

# Effect of Enerplant<sup>®</sup> doses on the development and nutrient use by cacao (*Theobroma cacao* L.) seedlings

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## ABSTRACT

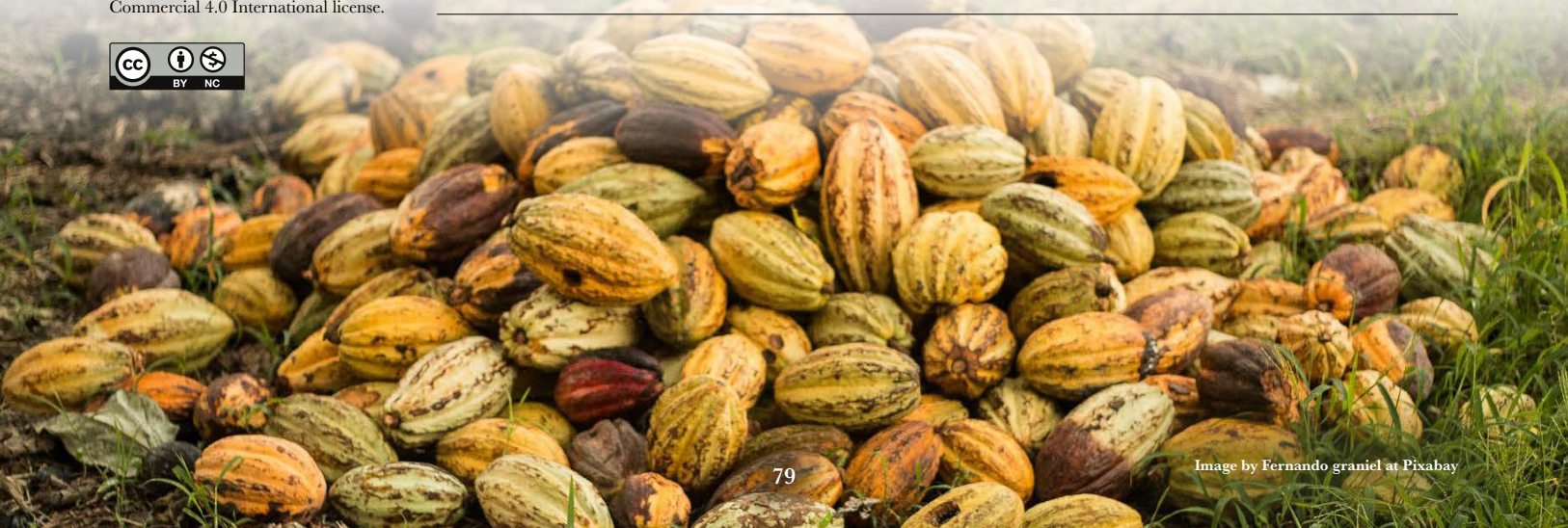
**Objective:** To evaluate the effect of the application of biostimulant doses on the development, absorption, and use efficiency of nitrogen, phosphorus, and potassium by cacao seedlings.

**Design/Methodology/Approach:** Five doses of Enerplant<sup>®</sup> (0, 1, 2, 3, and 4 mL ha<sup>-1</sup>) were applied to cacao seedlings grown under a saran mesh. The study was carried out using a completely randomized design. The foliar area received a monthly application of the biostimulant. In order to determine the nutrient absorption, 3 plants per treatment were divided into leaves, stems, and roots; they were subsequently dried for 72 h at 65 °C. Finally, they were ground, packaged, labeled, and sent to the laboratory, where the macronutrients of each component were analyzed.

**Results:** The cacao seedlings had a positive response to the biostimulant application. In average, the results of our two-year study were: 1 mL ha<sup>-1</sup> doses of Enerplant<sup>®</sup> significantly increased height (24%), dry mass (30%), quality index (58%), and foliar area (56%) of the cacao seedlings. Doses higher than 1- and 2-mL ha<sup>-1</sup> decreased plant growth. The nutrient absorption by cacao in the nursery stage followed the following pattern: K>N>P.

