International Journal of Recycling of Organic Waste in Agriculture (2023)12: 141-154
Doi: 10.30486/IJROWA.2022.1946944.1385

ORIGINAL RESEARCH

Soybean cultivars under the foliar application of a compounded bio-fertilizer in different plant phenological stages and doses

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Received: 22 December 2021 / Accepted: 06 June 2022 / Published online: 30 July 2022

Abstract

Purpose Soybean is critical in the global food scenario. The increase of grain productivity and sustainability are always wanted. This study evaluates the agronomic performance of soybeans with biofertilizer (BF) applications in different soybean phenological stages.

Method Experiments were performed in two regions and designed in randomized blocks using a 2×4 factorial scheme (two cultivars and four BF managements). Representative plants from each parcel were used. Plant biometric variables were assessed on plants at the full bloom stage. At the beginning of grain filling, the leaf and seed biochemical components were measured. Quantitative yield components were measured at harvest.

Results In both areas, the BF did not affect the soybean leaf area index, leaf dry biomass, and branch dry biomass. The BF application improved the total sugars, starch, and reducing sugars in area 1. In area 2, the chlorophyll was similar among the BF managements. Soybean cultivars and BF did not affect seed protein content in area 1. In area 2, the BF application presented a similar or greater soybean yield compared to control (no BF). The divergences observed between areas may be related to interactions between the soil-climatic conditions and the soybean cultivars.

Conclusion Choosing a more appropriate soybean cultivar is fundamental to obtaining higher yields. Nutritional management with leaf-applied BF is an alternative to increase soybean field performance. However, it is still necessary to consider the interactions of the BF with the crop genetics chosen for the region of cultivation and the best dose and time of application.

Keywords *Glycine max*, Foliar organic fertilization, Plant responses, Crop management

Introduction

The world's demands for food, especially protein, are constantly rising as the world's population continually increases. Unfortunately, the area available for crops is not rising with the population increase. Food production must increase between 30 and 60% by 2050 to attend to the population needs (van Dijk et al. 2021), preferably using the existing crop areas. Soybean (*Glycine max* L.) is the most important commodity in the current international market and thus has a relevant effect on the food industry for people and animals worldwide (Jia et al. 2020). Brazil and the United

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States are the biggest soybean crop producers representing over two-thirds of all soybean harvested (ourworldindata.org/soy).

In Brazil, the estimated soybean crop area will be 39.9 million hectares and will provide an expected grain production of 140.8 million tons in the 2021/22 harvest (CONAB 2021); in the United States, the soybean crop area was 35.1 million hectares and provided a grain production of 118.1 million tons in the 2021/22 harvest (USDA 2021).

The expansion of soybean production in these countries is mainly due to advances in scientific research to develop technologies able to increase adaptation and yield in the field. Among the most impacting technologies for improved crop production is the use of soluble fertilizers for foliar applications (Rauniyar 2020; Niu et al. 2021).

The progress in understanding plant biochemistry and physiology allowed the development and synthesis of new fertilizers, which are compounds destined to increase plant metabolic efficiency and the sustainability of crop activity (Bulgari et al. 2015). Plant biostimulants are compounds intended to improve plant performance, resistance, and productivity. Such crop fertilizers are significant tools considering the high genetic potential of modern crops and the limiting environmental factors (Jardin 2015); additionally, the organic fractions and microorganisms present in some of these compounds offer positive impacts on the soil-plant system (Yakhin et al. 2017; Sible et al. 2021).

The benefits of organic fractions are related to the accessibility to nutrients and the improvement of various metabolic pathways that synthesize primary and secondary metabolites (Onofrei et al. 2017).

The use of organic components such as chitin (Malerba and Cerana 2019) and microorganisms (Bargaz et al. 2018) associated with mineral nutrients (biofertilizers) can increase plant biomass and production. Such results were observed for various crops, as reported by Anli et al. (2020) in the palm, Rehman and Qayyum (2020) in the rice-wheat cropping system, El Maaloum et al. (2020) in tomato, Boutasknit et al. (2020) in garlic, Guerreiro et al. (2017), and Meyer et al. (2021) and Barros et al. (2022) in soybeans. All the positive effects reported from the application (primarily foliar) of biostimulants and/or biofertilizers are improved when the application occurs at the most appropriate time (period of application, hour, and plant phenology) and product dose. The results reported by Kocira (2019) illustrate that specific improvements can be achieved by changing the dose of the applied stimulus. The author indicated increased soybean yield and antioxidant activity when a higher biostimulant (complex of amino acids, macro, and micronutrients) dose (0.5%) was applied; however, a higher total fat and protein content occurred when a lower biostimulant dose (0.3%) was applied. These results indicate the intricate interactions and the potential that such amendments have to improve crop efficiency.

The lack of information on the use of alternative cropping products (biostimulants and biofertilizers) to soybean crop reveals the need to research and develop the efficacy of such products and the adequate management during the soybean crop cycle. Therefore, the objective was to evaluate the agronomic performance of soybean cultivars under the foliar application of a biofertilizer (BF) in different plant phenological stages and doses.

