Colegio de Postgraduados

Effects of different VIUSID Agro® concentrations on the growth of *Coffea arabica* L. seedlings

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ABSTRACT
Objective: The VIUSID Agro® biostimulant contains amino acids, vitamins, and minerals. It was subjected to a biocatalytic process of molecular activation to improve its biological activity. In Cuba, its benefits have been demonstrated mainly in vegetables, sugar cane, and tobacco.
Design/Methodology/Approach: The effect of the product on the morpho-agronomic traits of coffee seedlings was evaluated through experiments carried out in the nursery of the Instituto de Investigaciones Agro-Forestales of Tercer Frente, from December 2019 to July 2020 and from October 2020 to June 2021, using a saran shade cloth. In a completely randomized design, 6 concentrations of VIUSID Agro® (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL L^{-1} of water) were applied monthly on the second to the fifth pair of leaves. Seedling height, stem diameter, dry mass, leaf area, quality index, and efficiency were evaluated. Data were processed using a simple classification analysis of variance. The means were compared with Duncan's test. The concentrations and the leaf area were adjusted to several functions and the one with the highest coefficient of determination was selected.
Results: Coffee seedlings had a positive response to the biostimulant. The monthly foliar applications of VIUSID Agro® between the second and the fifth pair of leaves increased the morpho agronomic indicators of the seedlings.
Findings/Conclusions: VIUSID Agro® in concentrations ranging between 0.64 and 0.71mL L^{-1} of water caused significant increases in the leaf area of the seedlings.

Keywords: biostimulant, coffee tree, dose, growth promoter, nursery.

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INTRODUCTION

To increase crop production, several industries have developed nutritional complexes that contain micronutrients, amino acids, and plant extracts. These complexes have been called growth promoters or biostimulants [1].

In Cuba, the positive effect of biofertilizers and biostimulants on carrot (*Daucus carota* L.) [2], bean (*Phaseolus vulgaris* L.) [3], tomato (*Solanum lycopersicum* L.) [4], and soybean (*Glycine max* L.) [5] has been demonstrated. When the effect of FitoMas E on passion fruit

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(*Passiflora edullis* L.) was studied [6], the doses were found to have a similar effect on the variables analyzed and it was considered as a sign that an adequate concentration of the biostimulant promotes positive changes in the metabolism and physiology of plants and uniformly improves their agronomic traits.

When studying the effect of increasing doses of Enerplant in lettuce (*Lactuca sativa* L.), low concentrations of this product were found to induce organ growth, although not their number [7].

Likewise, when the effect of 6 doses of a biostimulant derived from algae in wine grape (*Vitis vinifera* L.) were analyzed, the best results were obtained with the 0.75 mL dose; however, the use of higher doses reduced the evaluated variables [8]. In the case of passion fruit, increasing doses of the Stimulate biostimulant on the leaf area had no effect [9].

Cuban researchers have studied the use of bioproducts in coffee (*Coffea arabica* L.) [10]. Increases in seedling height were reported in a range of 44 to 73% as a result of the application of a 250 mL L^{-1} m^2 concentration of FitoMas E [11], while its application in 0.2% concentrations led to 37% increases of this indicator with respect to the control [12].

VIUSID Agro® is also registered as one of the formulations used as a plant growth stimulant in Cuba. This product essentially contains amino acids, vitamins, and minerals and was subjected to a biocatalytic process of molecular activation to improve its biological activity and the biochemical reactivity of all its molecules. This process favored the vegetative and reproductive stage of the crops and increased the stem length and the number of leaves, flowers, and fruits, which had a positive influence on yields [1].

In Cuba, the positive effect of the foliar application of VIUSID Agro® has been demonstrated in *Solanum lycopersicum* L. [1], beans, radish (*Raphanus sativus* L.), *Beta vulgaris* L., lettuce [13], *Colocasia esculenta* Schott [14], *Nicotiana tabacum* L. [15], and *Saccharum* spp. [16]. In Mexico, productive increases were achieved in winter squash (*Cucurbita argyrosperma* Huber) [17]. Meanwhile, 2.0 and 4.0 mL doses of VIUSID Agro® dissolved in 5 L of water promoted highly significant results: more fruits per coffee plant, greater equatorial and longitudinal diameter of fruits, and higher yield per plant and per hectare of coffee than the control treatment [18].