**Findings/Conclusions:** The efficiency of nutrient use—regardless of the Enerplant<sup>®</sup> dose applied—was higher for P, followed by N and, finally, K. Applying 2 mL ha<sup>-1</sup> doses of the biostimulant guaranteed the highest absorption and use efficiency values of macronutrients by cacao seedlings.

**Key words:** biostimulant, efficiency, nutrition, *Theobroma*.



## INTRODUCTION

In Cuba, most cacao crops are located in the eastern region of the country. In order to maintain and increase the productive levels of cacao, Cuba has developed specific programs for the various companies involved. In these programs, complying with seedling production plans plays a major role.

Cuenca-Cuenca *et al.* (2019) carried out a summary of the researches about the positive effects of mineral fertilization on cacao seedlings, the edaphoclimatic conditions influence, the responses to each nutrient, and the different fertilizers doses. These authors suggest determining the efficient nutrient use for cacao clones and taking into consideration their interaction with the specific growing area.

In Cuba, there is a lack of literature about the nutrimental requirements of cacao in the nursery stage. In Peru, researches determined that cacao plants in greenhouses (2-6 months old) require 2.4 kg N ha<sup>-1</sup>, 0.6 kg P ha<sup>-1</sup>, and 2.4 kg K ha<sup>-1</sup> (García, 2018). Based on this information, a calculation of the costs required to meet the mineral fertilizer demand could be made—including the currently high costs of the said fertilizers. In view of this situation, a search for alternatives aimed to guarantee cacao nutrition should be carried out. Biofertilizers and biostimulants are one of these alternatives. The market for these products has seen a remarkable increase throughout the world (M&M, 2019).

Biostimulants are organic-based substances, which include vegetable regulators, as well as other substances that promote an indirect vegetal growth (such as carbohydrates and aminoacids) (Galindo *et al.*, 2019). Kumar and Alope (2020) provide other definitions, including the mandatory need to have their positive effects proven by scientific institutions; those effects should stimulate nutrient absorption, efficiency, abiotic stress tolerance, and harvest quality. Meanwhile, Rouphael and Colla (2020) mentioned the biostimulant effects, taking into account physiological and agronomic aspects, as well as the stress tolerance, and an increase in the efficient use of nutrients.

Enerplant<sup>®</sup> is a growth stimulating product of vegetable origin, produced by the Mexican company Biotec International S.A. de C.V. This product optimizes the macro and micronutrient assimilation, intensifies the growth, development, and fruit formation processes, offers significant production increases, and a higher disease and extreme temperature resistance (Biotec Internacional, 1996). Its chemical composition includes an active ingredient made up of an oligosaccharide mix (0.01%) and an inert ingredient made up of dextrose (80%), maltodextrine (19.68%), citric acid (0.15%), and a coloring agent (0.17%) (Noriega, 2009). Enerplant<sup>®</sup> is an organic agriculture certified product.

In Cuba, Cobas-Elías *et al.* (2016) recorded an increase of sugarcane yield when a 100% of SERFE+5.2 mL Enerplant<sup>®</sup> ha<sup>-1</sup> treatment was used; meanwhile, Núñez-Chávez *et al.* (2019) pointed out that an increase in the industrial yield of the spring remaining, ratooning, and sprout was obtained using a 2.6 mL ha<sup>-1</sup> Enerplant<sup>®</sup> dose and 4 mL ha<sup>-1</sup> of FitoMas-E. Alarcón *et al.* (2018) reported that using a 1.3 mL ha<sup>-1</sup> dose increased onion yields and bulb quality. Finally, while studying three biostimulant doses in lettuce, Baldoquin-Hernández *et al.* (2015) determined that a 1.5 mL ha<sup>-1</sup> dose had economic benefits.

There are few works about biostimulant use on coffee seedling grown in Brazil (Ferreira *et al.*, 2018); however, researches about the use of these products on coffee are available in Cuba (Bustamante and Ferrás, 2019). Nevertheless, this type of studies does not include cacao.

Therefore, a research aimed to establish the effect of Enerplant<sup>®</sup> doses on the development, foliar content, absorption, and use efficiency of N, P, and K by *Theobroma cacao* L. seedlings was carried out.

