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BIOSTIMULATION IN COWPEA BEAN (*Vigna unguiculata* L. WALP) AS AN ALTERNATIVE TO SYNTHETIC CHEMICAL FERTILIZERS IN MANABÍ, ECUADOR

Eduardo Fidel Héctor Ardisana[1]*, Antonio Torres García[2] and Jorge Leonardo Paguay Macías[3]

[1] Universidad Técnica de Manabí, Instituto de Posgrado, Ave. Urbina y Che Guevara, Portoviejo, Ecuador
eduardo.hector@utm.edu.ec
https://orcid.org/0000-0003-1371-7345
[2] Universidad Técnica de Manabí, Facultad de Ingeniería Agronómica, Santa Ana, Ecuador
https://orcid.org/0000-0001-7095-051X
[3] Universidad Técnica de Manabí, Programa de Maestría en Agronomía mención Producción Agrícola Sostenible, Instituto de Posgrado, Ave. Urbina y Che Guevara, Portoviejo, Ecuador
https://orcid.org/0000-0002-0949-0806
* Corresponding author: eduardo.hector@utm.edu.ec

ABSTRACT

Cowpea bean (*Vigna unguiculata* L. Walp) is a nutrient-rich crop that can be produced in dry periods. As an alternative to synthetic chemical fertilization (SCF) in this species, the effects of a biostimulant (bovine manure vermicompost leachate, BMVL) on growth and yield variables were studied. The research was carried out at the Las Maravillas site, Rocafuerte canton, province of Manabí, Ecuador. Foliar application of four dilutions of BMVL (1:10, 1:20, 1:30 and 1:40 v/v) were made at 10, 20, 30 and 40 days after sowing. Two control treatments, one with SCF (NPK 15-15-15), and the other without fertilization or biostimulant (absolute control) were included. Biostimulation with BMVL significantly increased plant length with respect to both controls. Grain weight and estimated yield had higher values in the plants that received biostimulants compared to the absolute control. However, values were lower than those in the control with SCF, which may indicate that the use of biostimulants cannot fully replace the contribution of nutrients by SCF. The estimated yields in the dilutions of BMVL 1:10, 1:30 and 1:40 v/v, which reached values between 89 and 94% of those obtained with SCF, show that BMVL can be used as an alternative to SCF to achieve reasonable production levels at low economic and environmental cost in the cultivation of cowpea bean.

Keywords: biostimulants, vermicompost leachates, sustainable agriculture, legumes, plant phisiology.

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INTRODUCTION

Beans are legumes that are widely distributed throughout the planet for human consumption. They are cultivated in numerous countries on five continents, reaching a world production of about 29 million tons in 2019 and an average yield of 0.87 t ha^{-1}, and recording the highest production and consumption in countries with low income per capita (FAOSTAT, 2021).

According to the Agricultural Public Information System of Ecuador (SIPA, 2021), beans occupied an area of about 19 000 ha in 2020, with a production of almost 12 000 t, and a yield below the world average (0.63 t ha^{-1}). In the province of Manabí, the same source reports that 701 ha have been planted, with a production of 584 t and yield close to the world average (0.83 t ha^{-1}).

Cowpea bean (*Vigna unguiculata* L. Walp) is rich in many nutrients, but figures on global consumption are much lower than those of common bean (*Phaseolus vulgaris* L.). In fact, it is difficult to find specific statistics on cowpea production and yield, which indicate that data of different species are included in a single analysis. Cowpea cultivation is recommended for its contribution to soil fertility (Pereira et al., 2011) and as a useful alternative for land use in dry periods (Chiquillo, 2017), which significantly contributes towards sustainable agriculture by small farmers.

The agricultural practices of the Green Revolution include the use of synthetic chemical fertilization (SCF), intending to obtain high yields. In Ecuador, the average application of these products reached 386.8 kg ha^{-1} in 2018 (Knoema, 2021), almost three times higher than the world figure of 136.8 kg ha^{-1}. The excess of SCF is not used by plants, and these elements become soil and water contaminants (Rai and Shukla, 2020) or form volatile compounds that are released into the atmosphere (Larios et al., 2021). Many farmers also apply SCF without a previous analysis of the nutrient needs of their land, which contributes to their accumulation in the soil.