The production of coffee seedlings is a vital task for the development of coffee growing in Cuba. Therefore, techniques that produce very highquality plants are required to ensure the durability of the plantations.

No information is available regarding the use of VIUSID Agro® in the production of coffee seedlings. Consequently, this research was carried out to study the effect of different biostimulant concentrations in the morpho-agronomic traits of coffee.

MATERIALS AND METHODS

The experiments were carried out in the nursery of the Instituto de Investigaciones Agro-Forestales, Unidad de Ciencia y Tecnología de Base - Tercer Frente (20° 08' 11.06" N y 76° 16' 22.27" W) located in the Consejo Popular Cruce de Los Baños, Tercer Frente municipality. The experiments took place in two periods: the first from December 2019 to July 2020 and the second from October 2020 to June 2021.

The experiment aimed to study the effect of 6 concentrations of VIUSID Agro® on the growth of *Coffea arabica* L. seedlings. In a completely randomized design, the following concentrations were studied: Without VIUSID Agro® (0 ml L^{-1}) - control; 0.2 ml L^{-1} of VIUSID Agro®; 0.4 ml L^{-1} of VIUSID Agro®; 0.6 ml L^{-1} of VIUSID Agro® 0.8 ml L^{-1} of VIUSID Agro®; 1.0 ml L^{-1} of VIUSID Agro®.

During the experimental period, VIUSID Agro® was applied monthly from the second to the fifth pair of leaves, for a total of 4 applications. The product was applied with a 16 L Matabi manual backpack sprayer, in the early hours of the morning. The control treatment was sprayed with water. To attenuate the sun effect, a saran shade cloth was used to regulate the shade to 60%.

Two "Isla 6-14" variety coffee seeds were sown in 2.5 cm × 25 cm polyethylene bags, which were filled with a 3:1 ratio of brown soil-filter cake substrate. During both years of the experimental period, the average temperature was around 24 °C, while the accumulated rainfall for 2020 and 2021 was 626 mm and 1,221 mm, respectively, mainly as a consequence of the rains in October and November (Figure 1).

Each treatment consisted of 45 seedlings arranged in 3 rows (15 seedlings per row). The following variables were evaluated in 10 plants per treatment:

- Plant height (cm), measured from the base of the stem to the apical meristem with a graduated ruler.
- Stem diameter (mm), measured with a Dijite brand digital caliper one cm above the soil.
- Leaf area (cm^2), estimated by the measurement of the linear dimensions of the leaves according to the following formula:

$$AF\left(\text{cm}^2\right) = length \times width \times 0.64 \quad [19].$$

- Aerial and root dry mass (g). Plants were divided by organs (leaves, stems, and roots) and washed with water, placed on paper, and dried in a forced-air oven at 70 °C until constant weight was achieved.

