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EFFECT OF APPLYING BIO-INPUTS ON PRODUCTION OF HIGH BUSH BLUEBERRY (*Vaccinium corymbosum L.*) cv. biloxi IN BRAZIL'S FEDERAL DISTRICT

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KEYWORDS

Sustainability

Biostimulants

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Productivity

ABSTRACT

Blueberry production is increasing in Brazil, and growers are turning to bio-inputs or biostimulants to be used on their growth. This has been due to the growing concern about sustainability in the food production chain and the necessity to increase the yield. The current experiment aimed at evaluating the effects of Samurai King, EM-1 and Brutal Plus (Minhofértil) biostimulants on the cultivation of Southern Highbush blueberries (*V. corymbosum* L.), cultivar 'Biloxi'. The parameters evaluated were plant height (cm), diameter of the main stem (mm), number of shoots, chlorophyll content, total number of leaves, leaf length and width. The total mass, number of fruits, average mass per fruit, the transversal and longitudinal diameters, and the total sugars (°Brix) were also measured. In 2020, the treatment of Samurai King + EM-1 showed the highest efficiency for the studied parameters related to fruits and yield but with no significant difference as compared to the other treatments. Regarding the plant growth, treatments 1 (Brutal Plus) and 5 (Brutal Plus + EM-1) were the most efficient. In 2021, treatment 7 (Brutal Plus + Samurai King + EM-1) had the highest yields, except for average mass per fruit and total sugars. In the two years of evaluation, although treatments obtained lower averages than the control, the effect observed was generally positive, revealing the efficiency of products containing microorganisms for the growth of blueberry plants. In conclusion, these bioproducts could remarkably affect plant biomass, production and fruit quality, resulting in better yields.

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1 Introduction

The global production of blueberries, which belongs to the *Ericaceae* family, has grown annually to match the increase in consumption. It produces small blue-black fruits and influences health conditions by improving vision, functioning as an anti-inflammatory and antioxidant. This genus, *Vaccinium spp*., contains around 450 species from Southeast Asia and the Americas.

Peru did not export blueberries in 2014, but it became the largest exporter in Latin America in 2019. It exported over one billion dollars (Ghezzi and Stein 2021). Looking at Brazil, close to 4 tons of blueberries were exported in 2002, earning Brazilian producers about 24,000 dollars (Cantuarias-Avilés et al. 2014). Since 2010, with the introduction of low-chill varieties from research at the University of Florida, the planted area has increased even more. In 2011, a total area of 142 ha produced about 59 tons of blueberries (Cantuarias-Avilés et al. 2014). Such production levels can be reached using Bio-inputs substances or microorganisms applied to plants to increase the efficiency of nutrition, tolerance to abiotic stresses and/or crop quality traits, regardless of their nutrient content (Aung et al. 2014; Halpern et al. 2015; Koza et al. 2022). Bio-inputs can also act as catalysts for nutrient uptake by accelerating natural processes and may enhance plant protection against pests and diseases through systemic induction of resistance (Olowe et al. 2020; Kumar et al. 2022). For example, the use of EM-1 at a dose of 1mL/100mL provided an increase in vegetative growth of four apricot (*Prunus armeniaca*) cultivars when compared to control treatment and organic fertilizer application (Al-Janabi et al. 2016). In fact, using bio-inputs in agriculture is a growing practice worldwide, which has also increased their market. The global bio-input market was valued at $3.2 billion in 2021, with an expected annual growth of 12.1%, and is forecasted to be valued at $5.6 billion in 2026 (Market Research Report 2022). Their increased use in agriculture is mainly due to concerns among consumers and producers regarding factors affecting the sustainability of the food production chain, such as global warming and soil degradation (Halpern et al. 2015). The bio-inputs are therefore employed to up production to meet demand.

In an experiment in Nanjing - China, the application of different species and strains of the genus *Bacillus* showed significant gains in blueberry production (Yu et al. 2020). The blueberries of the variety 'Lanmei No.1' presented an increase in chlorophyll content and photosynthetic rate, growth and productivity, as well as an increase in the amount of ammonia, nitrogen and organic matter in the soil (Yu et al. 2020). However, in the blueberry plantation, experiments with biological treatments, especially with the inoculation of microorganisms, are still scarce, leaving an information gap. The study, therefore, aimed to evaluate the effects of bio-inputs on the growth and production of blueberries.

