during this experimental period, which was double that of the previous experiment, potentially causing the washout of the product from the leaf surface. At the same time, it is known that applying bioproducts at doses higher than the plants' requirements can have a depressive effect on morphological variables. Bustamante *et al.* (2022) found this situation when studying Enerplant concentrations in cacao seedlings.

This difference in the efficiency index response of the coffee plants could also be related to the higher values of leaf area in the seedlings in 2021. By increasing the leaf

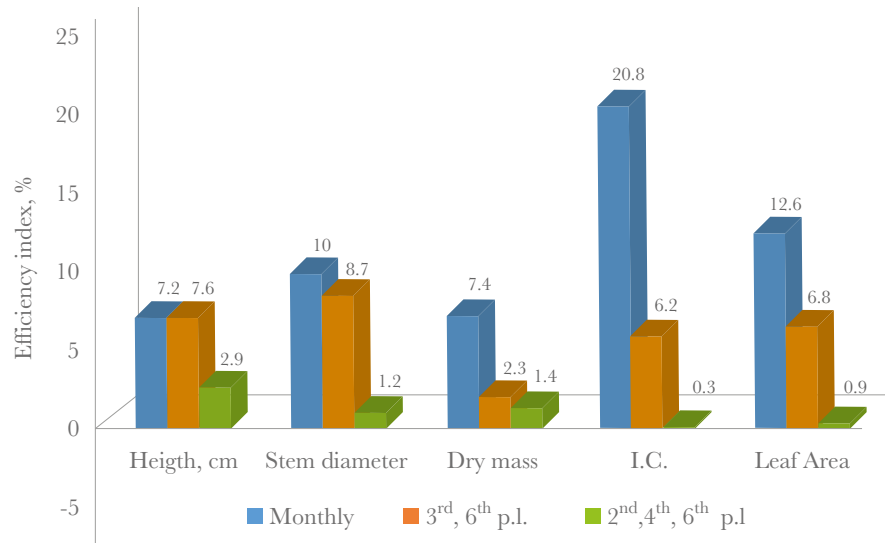


Figure 1. Efficiency index of the VIUSID application timings. 2020. IC: quality index.

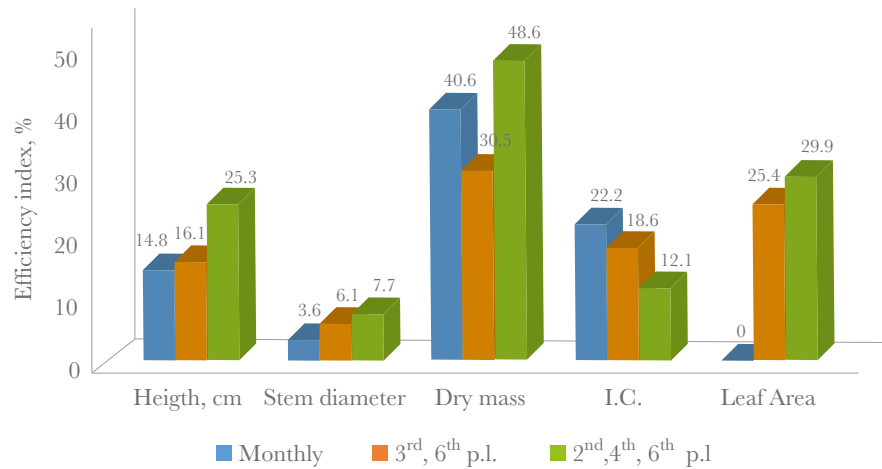


Figure 2. Efficiency Index of the timing of VIUSID application. 2021. IC: quality index.

area, conditions are created that enhance nutrient absorption from the solution (Fageria *et al.*, 2009).

When the product is applied at the third and fifth leaf pairs, production costs are reduced by 67% compared to its monthly application. This economic assessment aligns with the higher efficiency achieved with this timing of application, which supports its recommendation for use under the country’s productive conditions.

CONCLUSIONS

The application of VIUSID Agro[®] at the third and fifth leaf pairs resulted in the highest morpho-agronomic values for coffee seedlings on average over the two years, increased the product’s efficiency, and reduced production costs by 67% compared to its monthly application.

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