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Biostimulant spraying in the sowing furrow improves the initial growth of commom bean

A aplicação de bioestimulante no sulco de semeadura estimula o crescimento inicial do feijão-comum

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Abstract: The effects of doses of two biostimulants applied in the sowing furrow compared to the traditional seed treatment (ST) were evaluated through yield components. The experiment was carried out in randomized block design, with eight treatments [control (T1); doses of formulation 1 (0.2; 0.3; 0.4 L ha^{-1} – T2, T3, T4); doses of formulation 2 (0.25; 0.5; 0.75 L ha^{-1} – T5, T6, T7); commercial dose of formulation 1 as ST (T8)] and six replicates. The dose of 0.5 L ha^{-1} of formulation 2 promoted final plant stand 6.5% and 5.6% higher compared to the control and to the commercial standard, respectively, and the dose of 0.75 L ha^{-1} of the same formulation provided seedling emergence 21.4% faster than the commercial standard, which shows that these doses improved plant initial development. However, applying biostimulants in the sowing furrow and the seed treatment provided similar results for the yield components and productivity.

Keywords: Biostimulation. *Phaseolus vulgaris* L. Seed treatment.

Resumo: Os efeitos de doses de dois bioestimulantes aplicados no sulco de semeadura em relação ao tratamento padrão de sementes (TS) foram avaliados através dos componentes de produção. O experimento foi conduzido em blocos casualizados, com oito tratamentos [controle (T1); doses da formulação 1 (0,2; 0,3; 0,4 L ha^{-1} – T2, T3, T4); doses da formulação 2 (0,25; 0,5; 0,75 L ha^{-1} – T5, T6, T7); dose comercial da formulação 1 como TS (T8)] e seis repetições. A dose de 0,5 L ha^{-1} da formulação 2 promoveu estande final de plantas 6,5% e 5,6% superior ao verificado na testemunha e no padrão comercial, respectivamente, e a dose de 0,75 L ha^{-1} da mesma formulação proporcionou emergência 21,4% mais rápida que o padrão comercial, demonstrando que essas doses melhoraram o desenvolvimento inicial das plantas. No entanto, a aplicação de bioestimulantes no sulco de semeadura e no tratamento de sementes proporcionou resultados semelhantes para os componentes de rendimento e produtividade.

Palavras-chave: Bioestimulação. *Phaseolus vulgaris* L. Tratamento de sementes.

1 Introduction

The world's largest bean producers are Myanmar, India, Brazil, China, and Tanzania (Faostat, 2021). This is one of the main produced grains in Brazil, and its growth extends almost all over the territory. Production in the 2022/23 harvest was estimated at 3.07 million tons (Conab, 2023). In these circumstances, the importance of this crop in the Brazilian economic context is evident, but to meet the demand is necessary to maintain high productivity, even under adverse environmental conditions.

Common bean is essential in the population feed of developing countries in tropical and subtropical regions, such as Brazil, due to their high content of proteins, lysines, dietary fibers, carbohydrates, and mineral complexes, such as calcium, iron, and others, in addition to vitamins of the B complex (Conab, 2018; Araujo, Urbano, González-Andrés, 2020).

Biostimulants are the mixture of two or more products based on hormones, amino acids, seaweed, vitamins, humic substances, and micronutrients. The main effects of these products are based on its ability to improve the plant’s adaptive responses to environmental stresses since it increases the absorption and availability of nutrients, increases the efficiency of defense mechanisms, physiological activities, essential metabolites synthesis, and in the growth of shoot and root system, improving crop development and productivity (Povero, Mejia, Di Tommaso, Piaggesi, Warrior, 2016).