An alternative to the excessive application of SCF is the use of biostimulant products. These are substances or microbes that, when applied to plants, increase the absorption and assimilation of nutrients, improve stress tolerance or enhance their agronomic characteristics, regardless of the nutrient content they contain (Du Jardin, 2015). Diluted humic substances (Vujinović et al., 2020) and various microorganisms (Selvaraj, 2020) are examples of such products.

In recent years, research carried out in Mexico (Márquez et al., 2017), Colombia (Araujo et al., 2018), Peru (Cantaro et al., 2019) and Cuba (Torres et al., 2017; Calero et al., 2019) has shown the usefulness of organic products and microorganisms such as *Rhizobium* sp., *Trichoderma harzianum* and cyanobacteria to reduce nitrogen fertilization in common beans (*Phaseolus vulgaris* L.). A recent study conducted in Brazil revealed that cowpea beans can grow satisfactorily when wastewater from cattle farming is used for irrigation (Da Silva, 2021). In Ecuador, Ayón et al. (2017) managed to increase the vegetative growth of this species by applying STARLITE® biostimulant, which contains humic acids and microalgae extracts. Therefore, the objective of this research was to evaluate the effects of a biostimulant (vermicompost leachate from bovine manure) on the vegetative growth and estimated yield of cowpea beans (*Vigna unguiculata* L. Walp) in the province of Manabí, Ecuador.

MATERIALS AND METHODS

Location and description of the study area

The research was carried out from September to November 2019 at the Las Maravillas site, belonging to the Rocafuerte canton, province of Manabí, Ecuador, located at coordinates 0^{0}53’18” south latitude and 80^{0}27’20” west longitude, with an altitude of 8 meters above sea level. Its average temperature is 25.2^0 C, with an average rainfall of 540 mm and average relative humidity of 86 percent. The soil in which the study was carried out has a loamy-clay texture and shows a flat topography with slight undulations.

Description of experimental treatments

Two controls were used, one without fertilization or biostimulant (T_1, absolute control), and the other with the chemical fertilization commonly used by farmers in the area (T_2), consisting of NPK 15-15-15 applied at a rate of 10 g per plant, at 10 and 30 days after sowing (DAS). The biostimulant used was bovine manure vermicompost leachate (BMVL), which was produced at the La Cañita site, Charapotó Parish of the Sucre canton, province of Manabí, Ecuador, and supplied by the Ministry of Agriculture and Livestock. Four treatments (T_3-T_6) consisting of BMVL dilutions (1:10, 1:20, 1:30 and 1:40 v/v) were evaluated. Foliar applications of BMVL were performed at 10, 20, 30 and 40 DAS, using a backpack sprayer at a 1 000 L ha^{-1} spray application rate.

Experimental design

Seeds of the INIAP-463 variety (Mendoza and Linzán, 2005) were used. Seeds were sown at a row spacing of 0.5 m and 1 m between rows. A randomized complete block design was used, with six treatments and four replications. Each replication included 5 rows of 5 m length (20 m^2).

Agronomic management of the experiment

Sowing was done manually, using a spike at a depth of 5 cm. A drip irrigation system was used, with drippers located in the row 20 cm apart. Irrigation was carried out with a duration of 1.5 hours per week, distributed in 30 minutes three times per week, and a discharge of 2 L ha^{-1}. For the control of *Diabrotica* sp. and *Bemisia tabaci*, Actara® was applied in doses of 1 g L^{-1} at 11 and 23 DAS. For the control of *Oidium* sp, micronized sulfur was used in doses of 2 g L^{-1} of water with 20 ml of Agral at 43 DAS. Weed control was carried out manually, until completion of the experiment. Pods were harvested at 4-day intervals (62, 66 and 70 DAS) when formed grains were in the drying process.

Variables

Vegetative growth variables

Six plants were randomly selected (2 from each of the central rows of each replication) at 15, 25, 35 and 45 DAS, and the following variables were evaluated: plant length (cm), measured with a flex meter from the soil to the apex of the plant; stem diameter (cm), measured with a caliper at the base of the stem at 5 cm from the soil; and number of leaves, determined by counting the leaves of each plant.

At 62, 66 and 70 days (in the three harvest dates), 3 plants were randomly taken from those that were harvested in each repetition, and the volume of roots (cm^3) was determined by the glass cylinder method (Böhm, 1979).

