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Original article

Influence of biostimulants on growth and yield of short cycle crops in Manabí, Ecuador

Eduardo Héctor-Ardisana^{1*} 

Antonio Torres-García² 

Oswaldo Fosado-Téllez¹ 

Soraya Peñarrieta-Bravo² 

Jorge Solórzano-Bravo³ 

Vicente Jarre-Mendoza³ 

Fabián Medranda-Vera³ 

José Montoya-Bazán³ 

¹Instituto de Posgrado, Universidad Técnica de Manabí, Ecuador

²Facultad de Ingeniería Agronómica, Universidad Técnica de Manabí, Ecuador

³Estudiante de la Maestría en Agronomía, mención Producción Agrícola Sostenible, Facultad de Ingeniería Agronómica, Universidad Técnica de Manabí, Ecuador

*Author for correspondence: ehectorardisana@gmail.com

ABSTRACT

The agricultural model of the Green Revolution has led to the gradual degradation of soils, which is motivated by the impacts it causes, among which stands out the indiscriminate use of synthetic fertilizers, which while increasing yields to some extent, also acidify the soil, accumulate salts, provoke hydric stress and pollute water and crops. Biostimulants are an alternative to these practices, as they are microorganisms or products that favorably influence plants, not only due to the contribution of nutrients but also because they contain substances such as proteins, amino acids, humic and fulvic acids, growth regulators, and other beneficial molecules. Preliminary results of several investigations on the use of organic biostimulants (bioles, manure and vermicompost leachates, humic acids, efficient microorganisms) on four short-cycle species are presented: stevia (*Stevia rebaudiana* Bertoni), Swiss chard (*Beta vulgaris* L. subsp. cicla), peanuts (*Arachis hypogaea* L.) and sesame (*Sesamum indicum* L.). Variables that characterize the growth and yield of each of the studied species were evaluated, during a

crop cycle, in experimental designs of completely randomized blocks. In general, the use of any of the biostimulants tested allowed similar or superior results to those obtained with chemical fertilization in the four species studied. The results obtained stimulate the use of these biostimulants as substitutes or complements of conventional chemical fertilization in these species, contributing to the sustainability of agricultural production without prejudice to the environment.

Key words: Swiss chard, sesame, plant stimulation, peanuts, *Stevia*

INTRODUCTION

In South America, agricultural production is diverse and complex, resulting from the traditions of each country, and it is obtained at the cost of a deep ecological footprint, because of the need for the countries of the subcontinent to remain as producers and exporters of agricultural items that they play an important role in their trade balances ⁽¹⁾. Ecuador does not depart from the tendency to exploit the land in an intensive and indiscriminate way. Among the inputs used to achieve adequate yields are nitrogen, phosphoric and potassium fertilizers, of which the country applied an average of 124.03 kg ha⁻¹ in 2014, a figure slightly lower than the average for the subcontinent, which is of 135.46 kg ha⁻¹ ⁽²⁾ but undoubtedly very high. In addition, most farmers apply fertilizers without considering the amounts of nutrients present in the soils or the needs of the crops, leading to excessive use that has negative environmental consequences.

One of the organic alternatives to stimulate the growth and development processes of plants is the use of biostimulants, which are defined as substances or microorganisms that, applied to plants, increase the absorption and assimilation of nutrients, their tolerance to stress or improve their agronomic characteristics, regardless of the content of nutrients they provide ⁽³⁾. Among the most used biostimulants are vermicomposting, its leachates and efficient microorganisms ⁽⁴⁻⁷⁾.

Stevia (*Stevia rebaudiana* Bertoni), chard (*Beta vulgaris* L. subsp. Cicla) and sesame (*Sesamum indicum* L.) are short-cycle crops that are not among the predominant ones in Ecuadorian agriculture. Peanuts (*Arachis hypogaea* L.), also short-cycle, although occupying much higher agricultural areas, is not a high-impact item in the country's agricultural system. However, consumer appreciation for these species is increasing: stevia for its sweetening and medicinal use ⁽⁸⁾, chard for its antioxidant and anti-cancer properties ⁽⁹⁾, peanuts for its high content of oils, proteins and carbohydrates ⁽¹⁰⁾ and sesame for the anticancer power of the sesamol contained in its seeds ⁽¹¹⁾.