2 Materials and Methods

The present experimental work was carried out between March 2020 and April 2022 at the Biology Experimental Station, University of Brasília, Federal District, Brazil. According to the Köppen-Geiger criterion, the local weather is classified as tropical and type Aw, with dry winter and rainy summer (Cardoso et al. 2014). Three bio-inputs were used: Samurai King, EM-1 and Brutal Plus (Minhofertil). Samurai King and EM-1 bio-inputs are produced industrially by combining selected strains of efficient microorganisms. The biological composition of Samurai King® and EM1®, respectively, can be seen in Table 1 and 2, with appropriate adaptations (AL-Janabi et al. 2016). In contrast, Brutal Plus, currently registered as an organic fertilizer (Minho Fertil Co.,

Table 1 Microbiological characteristics of Samurai King® Brasília-DF, 2020

Bacteria	Quantification
*Bacillus cereus**	1.44 X 10^7
*Aeromicrobium kwangyangen sis***	2.8 X 10^7
*Bacillus subtilis***	1.4 X 10^7
Aeromicrobium sp. **	1.4 X 10^7
*Bradyrhizobium japonicum***	3.2 X 10^7
Acidovorax sp. **	8.8 X 10^7
*Stenotrophomonas sp.***	2.2 X 10^7
Sphingomonas fennica **	2.2 X 10^7
*Acinetobacter modestus***	2.2 X 10^7
*Methylobacterium adhaesivum***	1.8 X 10^7

*Spike metagenomics (%); **serial dilution (CFU/(ml/L or g))

Table 2 Composition of the EM1®

Photosynthetic Bacteria	*Rhodopseudomonas plustris* *Rhodobacter sphacerodes*
Lacticacid bacteria	*Lactobacillus plantarum* *Lactobacillus casei* *Streptococcus lactis*
Yeast	*Saccharomyces cerevisiae*
Fungi	*Aspergillus spp.* *Penicilium spp.*

Source: AL-JANABI et al. (2016)

Table 3 Contents of the natural rice husk substrate Brasília -DF, 2020

DD	TP	pH	EC	OM	Ca	Mg	Fe
kg m^{-3}		-	mS cm^{-1}	mg L^{-1}			
131.71	85.46	5.56	0.418	224.4	47.4	1.98	0.79

Source: de-LIMA et al. 2020; here Dry density (DD); total porosity (TP); pH; electrical conductivity (EC); organic matter (OM) and calcium (Ca); magnesium (Mg) and iron (Fe)

Brazil), is produced more artisanally; thus, the microbial composition might range according to the material used. The blueberry plants evaluated were in their third and fourth year of production. One plant in each black polyethylene pot contained 60 dm^3 of substrate (Table 3). The plants were covered with mesh to protect against birds, allowing solar incidence of 80% to penetrate (20% covering mesh). The pots containing plants were distributed in rows, with 0.4 m between plants and 1.5 m between rows. Substrate was covered with raw rice husk, without any burning process.

The soils were amended by fertigation, using ever-flow systems with four holes at the soil level, with a total flow rate of 4 L h^{-1} and one emitter per plant, totalling approximately 3 L of solution per plant per day. The quantities of nutrients were controlled, providing the following amounts on an annual basis: N - 250kg ha^{-1}, P_2O_5 - 160kg ha^{-1}, K_2O - 300kg ha^{-1}, CaO - 250kg ha^{-1}, MgO - 150kg ha^{-1}, and SiO_2 - 200kg ha^{-1}.

The experiment was organized in randomized blocks containing seven treatments: (i) Brutal Plus, (ii) Samurai King®, (iii) EM-1®, (iv) Brutal Plus + Samurai King®, (v) Brutal Plus + EM-1®, (vi) Samurai King ®+ EM-1®, (vii) Brutal Plus + Samurai King ®+ EM-1®. There was also a control treatment without application, considered treatment 0. The plants were arranged in three blocks, each containing the seven treatments and control, randomly arranged, with five plants from each treatment per block, totalling 40 plants per block and 120 in the complete experiment. The doses of bio-input applied were 3 mL L^{-1} for Brutal Plus and EM-1® and 4 mL L^{-1} for Samurai King® every 15 days throughout the study, ceasing only in December 2020, just before the plants were pruned in January 2021. No pesticides were applied during the analyses to control pests or plant diseases. Ten to twelve branches of each plant were chosen to evaluate the production and quality.

In the plants, the parameters evaluated were (i) plant height (cm) (Pl. Hgt), measured by the height of the previously marked main branch, (ii) diameter of the main stem (mm) (St. Diam), (iii) number of sprouts (NS), (iv) chlorophyll, using the ATLeaf equipment (Cl), (v) total number of leaves per plant (NL), (vi) leaf length (L. Leng), and (vii) leaf width (L. Wdt), and (viii) Yield. The date of the first harvest of that year was July 13, 2020, and the weekly measurements of total fruit mass, number of fruits and average mass per fruit were taken until November 4, 2020. From August to October of the same year, the transversal and longitudinal diameters of the fruits were measured monthly, and the total sugars in the pulp (°Brix) were also measured during this period.

In 2021, the aforementioned plant parameters were evaluated monthly between March and September. On the other hand, the fruit evaluations occurred from July 13, 2021, the date of the first harvest of that year. The fruit diameter and ºBrix assessment occurred every two weeks, while the data on the other variables were collected weekly.