Material and methods

Experimental area

Two soybean field experiments were performed in two separate experimental areas. One experiment was conducted in the municipality of 'Patos de Minas' (area 1) and in 'Uberlândia' (area 2), both in Minas Gerais state, Brazil (Fig. 1). Experimental area 1 was located in *Sertãozinho* Experimental Area, at 872 meters of altitude; experimental area 2 was located in *La Fuente* Farm), at 842 meters above sea level. The climate of both regions is classified as Aw (Savannah – hot, humid summer and cold, dry winter) according to Köppen-Geiger (Beck et al. 2018).

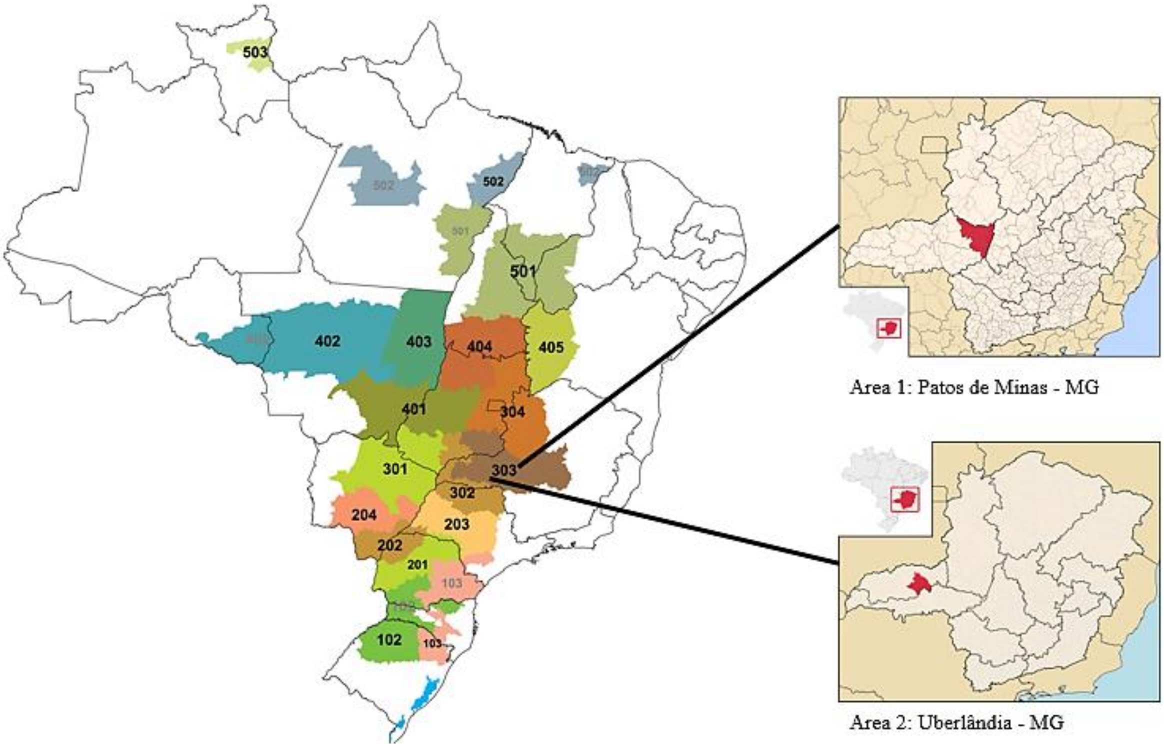

Fig. 1 Map of the edaphoclimatic regions for soybean planting in Brazil

The experimental areas are located in region 303, referring to *Triângulo Mineiro* (18º52'71.8"S 46º44'34.4"W) and *Alto Paranaíba* (18º58'14.7"S 48º12'53.8"W).

In area 1, the cultivars RK8115RR (genotype of excellent yield stability and high productive responses to investment) and BRS Desafio (genotype for high-tech environments, highly responsive to adequate planting window and population) were sown (crop cycle: November-March). In area 2, the cultivars RK6813RR (genotype of excellent yield stability, even in adverse conditions) and RK6719IPRO (genotype of early cycle and high productive potential) were sown (crop cycle: October-February). The planting line spacing was 0.5 m, and the planting stand was 17 plants per linear meter. The planting line spacing was 0.5 m, and the planting stand was 10 (RK8115RR) and 17 (BRS Desafio) plants per linear meter.The soil of the experimental areas is classified as eutrophic Red Latosol and dystrophic Red and Yellow Latosol in area 1 and area 2, respectively. The soil chemical and physical analyses are found in Table 1.

Biofertilizer and experimental design

The biofertilizer used originates from natural fungal extract, derived from mycelium cell walls and obtained from the fermentation of organic matter and additives, including defoaming agents, surfactant, pH corrector, and preservatives. Dry mycelium (raw material) was added to a container with water vapor. The mixture was heated to 100 °C for 3 hours then cooled to room temperature. The mixture was then centrifuged; the resulting solution was mixed with surfactant and homogenized to achieve the final product (biofertilizer).