Biostimulants can be applied via seed treatment, foliar, and sowing furrow (Kumari, Tzudir, Gohain, Singh, 2023). One of the main ways to use these products is in seed treatment, as they can be associated with micronutrients to minimize the problems arising from nutritional deficiency during germination and development (Castro, Bogiani, Silva, Gazola, Rosolem, 2008; Kumari *et al*., 2023). However, alternatives to this method are being studied. Although poorly reported, the application of biostimulants in the sowing furrow, in which spray tips are coupled to the grain seeder, leading to the simultaneous deposition of seeds and products in the planting furrow, has already promoted increases in the agricultural productivity of some crops, such as common bean and sugarcane (Dourado Neto, Dario, Barbieri, Martin, 2014; Santos *et al*., 2020).

In this context, the objective of this study was to evaluate the effect of doses of two biostimulants applied in the sowing furrow compared to the traditional seed treatment through evaluations of common bean crop yield components.

2 Materials and methods

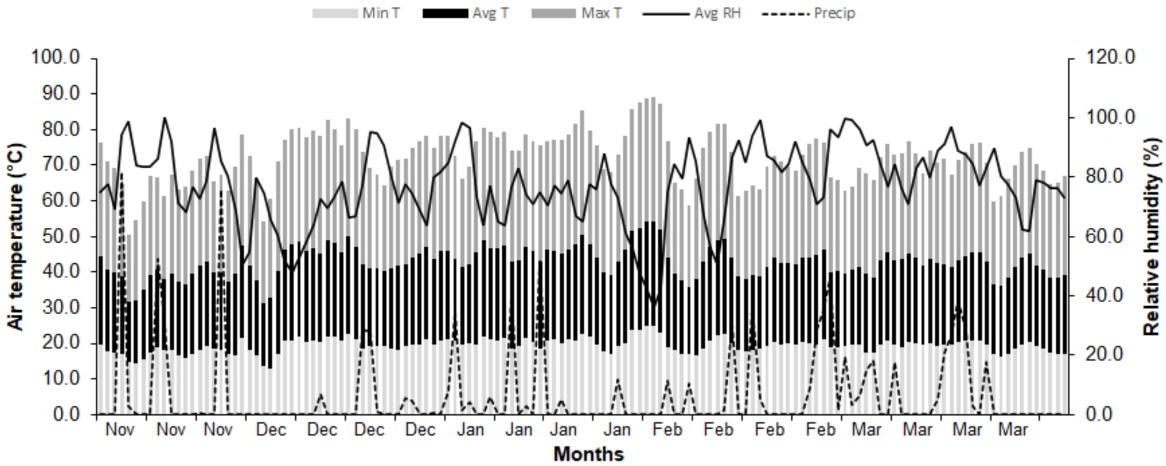
2.1. Description of the experimental area and treatments

The experiment was carried out in the 2018/19 season in an experimental area of Lageado Farm, belonging to the School of Agricultural Sciences of UNESP, located in Botucatu (22º50'30" S, 48º25'26" O and 788 m asl), in the state of Sao Paulo, Brazil.

The region's climate has been characterized by Thornthwaite's methodology as $B_2rB'_3a'$, a humid mesothermal climate with low water deficits in April, July, and August. The annual average air temperature is 20.3°C, and the yearly rainfall is 1,428.4 mm. The weather data recorded during the experiment conducting are demonstrated in Figure 1.

The soil of the experimental area has been classified as clayey texture Dystrophic Red Latosol (RLdf1). The soil chemical analysis was performed at 0−20 cm depth before the experiment was set up, and the basis fertilization used was 150 kg ha^{-1} of NPK in the formulation 00-20-20 (Raij, Cantarella, Quaggio, Furlani, 1996).

Figure 1. *Minimum, mean, and maximum air temperature mean relative humidity and precipitation from November 2018 to March 2019.*



Source: *Prepared by the author (2020)*

The cultivar IAC Imperador was used, and the planting occurred on November 21, 2018, with a plant density of 13 seeds per linear meter, in a no-till cropping system, using spacing between rows of 0.45 m, each experimental unit was constituted of plots with 2.25 m width and 10.0 m length, for a total of 22.5 m^2.