The Gaging model Origin Caldigital caliper with a precision of 0.01 mm was used to measure the diameter of the main stem and the transversal and longitudinal diameter of fruits. Plant height was measured with a measuring tape, and the branch was marked with coloured tape for identification. Leaf width and length were measured using a 30 cm-long millimetre-marked ruler. The chlorophyll content was measured using an ATLeaf CH Plus chlorophyll meter. The sugar content in the fruit was measured using a digital refractometer.

The parameters related to photosynthesis rate and stomatal conductance in the leaves were quantified using an evaluation with infrared gas analyzer (IRGA) equipment, which was performed on June 23, 2021. The measurements were taken twice daily (at 8 am and 12:00 pm). The IRGA equipment was used to measure photosynthetic activity, stomatal conductance, and transpiration rate, among other gas exchanges.

The data related to the different variables was run through variance analysis and Tukey's mean comparison test at the 5% probability level using SISVAR software (Ferreira 2011).

3 Results

For the analyses performed for 2020, the variables related to the fruits presented no significant difference between the treatments at the Tukey test of means at 5% probability (Table 4). Treatments 0 (control) and 6 (Samurai King + EM1) obtained the highest absolute values, with differences of 24% and 28.2%, respectively, in comparison to the least productive one (EM1) in the variable average mass of fruit per week (FMW). For the varying number of fruits per week (NFW), the highest values were obtained with the control and Samurai King ®+ EM-1® treatments, while the treatment EM1 showed the lowest yield. Although some differences were detected between them, they are not statistically significant.

The average mass per fruit (AMF) also varied little among the treatments, with an outstanding value for treatments 5 (Brutal Plus + EM1) and 6 (Samurai King + EM1), while the lowest one was detected in the treatment 4 (Brutal Plus + Samurai King). The total soluble sugars presented low variation among the treatments, with the highest values observed in treatments 0 and 5. The concentration of soluble solids was directly related to the cover used for cultivation, with a decrease in the value of °Brix when covering was done with a shading net compared to the use of polyethene cover and the control without any cover.

In 2021, the treatments showed greater differentiation and were significant at 5% probability in Tukey's Test for the mass of fruits per week (FMW) and number of fruits per week (NFW), with p-values of 0.0002 and 0.003, respectively. In the case of NFW, treatment 7 (Brutal Plus + Samurai King + EM1) showed the highest mass of 91.27g, and this was 67.4% higher than that presented by treatment 4 (Brutal Plus + Samurai King), which is 54.51g and found the least productive. The situation is repeated for NFS, with treatment 7 standing out, with an average value higher than 84 fruits per week. In contrast, treatment 5 (Brutal Plus + EM1) stood out for low production, showing average values of approximately 48 fruits per week.

There were no significant differences for AMF and BRIX, with very close values among the treatments (Table 4). The average mass per fruit was lower in 2021 compared to the previous year's values in most treatments. In compensation, total soluble sugars were higher in all treatments in 2021, showing that the products may have exerted some effect during the regular and ongoing application.

The graphs of the average mass of fruits per week (FMW) and the number of fruits per week (NFW) (Figures 1 and 2) for both years revealed a production peak between August and September. In the 2020 graph, a shorter peak can be observed, concentrated from the middle to the end of August, while in 2021, this higher production remained more stable from the beginning of August until the middle of September before falling again. Production after the peak felt in 2020 and did not recover until the end of the harvest at the end of November, remaining at a steady low. However, in 2021, after the peak production dropped, it rose again, revealing another production peak, even higher than the first, starting in the second half of October (Figure 1).

Table 4 Characteristics of High bush blueberry fruits in 2020 and 2021

	2020				2021			
Treatment	FMW	NFW	AMF	BRIX	FMW	NFW	AMF	BRIX
0	83.20^{a}	63.74^{a}	1.38^{a}	10.58^{a}	72.82^{ab}	70.33^{ab}	1.22^{a}	12.70^{a}
1	71.14^{b}	56.81^{a}	1.34^{a}	10.36^{a}	71.90^{ab}	64.92^{ab}	1.21^{a}	12.72^{a}
2	76.66^{ab}	55.93^{a}	1.39^{a}	9.99^{a}	73.99^{ab}	67.46^{ab}	1.26^{a}	12.52^{a}
3	67.06^{c}	52.40^{a}	1.32^{a}	9.27^{a}	69.27^{ab}	65.50^{ab}	1.26^{a}	12.50^{a}
4	70.75^{b}	56.12^{a}	1.24^{a}	9.90^{a}	54.51^{b}	49.91^{b}	1.31^{a}	12.88^{a}
5	77.86^{ab}	61.02^{a}	1.39^{a}	10.79^{a}	59.43^{b}	57.00^{ab}	1.22^{a}	12.80^{a}
6	85.99^{a}	63.19^{a}	1.40^{a}	9.82^{a}	55.44^{b}	47.98^{b}	1.27^{a}	12.94^{a}
7	69.84^{b}	53.17^{a}	1.33^{a}	10.45^{a}	91.27^{a}	84.62^{a}	1.25^{a}	12.75^{a}

