

Biostimulants: An innovation in agriculture for the cultivation of coffee (*Coffea arabica* L)

Article Data	ABSTRACT
<p>Universidad Estatal del Sur de Manabí (UNESUM), Faculty of Natural Sciences and Agriculture, km 1.5 via Noboa, Campus Los Ángeles, Jipijapa, Manabí, Ecuador.</p> <p>*Contact address: Universidad Estatal del Sur de Manabí (UNESUM). Faculty of Natural Sciences and Agriculture. km 11/2 via Noboa s/n Campus Los Angeles, Jipijapa, Ecuador. Tel. +05-2600229.</p> <p>Yhony Valverde-Lucio E-mail address: yhonny.valverde@unesum.edu.ec</p>	<p>The research was conducted in Jipijapa in the town of Andil, the objective was to evaluate the physiological and morphological behavior of arabica coffee in the nursery stage to the application of biostimulants: Starlite, Humega, Micorriza and Evergreen, compared to Urea. A completely randomized experimental design was applied, using a factorial arrangement of repetitions in time for the morphological variables, and Tukey's test was applied based on the -statistical differences found. The results obtained at the physiological level established a significant difference $p < 0.05$ in the variables dry matter, moisture and nitrogen (N), with Starlite and Evergreen biostimulants being the best in DM, and Humega and Evergreen in N content. There was a better response to chlorophyll (Cl) assimilation by all biostimulants, surpassing urea in general, with Micorriza and Starlite being the best, establishing a high positive correlation between N and chlorophyll. In terms of -morphological development,- urea showed a -better -response, and at the biostimulant level, Humega and Micorriza showed better results, all between 90 and 120 days.</p>
<p>Keywords:</p> <p>Dry matter, physiology, morphology, chlorophyll, nitrogen, correlation, measured over time.</p>	

Introduction

Coffee in Ecuador is a crop of great economic importance, since it has 199,215 cultivated hectares, 68% of which corresponds to *Coffea arabica* and 32% to *C. canephora*¹, distributed in 23 of the 24 provinces of the country, therefore, it is related to a broad social and economic fabric, the latter is based on the generation of employment for 105,000 producing families, as well as 700,000 families linked to the processes of marketing, industrialization, transport and export². Its production is concentrated in the provinces of Manabí (especially in the town of Jipijapa), Loja and the foothills of the Western Cordillera of the Andes³.

This production has shown a variable behavior in the last fifteen years. During the 2002-2011 period, a growing trend was observed, with a drastic change in 2012, producing a significant drop of 69% compared to 2011. This behavior was caused by an 8% decrease in cultivated area, with a 62% drop in yield, in that period. The advanced age of plantations and their renewal were the main causes of this productive decline⁴. Although Ecuador is one of the few countries that produces two types of coffee, Arabica and Robusta (*C. canephora*), coffee production has suffered a dizzying fall since the 1990's that has not been able to be recovered to date⁵.

A biostimulant is any substance or microorganisms that, when applied to plants, are able to improve their efficiency in absorption and assimilation of nutrients, tolerance to biotic and abiotic stresses or improve some of their agronomic characteristics, independently in the content of nutrients⁶, provides additional increases in crop yields, stimulates and invigorates from germination to fruiting. It reduces the crop cycle, enhancing the action of fertilizers, which allows to reduce between 30% and 50% the recommended doses⁷. These bioproducts are associated with nutrition, water relations, soil structure, pH, heavy metals and pathogens⁸. Thanks to biostimulants, plants obtain nutrients capable of reducing undesirable impacts on the environment, while ensuring that farmers obtain a higher return on their investments. They improve crop

quality: With their use, the crop has a higher quality (sugar content, color, firmness and nutrient absorption)⁹.

In a research conducted in Mexico, evaluated the response of coffee plants in nursery stage, managed under an ecological approach, they used three organic fertilizers (AO) (compost, bocashi and vermiabono) under different proportions (25, 50, 75 and 100%), indicate that the AO gave better benefits in the production of coffee plants, standing out 25 and 50%¹⁰. For the number of leaves, the highest proportion was at 50% compost, quantifying a higher dry and green weight of root, stem and leaves, which is considered as a relevant treatment for the production of coffee seedlings in the nursery stage¹⁰.

