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Vermicompost Leachate-Based Biostimulant and its Effects on Physiological Variables and Yield of Different Crops in Manabí, Ecuador

Bioestimulante a base de lixiviados de vermicompost y sus efectos en variables fisiológicas y el rendimiento de diferentes cultivos en Manabí, Ecuador

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Abstract: Using large volumes of synthetic chemical fertilizers is a general practice in modern agriculture at a high economic and environmental cost. Biostimulants have become an alternative to this routine due to their ability to stimulate plant physiological processes without polluting soil and water. In Ecuador, however, little research has been carried out on the effect of biostimulants on the yield of crops of agricultural interest. The objective of this research was to determine the effect of foliar application of a biostimulant based on bovine manure vermicompost (VCLB) leachates on physiological variables and the yield of corn, cotton, and peanuts under field conditions and chard and five pepper hybrids in semi-protected cultivation under the agro-climatic conditions of the province of Manabí, Ecuador. The nine experiments carried out with the species and hybrids included various dilutions of VCLB and controls consisting of fertilization with nitrogen, phosphorus, and potassium, depending on the species, and soil without fertilizer. In all species, VCLB-induced plant length, chlorophyll content, and crop yield exhibit equal or higher statistical differences than those achieved under chemical fertilization (NPK). These results demonstrate the potential of this biostimulant as a sustainable alternative to produce these crops, reducing potentially the adverse effects of production on the environment under the tropical conditions of Manabí, Ecuador. We recommend corroborating these results with production-scale research.

Keywords: climatic change, environment, fertilization, plant physiology, plant production, sustainable agriculture.

Resumen: El uso de grandes volúmenes de fertilizantes químicos sintéticos es una práctica general en la agricultura moderna, a un costo económico y ambiental elevado. Los bioestimulantes a base de sustancias naturales se han convertido en una alternativa a esta rutina por su capacidad de estimular procesos fisiológicos vegetales sin contaminar el suelo y el agua. En Ecuador, sin embargo, se han llevado a cabo pocos estudios sobre los efectos de los bioestimulantes en el rendimiento de cultivos de interés agrícola. El objetivo de esta investigación fue determinar el efecto de la aplicación foliar de un bioestimulante a base de lixiviados de vermicompost de estiércol bovino (VCLB) sobre variables fisiológicas y el rendimiento de maíz, algodón y maní en condiciones de campo, y de acelga y cinco híbridos de pimiento en cultivo semiprotegido, en las condiciones agroclimáticas de la provincia de Manabí, Ecuador. Los nueve experimentos realizados con las especies y los híbridos incluyeron diversas diluciones del VCLB y controles consistentes en la fertilización con nitrógeno, fósforo y potasio según la especie, y suelo sin fertilizante. En todas las especies, el empleo del VCLB indujo longitudes de plantas, contenidos de clorofila y rendimientos estadísticamente iguales o superiores a los alcanzados con la fertilización química con NPK. Estos resultados demuestran las potencialidades de este bioestimulante como alternativa sostenible para la producción de estos cultivos, lo que permitiría reducir las afectaciones al medio ambiente en las condiciones tropicales de Manabí, Ecuador. Se recomienda corroborar estos resultados con investigaciones a escala de producción.

Palabras clave: agricultura sostenible, fertilización, fisiología vegetal, medio ambiente, producción vegetal, cambio climático.

Introduction

In 2021, corn, cotton, peanuts, chard, and peppers, considered transitory crops, occupied an approximate area of 390,000 hectares in Ecuador, representing 7.37 % of the total cultivated area according to the National Institute of Statistics and Censuses (INEC, 2022). Corn stands out, with 366,138 ha planted that year (FAOSTAT, 2021). These crops constitute an essential product in the national market, being part of the human and animal diet or used as raw material for manufacturing other products demanded by the population.

A severe problem in modern agriculture is that large amounts of synthetic chemical fertilizers are consumed to sustain yields, increasing production costs (Matassa et al., 2015). Unfortunately, only 30–40 % of the fertilizers added to the crops are used by plants, and the remaining percentage is lost by leaching (Bouwman et al., 2009, 2013; Matassa et al., 2015). It is conceivable that the fertilizers used in agriculture have contributed significantly to climate change since the Green Revolution began (Murray et al., 2015; Norderhaug et al., 2015).