## MATERIALS AND METHODS

The research was carried out in the Estación Experimental Agro-Forestal del Tercer Frente nursery (20° 09' N and 76° 16' W, at 135 masl), Santiago de Cuba province, during two periods: January-May 2019, and February-June 2020.

During the first and second experimental periods, the average temperature was 24.5 °C and 25.9 °C, respectively. The average rainfall was 473.5 mm in 33 days, and 265.5 mm in 32 days, respectively.

The experiment was developed in a completely randomized design, where the effect of five Enerplant<sup>®</sup> doses (0, 1, 2, 3, and 4 mL ha<sup>-1</sup>) on seedlings of the *Theobroma cacao* L. UF 650 clon was studied. The seedlings were obtained from the hybrid seed bank of the institution.

Enerplant<sup>®</sup> was supplied by Biotech Internacional<sup>®</sup>. The product was monthly applied on the foliar area, before 10 am, from the second to the fifth leaf, using a 16-L Matabi sprayer, at a constant pressure.

Metal divisions were used to avoid interferences between the plots during the application. Control was watered on the same day as the rest of the treatments.

The seeds were sown in 12.5×25 cm black polyethylene bags. Each treatment was comprised of 34 plants; each plant was an experimental unit. Fifteen experimental units per treatment were used in the analysis.

In order to control bright sunshine, a black saran mesh was used. The mesh allowed 50% of the sunlight to pass through and was placed above the bags, as well as in one side of the greenhouse, in order to protect plants from direct radiation.

The plants were grown in a substrate (brown soil without carbonate/cattle manure, in a 3:1 ratio) with slightly acid pH values, high organic matter content, available phosphorus and potassium levels, and normal exchangeable cation values for this soil type (Table 1).

When the plant grew the sixth leaf, the following growth indicators were evaluated: height, dry mass, foliar area, and quality index. Subsequently, the plants were divided by organs (leaves, stems, and root) and were washed with water; they were placed in paper and

**Table 1.** Chemical properties of the soil and substrate (n=3).

	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	pH KCl	pH H <sub>2</sub> O	M.O. %	Ca <sup>+2</sup>	Mg <sup>+2</sup>	K <sup>+</sup>
	mg 100 g <sup>-1</sup> soil					cmol <sub>c</sub> kg <sup>-1</sup>		
Soil	34.21	35.05	6.04	6.97	2.51	48.40	13.14	0.82
Substrate	198.33	134.09	6.17	6.70	5.96	47.67	15.06	2.95

were dried at 70 °C in a forced-air stove, until they reached a constant weight. The plants were then crushed and filtered using a 0.2 mm sieve, before they were subject to a chemical analysis.

The chemical analysis was carried out at the lab of the Estación Territorial de la Caña de Azúcar of Palma Soriano, Santiago de Cuba province. N concentration was determined using the Kjeldahl method; P concentration was determined by colorimetry, using the molybdeno blue method; and K was determined by flame photometry.

Based on the dry matter values and the concentrations, in 2019, nutrient absorption was calculated, while the formula proposed by Siddiqi and Glass (1981) was used to estimate the nutrient use efficiency (EU).

During the statistical processing, the Kolmogorov-Smirnov Goodness of Fit test was used to corroborate the normality of the data and the Levene Test for Equality of the Variances was used for the variance homogeneity. Subsequently, a simple classification of variance analysis was carried out. The differences between treatments were determined using the Duncan's Multiple Range Test ( $p \leq 0.05$ ).

The effect of the product doses on the nutrient absorption and use efficiency was adjusted to several regression models, using the higher coefficient of determination ( $R^2$ ) as the selection criterion. The maximum values were determined calculating the first derivative from the regression equations.

## RESULTS AND DISCUSSION

### Seedling growth

The Enerplant<sup>®</sup> applications had a positive impact on the growth of the cacao seedlings (Table 2).

The treatments where the biostimulant was applied obtained the highest mean values; they statistically surpassed the control (without biostimulant applications) or their results were statistically similar. Overall, a dose of up to 2 mL ha<sup>-1</sup> of the product significantly increased the growth indicator of the cacao seedlings during both years (Table 2).