The biofertilizer is dark brown colored, pH 5.5 and 1.1 g dm^{-3} density (20 ºC). It contains (water-soluble): carbon (15.44%), nitrogen (0.15%), phosphorus (0.07%), potassium (0.05%), calcium (0.02%), magnesium (0.01%), sodium (0.09%), boron (0.01%), iron (8.1%), manganese (2%) and sugars (carbon and energy sources). Originating from recycled plant matter, the total sugar content has a predominance (> 97%) of hexoses (mannose, galactose, and glucose) and a low amount (< 0.5%) of N-acetyl glucosamine (GlcNAc) - the main component of chitin. Pentoses (arabinose and xylose) are also detected in small proportions. In addition to sugars, the proportion of peptides/proteins in the product extract was about 3%, providing an additional source of amino acid.

The experiments in each area were carried out in a complete randomized block design in both areas, in a factorial 2×4 scheme, using two soybean cultivars and four biofertilizer treatments, with four replications. The biofertilizer treatments consisted of foliar application in dose and time of application, according to Table 2.

Table 1 Soil chemical and physical analyses before the experiment implementation in *Uberlândia* (area 1) and *Patos de Minas* (area 2) at 0-20 cm depth

	pH	K	P	Al	Ca	Mg	H + Al	SB	t	T	V
	(H_2O)	--- mg dm^{-3} ---		cmolc dm^{-3}							%
Area 1	5.8	39.0	3.23	0.0	1.4	0.6	2.85	2.1	2.1	4.95	42.4
Area 2	5.8	39.0	55.6	0.1	2.0	0.9	5.0	3.0	3.1	8.0	57.5

	Zn	Cu	Mn	B	Fe
	mg dm^{-3}				
Area 1	0.4	0.5	6.0	0.15	45.0
Area 2	1.5	0.8	0.8	0.24	67.4

	Sand	Silt	Clay
	g kg^{-1}		
Area 1	40	56	4
Area 2	25	35	40

SB: sum of bases (Ca + Mg + K); t: effective cation exchange capacity (CEC); T: CEC at pH 7, V (%): percentage by base saturation .Soil P, K (HCl 0,05 mol L^{-1} + H_2SO_4 0,025 mol L^{-1}); Al, Ca, Mg = (KCl mol L^{-1}); organic matter = (Walkley-Black), B = [$BaCl_2.2H_2O$ a 0,125% hot water]; Cu, Fe, Mn, Zn = [DTPA 0,005 mol L^{-1}+CaCl 0,01 mol L^{-1} + TEA 0,1 mol L^{-1} a pH 7,3]; S-SO_4^{2-} = $Ca(H_2PO_4)$ 0,01 mol $L^{-1,}$ and granulometric parameters were performed according to EMBRAPA (2009).

The experimental plots consisted of six lines spaced by 0.5 m (three meters wide) and five meters long. The plant evaluations were assessed on the four central soybean lines discarding the initial and final 0.5 meters of each line resulting in a useful area of 8 m². The first biofertilizer application was performed when plants reached the R1 phenological stage (beginning of soybean flowering) - stage 60 in the BBCH scale (Meier 2018); the second application (only for treatment 3) was performed when plants reached the R3 phenological stage (end of soybean flowering) - stage 65 in the BBCH scale. The applications were carried out with a portable coastal spray (CO_2 pressurized), equipped with a spraying bar containing six fan-type spray tips spaced by 0.5 m. The established pressure was 40 PSI, providing a spray volume equivalent to 300 L ha^{-1}.

Phytosanitary care was performed according to crop monitoring for insect pests, diseases, and weeds. When necessary, registered (soybean) phytosanitary products were used and followed the dose recommended by the manufacturers.

Table 2 Treatments, doses, and stages of biofertilizer applications to soybean

Treatments	Dose applied* (L ha^{-1})	Soybean phenological stage
BF $^{0.5\text{-R1}}$	0.5	R1
BF $^{1\text{-R1}}$	1	R1
BF $^{0.5\text{-R1+R3}}$	0.5	R1 + R3
Control	-	-

*: spray pressure of 40 PSI, spray volume of 300 L ha^{-1}. BF 1-R1: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF 0.5-R1+R3: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied.

Evaluations

The leaf area index (LAI), fresh and dry biomass of leaves, main stem and branches, total and reducing sugar content, leaf chlorophyll content, protein content in grains, and yield components (number of pods with one, two and three grains, the biomass of thousand grains and final yield) were evaluated.

When the soybean plants reached R2 phenological stage (full bloom) - stage 69 on BBCH scale - the leaf area (LA) was determined by the product between the length (L) and the width (L) of the leaf (LA = L × W). The leaves of five representative plants per plot were measured using a ruler graduated (mm) determined in cm^2. The LAI was then calculated as the ratio of the mean LA and the respective mean ground area occupied by a plant.

The dry biomass of the soybean leaves, main stem, and branches was obtained by drying the samples in an oven (air-forced circulation) at 65 ºC until the stabilization of the sample dry biomass.

When soybean plants reached the R5 phenological stage (beginning of grain filling) - stage 75 on BBCH scale - ten plants from the two central lines of each plot were randomly selected to measure the leaf chlorophyll content using SPAD (Minolta brand, model SPAD-502) plant analyzer. The evaluations occurred in the upper third of the plant canopy, using perfect leaves free of damages or diseases and completely expanded.