Biostimulants are biopreparations based on natural substances that can replace or complement the application of chemical fertilizers in agriculture (Arumugam & Amalan, 2022). This stimulation can be carried out in various ways, including the contribution of growth-regulating substances (Aremu et al., 2015), the increase in the regulation of genes linked to hormonal processes (Barone et al., 2019), and the improvement of the physical and chemical properties of the soil (Pant et al., 2011, 2012), among others. Thus, biostimulants can improve crop tolerance to biotic and abiotic stress (Iwuagwu et al., 2022; Veobides et al., 2018).

Their regulatory effects on the physiological processes of plants, reducing problems caused by stress, stimulating growth and development, and ultimately increasing yield, have sparked the interest of researchers, academia, and industry, resulting in the use of these products as a global trend.

Among the most recently evaluated biostimulants in various crops are vermicompost leachates from animal manure. The leaching of the compost obtained with worms is a completely natural product, rapidly absorbed by crops, both through the roots or the foliar route, with an important contribution of humic and fulvic acids to the soil (Héctor et al., 2020), stimulating various physiological processes, including nutrient absorption (Anitha, 2020). The favorable effects of vermicompost and its leachates on plant physiology and yield have been evidenced in various crops like lettuce (Demir, 2019), pepper (Alcívar et al., 2021), chard and strawberries (Alemán et al., 2022). In Ecuador, a significant increase in sales of biostimulants from various origins has been registered in recent years due to their acceptance and proven usefulness, being included in the products registered for use in agriculture (AGROCALIDAD, 2020). However, few scientific studies have been carried out that compare the effects of biostimulants on the yield of crops of agricultural interest with those of synthetic chemical fertilizers.

The objective of this research was to determine the effect of foliar application of a biostimulant based on bovine manure vermicompost leachates on the yield of corn, cotton, and peanuts under field conditions and chard and five pepper hybrids in semi-protected cultivation under the conditions of the province of Manabí, Ecuador. According to the results reported in the

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literature on the properties of biostimulants, and in particular vermicompost leachates, an increase in the yields of crops treated with this biostimulant is expected.

Materials and Methods

Assays on corn, cotton, chard, and pepper hybrids were carried out at the "La Teodomira" Experimental Campus, located in the Lodana parish, Santa Ana canton, belonging to the Faculty of Agronomy Engineering of the Technical University of Manabí, Ecuador, located at 01º09' S and 80º21' W, 60 masl. A peanut experiment was carried out on the "Don Fabián" farm located at km 4 via Chone-Calceta, at coordinates 0°50' S and 80°15' W, 50 m a.s.l., in Manabí, Ecuador. The experiments were conducted under semi-protected (chard and pepper) and field (corn, cotton, and peanut) conditions in completely randomized block designs with four replications. Blocking and replications were used to minimize possible experimental errors due to fertility, slope, and soil moisture differences. The recommended fertilization doses with nitrogen, phosphorus, and potassium (NPK) from the technological chart for each species were used as controls, and control treatments without fertilization were also included.

The chemical composition of the soils where the peanut was grown was analyzed in the laboratory of the Pichilingue Experimental Station, province of Quevedo, belonging to the National Institute of Agricultural and Livestock Research (INIAP). For the remaining species and the vermicompost leachate biostimulant (VCLB), the analyses were performed in the Phytosanitary and Zoosanitary Regulation and Control Agency (AGROCALIDAD) laboratory. To categorize the number of nutrients in the soils (high, medium, or low), the regulations of the National Network of Soil Laboratories of Ecuador (RELASE, 2017) were followed.

The chard and pepper hybrid seeds were sown in germination trays with a mixture of sandy, clayey soil and well-decomposed plant residues in a 1:3 (v/v) ratio. After 25 days, the transplant was carried out in the semi-protected cultivation areas. The remaining species were sown directly in the field, and certified seeds were used for all species. The population density for each species was 62,500 plants ha^{-1} (chard), 25,000 plants ha^{-1} (pepper), 71,000 plants ha^{-1} (corn), 80,000 plants ha^{-1} (cotton), and 200,000 plants ha^{-1} (peanut).