Yield variables

At 62, 66 and 70 DAS, 6 randomly selected plants were harvested from the three central rows of each replicate, and the number of pods and grain weight (g) were determined. The values obtained were added cumulatively. The estimated yield of each repetition was obtained by converting grain weight data to t ha^{-1}.

Statistical analysis

After verifying the normality and homoscedasticity of the data by the Shapiro-Wilk and Levene tests, the data were processed through a simple variance analysis. For significant differences, the means were compared using the Tukey test with $p<0.05$. The IBM® Statistics® SPSS v.21 software was used.

RESULTS AND DISCUSSION

Effects of the biostimulant on vegetative growth variables

The results obtained for plant length, stem diameter, number of leaves and volume of the roots are shown in Tables 1-4. Regarding plant length, no significant differences were observed in the first sampling dates (Table 1). However, values obtained in all the BMVL treatments were higher than those in the control treatments (no fertilization and chemical fertilization) at 45 DAS. The concentrations of BMVL 1:10 and 1:30 (v/v) stood out at the same level of significance with BMVL 1:20 v/v and a second level BMVL 1:40 v/v, but being higher than both controls.

No significant differences were found between the experimental treatments in terms of stem diameter (Table 2), number of leaves (Table 3) or volume of roots (Table 4). However, Ayón et al. (2017) observed differences in plant length and stem diameter of cowpea due to the use of STARLITE® biostimulant, which contains humic acids. Furthermore, a study conducted by Santos et al. (2018) showed that the use of a biostimulant

Table 1. Effects of bovine manure vermicompost leachate on plant length of *Vigna unguiculata* L. Walp.

Experimental treatments	Plant length (cm)			
	15 DAS	25 DAS	35 DAS	45 DAS
T_1- No fertilized soil	12.31	34.73	44.86	53.15 c
T_2- NPK 15-15-15	12.32	36.94	47.02	53.90 c
T_3- BMVL 1:10 (v/v)	12.28	33.72	46.29	60.75 a
T_4- BMVL 1:20 (v/v)	12.56	34.18	49.48	59.97 ab
T_5- BMVL 1:30 (v/v)	12.29	12.13	48.54	61.00 a
T_6- BMVL 1:40 (v/v)	12.23	36.95	49.44	57.30 b

BMVL: bovine manure vermicompost leachate. DAS: days after sowing. Different letters in the same column indicate significant differences for Tukey test with p<0.05.

Table 2. Effects of bovine manure vermicompost leachate on stem diameter of *Vigna unguiculata* L. Walp.

Experimental treatments	Stem diameter (cm)			
	15 DAS	25 DAS	35 DAS	45 DAS
T_1- No fertilized soil	0.72	0.90	1.02	1.07
T_2- NPK 15-15-15	0.70	0.92	0.90	0.87
T_3- BMVL 1:10 (v/v)	0.65	1.00	1.05	0.90
T_4- BMVL 1:20 (v/v)	0.62	0.95	0.97	0.90
T_5- BMVL 1:30 (v/v)	0.60	1.00	1.05	1.05
T_6- BMVL 1:40 (v/v)	0.87	0.95	1.07	0.97

BMVL: bovine manure vermicompost leachate. DAS: days after sowing.

Table 3. Effects of bovine manure vermicompost leachate on the number of leaves of *Vigna unguiculata* L. Walp.

Experimental treatments	Number of leaves			
	15 DAS	25 DDS	35 DAS	45 DDS
T_1- No fertilized soil	5.47	8.60	15.50	20.80
T_2- NPK 15-15-15	5.32	8.15	15.42	20.77
T_3- BMVL 1:10 (v/v)	5.52	9.02	16.50	21.05
T_4- BMVL 1:20 (v/v)	5.62	8.82	16.07	21.60
T_5- BMVL 1:30 (v/v)	5.45	9.05	16.20	22.20
T_6- BMVL 1:40 (v/v)	5.95	8.87	16.07	22.20

BMVL: bovine manure vermicompost leachate. DAS: days after sowing.

Table 4. Effects of bovine manure vermicompost leachate on the volume of roots of *Vigna unguiculata* L. Walp.