Studies carried out in Manabí identified better productive characteristics in the varieties: Sarchimor (18%), Caturra (17%) and Catuai (14%)⁴. The promising coffee varieties and hybrids that are best adapted and present desirable morphological characteristics to the agro-ecological conditions of the southern zone of Manabí are the varieties: Pache, Caturra red-Paturra and Catuai: Pache, Caturra rojo-Pichilingue, Acawa, Hybrid Catimor 8664 and Hybrid Sarchimor 4260¹¹.

In Ecuador there are no scientifically rigorous studies that have evaluated organic biostimulants in the production of arabica coffee in the nursery stage, in this sense the objective of this work was to identify biostimulants that contribute to improve the morphological and physiological characteristics of arabica coffee plants in the nursery.

Materials and methods

The research work was carried out at the farm of the Universidad Estatal del Sur de Manabí (UNESUM) belonging to the Jipijapa canton, with a local steppe climate considered BSh, a variant of the dry subtropical climate and warm semi-arid, the average annual temperature is 23.7 °C, the average rainfall is 537 mm¹².

Seeds were selected from a Sarchimor 42-60 coffee crop (5 years old), which is from

UNESUM, 0.5 kg were used, sown in a seedbed -of 1 m x 1 m, the seeds germinated in an average of 45 days, after 60 days they were transplanted -to the bags (23 cm x 10 cm). The substrate was prepared with 40% black soil, 40% river sand and 20% compost in order to guarantee its texture and fertility. The substrate was disinfected with a commercial product (Imbio neen), of natural origin that acts as a fungicide and insecticide.

For the development of the trial, a nursery of guadua cane, cady, saran, with dimensions of 4 x 4 m, was built, and the beds were constructed for the -management of the -research.

The development of the research involved laboratory work, measuring DM dry matter, humidity (H), nitrogen (N), chlorophyll absorption (Cl), and data collection of morphological aspects, which measured the variables, plant height (PA), stem diameter (SD) and number of leaves (NH).

The equipment and methods used at -laboratory level - were: to measure DM and H, an average of 300 g of leaves per treatment -was required, a large capacity oven was used, with forced air ventilation, with an internal volume of 270 dm³ for the determination at 102 °C and 60 °C, -respectively, with perforated trays with 1 cm holes and located 20 cm apart between two successive trays-, considering a 24 h drying time. Depending -on the drying temperature, the -% DM - were obtained-: at 60 °C (MS60) and at 102 °C (MS102), by hot weighing¹³.

Laboratory tests were carried out at UNESUM, Bromatology laboratory, -N measurement was performed using the Kjeldahl method, a technique that digests nitrogens and other organic components of food in a mixture with sulfuric acid in the presence of catalysts-. -The reactions carried out in the Kjeldahl method were: digestion, catalyzation and titration¹⁴, the protein factor applied was 6.25.

Cl typically has two absorption groups in the visible spectrum: In the blue light region (400-500 nm). In the

red part of the spectrum (600-700 nm). Cl reflect the green middle part (500-600 nm), an: Ultrasonic Sonicator or glass mortar, countertop centrifuge: 3000 rpm, saturated MgCO₃ solution, the procedure consisted of adding 1 g of MgCO₃ to 100 mL of reagent water, filtering over glass fiber membrane of 1 µm pore, application of 90% acetone¹⁵.

To identify the biostimulant that favors -greater morphological development in arabica coffee (C. arabica) -seedlings at the nursery stage, data were collected on: AP (cm), using a graduated ruler, DT (mm) using a Vernier caliper, and NH. Statistical analysis. The completely randomized design was applied in the laboratory tests, and in the 20

morphological measurement, a -completely randomized experimental design was used-, with repetitions in time¹⁶. Considering the times as factor A: 30, 60, 90, and 120 days, factor B: the types of organic biostimulants, Humega, Evergreen-, Starlite, Micorriza, and urea as a control. Seventeen treatments were established, 16 by combination of factors and the control, which in this study was urea. Each treatment had 15 -experimental- units -(EU) (plants), using a total of 75 plants.

Prior analysis of variance, it was determined whether the -study variables had normal distribution and homogeneity of variances, which were ratified-. Therefore, ANOVA and Pearson's correlation were applied. The statistical analysis was carried out in the Infostat software; the comparison of means was performed using Tukey's test at 0.05% probability¹⁶.

Results

The evaluation of the physiological behavior (-N content) of arabica coffee (C. arabica) was carried out by taking samples of each of the treatments from the leaves of the plants, which were dehydrated to obtain DM and N content (Table 1).