Irrigation for chard, pepper, cotton, and corn was carried out by dripping, and sprinkling was used for peanuts according to the water requirements of each crop. The control of weeds was performed manually. The phytosanitary control relied upon chemical products, depending on the plant species and the pests that occurred.

The VCLB was produced at the Paraíso de los Ceibos Agricultural Association, located in La Cañita, Charapotó Parish, Sucre canton, Manabí, Ecuador, under technical advice from the Ministry of Agriculture and Livestock (procedure subject to intellectual property registration).
VCLB applications were made by foliar route between 15 and 90 days after sowing (DAS), every 15 days with a backpack SKU-BP01043 at a pressure of 43 PSI (295.55 kPa). The treatments consisted of different VCLB dilutions, depending on the species, and controls included chemical fertilization with NPK (16-16-16) or soil without fertilizer, as shown in Table 1. Chemical

fertilization in the treatment with NPK was done before planting superficially, except for corn, in which two variants were used (superficially at 5 cm from the sowing line and buried 10 cm deep in the sowing line).

Table 1. Experimental treatments with VCLB in the studied species

Species and cultivar	Sowing and harvest dates	Treatments (VCLB dilutions) (v/v)	Controls
Cotton (*Gossypium hirsutum* L.) Cv Alcalá 90	May–December 2019	2:10 3:10 2:10 + NPK 3:10 + NPK	NPK Soil without fertilizer
Corn (*Zea mays* L.) hybrid Pioneer 4039	July–December 2019	2:10 3:10	NPK (surface fertilization) NPK (buried fertilizer)
Peanut (*Arachis hypogaea* L.) var Criollo Caramelo	August–December 2017	1:10 2:10 3:10	NPK Soil without fertilizer
Chard (*Beta vulgaris* L. subsp. cicla) var Fordhook Giant	May–December 2018	1:10 1:20	NPK
Pepper (*Capsicum annuum* L) hybrid Salvador	May–December 2018	1:10 1:20 1:30	Soil without fertilizer
Pepper (*Capsicum annuum* L) hybrid Quetzal	May–December 2018	1:10 1:20 1:30	NPK Soil without fertilizer
Pepper (*Capsicum annuum* L) hybrid Canario	May–December 2018	1:10 1:20	NPK Soil without fertilizer
Pepper (*Capsicum annuum* L) hybrid Odín	May–December 2018	1:20 1:30	NPK Soil without fertilizer
Pepper (*Capsicum annuum* L) hybrid Nathalie	May–December 2018	1:10 1:20 1:30 1:40 1:50	NPK Soil without fertilizer

Source: Prepared by the authors

Plant length (cm) was determined with a flexometer at harvest time. Chlorophyll content (SPAD units) was measured with a portable SPAD502 Plus Chlorophyll Meter (Minolta) 15 days after the first VCLB application on the second fully developed leaf at 10 am. The measurements of both variables were taken in 6 plants of each replicate for a total of 24 plants.

Crop yield (t. ha^{-1}) was estimated from the production of 16–24 plants (depending on the species) and the area occupied by each plant.

The normality and homoscedasticity of the data obtained were verified using the Shapiro-Wilk and Levene tests, respectively. Data were processed by simple analysis of variance and means of treatments and controls for each variable, and species were compared with Tukey's test ($p < 0.05$). For the analysis, the IBM® SPSS® Statistics v.21 software was used.

Results and Discussion

Chemical Composition of Soils and Biostimulant

Generally, the soils in which the experiments were carried out can be considered suitable for agricultural use (RELASE, 2017). The composition of soils is shown in Table 2.

Table 2. Chemical composition of the soils used in the experiments

Parameter	Pepper and chard	Corn	Peanut
Ph	6.72 (AN)	6.79 (AN)	5.9 (MA)
Organic matter (%)	1.60 (L)	2.01 (L)	2.78 (L)
Nitrogen (%)	0.08 (L)	0.10 (L)	38 (M)
Phosphorus (ppm)	68.8 (H)	24.4 (L)	28 (L)
Potassium (cmol kg^{-1})	2.28 (H)	1.40 (H)	0.87 (H)
Calcium (cmol kg^{-1})	18.07 (H)	15.1 (H)	13 (H)
Magnesium (cmol kg^{-1})	4.82 (H)	5.55 (H)	3.2 (H)
Iron (ppm)	< 20 (H)	23.3 (M)	96 (H)
Manganese (ppm)	7.59 (M)	3.34 (L)	5.7 (H)
Copper (ppm)	4.63 (H)	3.24 (M)	6.6 (H)
Zinc (ppm)	1.60 (L)	1.60 (L)	7.5 (H)
Boron (ppm)	-	-	0.18 (H)