Given this increase in demand and consequently in the interest of producers it is necessary to propose alternatives that allow obtaining high yields without creating a dependence on

synthetic chemical fertilizers, which would affect the economic and ecological sustainability of these agroecosystems.

This research aimed to evaluate the effect of various biostimulants on the growth and performance of these species, in comparison with fertilizer products of chemical-synthetic origin.

MATERIALS AND METHODS

Location of the experiments

The tests on stevia and chard were developed in the experimental campus "La Teodomira" of the Faculty of Agronomic Engineering of the Technical University of Manabí, located in the Lodana parish, Santa Ana canton, at 01°14' LS and 80°23' LW with an altitude 60 meters above sea level, between June and August 2018, under semi-protected cultivation conditions. The peanut study was carried out at the "Don Fabián" farm, located on the Chone-Calaceta road, at 0°50' LS and 80°15' LW with an altitude of 50 m a.s.l, in the period between August and December 2017. The study with sesame, it was carried out on the Montoya Bazán family farm, located on the Babahoyo-Febres Cordero parish road, at 02°09' LS and 79°53' LW with an altitude of 20 meters above sea level, between June and September 2018. The first three localities belong to the province of Manabí and the last to the province of Guayas, Ecuador.

Vegetal material

All the seeds used were of certified origin. In the stevia, the Morita 2 variety was used; in chard, the Fordhook Giant variety was used; in peanuts the variety "Criolla Caramelo" was used and in sesame the variety "Portoviejo 1". Stevia, chard and sesame seeds were sown in germinating trays with alveoli of 5 x 5 cm (one seed per alveolus) with decomposed soil and plant residues, in a 1:3 (v/v) ratio, and once the plants were obtained with adequate vigor they were transplanted to the place of the experiment. The peanuts were sown directly, placing two seeds per site to guarantee germination.

Bioestimulants

All the biostimulants used in this research are products of organic composition, conceived to provide producers with sustainable alternatives to chemical fertilization. Two of them (Bioactivated and Humisil®) are commercial formulations based on microorganisms or humic acids. The rest (bovine manure leachate, bovine manure vermicomposting

leachate, chicken manure leachate, artisanal biol, and efficient microorganisms) are products of simple manufacture from crop residues or animal excrement, which can be made by producers in their own farms.

The bovine manure leachate (LEB) and the vermicompost leachate of bovine manure (LVEB) were supplied by the Ministry of Agriculture, and are produced at the Cañita site of the Charapotó Parroquia del Sucre canton, Manabí province, Ecuador. The preparation of the chicken manure (LG) leachate was carried out in a 100 L tank to which 25 kg of chicken manure and 50 L of water were added. The mixture was left to ferment for 45 days and subsequently the leachate was filtered through a fine cloth. Leachates were applied with a manual pump by foliar spraying, and four applications were made every 10 days after transplanting (DAT) or sowing the crop, with a product volume equivalent to 1000 L ha⁻¹.

The Bioactivated product was supplied by Bbo Agro, S. A. from Guayaquil, Ecuador, and is a liquid biopreparation based on *Saccharomyces* sp., *Bacillus* sp., *Lactobacillus* sp. and actinomycetes. The artisanal Biol was prepared with organic products and harvest residues in the following proportions: 18 kg of bovine manure, 13.5 kg of alfalfa and pea harvest residues, 0.9 kg of yeast, 9 kg of molasses, 13.5 kg of rice husk and 4 liters of bovine milk serum. All components were homogenized in a plastic tank up to 200 L with water. Subsequently, the tank was covered and the mixture was left to ferment for 45 days in the shade, at room temperature. After this time the contents of the tank were filtered through a fine cloth. Both products were applied by spraying with a manual pump on the ground, next to the plant; three applications were made every 10 DAT, with a volume of product equivalent to 1000 L ha⁻¹.

Humisil® is produced by Greentech® in Guayaquil, Ecuador, and contains 18 % humic acids, 4.02 % N-P-K and 1.5 % active silica. Five foliar applications were made by spraying with a manual pump, every 15 DAT, with a volume of product equivalent to 1000 L ha⁻¹.

