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ORIGINAL RESEARCH ARTICLE

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

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ABSTRACT

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 with 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.

Keywords: dry matter; physiology; morphology; chlorophyll; nitrogen; correlation; measured over time

1. Introduction

Coffee in Ecuador is a crop of great economic importance since it has 199,215 cultivated hectares, 68% of which correspond to *Cofféa 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 export[2]. 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 Andes[3].

This production has shown 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 and a 62% drop in yield in that period. The advanced age of plantations and their renewal were the main causes of this productive decline[4]. 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 1990s that has not been able to be recovered to date[5].

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A biostimulant is any substance or microorganism that, when applied to plants, is able to improve their efficiency in absorption and assimilation of nutrients, tolerance to biotic and abiotic stresses, or some of their agronomic characteristics, independently in the content of nutrients[6] , provide additional increases in crop yields, and stimulate and invigorate from germination to fruiting. It reduces the crop cycle, enhancing the action of fertilizers, which allows for a reduction of between 30% and 50% of the recommended doses[7]. These bioproducts are associated with nutrition, water relations, soil structure, pH, heavy metals, and pathogens[8] . 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 study conducted in Mexico that evaluated the response of coffee plants in the nursery stage, managed under an ecological approach, they used three organic fertilizers (AO) (compost, bocashi, and vermiabono) in different proportions (25%, 50%, 75%, and 100%) and indicated that the AO gave better benefits in the production of coffee plants, standing out at 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 a relevant treatment for the production of coffee seedlings in the nursery stage[10].

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 4260[11].

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 improving the morphological and physiological characteristics of arabica coffee plants in the nursery.

2. 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 and the average rainfall is 537 mm[12].

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

UNESUM, 0.5 kg were used and sown in a seedbed of 1 m × 1 m. The seeds germinated in an average of 45 days, and after 60 days they were transplanted to the bags (23 cm × 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, and saran with dimensions of 4 × 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 the 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; an internal

volume of 270 dm^3 for the determination at 102 °C and 60 °C, respectively; perforated trays with 1 cm holes were located 20 cm apart between two successive trays, considering a 24 h drying time. Depending on the drying temperature, the DM was obtained at 60 °C (MS60) and 102 °C (MS102) by hot weighing[13].

Laboratory tests were carried out at the UNESUM Bromatology Laboratory. N measurement was performed using the Kjeldahl method, a technique that digests nitrogen 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 titration[14], 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, a countertop centrifuge at 3000 rpm, and a and a saturated $MgCO_3$ solution. The procedure consisted of adding 1 g of $MgCO_3$ to 100 mL of reagent water, filtering over a glass fiber membrane with a 1 μm pore, and applying 90% acetone[15].

To identify the biostimulant that favors greater morphological development in Arabica coffee (*C. arabiga*) 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 measurements, a completely randomized experimental design was used, with repetitions in time[16]. 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 to the to the analysis of variance, it was determined whether the study variables had a 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% probability[16].

3. Results

The evaluation of the physiological behavior (N content) of arabica coffee (*C. arabiga*) 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
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, 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					

The significance test for the variable Cl, micorriza and starlite biostimulants were determined as the best treatments (**Figure 1**).

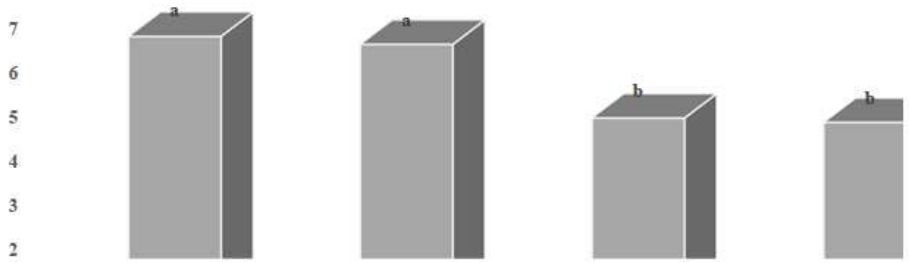

Figure 1. Analysis of variance for chlorophyll absorption.