Table 1 Results of laboratory tests and their significance

Biostimulants	Nitrogen	Humidity	MS
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Plants with Urea (Control)	2.9 ^a	70.23 ^a	30.4 ^b
Plants with Humega	2.88 ^{ab}	68.04 ^b	31.26 ^b
Plants with Evergreen	2.87 ^{abc}	68.25 ^{ab}	31.7 ^{ab}
Mycorrhizal Plants	2.84 ^{bc}	69.06 ^{ab}	31.12 ^b
Plants with Starlite	2.82 ^c	67.11 ^b	32.89^a
P value	0.0226	P= 0.0069	0.0418

The literals in the columns correspond to the significance test according to Tukey at P<0.05 probability. DM Dry matter.

The H analysis reported highly significant statistical differences between treatments, with a p-value of 0.0069. The results obtained led to the application of the Tukey test, which showed -that urea as a control presented a better -physiological response than the rest of the treatments, in order of importance were the biostimulants Humega and Starlite. In terms -of DM, the best results were presented by the biostimulants Starlite, Humega and Evergreen-, respectively. The ANOVA based on the p-value obtained of 0.0418, established a significant difference with 95% confidence, which led to the respective -significance analysis, which determined a high DM content in the biostimulants.

The results of the N analysis ratify those -obtained in

the determination of H, with a p-value of 0.0226, which implies a statistical difference between treatments with 95 % confidence. The analysis of the results by means of the -Tukey -significance test -established the control as the best treatment, followed by the biostimulants Humega and Evergreen, respectively.

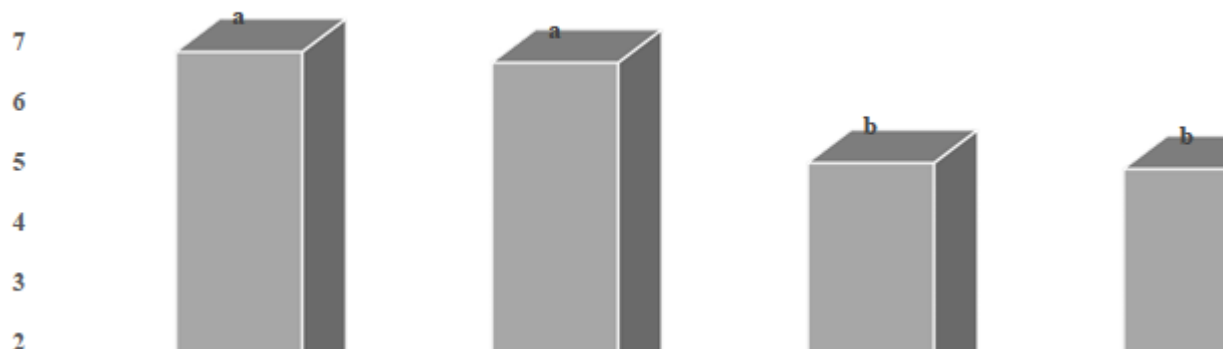
Regarding the determination of the biostimulant that affects a better absorption of Cl and its relationship with the N content, the respective laboratory- test was carried out using the absorbance method. The ANOVA resulted in high significance at the biostimulant level, with 99% confidence, Table 2 describes the highly significant difference between treatments with a p-value of 0.0039.

Table 2 Analysis of variance chlorophyll uptake

F.V.	gl	CS	CM	F	p-value
Biostimulants		12.46	3.12	7.9	0.0039**
Error		3.94	0.39		
Total		16.4			

CV: 12.06

Figure 1 Analysis of variance for chlorophyll absorption.



The significance test for the variable Cl, -Micorriza and Starlite biostimulants were -determined as the best treatments (Figure 1).

With regard to the identification of the biostimulant that favors greater morphological development in -Arabica coffee (C. arabica) seedlings, it was necessary to carry

out an integral analysis of the trial (Table 3), -performing a repeated measures analysis over time (30, 60, 90 and 120 days).

Table 3 Analysis of variance for morphological variables

F.V.	gl	CM	AP	NH	DT
Biostimulant		0.95	0.024*	0.8031ns	0.9806ns
Weather		112.55	<0.0001**	<0.0001**	0.0044**
Biostimulant*Time		2.79	0.994ns	0.1777ns	0.5433ns
Error		1.9			
Total					

ns= not significant. * Significant at $p < 0.05$ probability. ** Highly significant at $p < 0.01$ probability. AP Plant height, NH Number of leaves, DT Stem diameter.