Efficient microorganisms (EM) were produced at the Experimental Station of the Faculty of Agronomic Engineering of the Technical University of Manabí, cultivating *Lactobacillus plantarum* (104 UFC L⁻¹), *Lactobacillus casei* (104 UFC L⁻¹), *Rhodopseudomonas palutris* (103 CFU L⁻¹), *Saccharomyces cerevisiae* (103 CFU L⁻¹), molasses 10 % and pasteurized milk 15 %. After adjusting the pH to 3.5 the mixture was allowed to ferment at room temperature under anaerobic conditions for 15 days, and then air was permanently supplied by pumping for 15 days. Finally, the mixture was filtered through a fine cloth. 5 applications were made every 10 DAT. In each one of them, 150 ml of the efficient microorganism preparation were supplied to the soil, around the plant.

Chemical fertilizers

The fertilization with N-P-K in each crop was by application to the soil carried out, with the formulations and doses used by the producers on their farms. Calcium was applied by foliar spraying, as recommended by various authors ^(12,13).

Experimental design and variables evaluated

In the experiments carried out in the four species, randomized complete block designs were used.

In the experiment with stevia, four replications were included, each with 24 plants, of which the 6 central plants of the plot were evaluated. The experimental treatments consisted of two calcium concentrations (2 and 5 g L⁻¹) applied foliar (10 mL plant⁻¹, 5 applications every 15 DAT), two dilutions of Humisil[®] (1/10 and 1/20 v/v), chemical fertilization with NPK (18-18-18; 20 g plant⁻¹ at 15 DAT) and a treatment without fertilizers or biostimulants. At 45 DAT the height of the plant (cm), the diameter of the stem (cm) and the number of leaves per plant were determined. The harvest of leaves and stems was carried out at 60, 75 and 90 days, and based on the total dry mass of these, the yield (t ha⁻¹) of each treatment was estimated.

In the study carried out on chard, three repetitions were used. Each replication included 24 plants, of which the 6 central plants of the plot were evaluated. The experimental treatments were: chemical fertilization with NPK (15-15-15; 20 g plant⁻¹, of which 10 g as background fertilization and 10 g at 15 DAT), two dilutions of vermicompost leachate from bovine manure (1/10 and 1/20 v/v) and two from efficient microorganisms (1/10 and 1/20 v/v). At 60 days the length and width of the leaf (cm), and the dry mass (g) and root volume (cm³) were determined. Two harvests were carried out at 38 and 60 days, and with the total fresh mass (g) of the biomass of both, the yield (t ha⁻¹) of each treatment was estimated.

The study with peanut included four replicates. The treatments consisted of three dilutions of bovine manure leachate (1/10, 2/10 and 3/10 v/v), three of poultry manure leachate (1/10, 2/10 and 3/10 v/v), three of vermicomposting leachate from bovine manure (1/10, 2/10 and 3/10 v/v), chemical fertilization with NPK (100-25-50; 5 g plant⁻¹, as background fertilization) and a treatment without fertilizers or biostimulants. At 60 days, the height (cm) of three plants taken at random in the center of the plot was determined. At the time of harvest (120 days) the amount of nodules present in the same plants was counted; 100 pods were also taken at random in each plot to which

the dry mass (g) was determined to estimate the yield of dry pods (t ha^{-1}). The grains of these pods were determined the dry mass (g) to estimate the yield of dry grains (t ha^{-1}). In the sesame experiment, three replications were established. The treatments were: Bioactivated (1/20 v/v), artisanal biol (1/20 v/v), chemical fertilization with NPK (50-25-50; 125 g plant^{-1} at 20 and 45 DAT) and a treatment without fertilizers or biostimulants. In 20 plants selected at random in each experimental unit, the following variables were measured: plant height (cm) at 110 DAT; loading height -the height at which the lowest capsule is located- (cm) at 60 DAT; number of capsules per plant at 130 DAT; at this last moment the dry mass (g) of the seeds of these capsules was determined to estimate the performance of each treatment (kg ha^{-1}).

Statistical analysis

After verifying the normality and homoscedasticity of the data (with the Kolmogorov-Smirnov and Levene tests, respectively) the data of the variables were processed with a simple analysis of variance. The means of the treatments were compared with the Tukey test ($p < 0.05$). The IBM SPSS Statistic v.21 software was used.