With regard to the identification of the biostimulant that favors greater morphological development in Arabica coffee (*C. arabiga*) 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 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 exercise, 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	120	27.4^{a}	0.3^{a}	9.67^{b}
Urea	90	26.9^{ab}	0.23^{ab}	9.33^{bc}
Humega	120	25.97^{abc}	0.17^{abc}	12.33^{a}
Humega	90	25.53^{abc}	0.17^{abc}	11^{ab}
Starlite	120	25.33^{abc}	0.2^{abc}	8.67^{bc}
Urea	60	25.17^{abc}	0.1^{bc}	8^{c}
Starlite	90	24.8^{abc}	0.17^{abc}	8.33^{bc}
Humega	60	24.5^{bc}	0.13^{bc}	9.33^{bc}
Evergreen	120	24.3^{bc}	0.2^{abc}	11^{ab}
Starlite	60	24.23^{bc}	0.1^{bc}	7.67^{c}
Evergreen	90	24.23^{bc}	0.2^{abc}	11^{ab}
Mycorrhiza	120	23.8^{c}	0.2^{abc}	10^{abc}
Evergreen	60	23.5^{c}	0.1^{bc}	9.67^{bc}
Mycorrhiza	90	23.33^{c}	0.2^{abc}	9.67^{bc}
Urea	30	23.13^{c}	0.1^{bc}	5.33^{D}
Humega	30	22.33^{cd}	0.1^{bc}	3^{D}
Starlite	30	22.27^{cd}	0.1^{bc}	4.67^{D}
Mycorrhiza	60	22.17^{cd}	0.23^{ab}	9^{bc}
Evergreen	30	21.63^{cd}	0.1^{bc}	4.67^{D}
Mycorrhiza	30	18.93^{d}	0.1^{bc}	3^{D}

Literals in columns with different letters indicate significance 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).

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.

4. Discussion

The present research seeks to contribute to defining and understanding the contributions of biostimulants in coffee agriculture. However, these biostimulants are from diverse sources, are available on the market, and are mainly elaborated on 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"[17,18], The 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 preparations, plant conditioners, phytostimulators[21–25], in reference to urea, which is only a source of high N content. However, these biostimulants should not be considered 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 spraying[28]. 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 crops[29] The concentration of nitrogen is 30.94%, and up to 650 days after planting, absorption varies between 8.55 and 19.36 g/plant.

The laboratory tests, which allowed measuring the physiological results, coincide with Tello-Gómez[30], who points out that N is 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 2–4% of N; c) it is involved in the whole process of the 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 information[31]. The data reported on the level of N in DM coincides with the average in our research of 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 sources of aromatic AA and phenolic compounds (FC), terpenoids and isoprenoids, nitrogen-containing compounds (alkaloids), and sulfur-containing compounds (glucosinolate)[32,33].

Sanclemente & Peña[34], mention a general tendency to increase photosynthetic efficiency as N concentration increases. In the same sense, De Lima et al.[35] and Du Jardin[6] indicate that, in the leaves, there is a significant correlation between Cl contents and N concentration in the leaf, ranging from 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 fruits[36] 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, Acuña[38], applied the organic fertilizer microPlus and observed that the best absorption of nutrients, which influenced the agronomic response (AP, DT, root length), was 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 plant[39–41].

Conflict of interest

The authors declare no conflict of interest.

References

1. Espinoza Alarcón WL, Mosquera Yépez D. *Sectoral Analysis of Coffee and Processed Coffee and Its Importance in Ecuadorian Exports, Period 2012–2016* (Spanish) [Bachelor's thesis]. Universidad de Guayaquil; 2017.
2. Alarcón López A. *Productive Management Model for Coffee (Coffea arabica L.) Cultivation in Southern Ecuador* (Spanish) [Bachelor's thesis]. Universidad Politécnica de Madrid; 2011.
3. Aspiazu Villavicencio K, Navarro Moncayo J. *Project for the Commercialization of Bean Coffee (Organic Coffee), Acting as an Intermediary, for Local Consumption in the City of Guayaquil and as an Export Option* (Spanish) [Bachelor's thesis]. Escuela Superior Politécnica del Litoral; 2009.
4. Monteros Guerrero A. Yields of dry coffee beans in Ecuador 2016 (Spanish). Available online: https://docplayer.es/29340888-Rendimientos-de-cafe-grano-secoen-el-ecuador-2016.html (accessed on 22 October 2018).
5. Venegas Sánchez S, Orellana Bueno D, Pérez Jara P. The Ecuadorian reality in coffee production (Spanish). *RECIMUNDO* 2018; 2(2): 72–91. doi: 10.26820/recimundo/2.(2).2018.72-91
6. Du Jardin P. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* 2015; 196: 3–14. doi: 10.1016/j.scienta.2015.09.021
7. Díaz Medina A, Suárez Pérez C, Díaz Milanes D, et al. Influence of the biostimulant FitoMas-E on the production of coffee plant (*Coffea arabica* L.) seedlings. *Centro Agrícola* 2016; 43(4): 29–35.
8. González Vega M, Rosales Jenqui P, Castilla Valdés Y, et al. Effect of Bioenraiz® as a stimulant of germination and development of coffee tree (*Coffea arabica* L.) seedlings. Cultivos Tropicales 2015; 36(1): 73–79.
9. Ormeño MA, Ovalle A. Preparation and application of organic fertilizers. In: *Plant Science and Production*. INIA Divulga; 2007. pp. 29–31.