Treatments 0 – Control; 1 Brutal Plus; 2 Samurai King®; 3 EM-1®; 4 Brutal Plus + Samurai King®; 5 Brutal Plus + EM-1®; 6 Samurai King ®+ EM-1®; 7 Brutal Plus + Samurai King ®+ EM-1®; Average mass of fruit per week (FMW), Number of fruits per week (NFW), Average mass per fruit (AMF) and brix degrees (BRIX); Values followed by the same letters in the columns do not differ by the Tukey test at 5% probability level

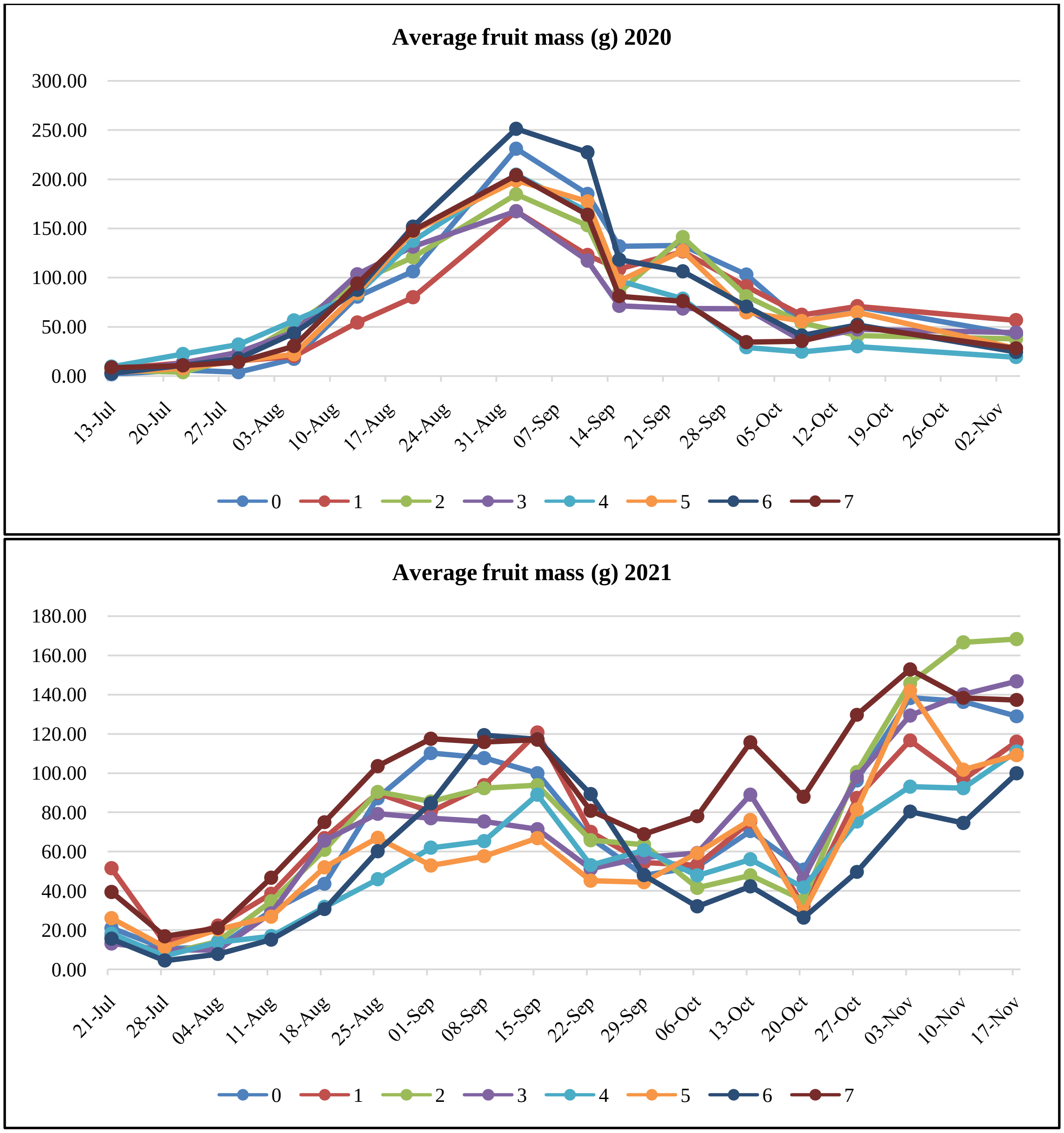
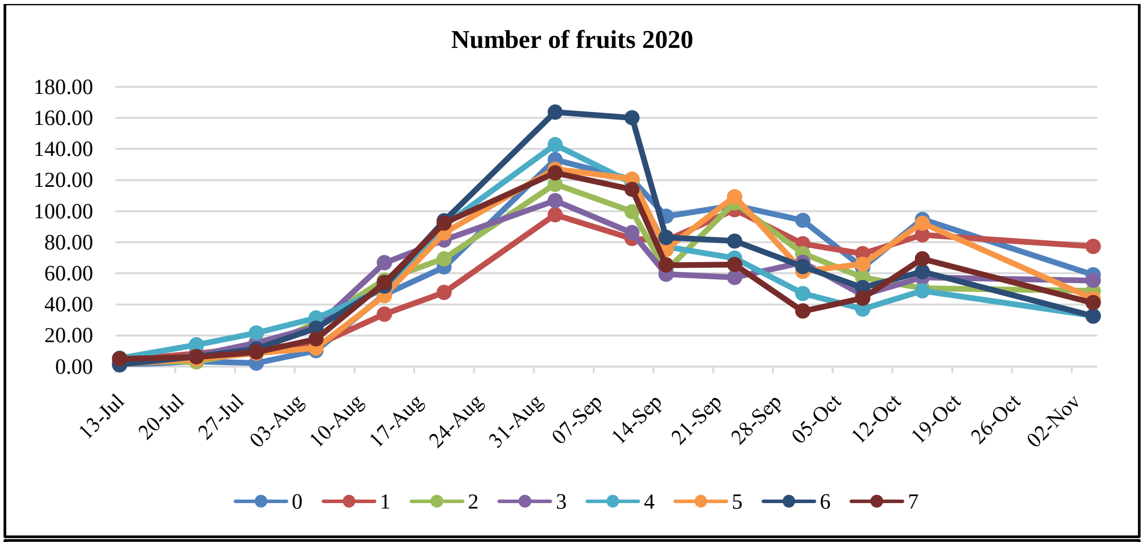