RESULTS AND DISCUSSION

Tables 1-4 show the results of the influence of the different products applied in each of the species on the growth and yield variables.

Table 1. Effect of the products applied on the growth and yield in stevia
(*Stevia rebaudiana* Bertoni)

Treatments	Height of the plant (cm)	Diameter of the stem (cm)	Amount of leaves	Yield (t ha^{-1})
Calcium 2 g L^{-1}	$45.04 \pm 0.25\text{ab}$	8.40 ± 0.56	$735.57 \pm 0.53\text{ab}$	$0.50 \pm 0.17 \text{ a}$
Calcium 5 g L^{-1}	$42.32 \pm 0.32 \text{ b}$	8.01 ± 0.52	$667.30 \pm 0.51 \text{ b}$	$0.38 \pm 0.11 \text{ c}$
Humisil® 1/10 (v/v)	$45.86 \pm 0.25 \text{ a}$	7.84 ± 0.50	$746.23 \pm 0.57 \text{ a}$	$0.48 \pm 0.16 \text{ a}$
Humisil® 1/20 (v/v)	$42.09 \pm 0.22 \text{ b}$	7.51 ± 0.50	$718.80 \pm 0.56\text{ab}$	$0.39 \pm 0.11\text{bc}$
NPK	$42.26 \pm 0.22 \text{ b}$	7.32 ± 0.50	$782.88 \pm 0.59 \text{ a}$	$0.46 \pm 0.15\text{ab}$
Soil without application	$40.31 \pm 0.20 \text{ c}$	7.12 ± 0.47	$659.20 \pm 0.50 \text{ b}$	$0.39 \pm 0.11\text{bc}$

Unequal letters indicate significant differences for Tukey's test with $p < 0.05$

In stevia, no significant differences were observed in the stem diameter variable. On the other hand, statistical differences were found in the remaining variables, highlighting in plant height Humisil® (1/10 v/v) and calcium (2 g L^{-1}); in the number of leaves, the best results were obtained with the treatment fertilized with NPK, with the two dilutions of Humisil® and with calcium (2 g L^{-1}). It is noteworthy that in these three variables the

treatments with Humisil® (1/10 v/v) and calcium (2 g L⁻¹) always produced statistically similar or superior results to those obtained with NPK. In yield, calcium (2 g L⁻¹), Humisil® (1/10 v/v) and fertilized treatment were at the same level, although the latter did not differ from the control without application.

In this species, studies on the use of biostimulants have focused on the inoculation of plants with growth-promoting rhizobacteria and arbuscular mycorrhizal fungi or combinations of these ^(14,15), and to a lesser extent microorganisms and organic products have been combined such as manure and vermicompost ⁽¹⁶⁾. The results obtained in these investigations, superior in all cases to those achieved with chemical fertilizers, have been attributed to an improvement in the absorption of nutrients by plants.

The yields obtained with the use of biostimulant products such as Humisil® are generally attributed not to the concentration of nutrients present in them, but to the contribution of humic acids. These substances activate plant metabolism, favorably affecting the absorption and assimilation of nutrients, the absorption of water, and increasing tolerance to different types of stress ^(3,4,6). As for calcium, this element plays an important role as a messenger in plant responses to environmental and hormonal signals ⁽¹⁷⁾. In stevia, in particular, calcium deficiencies cause necrosis in the leaf primordia, a reduction in the content of steviosides, brittle branches and short and thin roots ⁽¹⁸⁾. These effects can explain the results obtained in the variables height of the plant and number of leaves, determining components of the yield in this species.

Table 2. Effect of the products applied on the growth and yield of chard
(*Beta vulgaris* L. subsp cicla)

Treatments	Length of leaf (cm)	Width of leaf (cm)	Dry mass of the root (g)	Volume of root (cm ³)	Yield (t ha ⁻¹)
NPK	33.26 ± 3.68 c	17.71 ± 1.81 c	2.64 ± 0.48 c	22.60 ± 3.62 c	35.20 ± 0.92 b
LVEB 1/10 (v/v)	34.76 ± 3.15bc	18.16 ± 1.80bc	3.38 ± 0.93 b	26.53 ± 8.21bc	45.90 ± 1.09 a
LVEB 1/20 (v/v)	40.70 ± 5.94 a	19.47 ± 1.75ab	3.43 ± 0.88 b	28.26 ± 5.82ab	40.01 ± 1.27 a
EM 1/10 (v/v)	37.40 ± 3.77ab	18.89 ± 1.30bc	4.21 ± 1.29 a	30.33 ± 8.44ab	33.71 ± 1.62 b
EM 1/20 (v/v)	38.20 ± 4.76 a	20.61 ± 2.45 a	3.93 ± 0.63ab	32.66 ± 3.71 a	39.07 ± 1.02ab