All plots received standard seed treatment with the inoculant *Rhizobium tropici* (0.1 L 25 kg^{-1} of seeds) and the agrochemicals Carboxin + Tiram + Fipronil at doses of 0.3 L, 0.3 L, and 0.2 L 100 kg^{-1} of seeds, respectively.

For the spraying of biostimulants in the sowing furrow (T2 to T7), an application kit was used (Inocula Sulco, Mecmaq, Piracicaba, SP, Brazil) (Figure 2), adapted for application with CO_2 pressurization equipment, with solid stream spray nozzles (JS 0.78, KJF, Vinhedo, SP, Brazil) and flow rate of 50 L ha^{-1}. The equipment was fixed in the seeder chassis, and each sowing unit included a spray tip positioned between the planting furrow's opening discs.

Only T8 received traditional seed treatment with biostimulant; the seeds were packaged in a plastic bag, to which formulation 1 in the dose of 0.3 L 100 kg^{-1} of seeds was added, then the mixing was performed until achieving a homogeneous coverage.

Figure 2. *Equipment used for the application of biostimulants in the sowing furrow. The blue hose refers to spray tips coupled to the seeder.*

Source: *Prepared by the author (2020)*

2.2. Experimental design

The experiment was carried out in a randomized block design with eight treatments and six replicates. Treatments were constituted of a control (T1), three increasing doses of formulation 1 (F1) (T2, T3, and T4), three increasing doses of formulation 2 (F2) (T5, T6, and T7), and traditional seed treatment in the commercial dose of formulation 1 (T8), which was used as a commercial standard of comparison (Table 1).

Table 1. *Products, doses, and application methods of treatments.*

		Application method – Dosage	
	Treatments	**Sowing furrow (L ha^{-1})**	**Seed treatment (L 100 kg^{-1} of seeds)**
1	Standard	-	-
2	Formulation 1	0.2	-
3	Formulation 1	0.3	-
4	Formulation 1	0.4	-
5	Formulation 2	0.25	-
6	Formulation 2	0.5	-
7	Formulation 2	0.75	-
8	Formulation 1 (Commercial Standard)	-	0.3

Source: *Prepared by the author (2020)*

F1 and F2 concern a mixed mineral foliar fertilizer and a liquid organomineral fertilizer, respectively. F1 is constituted by 2.0% of N (nitrogen)-total, 0.4% of N-NH_4, 1.4% of N-NO_3, 1.0% of P_2O_5 (H_2O), 2.0% of K_2O, 1.3% of sulfur (S), 0.11% of boron (B), 0.4% of iron (Fe), 1.3% of manganese (Mn), 2.8% of zinc (Zn), 108 g L^{-1} of carbon (C) and density of 1110 kg m^{-3}. F2 is constituted by 1.0% of N-total, 0.4% of N-NH_4, 0.3% of N-NO_3, 1.0% of P_2O_5 (H_2O), 7.0% of K_2O, 3.8% of S, 0.02% of B, 5.0% of Zn, 105 g L^{-1} of C and density of 1200 kg m^{-3}.

2.3. Evaluations

The emergence speed index (ESI) was calculated using data from the daily counts of emerged seedlings until 15 days after sowing (DAS), according to the equation described by Maguire (1962).

The final plant stand (FPS) was determined one day before harvest. Counting the plants in two meters of the central rows in each experimental unit was performed, and the results were expressed in plants m^{-2}.

The total number of pods per plant (TNPP) was performed one day before harvest, evaluating eight plants per plot.

The plants were harvested manually, considering three central rows of each plot, and discarding 2.5 m from each end, corresponding to an inner plot of 6.75 m^2.

After harvest, the plants were tracked, and the 100-grain mass (100GM) and grain yield corrected to 14% humidity were determined, employing a precision balance (Shimadzu, BL-3200H, Piracicaba, SP, Brazil).