Ten newly mature trifoliate - corresponding to the third or fourth soybean leaf from the apex of the main branch - were collected (without petiole) from each plot for biochemical analyses. The total sugar content in the soybean leaves was determined according to Umbreit et al. (1957) method; the reducing sugars by Somogy and Nelson method (Nelson 1944); the starch by the Yemm and Willis (1954) method, and protein in grains according to the methodology proposed by the AOAC (2005).

Crop yield was estimated (kg ha^{-1}) at the end of the field experiment - stage 99 of the BBCH scale. The grains harvested from the useful plot (8 m^2) were weighed, and the moisture was adjusted to 13%. The weight of one thousand grains, the total pods per plant, and the number of pods with one, two, or three grains were also assessed. The experimental data were submitted to analysis of variance (ANOVA) at 5% significance ($p < 0.05$). The variable means were compared by Tukey's test of averages ($p < 0.5$). Both statistical analyses were performed using the Sisvar (v. 4.0) statistical software (Ferreira 2019).

Results and discussion

The application of the biofertilizer did not change ($p > 0.05$) the soybean LAI in both evaluated areas. There was variation among the cultivars, with the LAI higher for RK8115RR than for BRS Desafio in area 1 (58.8%) and higher for RK6719IPRO than for RK6813RR in area 2 (70.9%) (Table 3).

Table 3 Leaf area index as a function of biofertilizer treatments applied to the soybean crop in *Patos de Minas* (area 1) and *Uberlândia* (area 2)

Treatments	Area 1		
	8115	Desafio	Average
BF $^{0.5\text{-}R1}$	3.74	2.90	3.32 a
BF $^{1\text{-}R1}$	4.93	2.81	3.87 a
BF $^{0.5\text{-}R1+R3}$	5.52	3.49	4.51 a
Control	4.32	2.45	3.39 a
Average	4.62 a	2.91 b	
C.V. (%)	26.29		
Treatments	**Area 2**		
	6719	6813	Average
BF $^{0.5\text{-}R1}$	5.42	3.40	4.41 a
BF $^{1\text{-}R1}$	4.49	3.19	3.84 a
BF $^{0.5\text{-}R1+R3}$	6.26	3.13	4.69 a
Control	5.42	2.92	4.17 a
Average	5.40 a	3.16 b	
C.V. (%)	26.15		

*: Averages of the variables followed by distinct letters differ factor's levels from each other by the Tukey's test ($p < 0.05$). BF $^{0.5\text{-}R1}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{1\text{-}R1}$: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{0.5\text{-}R1+R3}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied. Soybean cultivars: 8115 - RK8115RR. Desafio - BRS Desafio. 6719 - RK6719IPRO. 6813 - RK6813RR. C.V. (%): coefficient of variation.

In area 1, the leaf and branch dry biomass were not influenced ($p > 0.05$) by the biofertilizer treatments or soybean cultivars. The highest main stem dry biomass referred to the BF $^{0.5\text{-}R1+R3}$ treatment, and it was 60.7% higher than the control treatment (Table 4).

The RK8115RR cultivar resulted in 25.7% higher main stem dry biomass than BRS Desafio. In area 2, the dry biomass of leaves, main stem, and branches were not affected ($p > 0.05$) by the biofertilizer application. Still, cultivar RK6719IPRO stood out against RK6813RR, with higher biomass accumulation in 40.5, 71, and 42.1% for leaf, main stem, and branch dry biomass, respectively. The main stem dry biomass was greater for both soybean cultivars area 1 only when double biofertilizer applications (BF $^{0.5\text{-}R1+R3}$) were implemented. This dry biomass stratification may be related to the integration of factors (Aluko et al. 2021), such as the edaphoclimatic conditions found in *Patos de Minas* (area 1) and the double application of nutrients presented in the biofertilizer. After the leaf absorption of nutrients and energy such as amino acids and sugars, plants can react in less time in interactions with the environment, reducing stress damage or falling plant performance (Du Jardin 2015). The double biofertilizer application (BF $^{0.5\text{-}R1+R3}$) improved the content of total sugars, starch, and reducing sugars in the soybean leaves cultivated in area 1, being 16.2; 18.1, and 16.8% higher than the control (no biofertilizer), respectively. In the same place, no difference ($p > 0.05$) was detected between the soybean cultivars (Table 5). There was a significant interaction between the biofertilizer management and the soybean cultivars in area 2 for total sugar, starch, and reducing sugar. No differences were observed between cultivars for the control (no biofertilizer) and the BF $^{0.5\text{-}R1}$ management. The performance of RK6813RR was higher than RK6719IPRO when the biofertilizer was applied at 1 L ha^{-1} dose (BF $^{1\text{-}R1}$); however, when the biofertilizer was applied twice (BF $^{0.5\text{-}R1+R3}$) the