Figure 1 Trends in the average fruit mass per week (FMW) for the different treatments in the years 2020 and 2021 (treatments: 0 – Control; 1 Brutal Plus; 2 Samurai King®; 3 EM-1®; 4 Brutal Plus + Samurai King®; 5 Brutal Plus + EM-1®; 6 Samurai King ®+ EM-1®; 7 Brutal Plus + Samurai King ®+ EM-1®)

The average fruit mass per week (FMW) also increased between August and September of 2020, dropping afterwards and maintaining stability until the end of the year. In 2021, the graph revealed a slight decrease in the same months, maintaining its level until the middle of October. Here, value began to rise, and treatment 2 (Samurai King®) reached the highest value on November 17, with an average of 197.7 fruits harvested per block. The highest average fruit mass per week (FMW) were observed in treatment 2 (Samurai King®) in 2021 (163.67 g) and in treatment 6 (Samurai King ®+ EM-1®) in 2020 (250 g).

For the parameters related to plant characteristics, in 2020, a statistical difference was observed in all variables evaluated except for plant height (Pl. Hgt) and yield per plant (Yield). The yield in 2020 returned a p-value of 0.7551 for the treatments and 0.8611 for the blocks, evidencing no difference between the blocks and treatments. The most productive treatment in 2020 was treatment 6 (Samurai King ®+ EM-1®), with an average yield of 401.27 g per plant. The least effective treatment was treatment 3 (EM-1®), with a yield of 312.96 g, a difference of 28.2% compared to the most productive treatment. In 2021, the yield showed a P-value of