2.4. Statistical analysis

Data were submitted to analysis of variance by the F-test, with subsequent comparison of means using the Tukey test ($p \leq 0.05$) through the statistics program Minitab®. The effects of doses of biostimulants were evaluated through a polynomial regression analysis, in which variables with $R^2 \geq 0.70$ were discussed, and treatment 1 was used as a comparative standard (Banzatto & Kronka, 2006).

3 Results and discussion

The treatments affected FPS and ESI, while TNPP, 100GM, and productivity of common bean plants were not significantly affected (Table 2).

The dose of 0.5 L ha^{-1} of F2 applied in the sowing furrow provided FPS, on average, 6.5% and 5.6% higher than the control and the commercial standard, respectively (Table 2).

Zinc (Zn) is an essential element for plants and can interfere with the growth and metabolism of plant species when there are toxic or insufficient levels of its concentration (Marschner, 2012). Some studies indicate a beneficial effect of zinc on germination and physiological quality of seeds for several species, such as common bean (Dörr *et al.*, 2017), maize (Itroutwar, Kasivelu, Vasantharaja, Malaichamy, Sevathapandian, 2020), and wheat (Rai-Kalal & Jajoo, 2021).

Thus, it is inferred that the higher FPS verified in the intermediate dose of F2 is related to the higher proportion of Zn found in the composition of this product, which may have led to the increase in germination, initial seedling growth and, consequently, in the final stand (Tamindžić *et al*., 2021).

The emergence speed is one of the parameters used to determine the vigor in a seed lot, which will result in seedlings with higher rate of development and ability to use the storage tissue reserves (Gonçalves, Cicero, Abud, 2017). Seed treatment with biostimulants, fertilizers, amino acids, and growth-promoting micro-organisms is one of the techniques used to promote quick germination and emergence and standardize crop canopy formation (Binsfeld, Barbieri, Huth, Cabrera, Henning, 2014).

The highest dose of F2 (0.75 L ha^{-1}) presented the greatest ESI among the treatments. It promoted seedling emergence 21.4% faster than the lowest dose of F1 and the commercial standard (Table 2).

Solino, Oliveira, Schmitt, Silva, and Schwan-Estrada (2020), studying the germination and emergence of common bean seedlings under inoculation with biostimulators, found that seeds treated with *B. subtilis* BV02 and *T. asperellum* BV10 showed ESI 45% higher than untreated seeds. Similarly, Melo *et al*. (2018), studying peanut seeds, and Badawi, Seadh, and Emhimmid (2020), studying wheat seeds, verified that phytohormones provided positive effects when applied via seeds, with a growing increase in ESI.

The fact that the use of biostimulants improves the water use efficiency, nutrient absorption, and increases the growth of the shoot and root system, allows better growth and initial crop development, favoring the formation and establishment of a good plant stand (Albrecht *et al*., 2011), as verified in the present study.

In addition, Wijewardana *et al*. (2018) verified that biostimulants are more efficient when plants are subjected to stress conditions because it stimulates strategies in plants that allow partial maintenance of their growth and development under water deficiency. At the beginning of the cultivation, there was low rainfall (Figure 1), which may have enabled the potential effects of the formulations applied in the sowing furrow to be noticed in the ESI and FPS.

Table 2. *Final plant stand (FPS), emergence speed index (ESI), total number of pods per plant (TNPP), 100-grain mass (100GM), and grains yield of common bean plants under the application of biostimulants in the sowing furrow.*