10. Aguilar Jiménez CE, Alvarado Cruz I, Martínez Aguilar FB, et al. Assessment of three organic fertilizers on coffee (*Coffea Arabica* L.) in nursery stage. *Siembra* 2016; 3(1): 11–20.
11. Parrales Marcillo G, Castro Piguave C. Determination of the morphological characteristics of 20 varieties of arabica coffee hybrids of high genetic value (Spanish). Available online: https://repositorio.unesum.edu.ec/handle/53000/1280 (accessed on 26 July 2019).
12. Provincial Government of Manabí. *Canton Jipijapa*. Provincial Government of Manabí; 2019.
13. De la Roza Delgado B, Martínez Fernández A, Argamentería Gutiérrez A. Determination of dry matter in pastures and forages from drying temperature for analysis (Spanish). *Rev Pastures* 2002; 32(1): 91–104.
14. Selecta JP. Barcelona: Laboratory apparatus factory. Available online: https://grupo-selecta.com/en/?avia_forced_reroute=1 (accessed on 10 October 2019).
15. Cofes. Chlorophyll a quantification. Buenos Aires: Federal council of health services entities. Available online: http://www.xn--cofs-dpa.org.ar/downloads/relas/5jomada/4CLO-ROFLA.pdf. (accessed on 1 October 2018).
16. Gabriel J, Indaco-chea Ganchozo B, Valverde A, Castro Piguave C. Experimental designs: Theory and practice for agricultural experiments (Spanish). Available online: http://142.93.18.15:8080/jspui/handle/123456789/116 (accessed on 22 October 2018).
17. Yakhin OI, Lubyanov AA, Yakhin IA, et al. Biostimulants in plant science: A global perspective. *Frontiers in Plant Science* 2017; 7: 2049. doi: 10.3389/fpls.2016.02049
18. Huang B. Plant growth regulators: What and why. *Golf Course Management* 2007; 75(1): 157–160. doi: 10.1007/s00572-010-0337-z
19. Basak A. Biostimulants: Definitions, classification and legislation. In: Gawroñska H (editor). *Biostimulants in Modern Agriculture: General Aspects*. Editorial House Wieú Jutra, Limited; 2008. pp. 7–17.
20. Bulgari R, Cocetta G, Trivellini A, et al. Biostimulants and crop responses: A review. *Biological Agriculture & Horticulture* 2014; 31(1): 1–17. doi: 10.1080/01448765.2014.964649
21. Russo RO, Berlyn GP. The use of organic biostimulants to help low input sustainable agriculture. *Journal of Sustainable Agriculture* 1991; 1(2): 19–42. doi: 10.1300/J064v01n02 04
22. Karnok KJ. Promises, promises: Can biostimulants deliver. *Golf Course Management* 2000; 68(8): 67–71.
23. Banks JM, Percival GC. Evaluation of biostimulants to control Guignardia leaf blotch (*Guignardia aesculi*) of horsechestnut and black spot (*Diplocarpon rosae*) of roses. *Arboriculture and Urban Forestry* 2012; 38(6): 258–261. doi: 10.13140/rg.2.1.2897.2883
24. Hamza B, Suggars A. Biostimulants: Myths and realities. *TurfGrass Trends* 2001; 10(8): 6–10.
25. Jardin PD. The science of plant biostimulants—A bibliographic analysis. Available online: https://www.researchgate.net/publication/288264889_The_science_of_plant_biostimulants_-_a_bibliographic_analysis (accessed on 15 October 2019).
26. Torre LA, Battaglia V, Caradonia F. Legal aspects of the use of plant strengtheners (biostimulants) in Europe. *Bulgarian Journal of Agricultural Science* 2013; 19(6): 1183–1189.