Experimental treatments	Volume of roots (cm^3)		
	62 DAS	66 DAS	70 DAS
T_1- No fertilized soil	120.75	136.50	140.50
T_2- NPK 15-15-15	139.50	138.50	142.00
T_3- BMVL 1:10 (v/v)	121.05	125.75	130.75
T_4- BMVL 1:20 (v/v)	124.90	131.25	134.25
T_5- BMVL 1:30 (v/v)	133.55	137.25	141.25
T_6- BMVL 1:40 (v/v)	100.35	134.50	137.50

BMVL: bovine manure vermicompost leachate. DAS: days after sowing.

made from poultry manure inoculated with diazotrophic bacteria resulted in an increase in plant length and mass of the aerial parts and roots. The presence of humic substances and growth regulators in the BMVL could be associated with the results obtained.

Effects of the biostimulant on yield variables

No differences were found in the number of pods per plant between the experimental treatments (Table 5). Instead, the use of SCF led to the highest result in grain weight and estimated yield, which differed significantly from the other treatments. The second place in order of significance was occupied by BMVL 1:40 (v/v), followed by BMVL 1:10 and 1:30 v/v, and the concentration of BMVL 1:20 (v/v), which was at the same level of the non-fertilized control.

Table 5. Effects of bovine manure vermicompost leachate on yield variables from plants of *Vigna unguiculata* L. Walp.

Experimental treatments	Number of pods	Grain weight (g)	Estimated yield (t ha^{-1})
T_1- No fertilized soil	29.22	142.15 d	0.56 d
T_2- NPK 15-15-15	27.15	175.42 a	0.70 a
T_3- BMVL 1:10 (v/v)	28.93	155.35 c	0.62 c
T_4- BMVL 1:20 (v/v)	28.35	148.20 d	0.59 d
T_5- BMVL 1:30 (v/v)	29.42	156.75 c	0.62 c
T_6- BMVL 1:40 (v/v)	30.92	164.92 b	0.66 b

BMVL: bovine manure vermicompost leachate. DAS: days after sowing. Different letters in the same column indicate significant differences for Tukey test with $p<0.05$.

Cowpea has significant demands for macroelements (Santos et al., 2018) and microelements, such as iron and zinc (Guillén et al., 2021). It is known that bovine manure vermicompost leachates, such as that used in this research, do not provide nutrients in substantial amounts (Héctor et al., 2020). Accordingly, the most outstanding results have been obtained with SCF.

The presence of growth regulators (cytokinins, auxins, abscisic acid, gibberellins and brassinosteroids) in biostimulants has been demonstrated by Aremu et al. (2015), while there is evidence that these substances play a role on the regulation of genes linked to hormonal response (Barone et al., 2019). Unlike the large amounts of nutrients provided by SCF, these are the effects that can explain the effect caused by biostimulants.

It is interesting that even when the highest results in the estimated yield were obtained with SCF (0.70 t ha^{-1}), 0.66 t ha^{-1} was reached with BMVL 1:40 v/v, which represents approximately 94% of that performance. The yields obtained with BMVL 1:10 and 1:30 v/v are not negligible either, approaching 89% of those obtained with SCF. In those cases, the use of biostimulants led to higher estimated yields with respect to the non-fertilized control by 10,7-25%. A study conducted by Márquez et al. (2017) in *Phaseolus vulgaris* L. showed that the use of *Trichoderma harzianum* allowed reducing nitrogen fertilization to 50 %, reaching yields with respect to SCF that were similar to those obtained in the present study. In addition, Cantaro et al. (2019) used *Rhizobium* sp., recording 90 % of the yields obtained with nitrogen fertilization in the same species. More recently, Harireddy and Dawson (2021) found stimulatory effects on cowpea yield by applying a mixture of vermicompost, *Rhizobium*, and *Azospirillum*. In the present study, although the use of BMVL alone in cowpea does not allow achieving yields similar to those produced with SCF, it could become an alternative to achieve reasonable production levels at a low economic and environmental cost.

CONCLUSIONS

The application of a bovine manure vermicompost leachate (BMVL) in cowpea (*Vigna unguiculata* L. Walp) stimulated plant length with respect to the non-fertilized control and the control with synthetic chemical fertilization. In the plants treated with BMVL, values for grain weight and estimated yield were higher than those of the untreated plants, but lower than those obtained in the plants to which synthetic chemical fertilizer was applied.

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