		Analyzed variables				
	Treatments	**FPS**	**ESI**	**TNPP**	**100GM (g)**	**Yield (kg ha^{-1})**
1	Standard	10.1 b	5.0 ab	10.8 a	24.1 a	2,586.7 a
2	Formulation 1	10.3 ab	4.4b	12.3 a	24.4 a	2,610.0 a
3	Formulation 1	10.6 ab	5.1 ab	11.3 a	24.3 a	2,634.7 a
4	Formulation 1	10.6 ab	5.4 ab	11.7 a	24.5 a	2,628.8 a
5	Formulation 2	10.4 ab	4.8 ab	11.0 a	24.5 a	2,618.8 a
6	Formulation 2	10.8 a	4.8 ab	11.7 a	24.8 a	2,684.2 a
7	Formulation 2	10.3 ab	5.6 a	11.7 a	24.6 a	2,698.2 a
8	Formulation 1 (Commercial Standard)	10.2 b	4.4 b	11.3 a	24.3 a	2,608.5 a
	F values	3.27*	3.08*	1.42 ns	1.10 ns	1.27 ns
	CV (%)	3.16	13.46	8.35	2.07	3.17

Source: *Prepared by the author (2020)*

The application of F1 in the sowing furrow provided an absolute value of TNPP, on average, 7.7% and 3.4% higher compared to plants developed in the control and in the commercial standard, respectively, and the application of F2 provided absolute value of TNPP similar to the commercial standard and, on average, 5.3% higher than plants developed in the control (Table 2). Dourado Neto *et al*. (2014), analyzing the efficiency of biostimulants in the common bean crop, also did not observe significant differences between treatments for the number of pods per plant.

The 100GM absolute values verified for the doses of F1, F2, control, and commercial standard were similar (Table 2). Bossolani *et al.* (2017) and Frasca, Nascente, Lanna, Carvalho, and Costa (2020) also did not verify increases in 100GM in relation to the treatments, or differentiation with the control, when analyzing the effect of biostimulants on plant growth and agronomic performance of common bean. This suggests that the formulations did not interfere with photoassimilate transport to the seeds. However, 100GM is an inherent characteristic of the cultivar and may be slightly affected by moderate environmental variations, such as those observed during this experiment (Figure 1).

There was no difference in grain yield between treatments (Table 2). Still, the application of F2 in the sowing furrow provided an absolute value, on average, 3.0% and 2.2% higher than the control and the commercial standard, respectively (Table 2).

It can be explained by the fact that productivity in common bean crop is highly related to yield components, which also did not differ between treatments.

Effect of doses

FPS, 100GM, and productivity were significant ($R^2 \geq 0.70$) for the factor doses of formulation 1.

Applying F1 in the sowing furrow promoted a linear increase in FPS (Figure 2A). The doses of 0.3 and 0.4 L ha^{-1} provided FPS, on average, 2.8% and 4.7% higher than the dose of 0.2 L ha^{-1} and the control, respectively.

There was a quadratic effect of F1 doses on 100GM. Although the values between treatments were similar, the highest average was achieved at the highest dose (0.4 L ha^{-1}) (Figure 2B). And the optimal dose that would provide the greatest 100GM is 0.358 L ha^{-1}.

Using 0.3 and 0.4 L ha^{-1} doses of F1 allowed obtaining plants with higher absolute values of grain productivity, with quadratic behavior of the means (Figure 2C). These doses provided productivity, on average, 1.4% higher than the control, but the optimal dose that would provide the best result is 0.717 L ha^{-1}.

Different doses of F1 evidenced no difference for FPS, 100GM, and productivity, although the dose of 0.4 L ha^{-1} has provided the highest averages for these variables. Thus, it is inferred that the highest dose provided a greater amount of nutrients such as Zn, nitrogen (N), and potassium (K), which act in germination, flowering, and grain filling and formation of pods, respectively (Marschner, 2012).

Furthermore, although in low concentrations, F1 is constituted by Fe and Mn. Fe is an essential trace element for plant growth and development. It is a cofactor for several enzymes and proteins that play vital roles in different physiological processes, including photosynthesis, protein synthesis, nitrogen metabolism, and respiration (Kaya, Akram, Ashraf, 2019; Mahawar, Ramasamy, Pandey, Prasad, 2023). Fe is very important in the synthesis of heme molecules, mainly chlorophyll, cytochromes, electron transport chain, and central proteins in the stroma (Salhi, Hajlaoui, Krouma, 2022), which makes it essential for the activity and efficiency of photosystem II (FSII) (Samborska-Skutnik, Kalaji, Sieczko, Baba, 2020).