AN:almost neutral; MA: moderately acid; L: low; M: medium; H: high.
Source: Laboratories of AGROCALIDAD (pepper, chard, and corn) and INIAP (peanut)

The sandy, clayey soil in which the experiments were carried out with hybrids of pepper and chard, under semi-protected conditions, contains high amounts of several macro-elements (P, K, Ca, Mg, and Cu), with a medium amount of Mn, and the content of N, Fe, Zn, and organic matter was low. The sandy, clayey soil analysis where the corn experiment was carried out

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showed high amounts of K, Ca, and Mg, with medium content of Fe and Cu, while N, P, Mn, Zn, and the organic matter appeared in low proportions. The analysis of the clay loam soil for the peanut experiment reflects high contents for the nutrients K, Ca, Mg, Fe, Mn, Cu, Zn, and, in average quantity, N. At the same time, low amounts were recorded for organic matter, P, and B.

The chemical analysis of the VCLB applied in the experiments showed that all the determined mineral elements were found in low amounts (Table 3).

Table 3. Chemical composition of VCLB

Parameter (%)	Value
Nitrogen	0.18 (Low)
Phosphorus	0.0015 (Low)
Potassium	0.2780 (Low)
Calcium	0.0180 (Low)
Magnesium	0.0503 (Low)
Iron	0.0003 (Low)
Zinc	0.0019 (Low)

Source: AGROCALIDAD

From these results, it can be inferred that any beneficial effect that VCLB can produce is not the result of the supply of nutrients but other substances that contribute to the physiological processes of the plants. Although some authors, such as Verma et al. (2018), have reported the presence of nutrients in vermicompost leachates, other found molecules such as humic and fulvic acids, amino acids, and the growth regulators were responsible for the stimulation (Esakkiammal et al., 2015; Héctor et al., 2020). This stimulating effect can help the plant better use the soil's inorganic nutrients and organic matter.

Effect of Biostimulants on Physiological Variables

The behavior of the plant length and the chlorophyll content of the plants in the different treatments is shown in Tables 4 and 5.

The longest plant length in cotton was reached in the combination of VCLB 1:20 (v/v) + NPK, significantly exceeding the other treatments. In corn, the plants treated with VCLB statistically equaled in height the ones fertilized on the surface, but not those that received the fertilizer buried. Using VCLB in the 3:10 v/v dilution in peanuts resulted in significantly taller plants than those fertilized with NPK. Similar results were found in chard when using VCLB in the 1:20 (v/v) dilution. In the Odín and Nathalie pepper hybrids, no significant differences were found between the treatments. In the three remaining hybrids, at least one dilution of VCLB equaled or statistically exceeded the control.

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Table 4. Effects of the VCLB on the studied species' plant length (cm)