The tallest cacao seedlings were obtained with the application of 2 mL ha<sup>-1</sup> doses of Enerplant<sup>®</sup>; however, these results were not statistically different from those achieved with the application of 1 mL ha<sup>-1</sup> doses of the biostimulant, a treatment which significantly

**Table 2.** Effect of the Enerplant<sup>®</sup> doses in the growth of cacao seedlings.

Enerplant, mL ha <sup>-1</sup>	Height (cm)		Total dry mass (g)		Quality index		Leaf area (cm <sup>2</sup> )	
	2019	2020	2019	2020	2019	2020	2019	2020
0	27,27 d	32,41 b	2,85 e	4,72 bc	0,267 c	0,503 b	620,73 b	636,34 c
1	34,21 ab	39,59 a	3,93 b	5,74 a	0,559 a	0,541 a	1094,66 a	858,80 a
2	35,25 a	40,45 a	4,45 a	5,17 b	0,379 b	0,465 c	1009,30 a	789,04 ab
3	32,87 bc	38,80 a	3,71 c	4,54 c	0,326 bc	0,330 e	747,09 b	832,77 ab
4	31,33 c	34,61 b	3,20 d	4,56 c	0,274 c	0,415 d	710,03 b	748,15 b
SE, $\bar{x}$	0,56*	0,96*	0,13*	0,19*	0,02*	0,02*	59,51*	31,72*

SE=Standard error. Means with different letters in each column are statistically different (Duncan,  $p \leq 0.05$ ).

increased height in 2019 and 2020 (25% and 22% more than control, respectively) (Table 2). A downwards trend for this indicator was identified when higher doses were applied (3 and 4 mL ha<sup>-1</sup>). These height values were slightly higher than those reported by Suparno *et al.* (2015) for cacao seedlings evaluated for a longer time (180 days) in Indonesia.

Statistically higher dry mass values were recorded with the 2 mL ha<sup>-1</sup> (2019) and the 1 mL ha<sup>-1</sup> (2020) doses (37% and 22% more than other treatments, respectively). Likewise, a downwards trend for the height variable was recorded when higher biostimulant doses were applied (Table 2).

Gonçalves *et al.* (2018) explain that the ideal balance for the growth of different vegetative organs is variable: a certain endogenous concentration of a given nutrient might favor the growth of one organ and inhibit the growth of another.

During this stage of the cultivation, the leaves, the stem, and the roots account for 59%, 27%, and 14% of the dry mass, respectively (data not shown), when the study doses were applied. These figures are higher than those reported by Osorio *et al.* (2017) who studied various container sizes and established that, 305 days after their emergence, the accumulated biomass of cacao seedlings was mainly distributed among leaves (48%) and the stem (33%), and, to a lesser extent, in the root.

Applying 1 mL ha<sup>-1</sup> doses of Enerplant<sup>®</sup> favoured the success of cacao seedlings. The highest quality index was obtained during both years with this dose. They were statistically superior to every other of the study treatments (109% and 7% increases during the first and second years, respectively). The 4-mL dose of the biostimulant reduced this indicator during the second year by 17%, compared with the treatment without application (Table 2).

Applying a 1 mL ha<sup>-1</sup> dose achieved the highest leaf area values for both years (Table 2). These values were statistically superior ( $p \leq 0.05$ ) than the control by 76% and 35%, during the first and second years, respectively. Likewise, an increased dose showed a downwards trends for this variable. The leaf area values were higher than those reported by García (2018) who studied the application of water-soluble fertilizers to seedlings of this crop in Peru.

The positive results of the application of Enerplant<sup>®</sup> —which are higher than the control treatment corroborate that the product biostimulates vegetative growth, significantly increasing the morphological variables of cacao seedlings. Remarkably, this effect can disappear when doses higher than 1- and 2-mL ha<sup>-1</sup> are applied.