And Mn is a metal cofactor for approximately 6% of all known metalloenzymes, in addition to being an essential element of the metalloenzyme cluster of the oxygen evolution complex in PSII (Waldron, Rutherford, Ford, Robinson, 2009; Alejandro, Höllet, Meier, Peiter, 2020), which makes it essential for seed germination and photosynthetic process.

It implies that Fe and Mn can improve plant growth and physiology, increasing photosynthetic efficiency and, consequently, crop yield (Kaya *et al*., 2019; Alejandro *et al*., 2020; Singh *et al*., 2021). This may be one of the explanations for the better results provided by the dose of 0.4 L ha^{-1} of F1.

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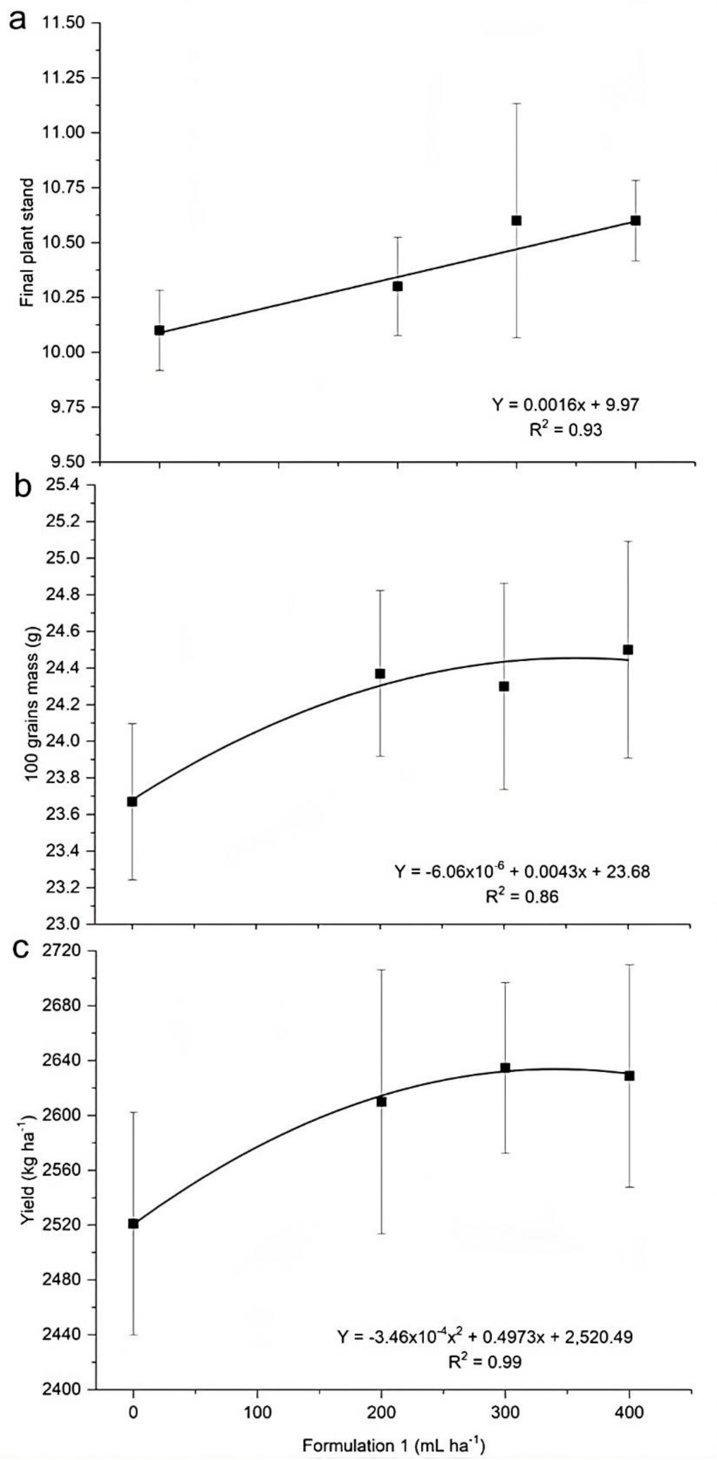
Figure 2. *Final plant stand (A), 100-grain mass (B), and yield (C) of common bean plants under different doses of formulation 1 applied in the sowing furrow.* ***Source:*** *Prepared by the author (2020)*