RK6719IPRO soybean cultivar stood out. The accumulation of total sugar, starch, and reducing sugar in RK6813RR and RK6719IPRO cultivars (area 2) were improved for the BF $^{0.5\text{-}R1}$ and BF $^{05\text{-}R1+R3}$ managements, respectively (Table 5). In area 1, no differences ($p > 0.05$) for the SPAD chlorophyll content were detected between cultivars for the BF $^{0.5\text{-}R1+R3}$ treatment (Table 6). The cultivar RK8115RR presented the highest chlorophyll content when BF $^{0.5\text{-}R1}$ and BF $^{0.5\text{-}R1+R3}$ management were applied. For BRS Desafio, the biofertilizer used once (BF $^{0.5\text{-}R1}$ or BF $^{1\text{-}R1}$) presented the highest chlorophyll content. In area 2, the chlorophyll content was similar ($p > 0.05$) among the biofertilizer managements and averaged 42.14 SPAD chlorophyll content. The RK6719IPRO soybean cultivar presented 5.7% more SPAD chlorophyll content than the RK6813RR soybean cultivar.

Table 4 Soybean leaf, branches, and main stem dry biomass as a function of the biofertilizer treatments applied in *Patos de Minas* (area 1) and *Uberlândia* (area 2)

Area 1									
Treatment	Leaf dry biomass (g)			Branch dry biomass (g)			Stem dry biomass (g)		
	8115	Desafio	Average	8115	Desafio	Average	8115	Desafio	Average
BF $^{0.5\text{-}R1}$	10.86	14.61	12.73 a*	6.53	7.94	7.23 a	3.96	2.78	3.37 b
BF $^{1\text{-}R1}$	14.23	13.01	13.62 a	6.36	6.81	6.59 a	4.00	2.58	3.28 b
BF $^{0.5\text{-}R1+R3}$	13.69	15.83	14.77 a	7.23	8.14	7.69 a	4.54	4.38	4.45 a
Control	11.85	12.26	12.06 a	5.72	6.46	6.09 a	2.98	2.58	2.77 b
Average	12.66 a	13.93 a		6.46 a	7.34 a		3.86 a	3.07 b	
C.V. (%)		18.18			19.95			18.23	
Area 2									
Treatment	Leaf dry biomass (g)			Branch dry biomass (g)			Petiole dry biomass (g)		
	6719	6813	Average	6719	6813	Average	6719	6813	Average
BF $^{0.5\text{-}R1}$	5.30	4.91	5.11 a	4.33	2.71	3.52 a	4.33	3.48	3.90 a
BF $^{1\text{-}R1}$	5.88	3.69	4.79 a	4.33	2.55	3.44 a	4.83	3.25	6.08 a
BF $^{0.5\text{-}R1+R3}$	6.69	3.69	5.19 a	4.99	2.50	3.75 a	5.24	3.41	4.33 a
Control	4.22	3.45	3.84 a	3.58	2.33	2.96 a	4.25	3.00	3.63 a
Average	5.52 a	3.93 b		4.31 a	2.52 b		4.66 a	3.28 b	
C.V. (%)		25.94			16.78			14.90	

*: Averages of the variables followed by distinct letters, lowercase in the column, differ factor's levels from each other by the Tukey's test ($p < 0.05$). BF $^{0.5\text{-}R1}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{1\text{-}R1}$: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{0.5\text{-}R1+R3}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied. Soybean cultivars: 8115 - RK8115RR. Desafio - BRS Desafio. 6719 - RK6719IPRO. 6813 - RK6813RR. C.V. (%): coefficient of variation.

The application of the glyphosate herbicide, even in tolerant soybean (RR), can cause leaf yellowing and chlorotic symptoms. This symptom can cause farmers and technicians to confuse the visual symptom with manganese (Mn) deficiency, which has been proved not to be the direct cause of the leaf yellowing (Basso et al. 2011). However, the biofertilizer studied here has a series of nutrients such as Mn, sugar, and amino acids that can help plants recover from the stresses caused by the presence of the herbicide. Also, Mn is an activator of different plant enzymes, essential for the water-splitting reaction in photosystem II (PSII), ROS (reactive species of oxygen) scavenging, and plant growth regulation (Alejandro et al. 2020). In area 1, for all biofertilizer managements, the highest numbers of pods with three grains were observed for the BRS Desafio cultivar. However, none of the treatments affected the number of pods in this cultivar. A low number of pods containing three grains was observed in cultivar RK8115RR when the low dose of biofertilizer (0.5 L ha^{-1}) was applied at the beginning of soybean flowering (BF $^{0.5\text{-R1}}$). The number of two-grain pods was not affected by the treatments, but the number of pods containing two grains of the BRS Desafio soybean cultivar was 215% bigger than the number of the RK8115RR soybean cultivar (Table 7).