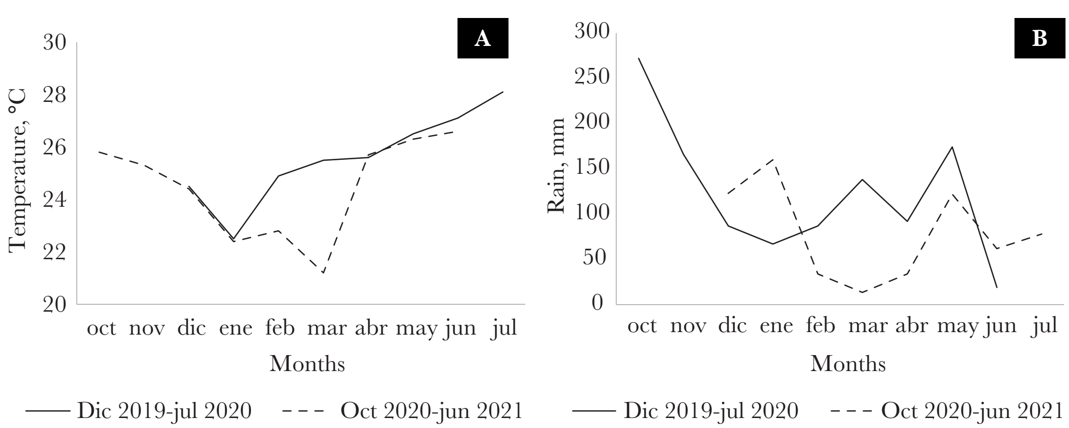

Figure 1. A: Monthly temperature and B: rainfall during the experimental period.

- Quality index [20].
- Efficiency index [21], calculated for the variables evaluated in the experiment, based on the following formula:

$$Efficiency\ index = \left(\frac{treatment\ value\ c - control\ value}{control\ value} \right) \times 100$$

The simple classification analysis of variance was carried out using a completely randomized design, according to the fixed-effects linear model. The data was processed using the Statistica software for Windows. The normality of the data and the homogeneity of the variance were verified by the Kolmogorov-Smirnov test and by the Levene test, respectively. Duncan's multiple-range test ($p \leq 0.05\%$) was used to determine the differences between the treatments.

To recommend the biostimulant concentration, the data of the relative increase in leaf area were correlated with the different concentrations and the function with the highest coefficient of determination was selected.

RESULTS AND DISCUSSION

Coffee seedling height

Plant height is a trait that is strongly modified by environmental conditions. Humidity, mineral nutrition, and light intensity are some of the factors that exercise the greatest influence on it.

VIUSID Agro® concentrations had a positive effect on height (Table 1). In 2020, this variable gradually increased until it reached a concentration of 0.4 mL L^{-1}; afterwards, a significant decrease trend was recorded. The greatest growth with respect to the control (28.9%) was found with the application of the 0.8 mL L^{-1} concentration, although it was not statistically different from the growth achieved with 0.4 mL L^{-1}.

In 2021, a positive result (like that of 2020) was found regarding the increase of the VIUSID Agro® and the coffee seedling height. A tendency towards an almost linear height growth with increasing concentrations was observed (Table 1). The action of 0.2-0.6

Table 1. Effect of VIUSID Agro® concentrations on the height and diameter stem of coffee seedlings.

Concentration (mL L^{-1})	Height (cm)		Stem diameter (mm)	
	2020	2021	2020	2021
0.0	16.30 d	21.07 d	2.82 bc	3.37 b
0.2	18.50 c	23.14 c	2.65 c	3.35 b
0.4	20.07ab	23.75 c	2.89 abc	3.39 b
0.6	19.53 b	22.87 c	2.99 ab	3.38 b
0.8	21.02 a	26.35 a	3.10 a	3.75 a
1.0	17.58 cd	25.30 b	2.73 c	3.51 b
E, E, $\bar{x}$	0.20*	0.31*	0.04	0.08*

* Means with different letters in the same column differ from each other according to Duncan's test for $p<0.05$.

concentrations was statistically similar and caused a 9.8-8.50% increase in this indicator. The greatest increase (25%) in this indicator with respect to the control was found with the 0.8 mL L^{-1} concentration of VIUSID Agro®.

As a consequence of the application of 250 mL L^{-1} m^2 of FitoMas E, a 44-73% increases in coffee seedling height have also been recorded [11].

Stem diameter

In both years, the highest absolute value of the stem diameter was reached with the application of 0.8 mL L^{-1} (Table 1) and it was significantly different from the control. The increase in this indicator was lower than the increase in height and it amounted to 10% and 12%, in 2020 and 2021, respectively.

Dry mass

Biomass is considered an important indicator of the ecological and management processes that occur in the vegetation and it reflects the conditions of the site and the soil, water, and solar radiation resources available in it [22].