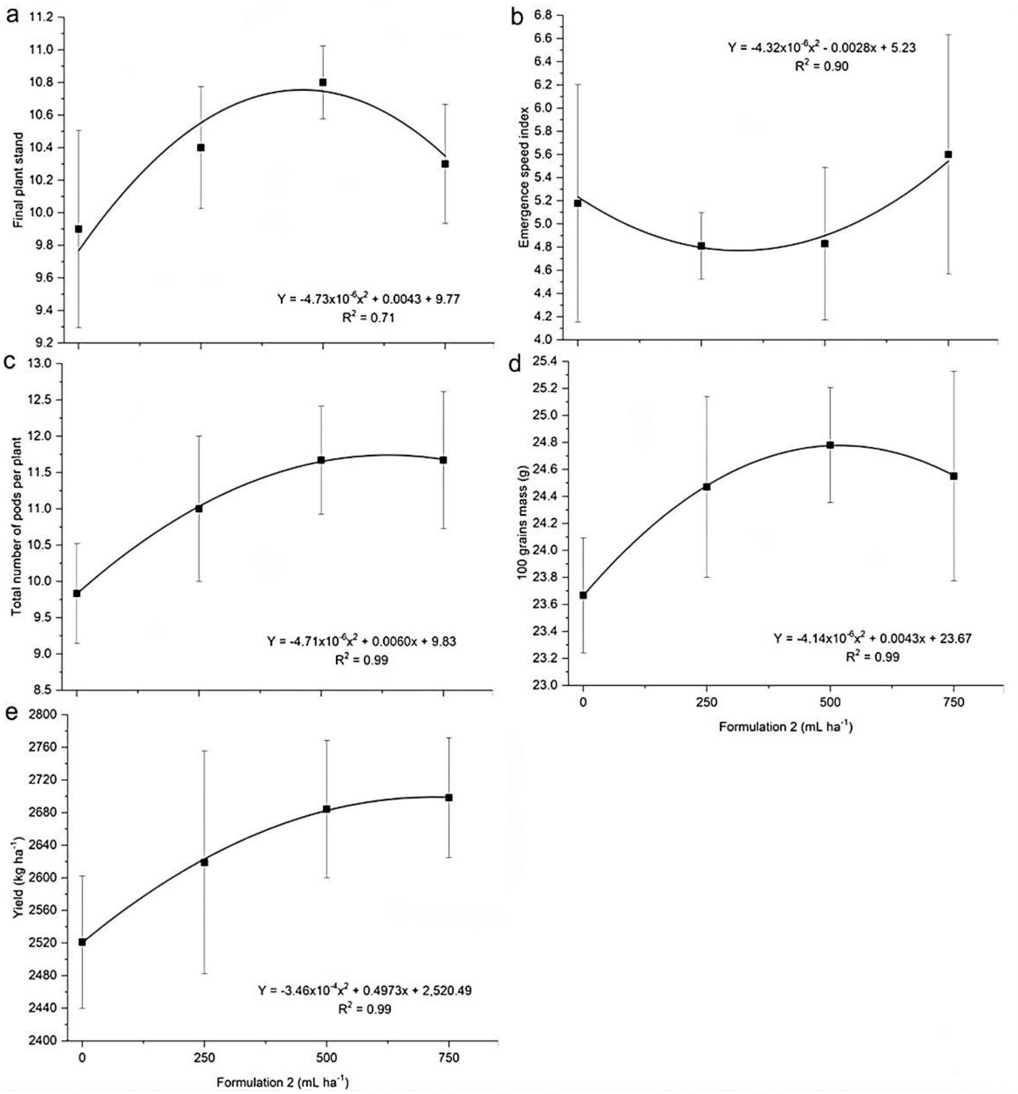


All variables evaluated were significant ($R^2 \geq 0.70$) for the factor doses of formulation 2.

FPS was adjusted to the quadratic model, with increases of means until the dose of 0.5 L ha^{-1}, when the highest value was found, which was 3.7% and 6.5% higher than the dose of 0.25 L ha^{-1} and the control, respectively (Figure 3A). And the 0.454 L ha^{-1} dose would be responsible for the highest FPS.

There was a quadratic effect of F2 doses on the ESI, in which the lowest doses resulted in a decrease of 4% in the emergency speed compared to the control treatment. In contrast, the dose of 0.75 L ha^{-1} promoted seedling emergence, on average, 14.3% and 10.7% faster than the other doses and the control, respectively (Figure 3B).

Figure 3. *Final plant stand (A), emergence speed index (B), total number of pods per plant (C), 100-grain mass (D), and yield (E) of common bean plants under different doses of formulation 2 applied in the sowing furrow.*



Source: *Prepared by the author (2020)*

It was verified quadratic adjustment of F2 doses on the TNPP (Figure 3C). The highest values were observed in the doses of 0.5 and 0.75 L ha^{-1}, which provided an increase of 6.0% and 7.7% in TNPP compared to the lowest dose and the control, respectively. The optimal dose that would provide the highest TNPP is 0.637 L ha^{-1}.

There was a quadratic effect of the F2 doses on 100GM, with an increase in values until the dose of 0.5 L ha^{-1}, when it achieved the highest average, which was 2.8% higher compared to the control (Figure 3D). And the dose of 0.519 L ha^{-1} would provide the greatest 100GM.