### Nutrient concentration

Compared with control without Enerplant<sup>®</sup> application, the N and K concentration on the leaves did not increase when the biostimulant was applied (Table 3). Rather, N and K concentrations decreased as the doses increased. However, phosphorous concentration in the three organs analyzed showed a gradual increase and the highest phosphorous values were recorded with the highest biostimulant dose.

The values of N were higher than those reported in Colombia by Puentes-Páramo *et al.* (2016) for the ICS-95, CCN-51, TSH-565, and ICS-39 clones and by De Oliveira *et al.*

**Table 3.** Effect of Enerplant<sup>®</sup> doses in the nutrient concentrations of cacao seedling organs (experiment carried out in 2019).

Enerplant, mL ha <sup>-1</sup>	Leaves			Stem			Root		
	% N	% P	% K	% N	% P	% K	% N	% P	% K
0	22,3 a	2,2 d	25,8 a	16,1 b	4,6 e	21,4 d	18,4 a	3,0 e	15,9 d
1	22,6 a	2,2 d	25,4 a	16,0 c	5,0 d	26,1 b	17,0 b	3,1 d	18,3 c
2	20,9 b	2,4 b	25,4 a	16,8 a	5,5 c	26,5 a	15,7 c	3,6 b	18,8 b
3	20,4 c	2,3 c	23,8 b	15,4 d	5,6 b	26,5 a	16,1 c	3,3 c	19,2 a
4	19,9 d	2,5 a	24,0 b	16,1 b	5,9 a	25,1 c	14,3 d	3,7 a	19,0 b
SE, $\bar{x}$	0,13*	0,019*	0,15**	0,01	0,001	0,01	0,14	0,028	0,07

SE=Standard Error. Means with different letters in each column are statistically different (Duncan,  $p \leq 0.05$ ).

(2019) for the CCN 51 and PS1319 clones in Brazil. The P and K concentrations in this experiment were likewise higher than the concentrations reported by the said authors. The concentrations fell within the adequate range for N (22-25 g kg<sup>-1</sup>) and P (1.5-2.5 g kg<sup>-1</sup>) concentrations established for high-productivity cultivation areas in Brazil (Marrocos *et al.*, 2020). The high nutrient values determined in this experiment could be related to the substrate fertility, as well as to the age of the material used (4 months), while the values reported by Puentes-Páramo *et al.* (2016) belong to established plantations and have therefore greater dry mass, which might have been the result of the lixiviation effect.

### Nutrient absorption and use efficiency

The nutrient absorption by cacao during the nursery stage showed a K>N>P pattern, regardless of the organ that was analyzed and the Enerplant<sup>®</sup> doses that was applied (Table 4).

Increasing the Enerplant<sup>®</sup> doses (up to 2 mL ha<sup>-1</sup>) increased nutrient absorption. Greater doses resulted in a diminishing trend of absorption values (Table 4) —except for K values in the root.

In their study about the use efficiency of N, Ribeiro *et al.* (2008) did not find differences between the study's cacao clones, although they did notice that the efficiency of that indicator increased along with the nitrogen doses.

**Table 4.** Effect of the Enerplant<sup>®</sup> doses in nutrient absorption by cacao seedlings (mg organ<sup>-1</sup>), during the 2019 experiment.

Enerplant, mL ha <sup>-1</sup>	Leaves			Stem			Root		
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
0	36,22 d	4,40 e	95,95 c	12,71 c	4,38 e	38,70 d	7,82 c	1,53 c	15,64 b
1	56,59 a	6,89 b	145,68 a	12,60 c	4,74 d	47,08 c	10,78 a	2,37 ab	26,60 a
2	53,06 b	7,62 a	147,69 a	21,60 a	8,52 a	78,07 a	9,88 ab	2,71 a	27,06 a
3	46,18 c	6,51 c	123,43 b	13,98 b	6,13 c	55,13 b	8,61 bc	2,13 b	23,55 a
4	36,32 d	5,69 d	100,32 c	14,65 b	6,47 b	52,33 b	7,60 c	2,37 ab	23,14 a
SE, $\bar{x}$	0,71*	0,09*	1,82*	0,29*	0,12/*	1,06*	0,53*	0,13*	1,35*

SE=Standard error. Means with different letters in each column are statistically different (Duncan,  $p \leq 0.05$ ).