The application of a 0.6-mL L^{-1} concentration of VIUSID Agro® in 2020 increased the total dry mass by 17% with respect to the control, although no significant difference was found with the action of the biostimulant in the 0.4 and 0.8 mL L^{-1} concentrations (Table 2).

The effect of the biostimulant on the dry mass of the leaves in 2020 did not show a consistent pattern (Table 2). For the stem dry mass, the concentrations increased this variable, although no differences were found between them. The application of 0.4, 0.6, and 0.8 mL L^{-1} concentrations of VIUSID Agro® significantly increased the dry mass of the root with respect to the control and the 0.2 mL concentration.

The ideal balance for the growth of the different plant organs is variable: a certain endogenous concentration can favor the growth of one organ and inhibit the growth of another [23]. This last behavior was observed in these experiments during the evaluation of the response of coffee seedlings to the biostimulant.

Table 2. Effect of VIUSID Agro® concentrations on the dry mass of coffee seedlings and their composition (2020).

Concentration (mL L^{-1})	Dry matter (g)			
	Leaf	**Stem**	**Root**	**Total**
0.0	1.75 ab	0.28 b	0.44 b	2.47 cd
0.2	1.50 b	0.36 a	0.48 b	2.34 d
0.4	1.60 ab	0.36 a	0.66 a	2.62 abc
0.6	1.84[a]	0.36 a	0.62 a	2.82 a
0.8	1.77 a	0.41 a	0.60 a	2.78 ab
1.0	1.67 ab	0.37 a	0.50 b	2.54 bcd
E, E, $\bar{x}$	0.03*	0.01*	0.01*	0.04*

* Means with different letters in the same column differ from each other according to Duncan's test for $p<0.05$.

During the evaluation of the effect of the concentrations on the seedlings dry mass in 2021 (Table 3), the application of 0.8 mL L^{-1} of VIUSID Agro® was found to significantly increase this variable, unlike the rest of the treatments (98.4% with respect to the control).

Quality index

With the application of increasing doses of VIUSID Agro® in 2020, the quality index of the seedlings tended to increase as well, with similar values for the 0.4, 0.6, and 0.8 mL L^{-1} concentrations. The 1 mL L^{-1} concentration of the biostimulant resulted in values similar to those of the control treatment. In 2021, this indicator recorded the highest value with the application of 0.8 mL, which differed from the rest of the treatments (Table 4).

Leaf area

Leaf area is an important variable in most agricultural and physiological studies about plant growth, light uptake, photosynthetic efficiency, respiration, transpiration, response to irrigation and fertilization, nutrient use, and especially carbon assimilation during the plant life cycle [24-26].

The leaf area is an index that adequately expresses the response of the integrated growth of the coffee seedlings and has higher quantitative values than the other variables [27,28].

Table 3. Effect of VIUSID Agro® concentrations on the dry mass of coffee seedlings and their composition (2021).

Concentration (mL L^{-1})	Dry matter (g)			
	Leaf	Stem	Root	Total
0.0	1.75 c	0.46 d	0.52 e	2.73 d
0.2	2.473 b	0.90 b	0.93 b	4.39 b
0.4	2.58 b	1.10 a	0.81 c	4.49 b
0.6	2.57 b	0.75 bc	0.99 b	4.31 b
0.8	3.12 a	1.20 a	1.11 a	5.43 a
1.0	2.47 b	0.63 c	0.67 d	3.77 c
E, E, $\bar{x}$	0.074*	0.046*	0.032*	0.13*

*Means with different letters differ by $p \leq 0.05$ according to Duncan's test.

Table 4. Effect of VIUSID Agro® concentrations on the quality index and leaf area of coffee seedlings.

Concentration (mL L^{-1})	2020		2021	
	Quality index	Leaf area (cm^2)	Quality index	Leaf area (cm^2)
0	0.24 c	305.37 b	0.26 d	402.7 d
0.2	0.22 c	298.42 b	0.41 b	409.2 d
0.4	0.27 ab	388.62 a	0.38 b	453.0 c
0.6	0.28 a	396.26 a	0.42 b	461.7 bc
0.8	0.27 ab	403.05 a	0.52 a	528.0 a
1.0	0.24 bc	317.54 b	0.31 c	487.2 b
E, E, $\bar{x}$	0.004*	7.42*	0.015*	10.85*

*Means with different letters differ by $p \leq 0.05$ according to Duncan's test.