A completely randomized design with -observations over time was applied-, and the time factor was added to the biostimulant factor. The variables analyzed that are related to the morphological development of the plant were: DT, AP, and NH.

The ANOVA-factorial ANOVA, at the PA, DT and NH levels, -presented a value of $p < 0.05$ and did not identify interaction between the factors: biostimulants and time. However, -in the ANOVA for this type of -experimental exercises, it is understood that when the biostimulant*time interaction is significant ($p < 0.05$), it

is better to analyze each date separately-, understanding that this interaction can -conceal true differences between biostimulants. In view of the results obtained, it was decided to apply Tukey's significance test (Table 4), which determined statistical differences between biostimulants. It was found that the control (urea) expressed a better morphological response-, and at the biostimulant level, Humega and Mycorrhiza, all between 90 and 120 days, which gives the guideline to understand that, the longer the time, the better the response of the fertilizer and biostimulants is expressed in the coffee plant in the nursery stage.

Table 4 Results of morphological variables, with Tukey's significance test.

Biostimulants	Time (min)	AP	DT	NH
Urea		27.4 ^a	0.3 ^a	9.67 ^b
Urea		ab	0.23 ^{ab}	9.33 ^{bc}
Humega		25.97 ^{abc}	0.17 ^{abc}	12.33 ^a
Humega		25.53 ^{abc}	0.17 ^{abc}	ab
Starlite		25.33 ^{abc}	0.2 ^{abc}	8.67 ^{bc}
Urea		25.17 ^{abc}	0.1 ^{bc}	c
Starlite		24.8 ^{abc}	0.17 ^{abc}	8.33 ^{bc}
Humega		24.5 ^{bc}	0.13 ^{bc}	9.33 ^{bc}
Evergreen		24.3 ^{bc}	0.2 ^{abc}	ab
Starlite		24.23 ^{bc}	0.1 ^{bc}	7.67 ^c
Evergreen		24.23 ^{bc}	0 ^{nc}	ab
Mycorrhiza		23.8 ^c	0 ^{nc}	abc
Evergreen		23.5 ^c	0.1 ^{bc}	9.67 ^{bc}
Mycorrhiza		23.33 ^c	0.1	9.67 ^{bc}
Urea		23.13 ^c	0.1 ^{bc}	5.33 ^D
Humega		22.33 ^{cd}	0.1 ^{bc}	D
Starlite		22.27 ^{cd}	0.1 ^{bc}	4.67 ^D
Mycorrhiza		22.17 ^{cd}	0.23 ^{ab}	bc
Evergreen		21.63 ^{cd}	0.1 ^{bc}	4.67 ^D
Mycorrhiza		18.93 ^o	0.1 ^{bc}	D

Literals in columns with different letters indicate significance at $p < 0.05$ probability. AP Plant height, NH Number of leaves, DT Stem diameter.

Table 5 Pearson Correlation

	Humidity	MS	Nitrogen	Chlorophyll	AP	NH	DT
Humidity	1.00	-0.68*	0.52*	0.45	0.12	0.04	0.09
Dry matter		1.00	-0.57*	0.58	0.18	0.10	0.39
Nitrogen			1.00	-0.78*	0.44	0.29	0.25
Chlorophyll				1.00	0.21	0.47	0.21
Height					1.00	0.22	0.16
Number of sheets						1.00	0.15
Diameter							1.00

*: Significant at $p < 0.05$ probability. AP Plant height, NH Number of leaves, DT Stem diameter.

A significant negative correlation (Table 5) was established between moisture and DM (-0.68) and a -significant positive correlation -between H and N content (0.52). Likewise-, a significant negative correlation was observed between DM and N content (-0.57) and finally a negative -correlation was determined

between N content and Cl content (-0.78).

Discussion

The present research seeks to contribute to define and understand the contributions of biostimulants in coffee

agriculture. However, these biostimulants are from diverse sources and are available on the market, and are mainly elaborated based on bacteria, fungi, algae, plants, animals and raw materials containing humates. It is therefore proposed to distinguish biostimulants as "a formulated product of biological origin that improves plant productivity as a consequence of novel or emerging properties of the constituent biochemical complexes, and not as a sole consequence of the presence of known essential plant nutrients, The¹⁷¹⁸-definition provided here is important as it emphasizes the principle that biological function can be positively modulated by the application of molecules, or mixtures of molecules, for which an explicit mode of action has not been defined^{19,20}.