LVEB: vermicompost leachate from bovine manure; EM: efficient microorganisms
 Unequal letters indicate significant differences for Tukey's test with p<0.05

In chard (Table 2), the application of biostimulants produced results that equaled or surpassed NPK fertilization in all the variables related to vegetative growth. Regarding the yield, the two dilutions of vermicompost leachates from bovine manure were significantly superior to fertilization with NPK; the treatment with efficient 1/20 (v/v) microorganisms did not differ from the leachate treatments or the NPK-fertilized

treatment; in turn, NPK fertilization did not show significant differences with the 1/10 (v/v) dilution of efficient microorganisms. That is, the application of either of the two biostimulants always led to yields equal to or greater than those obtained with NPK. The most widely used biostimulants in chard have been Kelpak, rich in auxins and cytokinins, produced from the seaweed *Ecklonia maxima*, and which increases the dry mass of leaves and roots ⁽¹⁹⁾, VIUSID, with a high content of various amino acids, which increases yields by 29.50 % ⁽²⁰⁾. Besides, efficient microorganisms, which lead to increases in chlorophyll content and improvements in the soil, but not to significant increases in biomass ⁽²¹⁾.

Table 3. Effect of the products applied on the growth and yield of peanuts
(*Arachis hypogaea* L.)

Treatments	Height of the plant (cm)	Nodules per plant	Dry pod yield (t ha ⁻¹)	Yield dry beans (t ha ⁻¹)
LEB 1/10 (v/v)	35.6 ± 3.84 c	75.7 ± 5.32 b	6.72 ± 0.23 b	3.99 ± 1.20 c
LEB 2/10 (v/v)	38.5 ± 2.91bc	82.3 ± 4.81 b	6.72 ± 0.37 b	4.27 ± 0.99bc
LEB 3/10 (v/v)	39.6 ± 3.64 b	84.3 ± 3.62 b	7.07 ± 0.45 ab	4.90 ± 1.11bc
LG 1/10 (v/v)	37.1 ± 3.40 c	82.2 ± 5.00 b	6.86 ± 0.61 b	5.04 ± 1.17 b
LG 2/10 (v/v)	41.4 ± 2.83 b	90.2 ± 5.23 ab	7.07 ± 0.39 ab	5.04 ± 0.94 b
LG 3/10 (v/v)	43.0 ± 3.67 a	92.1 ± 4.91 a	7.70 ± 0.33 a	5.32 ± 0.89ab
LVEB 1/10 (v/v)	39.1 ± 3.53 b	91.5 ± 4.97 a	6.72 ± 0.27 b	5.11 ± 1.02 b
LVEB 2/10 (v/v)	45.8 ± 4.01 a	94.0 ± 4.60 a	7.42 ± 0.39 ab	5.88 ± 1.16 a
LVEB 3/10 (v/v)	53.6 ± 3.82 a	95.0 ± 5.33 a	9.17 ± 0.40 a	6.86 ± 1.12 a
NPK	42.6 ± 3.65ab	86.2 ± 5.40ab	6.93 ± 0.22 ab	4.90 ± 1.04bc
Soil without application	34.6 ± 3.21 c	74.0 ± 4.38 b	5.60 ± 0.39 c	3.78 ± 1.21 c

LEB: leachate from bovine manure; LG: leachate from chicken manure; LVEB: vermicompost leachates from bovine manure.