Species and Cultivar	**Treatments (VCLB) and Controls**										
	1:10 (v/v)	**2:10 (v/v)**	**3:10 (v/v)**	**1:20 (v/v)**	**1:30 (v/v)**	**1:40 (v/v)**	**1:50 (v/v)**	**2:10 (v/v) + NPK**	**3:10 (v/v) + NPK**	**NPK**	**Soil Without Fertilizer**
Cotton (*Gossypium hirsutum* L.) Cv Alcalá 90	NP	68.3 ± 3.1 b	67.6 ± 3.1 b	NP	NP	NP	NP	87.8 ± 4.0 a	76.9 ± 3.8 b	72.7 ± 3.3 b	23.6 ± 0.3 b
Corn (*Zea mays* L.) hybrid Pioneer 4039	NP	2.01 ± 0.21 b	1.94 ± 0.28 b	NP	NP	NP	NP	NP	NP	2.28 ± 0.17 a (buried) 2.17 ± 0.18 ab (superficial)	NP
Peanut (*Arachis hypogaea* L.) var Criollo Caramelo	39.11 ± 2.91 c	45.79 ± 2.63 b	53.63 ± 3.48 a	NP	NP	NP	NP	NP	NP	42.59 ± 2.85 bc	NP
Chard (*Beta vulgaris* L. subsp. cicla) var Fordhook Giant	34.76± 3.68 bc	NP	NP	40.70± 5.94 a	NP	NP	NP	NP	NP	33.26 ± 3.68 c	NP
Pepper (*Capsicum annuum* L.) hybrid Salvador	59.0 ± 1.32 b	NP	NP	62.42 ± 1.86 a	62.42 ± 1.91 a	NP	NP	NP	NP	NP	55.08 ± 3.12 c
Pepper (*Capsicum annuum* L.) hybrid Quetzal	69.0 ± 1.93 ab	NP	NP	65.61± 1.43 bc	73.78± 2.65 a	NP	NP	NP	NP	61.00 ± 1.05 c	52.06 ± 2.13 d
Pepper (*Capsicum annuum* L.) hybrid Canario	105.8± 4.35 a	NP	NP	107.0± 3.31 a	NP	NP	NP	NP	NP	105. 8 ± 7.24 a	10.9 ± 4.22 b
Pepper (*Capsicum annuum* L.) hybrid Odín	NP	NP	NP	58.75 ± 11.7	63.19 ± 11.4	NP	NP	NP	NP	64.94 ± 9.12	NP
Pepper (*Capsicum annuum* L.) hybrid Nathalie	69.9 ± 4.87	NP	NP	69.9 ± 4.56	68.4 ± 5.23	71.0 ± 5.88	66.0 ± 5.32	NP	NP	67.0 ± 6.18	70.0 ± 3.86

Different letters in the row indicate significant differences for Tukey's test with $p < 0.05$ NP: Not performed. Source: Prepared by the author

Table 5. Effects of the VCLB on the chlorophyll content (SPAD units) of the studied species

Species and Cultivar	Treatments (VCLB) and Controls										
	1:10 (v/v)	2:10 (v/v)	3:10 (v/v)	1:20 (v/v)	1:30 (v/v)	1:40 (v/v)	1:50 (v/v)	2:10 (v/v) + NPK	3:10 (v/v) + NPK	NPK	Soil Without Fertilizer
Cotton (*Gossypium hirsutum* L.) Cv Alcalá 90	ND	10.3 ± 0.4 a	10.3 ± 0.2 a	NP	NP	NP	NP	7.7 ± 0.3 b	10.6 ± 0.4 a	9.6 ± 0.4 a	6.3 ± 0.3 b
Corn (*Zea mays* L.) hybrid Pioneer 4039	NP	42.14 ± 8.41 b	41.83 ± 7.84 b	NP	NP	NP	NP	NP	NP	52.87 ± 7.26 a (buried) 41.85 ± 5.1 b (superficial)	NP
Peanut (*Arachis hypogaea* L.) var Criollo Caramelo	46.71 ± 1.98 bc	7.70 ± 1.81 b	50.3 ± 1.14 a	NP	NP	NP	NP	NP	NP	45.78 ± 1.32 c	NP
Chard (*Beta vulgaris* L. subsp. cicla) var Fordhook Giant	45.13± 3.04 b	NP	NP	47.8 ± 3.74 a	NP	NP	NP	NP	NP	45.13 ± 3.46 b	NP
Pepper (*Capsicum annuum* L.) hybrid Salvador	47.65 ± 0.96 ab	NP	NP	46.53 ± 0.72 bc	48.2 ± 0.74 a	NP	NP	NP	NP	NP	46.18 ± 0.79 c
Pepper (*Capsicum annuum* L.) hybrid Quetzal	35.47± 0.47 c	NP	NP	48.72± 0.51 ab	51.56± 0.74 a	NP	NP	NP	NP	47.97 ± 0.38 b	47.43 ± 0.31 bc
Pepper (*Capsicum annuum* L.) hybrid Canario	50.69 ± 0.79 a	NP	NP	50.82± 0.57 a	NP	NP	NP	NP	NP	50.01 ± 1.24 b	49.51 ± 1.39 c
Pepper (*Capsicum annuum* L.) hybrid Odín	NP	NP	NP	48.42 ± 4.60	48.63 ± 4.42	NP	NP	NP	NP	48.49 ± 2.60	NP
Pepper (*Capsicum annuum* L.) hybrid Nathalie	47.33 ± 1.42 c	NP	NP	48.80 ± 2.18 c	47.67 ± 1.84 c	60.8 ± 2.11 ab	59.9 ± 1.36 ab	NP	NP	62.08 ± 1.85 a	59.55 ± 1.25 b