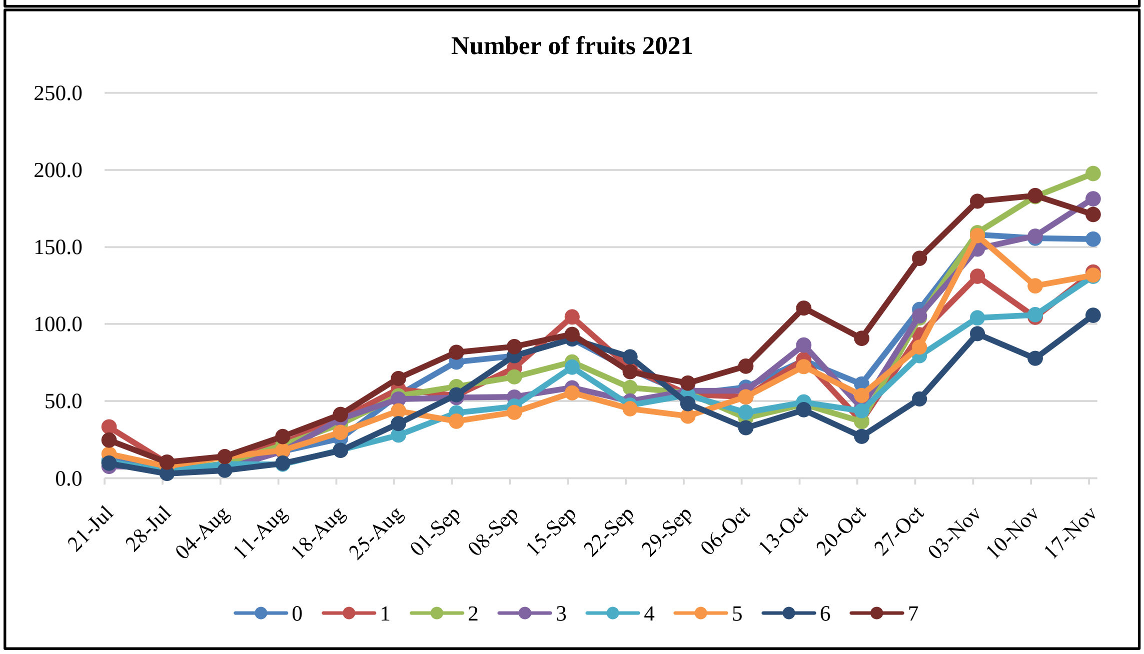


Figure 2 Trend of the Number of fruits per week (NFW) for the different treatments in the years 2020 and 2021 (treatments: 0 – Control; 1 Brutal Plus; 2 Samurai King®; 3 EM-1®; 4 Brutal Plus + Samurai King®; 5 Brutal Plus + EM-1®; 6 Samurai King ®+ EM-1®; 7 Brutal Plus + Samurai King ®+ EM-1®)

0.0498 for the different treatments at the 5% probability level. The highest yield was reported in treatment 7 (Brutal Plus + Samurai King ®+ EM-1®), with an average yield of 328.59 g per plant, and this was 67.4% higher than the average of the least effective treatment (Brutal Plus + Samurai King®). The analysis of variance also showed a significant difference between the blocks, with a P-value of 0.0031. In the spacing adopted, the productivity per hectare was 5.47 tons, with 16.67 plants. Although yield was lower in this case than that reported by large blueberry producers, this mode of cultivation is cheaper because it eliminates the use of chemical pesticides, which can add substantially to the cost of production.

In the second year of evaluation, no significant differences were observed for the variables number of shoots (NS), number of leaves per plant (NL) and chlorophyll in leaves (Cl). For all other variables, ANOVA demonstrated a difference between treatments. The chlorophyll index in leaves was higher for treatments 6 (Samurai King ®+ EM-1®) and 5 (Brutal Plus + EM-1®) in 2020 and 2021, respectively. The difference between treatments was observed only in 2020, when treatment 6 returned average values of 63.21, 12.1% higher than treatment 1 (Control), which was the least productive treatment in that year (56.38) (Table 5).

Table 5 High bush blueberry growth parameters evaluated in 2020 and 2021

Treatment	Pl Hgt	St Diam	NS	NL	Cl	L Leng	L Wdt	Yield
2020								
0	29.82^{a}	7.87^{ab}	10.67^{a}	458.33^{bc}	60.97^{ab}	4.28^{b}	2.48^{b}	388.28^{a}
1	29.81^{a}	6.55^{c}	11.19^{a}	548.67^{a}	56.38^{b}	4.83^{a}	2.84^{a}	332.00^{a}
2	30.55^{a}	6.58^{c}	8.26^{b}	344.56^{e}	62.67^{a}	4.56^{ab}	2.61^{ab}	357.75^{a}
3	34.63^{a}	7.09^{bc}	9.81^{ab}	432.67^{bcd}	61.41^{ab}	4.50^{ab}	2.50^{b}	312.96^{a}
4	30.47^{a}	6.35^{c}	9.22^{ab}	387.56^{cde}	62.93^{a}	4.71^{ab}	2.53^{b}	330.18^{a}
5	32.72^{a}	8.35^{a}	8.19^{b}	442.00^{bcd}	59.61^{ab}	4.75^{ab}	2.48^{b}	363.35^{a}
6	32.57^{a}	6.97^{bc}	8.19^{b}	360.33^{e}	63.21^{a}	4.50^{ab}	2.49^{b}	401.27^{a}
7	29.28^{a}	7.94^{ab}	9.96^{ab}	502.78^{ab}	60.29^{ab}	4.67^{ab}	2.60^{ab}	325.91^{a}
2021								
0	65.32^{ab}	6.01^{ab}	15.10^{a}	739.33^{a}	60.51^{a}	6.28^{a}	3.59^{a}	262.18^{ab}
1	74.66^{a}	6.68^{a}	13.90^{a}	633.44^{a}	62.55^{a}	6.39^{a}	3.61^{a}	258.85^{ab}
2	63.61^{b}	5.66^{b}	14.94^{a}	730.06^{a}	62.27^{a}	5.73^{b}	3.16^{b}	266.40^{ab}
3	68.52^{ab}	5.60^{b}	15.94^{a}	744.11^{a}	61.90^{a}	6.23^{a}	3.64^{a}	249.39^{ab}
4	65.53^{ab}	5.51^{b}	14.25^{a}	665.33^{a}	59.10^{a}	6.23^{a}	3.59^{a}	196.25^{b}
5	62.23^{b}	5.47^{b}	15.33^{a}	725.22^{a}	63.34^{a}	6.28^{a}	3.63^{a}	213.98^{ab}
6	60.87^{b}	5.21^{b}	16.25^{a}	760.89^{a}	62.84^{a}	6.07^{ab}	3.41^{ab}	199.59^{ab}
7	66.72^{ab}	5.75^{b}	16.04^{a}	805.33^{a}	62.91^{a}	6.14^{ab}	3.63^{a}	328.59^{a}