The nitrogen absorption values recorded in this study for the above-ground areas (leaves+stems) were similar to the findings of Ribeiro *et al.* (2008) for seven-month-old cacao organs fertilized with a doses equivalent to 120 mg of N per 6-kg pot; however, the estimated nitrogen absorption values recorded for the root were lower than the results of the same authors.

Cabala-Rosand and Mariano (1985) discovered a close relationship between the phosphorous content in the roots of cacao seedlings and the size of the roots; they also found that longer cultivars with greater root area absorbed more environmental P. However, Ribeiro *et al.* (2008) believed that, in the case of perennial cacao crops, nutrient concentration in the roots may not be a proper indicator for the selection of efficient genotypes, at least in the case of young plants, whose nutrient concentration is also influenced by their growth conditions and size.

Bulgari *et al.* (2019) and Yakhin *et al.* (2017) explain that the complex nature of the composition of biostimulants and the wide range of molecules that they include make it difficult to understand and define which are the most active compounds. The action of biostimulants is a consequence of the synergic action of the various bioactive molecules.

Regardless of the Enerplant<sup>®</sup> dose applied, the nutrient use efficiency was higher for P, followed by N and finally by K (Table 5). The highest nitrogen and potassium use efficiency values were higher when a 2 mL ha<sup>-1</sup> dose was applied, while the highest P values were obtained with the 1 mL ha<sup>-1</sup> dose.

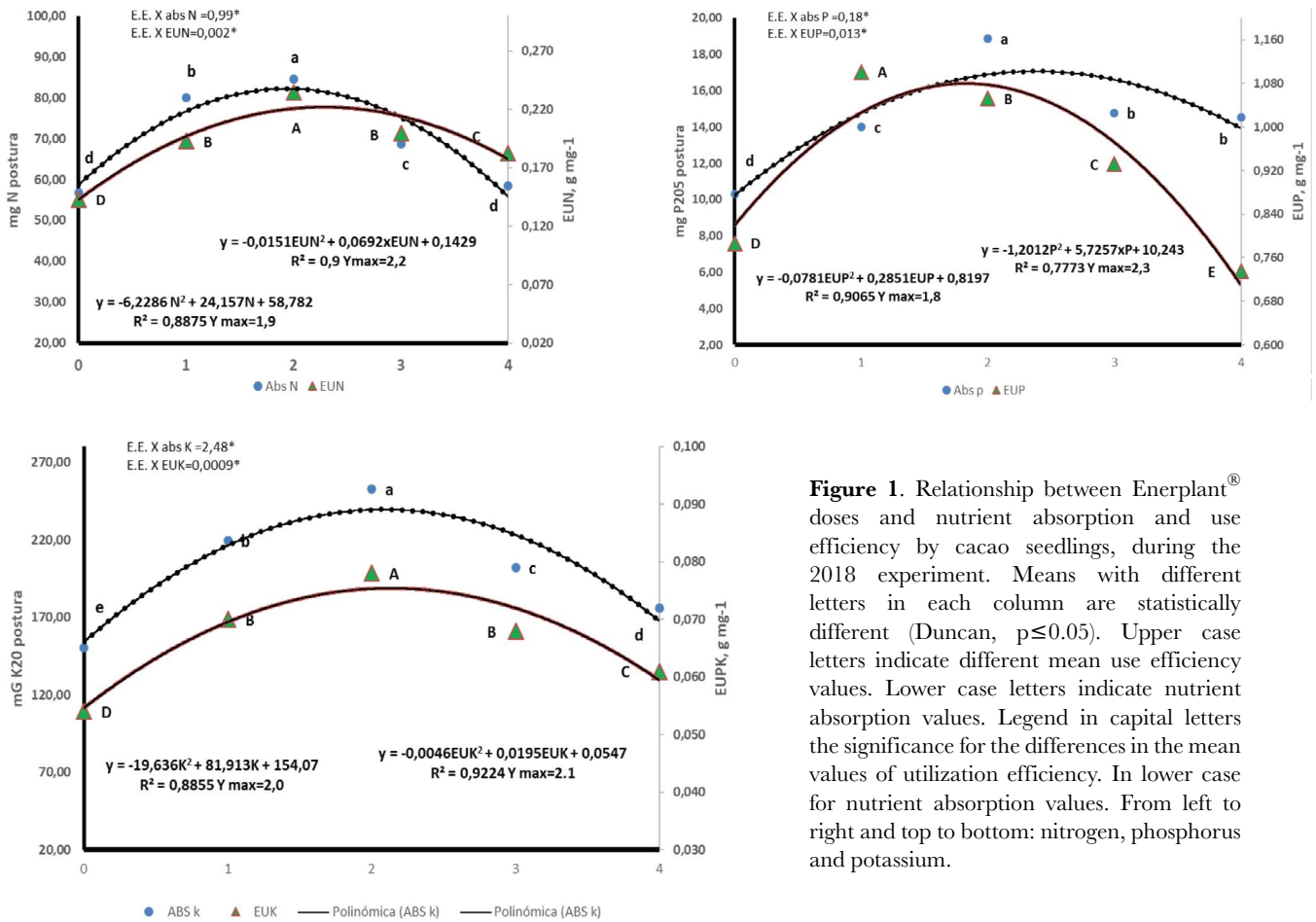
Correlating the biostimulant doses with the absorption values and the use efficiency showed the highest adjustment to the quadratic functions (Figure 1). Applying a 2 mL dose of Enerplant<sup>®</sup> per hectare resulted in the highest nutrient absorption and use efficiency values by cacao seedlings, which were statistically different from the values recorded when the rest of the doses were applied. Applying higher biostimulant doses resulted in a diminishing trend for both variables.