In 2020, the VIUSID Agro® concentrations significantly increased the leaf area of the coffee seedlings, starting from the 0.4 mL L^{-1} concentration. This increase was 27% higher than the control (Table 4) and did not differ from the effect achieved with concentrations of up to 0.8 mL. When applying a 1 mL L^{-1} concentration of VIUSID Agro®, the leaf area decreased significantly reaching a statistically similar value to the control.

In 2021, a similar increase in leaf area was recorded after the application of VIUSID Agro® (Table 4). The highest average value was found when a concentration of 0.8 ml L^{-1} of the biostimulant was applied, causing a 31% increase with respect to the control; this result significantly differed from the rest of the treatments. A decrease in this variable was also found when the highest concentration was applied.

VIUSID Agro® had a positive effect on the growth of tobacco seedlings and increased the fresh mass, dry mass, and the stem diameter by 28.4%, 30.5%, and 41.2%, respectively. Therefore, the product should be applied in doses of 0.5, 0.7, and 1.0 L ha^{-1} [15]. The correlation of the relative increases in the leaf area with the concentrations of the biostimulant showed the greatest adjustments to the quadratic functions, recording maximum values of 0.64 (2020) and 0.71 (2021) (Figure 2). Therefore, the use of VIUSID Agro® can be recommended as a biostimulant for Cuba's coffee seedlings production.

The results of the biostimulant application can be attributed to its composition: Its formula includes potassium phosphate (which favors the formation of carbohydrates and is necessary for the transfer and storage of energy in plants [29]), zinc sulfate (which favors the formation and development of new tissues), and glycine (which is a vital amino acid for the growth process and photorespiration).

Another equally important component is folic acid —an important coenzyme in the metabolism of amino acids and in the synthesis of the nitrogenous bases required for the formation of new tissues.

VIUSID Agro® and other products with amino acids [29] have three types of effects on the plants. One of these effects is hormonal: when amino acids enter the plants, they stimulate the formation of chlorophyll and indoleacetic acid (IAA), as well as the production of vitamins and the synthesis of numerous enzymatic systems [30].

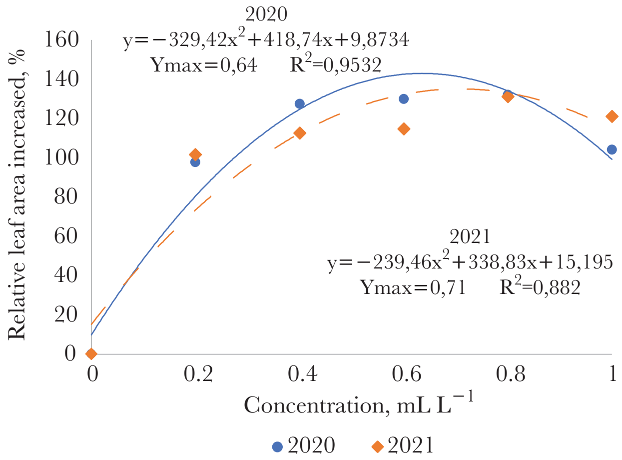

Figure 2. Relative increase in leaf area due to the effect of VIUSID Agro® concentrations.

Amino acids are stimulants that allow the plant to "save energy" and catalyze the synthesis of sugars, starch, and other components of leaves, flowers, and fruits. They contribute to the increase of chlorophyll in the leaves and delay aging, thereby intensifying the performance of photosynthesis [18].

CONCLUSIONS

The monthly application of VIUSID Agro® between the second and fifth pair of leaves favored the morpho-agronomic indicators of coffee seedlings. The VIUSID Agro® in 0.64 to 0.71 mL L^{-1} concentrations of water favored the greatest increases in the leaf area of the coffee seedlings.

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