Different letters in the row indicate significant differences for Tukey's test with $p < 0.05$. NP: Not performed. Source: Prepared by the authors

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Biostimulants, and particularly vermicompost leachates, tend to boost plant length, as occurs in *Cucumis sativus* (El-Nemr et al., 2012), *Lactuca sativa* (Dudaš et al., 2016), and *Ocimum basilicum* (Kosem et al., 2022). These augments are generally associated with increases in yield due to the plant's biomass increase, which results in a greater production area.

Regarding the chlorophyll content, in cotton, the value reached for NPK was statistically similar to that achieved with VCLB (2:10 or 3:10 v/v) or with the combination of VLCB 3:10 v/v + NPK; the chlorophyll content for those treatments significantly exceeded that of plants grown in unfertilized soil. For corn, the chlorophyll content obtained after the two VCLB dilutions was statistically similar to surface chemical fertilization (possibly due to a low nitrogen availability in the soil), and these three treatments were lower than buried fertilization. Two VCLB treatments in peanuts (2:10 and 3:10 v/v) significantly outperformed chemical fertilization for this variable. One of the VCLB treatments (1:20 v/v) was statistically superior to NPK fertilization in chard. In the pepper hybrids, at least one of the VCLB dilutions equaled or exceeded the control, even in Odín, where no significant differences between the treatments were found.

The increase in the chlorophyll content by the action of various types of biostimulants has been demonstrated in several species such as *L. sativa* (Amanda et al., 2009), *Capsicum annuum* (Parađiković et al., 2011), *Zea mays* (Ertani et al., 2013), and *Solanum tuberosum* (Mystkowska, 2022). Unsuccessful attempts have been made to associate this event with changes in yield, probably because the physiological processes are much more complex due to the number of genes, enzymes, and metabolites involved, as pointed out by (Bulgari et al., 2015).

Effect of Biostimulant on Yield

Table 6 shows the results of applying the VCLB on crop yield. In cotton, the highest results, without statistical differences between them, were obtained with VCLB (3:10 v/v), the combinations of VCLB 2:10 v/v + NPK and VCLB 3:10 v/v + NPK, and the chemical fertilization. The VCLB (2:10 v/v) was located in a second level of statistical significance, and the control without fertilization obtained the poorest results. In this species, using vermicompost as a substitute or complement to inorganic fertilization has led to favorable results (El-Sayed & Abd El All, 2020; Fergusson, 2016; Jan et al., 2020).

The yield in corn in the two treatments with VCLB (2:10 and 3:10 v/v) did not show statistical differences with plants fertilized with NPK (buried or superficial). Calderín et al. (2016), using an extract of liquid humus by foliar route, increased the yield in this crop by approximately 28 %. García et al. (2020) used liquid livestock fertilizer by foliar route, obtaining results that exceeded the control without application. Álvarez et al. (2020) obtained similar results with bovine manure leachates at very low dilutions.

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Table 6. Effects of the VCLB on the estimated yields (t ha^{-1}) of the studied species