Treatments: 0 – Control; 1 Brutal Plus; 2 Samurai King®; 3 EM-1®; 4 Brutal Plus + Samurai King®; 5 Brutal Plus + EM-1®; 6 Samurai King ®+ EM-1®; 7 Brutal Plus + Samurai King ®+ EM-1®; Plant height (Pl. Hgt); Main stem diameter (St. Diam.); Number of sprouts (NS); Number of leaves per plant (NL); Chlorophyll content (Cl); Leaf length (L. Leng); Leaf width (L. Wdt); and Yield. Values followed by the same letters in the columns do not differ by the Tukey test at 5% probability level

The main stem diameter (St Diam) showed similar results in both years, decreasing in the second year. In 2020, the treatment that presented the largest diameter was treatment 5 (Brutal Plus + EM-1®), with an average of 8.35 mm, while in 2021, the best performance occurred for treatment 1 (Control), with an average diameter of 6.39 mm. The height of plants was measured in the first year, counting from the second growth flush (first secondary shoots). In 2021, the measurement was made from the first shoots after pruning, which is why there is a difference in values between the two years. The difference between treatments was observed only in the second year, in which treatment 1 (Control) was the fastest growing, with an average height of 74.66 cm.

For the number of sprouts (NS), the treatments differed in 2020, and among the tested treatments, treatment 1 (Control) presented more primary shoots from the crown of the plant. There was no significant difference in 2021, but the absolute values increased compared to the previous year, with treatment 6 (Samurai King ®+ EM-1®) producing an average of 16.25 shoots per plant. The control treatment also stood out in the number of leaves (NL) per plant in 2020, with an average of 548.67 leaves. In 2021, this parameter was observed for treatment 7 (Brutal Plus + Samurai King ®+ EM-1®), with a value 46.7% higher than the best treatment in 2020. For the number of shoots (NS), leaf length (L. leng) and leaf width (L. Wdt), the absolute values increased from 2020 to 2021. In both years, treatment 1 showed higher leaf sizes, except for leaf width in 2021, when it was exceeded by treatments 3 (EM-1®), 5 (Brutal Plus + EM-1®) and 7 (Brutal Plus + Samurai King ®+ EM-1®), which showed values of 3.64 cm, 3.63 cm and 3.63 cm respectively (Table 5).

In the photosynthesis and stomatal conductance analyses performed using the IRGA equipment, no differences were observed between the treatments for any of the parameters evaluated at either of the two evaluation seasons.

4 Discussion

The application of biostimulants on crops has shown varied results, depending on the crop, the composition of the input, and other factors. In the present study, fruit biomass showed no effects of the applied treatments, but previous studies demonstrated the efficiency of foliar or seed application of similar bioproducts, especially plant hormones, on soybean production (Bertolin et al. 2010). Foliar application proved effective, especially in the crop's vegetative phase (Bertolin et al. 2010). On the other hand, apples in Italy received bio-inputs based on algae, amino acids and humic and fulvic acids, but no gains were observed in the harvest, the fruit quality, and did not show any observable difference in the photosynthetic rate of the crop (Thalheimer and Paoli 2001). The use of efficient microorganisms, such as bacteria and mycorrhiza, has already revealed an increase in crop plant yields and has a unique role in abiotic stress tolerance

A previous study showed increased harvest weight in conventional and organic carrot crops. Bio-inputs applied were based on bacteria such as *Bacillus subtilis*, *Lactobacillus*, *Saccharomyces cerevisiae* yeast, humic and fulvic acids, among other compounds common to the formulations used in this study's experiments, and administered at low doses of 2L/ha. Besides the increase in carrot weight, a greater accumulation of monosaccharides, carotenoids, and phenols and increased antioxidant activity were also observed (Gavelienė et al. 2021).

The total soluble sugars and soluble solids showed low variation among the treatments in the present study. It is also noteworthy that in the case of higher temperatures, the shade should be removed to allow for better fruit development (Pereira et al. 2021). According to these authors, a shading mesh could reduce the harvest period of the crop.

As regards the fruit mass and the number of fruits per week, different types of biostimulants showed different actions, and their combination may prove effective in some cases. The main variations in bio-inputs are associated with the origin of the material used to manufacture the product, how this material is processed, and the mixtures of other substances to make the commercial product. In addition to the increase in yield, the use of biological inputs can increase the resistance of the fruits to cracking, as observed by Rodrigues et al. (2020) in pomegranate crops. Little or no relationship has been reported between applying efficient microorganisms and total soluble solids (Auriga et al. 2018). In grapes, total soluble solids, total titratable acidity, and the amount of polyphenols present in the fruit were not influenced by the application of MS, with the amount of polyphenols altered only when a different cultivar was used and when the number of buds per branch increased (Auriga et al. 2018).