Table 5 Total sugar content, starch content, and reducing sugar content in soybean leaves (as a function of the biofertilizer treatments applied in *Patos de Minas* (area 1) and *Uberlândia* (area 2)

Treatment	Total sugar (mg sucrose g^{-1} dry biomass)			Starch (mg starch g^{-1} dry biomass)			Reducing sugar (g glucose g^{-1} fresh biomass)		
Area 1	8115	Desafio	Average	8115	Desafio	Average	8115	Desafio	Average
BF $^{0.5\text{-R1}}$	20.4	22.5	21.5 b*	30.9	29.9	30.5 bc	5.7	5.8	5.7 b
BF $^{1\text{-R1}}$	23.4	21.9	22.7 ab	31.7	32.7	32.2 ab	5.6	6.2	5.9 b
BF $^{0.5\text{-R1+R3}}$	24.8	23.9	24.3 a	34.7	32.7	33.7 a	6.6	6.5	6.5 a
Control	20.4	21.5	20.9 b	28.1	29.5	28.8 c	5.5	5.5	5.5 b
Average	22.2 a	22.5 a		31.4 a	31.2 a		5.8 a	6.0 a	
C.V. (%)		8.1			5.8			5.5	
Area 2	6719	6813	Average	6719	6813	Average	6719	6813	Average
BF $^{0.5\text{-R1}}$	22.1 Ab	23.0 Ab	22.5	29.7 Ab	30.6 Ab	30.1	4.4 Ab	4.8 Ab	4.6
BF $^{1\text{-R1}}$	18.6 Bc	25.6 Aa	22.1	26.4 Bc	33.9 Aa	30.1	4.2 Bb	5.9 Aa	5.1
BF $^{0.5\text{-R1+R3}}$	24.5 Aa	11.5 Bd	17.9	33.7 Aa	15.4 Bc	24.6	5.9 Aa	2.7 Bc	4.3
Control	19.3 Ac	20.1 Ac	19.7	28.6 Abc	29.0 Ab	28.8	4.3 Ab	4.5 Ab	4.4
Average	21.1	20.1		29.6	27.21		4.7	4.5	
C.V. (%)		4.6			4.6			6.6	

*: Averages of the variables followed by distinct letters, uppercase in the row and lowercase in the column, differ factor's levels from each other by the Tukey's test ($p < 0.05$).

BF $^{0.5\text{-R1}}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{1\text{-R1}}$: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{0.5\text{-R1+R3}}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied. Soybean cultivars: 8115 - RK8115RR. Desafio - BRS Desafio. 6719 - RK6719IPRO. 6813 - RK6813RR. C.V. (%): coefficient of variation.

Table 6 SPAD chlorophyll content in soybean leaves in *Patos de Minas* (area 1) and *Uberlândia* (area 2)

Treatments	Area 1		
	8115	Desafio	Average
BF $^{0.5\text{-}R1}$	43.30 Ba*	46.92 Aa	45.11
BF $^{1\text{-}R1}$	38.32 Bb	47.52 Aa	42.92
BF $^{0.5\text{-}R1+R3}$	43.97 Aa	43.72 Ab	43.85
Control	38.80 Bb	42.30 Ab	40.55
Average	41.1	45.11	
C.V. (%)		2.56	
Treatments	Area 2		
	6719	6813	Average
BF $^{0.5\text{-}R1}$	43.79	41.57	42.69 a
BF $^{1\text{-}R1}$	43.83	41.70	42.77 a
BF $^{0.5\text{-}R1+R3}$	42.85	39.31	41.08 a
Control	42.77	41.28	42.03 a
Average	43.31 a	40.96 b	
C.V. (%)		3.14	

*: Averages of the variables followed by distinct letters, uppercase in the row and lowercase in the column, differ factor's levels from each other by the Tukey's test ($p < 0.05$). BF $^{0.5\text{-}R1}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{1\text{-}R1}$: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{0.5\text{-}R1+R3}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied. Soybean cultivars: 8115 - RK8115RR. Desafio - BRS Desafio. 6719 - RK6719IPRO. 6813 - RK6813RR. C.V. (%): coefficient of variation.

In area 2, the number of pods with three grains was not affected ($p > 0.05$) by the biofertilizer management or soybean cultivars. Pods with two and one grains showed an interaction between the managements and cultivars. Better results of the RK6813RR soybean cultivar were observed when the BF $^{0.5\text{-}R1}$ or BF $^{1\text{-}R1}$ managements were applied (Table 7). For pods with only one grain, the RK6813RR stood out mainly when the biofertilizer was applied once (BF $^{0.5\text{-}R1}$ or BF $^{1\text{-}R1}$). There were no differences among the biofertilizer managements in both areas for the total number of pods (Table 8). In area 1, the BRS Desafio soybean cultivar produced 35.4% more pods than the RK8115RR soybean cultivar. The weight of 1,000 grains showed no difference between nutritional management in area 2, and RK6813RR was 8.8% more responsive in PMG than RK6719IPRO. In area 1, the application of BF $^{0.5\text{-}R1}$ and BF $^{0.5\text{-}R1+R3}$ increased plant height by 18.2 and 18.1% higher than the control, respectively. Biofertilizer management and the soybean cultivars did not interfere with the seed protein content in area 1. In area 2, there was an interaction between these factors, and the RK6719IPRO presented a content 11.7% higher than RK6813RR comparing the control treatment (no biofertilizer). The application of the BF $^{0.5\text{-}R1}$ treatment to the RK6813RR soybean cultivar improved the seed protein content by 11.4% compared to the control treatment. In area 1, a significant interaction ($p < 0.05$) was detected between the biofertilizer management and the soybean cultivars (Table 9). The BRS Desafio soybean cultivar presented superior production performance than the RK8115RR soybean cultivar, except for the BF $^{0.5\text{-}R1+R3}$. The biofertilizer management that stood out productivity for BRS Desafio soybean cultivar was BF $^{1\text{-R1}}$, producing 34% more yield than the control. The RK8115RR produced more grains when BF $^{1\text{-R1}}$ and BF $^{0.5\text{-R1+R3}}$ management was implemented and were 31.5 and 43.3% more productive than where no bio-fertilizer was applied (control).