27. La Torre A, Battaglia V, Caradonia F. An overview of the current plant biostimulant legislations in different European Member States. *Journal of the Science of Food and Agriculture* 2016; 96(3): 727-734. doi: 10. 1002/jsfa.7358
28. Cenicafe. Physiological and nutritional disturbances of coffee. Available online: https://www.cenicafe.org/es%20/index.%20php/nuestras_publicaciones/cartillas (accessed on 10 October 2018).
29. Ramirez Builes VH, Moreno Berrocal AM, Lopez Ruia JC. Early evaluation of nitrogen deficiency in coffee and applications. Available online: http://www.biblioteca.cenicafe.org (accessed on 15 October 2018).
30. Tello Gómez E. Effect of the application of biostimulants, foliar fertilizers and kaolin, on the agronomic behavior and production of the rice variety (Oryza sativa) CR-4477 on La Vega farm, San Carlos, Alajuela, Costa Rica (Spainish). Available online: https:// repositoriotec.tec.ac.cr/handle/2238/3321 (accessed on 26 July 2019).
31. Sadeghian Khalajabadi S, Gonzales Osorio H. Technical advances: General fertilization alternatives for coffee plantations in the production stage. Available online: https://www. cenicafe.org › publications › avt04241 (accessed on 15 October 2018).
32. Aharoni A, Galili G. Metabolic engineering of the plant primary—Secondary metabolism interface. *Current Opinion in Biotechnology* 2011; 22(2): 239–244. doi: 10.1016/j.copbio.2010.11.004
33. Rafiee H, Naghdi Badi HA, Mehrafarin A, et al. Application of plant biostimulants as new approach to improve the biological responses of medicinal plants—A critical review. *Journal of Medicinal Plants* 2016; 15(59): 6–39.
34. Sanclemente MA, Peña EJ. Growth and photosynthetic efficiency of Ludwigia decurrens walter (Onagraceae) under different concentrations of nitrogen. *Acta Biológica Colombiana* 2008; 13(1): 175–186.
35. De Lima Vasconcelos R, de Mello Prado R, Reyes Hernandez A, Caione G. Effect of measurement time, position and leaf portion on chlorophyll indices in potato (Spanish). *Idesia* 2014; 32(4): 23–28. doi: 10.4067/S0718-34292014000400004
36. Sadeghian Khalajabadi S, Salamanca Jimenez A. Micronutrients in coffee fruits and leaves. *Ceni-Coffee* 2015; 66(2): 73–87.

37. Utria Borges E, Rodríguez Oquendo V, Moisés Medina LG, et al. Response of coffee seedlings (*Coffea arabica* L.) to brassinosteroid application at different concentrations and stages of development (Spanish). *Rev Chapingo Ser Hortic* 2004; 10(1): 11–14. doi: 10.5154/r.rchsh.2000.12.084
38. Acuña P. Influence of foliar fertilizer application at nursery level of arabica coffee (*Coffea arabica*) in Cabo de Hacha site of Jipijapa canton 2013 (Spanish). Universidad Estatal del Sur de Manabí; 2013.
39. University of Lodz. Biostimulators in modern agriculture. Retrieved from: https://ususosweb.uni.lodz.pl/kontroler.php?_action=actionx:katalog2/przedmioty/pokazPrzedmiot(prz_kod:0400-BE707D)&lang=2 (accessed on 5 October 2019).
40. Przybysz A, Wrochna M, Slowinski A, Gawronska H. Stimulatory effect of Asahi SL on selected plant species. *Acta Sci Pol Hortoru* 2010; 9(2): 53–64.
41. Truba M, Jankowski K, Sosnowski J. The plants reaction on biological preparations treatment. *Ochrona Środowiskai Zasobów Naturalnych* 2012; 53: 41–52.