Yield was adjusted to the quadratic model, with an increase in values until the dose of 0.75 L ha^{-1}, when the highest average was verified, and it was 3.0% and 4.1% higher than the lowest dose and the control, respectively (Figure 3E). The optimal dose that would provide the highest productivity is 0.718 L ha^{-1}.

The highest doses of F2 were efficient in increasing the initial seedling development. F2 does not have Fe and Mn in its constitution and has N in a lower concentration than F1, but it has higher concentrations of K, S, and Zn.

Potassium, found in high concentration in F2, activates more than 60 enzymes and directly participates in plant protein synthesis (Barker & Pilbeam, 2015; Prado, 2021). This nutrient stimulates vegetative growth in common bean plants (Sarah *et al*., 2021). Zinc benefits bean seeds' germination and physiological quality (Dörr *et al*., 2017). And sulfur deficiency can impair seed germination and vigor (Popinigis, 1985).

Therefore, it is suggested that higher doses of F2 provided greater amounts of K, Zn, and S to seeds and, consequently, increased ESI and FPS.

4 Conclusions

Biostimulants influenced the initial development of the common bean crop, but the same did not happen for the yield and productivity components. Applying 0.5 and 0.75 L ha^{-1} doses of formulation 2 in the sowing furrow promoted increases in the final plant stand and in the emergence speed index, respectively. In contrast, formulation 1 applied in the planting furrow showed results similar to the commercial standard and the control. Therefore, applying the highest doses of formulation 2 in the sowing furrow can be recommended to stimulate the initial growth of the common bean.

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Conflict of interest
The authors declare no conflict of interest.