Unequal letters indicate significant differences for Tukey's test with p<0.05

Several biostimulants (3/10 v/v chicken manure leachate, and 2/10 and 3/10 v/v bovine manure vermicompost leachates) reached high values in the variables of plant height, nodules per plant and dry pod yield obtained in peanuts (Table 3), similar to fertilization with NPK. In particular, with the 3/10 (v/v) chicken manure leachate and with the three dilutions of vermicompost leachate from bovine manure, nodulation levels were significantly higher than those of the soil to which no product was applied, although it is evident that in the soil there are populations of rhizobia compatible with the crop. Other authors have found similar increases in this symbiotic process in the presence of vermicompost ⁽²²⁻²⁴⁾.

The yield of dry grains with NPK was significantly by the vermicompost leachate exceeded from bovine manure in the 2/10 and 3/10 dilutions (v/v). In this species, the application of the biostimulant Stimulate[®], rich in auxins, cytokinins and gibberellins,

favors the production of vigorous and healthy seeds, alone or combined with chemical elements ^(25,26). The biostimulant Nutrifer® 202, although it includes several types of micronutrients of inorganic origin, also contains extracts of seaweed (*Ascophyllum nodosum*) that provide amino acids, vitamins and phytohormones, and acts on the general metabolism of peanut plants, favoring their growth, development and nodulation, especially in combination with *Bradyrhizobium* sp. ⁽²⁷⁾. The use of vermicompost has caused increases in the yields in this species ^(28,29) so similar effects can be expected in its leachates, as those used in this experiment.

Table 4. Effect of the products applied on the growth and yield in sesame
(*Sesamum indicum* L.)

Treatments	Height of the plant (m)	Height of load (cm)	Number of capsules per plant	Yield (kg ha ⁻¹)
Bioactivated	2.56 ± 0.18	1.26 ± 0.45	86.58 ± 2.81c	687.0 ± 6.23c
Artisanal Biol	2.66 ± 0.23	1.30 ± 0.40	103.55 ± 3.63b	796.0 ± 7.43b
NPK	2.59 ± 0.31	1.30 ± 0.46	119.68 ± 3.44a	1011.7 ± 6.81a
Soil without application	2.52 ± 0.60	1.23 ± 0.39	43.73 ± 4.01d	617.0 ± 5.98d

Unequal letters indicate significant differences for Tukey's test with p<0.05

No differences were observed in the height of the plants or in the loading height of sesame for the experimental treatments (Table 4). In the number of capsules per plant and yield, NPK fertilization significantly surpassed the other treatments. In this species, the use of marine algae (*Kappaphycus* and *Gracilaria*) or extracts of these, which contain amino acids, phytohormones and other organic compounds and which have increased yield, stands out ^(30,31). In another study the foliar application of extracts of seaweed and leaves of terrestrial plants exerted effects similar to those caused by the use of synthetic growth regulators ⁽³²⁾. The fact that no stimulant effects were found in the present research suggests the need to broaden the spectrum of biostimulants and their doses, in order to find a sustainable alternative for production. However, it is noteworthy that with the artisanal biol, 78.67 % of the yield obtained with NPK was reached, and that reached in the soil without application was exceeded by 22.48 %. It supports the possibility of its use as a friendly alternative to the environment, and encourages research with other doses of this product.

At present, there are no detailed studies on the physiological mechanisms of action of the large number of natural substances, microorganisms and more or less elaborated products that are considered biostimulants. This is largely due to the high costs of analysis of the composition of biostimulants, which conspire against the profitability of their use,

especially if the agroecological and sustainable approach to their use is taken into account (that the peasants produce their own biostimulants). On the other hand, it is known that biostimulants favor the growth and development processes of plants through the improvement of the availability of nutrients in the soil, the increase in the absorption and assimilation of mineral elements ⁽³³⁾, and the increase of the tolerance of vegetables to stress ⁽³⁴⁾, all of which leads to obtaining higher yields. The effects observed in this research are consistent with expectations, according to the definition of biostimulants offered by other authors ^(3,35).

CONCLUSION

In general, the biostimulants tested in the present study produce encouraging results, since they manage to equal or exceed those obtained with synthetic chemical fertilization in the species in which they were used.

RECOMMENDATIONS

- It would be convenient to carry out studies on a larger scale to corroborate these results, with a view to their future introduction in agricultural production as a sustainable alternative as total or partial substitute for fertilization with synthetic chemicals.
- It would also be useful to investigate the physiological mechanisms of action of these biostimulants in the species tested, as a way for the future formulation of other products of this type.

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