Table 7 The number of pods with three, two, and one grain as a function of the biofertilizer management applied in *Patos de Minas* (area 1) and *Uberlândia* (area 2)

				Area 1					
Treatment	Three-grain pod			Two-grain pod			One-grain pod		
	8115	Desafio	Average	8115	Desafio	Average	8115	Desafio	Average
BF $^{0.5\text{-R1}}$	8.75 Bb*	31.75 Aa	20.25	2.25	10.69	6.47a	0.75	0.94	0.84 ab
BF $^{1\text{-R1}}$	17.25 Ba	30.50 Aa	23.88	4.00	12.06	8.03a	0.94	1.38	1.15 a
BF $^{0.5\text{-R1+R3}}$	18.00 Ba	30.25 Aa	24.13	4.06	12.44	8.25a	0.19	0.06	0.12 b
Control	14.00 Bab	34.25 Aa	24.13	5.13	13.31	9.22a	0.25	0.25	0.25 ab
Average	14.50	31.68		3.85 b	12.12 a		0.53 a	0.66 a	14.50
C.V. (%)		18.44			28.71			113.56	
				Area 2					
Treatment	Three-grain pod			Two-grain pod			One-grain pod		
	6719	6813	Average	6719	6813	Average	6719	6813	Average
BF $^{0.5\text{-R1}}$	27.70	25.67	26.69 a	6.30 Ba	12.27 Aa	9.29	0.27 Ba	1.20 Aa	0.74
BF $^{1\text{-R1}}$	23.30	24.85	24.08 a	5.17 Ba	11.00 Aa	8.08	0.40 Ba	1.00 Aab	0.70
BF $^{0.5\text{-R1+R3}}$	27.02	19.97	23.49 a	7.80 Aa	8.27 Aa	8.04	0.60 Aa	0.35 Ab	0.48
Control	24.32	24.20	24.26 a	7.80 Aa	9.87 Aa	8.84	0.32 Aa	0.35 Ab	0.34
Average	25.58 a	23.67 a		6.77	10.36		0.40	0.73	
C.V. (%)		23.81			26.92			63.78	

*: Averages of the variables followed by distinct letters, uppercase in the row and lowercase in the column, differ factor's levels from each other by the Tukey's test ($p < 0.05$). BF $^{0.5\text{-R1}}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{1\text{-R1}}$: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{0.5\text{-R1+R3}}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied. Soybean cultivars: 8115 - RK8115RR. Desafio - BRS Desafio. 6719 - RK6719IPRO. 6813 - RK6813RR. C.V. (%): coefficient of variation.

The soybean development parameters responses mainly were related to the natural genetic contrasts between the cultivars, especially in *Patos de Minas* (area 1). The interaction between plant genetics and the environment determines grain yield (Khaki et al. 2020); therefore, choosing the most appropriate soybean cultivar is fundamental to obtaining higher productive ceilings. In area 2, there was no difference between cultivars (Table9). Regarding the biofertilizer management, the application that stood out was BF $^{1\text{-R1}}$, with productivity 15.3% higher than the control. The average productivity achieved in area 2 was higher than that observed for the Minas Gerais state in the 2018/19 harvest (3,222 kg ha^{-1}), according to CONAB (2021).

Table 8 The total number of pods, the weight of 1,000 grains, and protein content in soybean grain as a function of the biofertilizer management applied in *Patos de Minas* (area 1) and *Uberlândia* (area 2)

	Area 1								
Treatment	Total pod number			1,000 grain weight (g)			Seed protein (%)		
	8115	Desafio	Average	8115	Desafio	Average	8115	Desafio	Average
BF $^{0.5\text{-}R1}$	11.75	43.40	27.56 a*	138.90	135.24	137.07 a	35.38	34.90	35.14 a
BF $^{1\text{-}R1}$	22.20	43.98	33.06 a	119.91	121.69	120.79 b	35.75	35.45	35.60 a
BF $^{0.5\text{-}R1+R3}$	22.28	42.80	32.50 a	139.57	134.13	136.85 a	35.65	35.33	35.49 a
Control	19.38	47.83	33.59 a	114.52	117.17	115.84 b	34.53	34.78	34.65 a
Average	18.90 b	44.50 a		128.23 a	127.06 a		35.33 a	35.11 a	
C.V. (%)		18.31			6.76			5.54	
	Area 2								
Treatment	Total pod number			1,000 grain weight (g)			Seed protein (%)		
	6719	6813	Average	6719	6813	Average	6719	6813	Average
BF $^{0.5\text{-}R1}$	34.27	39.15	36.71 a	126.00†	140.50	133.25 a	34.90 Aa	36.12 Aa	35.51
BF $^{1\text{-}R1}$	28.87	36.85	32.86 a	128.75	139.50	134.13 a	33.82 Aa	34.90 Aab	34.39
BF $^{0.5\text{-}R1+R3}$	35.42	28.60	32.01 a	123.00	136.00	129.50 a	36.32 Aa	35.20 Aab	35.74
Control	32.45	34.42	33.44 a	126.50	132.75	129.63 a	36.22 Aa	32.40 Bb	34.33
Average	32.76 a	34.76 a		126.06 b	137.18 a		35.32	34.67	
C.V. (%)		19.92			6.12			5.32	