In the present study, the production peak occurred in August and September. According to Tuell and Isaacs (2010) climate is a determining factor in the production of blueberries, and colder climates prove most suitable for higher production. Colder climates also directly influence the presence of pollinating insects essential for increased crop production (Tuell and Isaacs 2010). Covering the crop and the absence of such insects may influence an earlier start for the harvest period. In an experiment with different covers for blueberry production in Portugal, an earlier harvest was observed using polyethene cover, and a later harvest was reported using shade netting cover (Pereira et al. 2021). In the case of this experiment, covering the crops with shade netting may have prevented the entry of birds, protecting the crop, but it probably also decreased the entry of pollinating insects. Various species of pollinators play a significant role in blueberry yields. In Vermont, United States, various wild bee species provided 12% increases in fruit mass and fruit set compared to treatment with hand pollination alone (Nicholson and Ricketts 2019).

An essential factor in the setting of flowers is the correct management of pruning to maintain the entry of light through the crown of the plant. The production of floral buds increases with more radiation, concentrated mainly in the first 60 cm from the crown, where light penetrates with greater intensity. In an experiment with the Chile cultivar' Choice', 63.7% of the floral clusters were present in the first 60 cm, indicating that light intensity caused a considerable increase in flower production and fruit production (Yáñez et al. 2009). The increase in productivity with soil microorganisms is linked to several factors, such as the production of auxins, one of the most important hormones for plant development, which are present in them in larger quantities. Further, biosynthesis of indol-acetic acid (IAA) can be performed by bacteria and fungi. Some of the said organisms were present in the products used here, such as *Bradyrhizobium* and can even be stimulated by the exudation of secondary compounds from the plants themselves (Theunis et al. 2004; Ortíz-Castro et al. 2012).

The use of microorganisms to increase the chlorophyll indexes and photosynthetic rate of plants has already been studied. In an experiment, Li et al. (2020) reported that the application of biological fertilizer composed of a mixture of microorganisms and biochar on tobacco plants has a positive difference of approximately 3.47% to 69% on the SPAD index and 8 to 107% on the rate of photosynthesis. This was in addition to a significant increase in stomatal conductance and transpiration rate, indicating a beneficial effect of fertilizer application on the rate of photosynthesis and other relevant characteristics (Li et al. 2020). The photosynthesis rate is also influenced by the water potential in the leaf, which is directly related to the correct irrigation of the plants (Rho et al. 2012). Improved plant development and stem enlargement may be related to the increased resistance of plants to different environmental and weather conditions. Through suppression of oxidative stress with increased antioxidant levels, the application of corn and propolis extracts increased the resistance of fava bean plants (*Vicia faba* L.) to water, salt, and cadmium (Cd^{2+}) stress. These extracts have been reported to increase the efficiency of photosynthesis and gas exchange, as well as plant growth and yield under the mentioned stresses and optimal conditions (Desoky et al. 2021; Koza et al. 2022).

De Silva et al. (2000) reported increased stem diameter and leaf area with soil inoculation of a *Pseudomonas fluorescens* isolate. Furthermore, this bacterium also increased plant growth and nutrition and functioned as a biocontrol agents (Lally et al. 2017; Sah et al. 2021). Further, the application of magnesium also helps in nutrient uptake and makes chlorophyll development more efficient, which helps increase plant yield (Awad and El-Ghamry 2007). Increased yield in wheat was studied in a long-term experiment in which the continued action of microorganisms improved organic fertilizer uptake efficiency (Hu and Qi 2013).

Photosynthesis and stomatal conductance showed no effect arising from the treatments. In contrast, the use of MS, besides increasing indices of stomatal conductance, intercellular CO_2 concentration, transpiration rate, and photosynthetic rate, can also increase, which is associated with the induction of pathogen resistance (Hamid et al. 2021; Li et al. 2020) and are also indirectly affecting plant photosynthesis. Leaf damage caused by pathogens such as *Septoria albopuncata* has greatly decreased CO_2 assimilation and transport rates, resulting in decreased photosynthesis (Roloff et al. 2004). Thus, resistance to pathogens is crucial in improved production.

Conclusions

The analyses revealed significant differences between treatments for some parameters in the two years of evaluation of Blueberry plants, particularly in 2020, when Samurai King + EM-1 showed a remarkable effect on plant growth. Additionally, in 2021, the Brutal Plus + Samurai King + EM-1 treatment was the most productive and showed better yields in the variables related to fruit, except in AMF and BRIX. In both years, the effect was generally positive, revealing the efficiency of applying bio-inputs to develop blueberry plants. It is recommended that these products use be continued, as their efficiency in improving fruit production in the long term was demonstrated.

Authors contributions

Conceptualization: Yamanishi OK. Data curation: Pinho GP, Lima FN. Formal analysis: Data curation: Pinho GP, Lima FN. Funding acquisition: Yamanishi OK. Investigation: Murakami K. Methodology: Pinho GP, Lima FN. Project administration: Lima FN. Resources: Yamanishi OK. Supervision: Lima FN. Writing-original draft: Murakami K., Cruz AF. Writing-review & editing: Cruz AF.

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