In our research we were able to observe a significant physiological response of the coffee plant to biostimulants, possibly because they are composed of biogenic stimulants, metabolic enhancers, plant strengtheners, positive plant growth regulators, generators, allelopathic preparation, plant conditioners, phytostimulators²¹⁻²⁵, in reference to urea, which is only a source of high N content. However, these biostimulants should not be considered as pesticides or fertilizers^{26,27}. Urea is used very frequently by growers in our sector, but it is commonly used indiscriminately and therefore without considering the consequences it may have on the plant. Biuret is a chemical compound found in urea; Biuret toxicity increases when urea is used in foliar spraying²⁸. Nitrogen is one of the nutrients that most limits plant growth, since, together with potassium, it has the highest level of demand per unit of DM of crops²⁹, the concentration of N is 30.94% and up to 650 days after planting, absorption varies between 8.55 to 19.36 g/plant.

The laboratory tests, which allowed measuring the physiological results, coincide with Tello-Gómez³⁰, who points out that N, an important structural constituent of Cl, and important in photosynthesis. In addition, it is part of amino acids and nucleic acids, its functions are: a) it is part of Cl, b) the DM of plants contains 2 to 4% of N, c) it is involved in the whole process of formation of tissues for plant growth, d) it is

the element that gives the greatest response to the production of coffee plants and e) it is a constituent of nucleic acids, and therefore responsible for the genetic information³¹. The data reported on the level of N in DM coincide with the average in our research with 2.86%. However, we should mention that biostimulants can be primary metabolites such as amino acids-, sugars, nucleotides and lipids^{32,33} or secondary metabolites-, including glycolysis, tricarboxylic acid (TCA), aliphatic amino acids (AA), pento-saphosphate and shikimic acid pathways which are mainly the source of aromatic AA and phenolic compounds (FC), terpenoids/isoprenoids, nitrogen-containing compounds (alkaloids), sulfur-containing compounds (glucosinolate)^{32,33}.

Sanclemente & Peña³⁴, mention a general tendency to increase photosynthetic efficiency as N concentration increases. In the same sense De Lima et al.³⁵, Du Jardin⁶ indicate that, in the leaves, there is a significant correlation between Cl contents with N concentration in the leaf, 50 to 70%, in any case both authors contrast with our results, we observed a significant negative correlation.

Coffee leaves complete their expansion and become potential exporters of nutrients. The degradation of compounds contained in the mature leaf cells leads to the migration of photoassimilates and mobile mineral nutrients, especially N and K to landfills such as roots and fruits³⁶, the laboratory analysis carried out on coffee leaves determined the DM with a p-value of 0.041 defined by ANOVA, a significant difference was established between treatments, presenting the Starlite and Evergreen biostimulants as those with the best physiological response.

Regarding the morphological development of coffee in the nursery stage, the variables DT, AP, and NH were considered, in all cases the development was uniform, with an increasing trend line during the time of the experiment, only statistical differences by means of significance test, greater assimilation is visualized in the fourth and fifth month of age of the plants, observing however a better response with urea and humic acid, results that are opposed to those of Utria et al.³⁷ who reported favorable responses with the use of

brassinosteroid in the coffee development process, observing a -vigorous development -of coffee seedlings when they were embedded -in the biostimulant, but with a tendency to have better behavior when it was applied in the second pair of true leaves and with concentrations of 0.01 and 0.05 mg L⁻¹ .

Likewise, Acuña³⁸ , applied the organic fertilizer microPlus, and observed that the best absorption of nutrients, which influenced the agronomic response - (AP, DT, root length) were presented at 180 days of plant age, coinciding with the results of the research, where better responses of both urea and biostimulants - were observed at 120 days, giving the guideline for further research in this regard. It is worth noting that these biostimulants activate -organic compounds (phenols, vitamins, polysaccharides, betaines, etc.), growth regulators, and also macro and micro elements in the -plant³⁹⁻⁴¹ .

Conflicts of interest

The authors declare that this research does not generate conflicts of interest.

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Ethical aspects

Approval of the research by the Ethics Committee, Vice Rectorate of Research of the -Universidad Estatal del Sur de Manabí (UNESUM), (-Cantón Jipijapa) and followed the guidelines established for this committee.

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