Species and Cultivar	Treatments (VCLB) and Controls									
	1:10 (v/v)	**2:10 (v/v)**	**3:10 (v/v)**	**1:20 (v/v)**	**1:30 (v/v)**	**1:40 (v/v)**	**1:50 (v/v)**	**2:10 (v/v) + NPK**	**3:10 (v/v) + NPK**	**NPK**	**Soil Without Fertilizer**
Cotton (*Gossypium hirsutum* L.) Cv Alcalá 90	NP	5.6 ± 0.3 b	6.3 ± 1.4 a	NP	NP	NP		7.0 ± 1.2 a	6.5 ± 1.1 a	6.1 ± 0.9 a	4.3 ± 0.9 c
Corn (*Zea mays* L.) hybrid Pioneer 4039	NP	8.83 ± 2.94	7.91 ± 3.71	NP	NP	NP	NP	NP	NP	9.63 ± 2.61 (buried) 8.83 ± 2.94(superficial)	NP
Peanut (*Arachis hypogaea* L.) var Criollo Caramelo	5.11 ± 1.02 b	5.88 ± 1.16 a	6.86 ± 1.12 a	NP	NP	NP	NP	NP	NP	4.90 ± 1.04 bc	3.78 ± 1.21 c
Chard (*Beta vulgaris* L. subsp. cicla) var Fordhook Giant	7.68 ± 1.09 a	NP	NP	7.67 ± 1.27 a	NP	NP	NP	NP	NP	6.68 ± 0.92 b	NP
Pepper (*Capsicum annuum* L.) hybrid Salvador	19.92 ± 0.94 b	NP	NP	23.21 ± 0.67 a	23.20 ± 0.46 a	NP	NP	NP	NP	NP	19.09 ± 0.72 c
Pepper (*Capsicum annuum* L.) hybrid Quetzal	7.41 ± 0.37 d	NP	NP	7.90 ± 0.27 ab	8.92 ± 0.32 a	NP	NP	NP	NP	7.64 ± 0.25 bc	7.48 ± 0.28 cd
Pepper (*Capsicum annuum* L.) hybrid Canario	33.65 ± 10.13 bc	NP	NP	38.33 ± 10.45 a	ND	NP	NP	NP	NP	32.10 ± 5.13 bc	26.07 ± 4.05 c
Pepper (*Capsicum annuum* L.) hybrid Odín	NP	NP	NP	26.99 ± 9.04	28.49 ± 5.19	NP	NP	NP	NP	25.09 ± 5.89	NP
Pepper (*Capsicum annuum* L.) hybrid Nathalie	43.14 ± 1.63 ab	NP	NP	43.21 ± 1.63 ab	44.72 ± 1.63 ab	41.6 ± 0.1 b	40.7 ± 0.1 b	NP	NP	45.94 ± 1.69 a	35.67 ± 1.75 c

Different letters in the row indicate significant differences for Tukey's test with $p < 0.05$. NP: Not performed Source: Prepared by the authors

Two of the VCLB treatments (2:10 and 3:10 v/v) led to significantly higher yields in peanuts than those obtained with NPK or unfertilized soil. The third treatment with VCLB (1:10 v/v) showed yields statistically similar to inorganic fertilization and significantly higher than those of the soil without fertilization. For peanuts, vermicompost was used instead of leachates as a substitute or complement to chemical fertilization. Mycin et al. (2010) reported high yields with the application of 4 t ha^{-1} of vermicompost; Ramos et al. (2019) found no differences between the yields obtained with vermicompost and with chemical fertilizers; Bekele et al. (2019) increased yields by combining vermicompost and the contribution of inorganic nutrients (N and P_2O_5).

The two treatments with VCLB in chard (1:10 and 2:10 v/v) significantly exceeded the yield obtained with NPK fertilization. Alemán et al. (2022) evaluated the effect of equine feces leachates on several agricultural species, including chard. They concluded they constitute an eligible alternative as organic fertilizer to replace inorganic fertilizers.

In the five hybrids of pepper, the average yield ranged between 19 and 24 t ha^{-1} in the Salvador hybrid, with significant differences in the 1:20 and 1:30 v/v dilutions compared to the 1:10 v/v dilution and to the plants that did not receive any application. The low values in the yield of the hybrid Quetzal (between 7.41 and 8.92 t ha^{-1}) compared to other hybrids were due to the effect of insect pests in the experiment; even so, the yield obtained with VCLB 1:30 (v/v) exceeded the other treatments, and no statistical differences were found between VCLB 1:20 (v/v) and chemical fertilization. In the Canario hybrid, the yields were between 26 and 39 t ha^{-1}, highlighting the VCLB 1:20 (v/v) above all the treatments. In the Odín hybrid, no significant differences were observed between yields (values between 25 and 29 t ha^{-1}) resulting from applying VCLB in the dilutions of 1:20 and 1:30 v/v and those achieved with NPK. In the Nathalie hybrid, the mean yield values ranged between 35 t ha^{-1} (soil without fertilizer application) and 46 t ha^{-1} (soil with NPK); however, the yields achieved with the VCLB dilutions 1:10, 1:20, and 1:30 (v/v) did not show statistical differences with those obtained under chemical fertilization.