Ferreira *et al.* (2017) studied the effect of five concentrations of a biostimulant made of algae extract in the vegetative growth of grape seedlings and determined that increasing the dose reduced the evaluated variables. The increase in the high doses of biostimulants diminished metabolic processes (Ferreira *et al.*, 2018).

**Table 5.** Nitrogen, phosphorous, and potassium efficiency use by cacao seedlings in 2018 (g dry mass mg nutrient<sup>-1</sup>).

Enerplant <sup>®</sup> , mL ha <sup>-1</sup>	NUE	PUE	KUE
0	0,143 d	0,787 d	0,054 d
1	0,193 b	1,101 a	0,070 b
2	0,235 a	1,052 b	0,078 a
3	0,200 b	0,931 c	0,068 b
4	0,182 c	0,735 e	0,061 c
SE, $\bar{x}$	0,002*	0,013*	0,0009*

SE=Standard Error. \*Means with the same letters do not differ for  $p \leq 0.05$  according to Duncan's test. NUE=nitrogen utilization efficiency; PUE=phosphorus utilization efficiency and KUE=potassium utilization efficiency).



**Figure 1.** Relationship between Enerplant<sup>®</sup> doses and nutrient absorption and use efficiency by cacao seedlings, during the 2018 experiment. Means with different letters in each column are statistically different (Duncan,  $p \leq 0.05$ ). Upper case letters indicate different mean use efficiency values. Lower case letters indicate nutrient absorption values. Legend in capital letters the significance for the differences in the mean values of utilization efficiency. In lower case for nutrient absorption values. From left to right and top to bottom: nitrogen, phosphorus and potassium.

## CONCLUSIONS

*Theobroma cacao* L. seedlings had a positive response to the application of Enerplant<sup>®</sup>. Likewise, it significantly increased the morphological variables of cacao seedlings. In average, during the two years of the study, a 1 mL ha<sup>-1</sup> dose of Enerplant<sup>®</sup> significantly increased the height (24%), dry mass (30%), quality index (58%), and leaf area (56%) of the cacao seedlings. Doses above 1- and 2- mL ha<sup>-1</sup> diminished seedling growth. Finally, applying a 2 mL ha<sup>-1</sup> dose of biostimulant guaranteed greater N, P, and K absorption and use efficiency values by cacao seedlings.

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