*: Averages of the variables followed by distinct letters, uppercase in the row and lowercase in the column, differ factor's levels from each other by the Tukey's test ($p < 0.05$). BF $^{0.5\text{-}R1}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{1\text{-}R1}$: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{0.5\text{-}R1+R3}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied. Soybean cultivars: 8115 - RK8115RR. Desafio - BRS Desafio. 6719 - RK6719IPRO. 6813 - RK6813RR. C.V. (%): coefficient of variation.

Chlorophyll content and the productivity components are factors that significantly increase crop productivity. Silva et al. (2017) attributed photosynthetic tissue and chlorophyll biomass to the improved soybean production, fertilized with macro and micronutrients during the vegetative period. The authors reported that the application of K, Mg, S, and B during the grain filling phase also had a positive effect on grain production. The current study results and the information found in the literature indicate that adequate nutrient positioning to meet crop demand determines the most efficient use of the fertilizers applied (Fernández et al. 2015; Guerreiro et al., 2017; Meyer et al. 2021). Carvalho et al. (2013) also observed that the improved soybean grain yield when biostimulants were applied to the leaves. Regarding the time of application, it is important to consider the composition of the leaf spray product to be applied due to the interaction with other activities necessary during crop management. Prieto et al. (2017), for example, highlighted the importance of this aspect and reported a negative effect of biostimulant application on the soybean reproductive stage inoculated with *Bradyrhizobium* spp. The divergences observed between the areas in the present study, where the same biofertilizer was tested and at the same doses, may be related to the interaction between soil-climatic conditions and soybean cultivars.

Table 9 Soybean grain productivity as a function of the biofertilizer management applied in *Patos de Minas* (area 1) and *Uberlândia* (area 2)

			Area 1			
Treatments	Productivity (kg ha^{-1})			Productivity (60 kg-bag ha^{-1})		
	8115	Desafio	Average	8115	Desafio	Average
BF $^{0.5\text{-}R1}$	2814 Ba*	3295 Aab	3054.73	46.90 Ba	54.92 Aab	50.91
BF $^{1\text{-}R1}$	2651 Bab	3663 Aa	3157.77	44.19 Bab	61.06 Aa	52.63
BF $^{0.5\text{-}R1+R3}$	3067 Aa	3010 Ab	3039.33	51.13 Aa	50.17 Ab	50.65
Control	2140 Bb	2734 Ab	2437.52	35.67 Bb	45.57 Ab	40.62
Average	2668.2	3175.8		44.47	52.93	
C.V. (%)			10.65			
			Area 2			
Treatments	Productivity (kg ha^{-1})			Productivity (60 kg-bag ha^{-1})		
	6719	6813	Average	6719	6813	Average
BF $^{0.5\text{-}R1}$	4389.90	4700.00	4544.95 ab	73.16	78.33	75.74 ab
BF $^{1\text{-}R1}$	5094.90	4872.39	4983.69 a	84.91	81.20	83.06 a
BF $^{0.5\text{-}R1+R3}$	4811.25	4761.25	4786.25 ab	80.18	79.35	79.77 ab
Control	4237.44	4409.85	4323.64 b	70.62	73.49	72.06 b
Average	4633.37	4685.87		77.22 a	78.09 a	
C.V. (%)			7.61			

*: Averages of the variables followed by distinct letters, uppercase in the row and lowercase in the column, differ factor's levels from each other by the Tukey's test ($p < 0.05$). BF $^{0.5\text{-}R1}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{1\text{-}R1}$: 1 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) soybean phenological stage. BF $^{0.5\text{-}R1+R3}$: 0.5 L ha^{-1} of biofertilizer applied at R1 (beginning of flowering) and R3 (end of flowering) soybean phenological stage. Control: no biofertilizer applied. Soybean cultivars: 8115 - RK8115RR. Desafio - BRS Desafio. 6719 - RK6719IPRO. 6813 - RK6813RR. C.V. (%): coefficient of variation.

Conclusion

The foliar application of BF presented similar or superior soybean crop yield compared to the control (no BF). Choosing the most appropriate soybean cultivar for the prevailing soil-climatic conditions is fundamental to obtaining higher yields.Nutritional management with leaf biofertilizer is a viable and efficient alternative to increase soybean yield.

It is still necessary to consider the interactions of the biofertilizer with the crop genetics chosen for the region of cultivation and the best dose and time of application.

Acknowledgment Universidade Federal de Uberlândia (UFU).Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Compliance with ethical standards Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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