Other authors have studied the effect of vermicompost and its leachates on pepper. Arancon et al. (2005) demonstrated that vermicompost made from bovine manure or paper waste can exceed the yields produced by inorganic fertilization. Álvarez et al. (2016) increased the yield of jalapeño pepper and onion by combining bokashi and vermicompost leachates. Alcívar et al. (2021) found that treatments based on leachates from vegetable and agro-industrial residues and vermicompost represent an alternative to enhance yield.

Biostimulants are substances or mixtures of them, including microorganisms, that stimulate physiological processes in plants (Du Jardin, 2015) and consequently allow them to behave efficiently in their environment. The low nutrient contents detected in the VCLB indicated that the yields obtained were not a direct contribution of mineral elements by the VCLB but to the stimulation of the plant's functioning.

The mode of action of biostimulants is mainly attributed to the presence of bioactive substances or secondary metabolites, which trigger a wide range of biochemical, physiological, and molecular responses, including an increase in nutrient uptake and efficient use (Rouphael &

Colla, 2020). The substances present in biostimulants include hormones, phenolic compounds, saccharides, and other organic components (Esakkiammal et al., 2015; Kocira et al., 2020) that collectively promote plant growth and reduce production costs.

The explanation for the complexity of these responses has been suggested by Yakhin et al. (2017), who observed it not only as a consequence of nutritional contributions, growth regulators, or protective substances separately but also due to interactions between these effects. Differences between plant species also affect the results of the application of biostimulants, which is why Baltazar et al. (2021) point out that their study at the molecular level, mainly in gene expression, could clarify the panorama and facilitate a more orderly and precise use of biostimulants.

In the long term, it seems unlikely that only with the application of VCLBs will it be possible to maintain high crop yields since they do not provide significant amounts of helpful chemical elements to plants (Esakkiammal et al., 2015) and plant absorption would gradually deplete soil nutrients. However, vermicompost leachate's favorable effects on yield and other organic or inorganic fertilizers have also been reported (Bidabadi et al., 2016). Consequently, applying biostimulants, such as the one used in this study, could reduce the employment of chemical fertilizers and contribute to a more efficient use of nutrients by plants. From an economic point of view, growers would also benefit from the low production cost of biostimulants compared to synthetic fertilizers and the possibility of producing biostimulants on their farms, adding value to their crop residues.

It is necessary to corroborate these studies, which are conducted on small plots of experimental design based on the estimation of yields on a larger scale at the production level, which would provide information on real yields. In this way, it would be possible to stimulate producers to use biostimulants as partial substitutes for synthetic chemical fertilizers.
Furthermore, developing a training program for this purpose would be helpful given the possibility that growers themselves can produce biostimulants on their farms, using crop residues as raw material.

Conclusions

Foliar application of VCLB induced similar or higher plant lengths and chlorophyll contents than chemical fertilization with NPK in cotton, corn, and peanut crops under field conditions and in chard and five pepper hybrids under semi-protected conditions.
The yields obtained with the VCLB applied by foliar route equal or exceed those achieved with chemical fertilization with NPK for all species.

These results demonstrate the potential of this biostimulant as a sustainable alternative to produce these crops, reducing the applications of synthetic chemical fertilizers and consequently reducing the effects of production on the environment under the tropical conditions of Manabí, Ecuador.

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Authors' Contributions

Torres-García, Antonio: design of methodologies, registration of information in the field, construction of databases, preparation of the manuscript; Héctor-Ardisana, Eduardo Fidel: registration of information in the field, construction of databases, supervision of activities, preparation of the manuscript; Zambrano-Gavilanes, Freddy Eli: registration of information in the field; León-Aguilar, Rolando: registration of information in the field; Fosado-Téllez, Osvaldo Alberto: analysis of information.

Ethical Implications

This study has no ethical implications.

Conflict of Interest

The authors declare no conflicts of interest in this study.

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