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Improved nutrient uptake in three *Crotalaria* species inoculated with multifunctional microorganisms[1]

Melhoria da absorção de nutrientes em três espécies de *Crotalaria* inoculadas com microorganismos multifuncionais

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HIGHLIGHTS:

Multifunctional microorganisms promote the nutrient enrichment in Crotalaria *plants.*
Cover crop residues are vital in managing soil fertility.
Nutritionally improved cover crops increase soil nutrient levels for the subsequent crop.

ABSTRACT: Cover crops are essential in recovering soil productivity. *Crotalaria* is one of the most efficient legume species in terms of biomass production and nitrogen fixation. This study aimed to assess the effect of multifunctional microorganisms on the agronomic performance of *Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*. The experiment was conducted under greenhouse conditions, in a completely randomized design, with four replicates. Treatments consisted of six rhizobacterial isolates (BRM 32109 and BRM 32110 (*Bacillus* spp.), BRM 32111 and BRM 32112 (*Pseudomonas* spp.), BRM 32113 (*Burkholderia* spp.), BRM 32114 (*Serratia* spp.)), and one fungal isolate (*Trichoderma* spp. (T-26)), in addition to a control treatment (no microorganism). The main effect of multifunctional microorganisms on the three *Crotalaria* species was macro and micronutrient concentration increased. Sulfur and zinc concentrations increased in C. *juncea* roots, calcium and sulfur in *C. spectabilis* shoots, and *C. ochroleuca* exhibited higher concentrations of phosphorus and copper in shoots and zinc and copper in roots. In summary, improved nutritional status in *Crotalaria* directly affects nutrient availability for the subsequent crop.

Key words: plant growth promotion, biomass, nutrient, gas exchange

RESUMO: Plantas de cobertura são essenciais na recuperação da produtividade do solo. *Crotalaria* é uma das mais eficientes espécies de leguminosas em termo de produção de biomassa e fixação de nitrogênio. Objetivou-se neste estudo avaliar o efeito de microorganismos multifuncionais no desempenho agronômico de *Crotalaria juncea, C. spectabilis* e *C. ochroleuca*. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado, com quatro repetições. Os tratamentos consistiram em seis isolados de rizobactérias (BRM 32109 e BRM 32110 (*Bacillus* sp.), BRM 32111 e BRM 32112 (*Pseudomonas* sp.), BRM 32113 (*Burkholderia* sp.), BRM 32114 (*Serratia* sp.)) e um isolado fúngico (*Trichoderma* sp. (T-26)), além do tratamento controle (sem microrganismo). O principal efeito dos microorganismos multifuncionais sobre as três espécies de *Crotalaria* foi o aumento da concentração de macro e micronutrientes. Enxofre e zinco aumentaram na raiz de plantas de *C. juncea*; cálcio e enxofre na parte aérea de plantas de *C. spectabilis*; e plantas de *C. ochroleuca* apresentaram maior concentração de fósforo e cobre na parte aérea e de zinco e cobre na raiz. Em resumo, o melhor *status* nutricional em plantas de *Crotalaria* afeta diretamente a disponibilidade de nutrientes para a cultura subsequente.

Palavras-chave: promoção do crescimento vegetal, biomassa, nutrientes, trocas gasosas

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Introduction

Crotalaria spp. is a legume used as a cover crop in a no-till systems in order to protect the soil against erosion and increase organic matter and nutrient accumulation (Aita & Giacomini, 2006; Pereira et al., 2016). *Crotalaria* species are also used to reduce the incidence of phytonematodes in the soil (Pacheco et al., 2015) and break up compacted layers due to their deeper and more branched root system (Bonfim-Silva et al., 2012), in addition to fixing nitrogen (200 to 300 kg ha^{-1}) through a symbiotic relationship with bacteria of the genus *Rhizobium* (Dourado et al., 2001). Rotating cash crops with leguminous cover crops in the off season (March to September) is widespread in Brazilian Cerrado agricultural systems (Boer et al., 2007).

The use of multifunctional microorganisms in symbiosis with the cover crop is essential for sustainable intensification of agricultural systems in the Brazilian Cerrado region. These microorganisms usually inhabit the rhizosphere of plants (Graças et al., 2015) and improve crop systems resilience by promoting plant growth through direct and indirect mechanisms, in addition to increasing plant protection against pathogens and insects (Ahemad & Kilbret, 2014).

Previous studies conducted at Embrapa Rice and Beans research center demonstrated the efficiency of microorganisms, rhizobacteria and fungi in increasing biomass production and disease resistance in upland rice (Filippi et al., 2011; Silva et al., 2012; França et al., 2015; Nascente et al., 2017). In a more recent study, micronutrients, fulvic acid and *Ascophyllum* promoted total dry matter accumulation and a larger number of pods per plant in the common bean, an important legume crop in Brazil (Frasca et al., 2020). Our hypothesis is that these microorganisms may also significantly increase biomass production in *Crolataria*. As such, this study aimed to assess the effect of multifunctional microorganisms on the agronomical performance of three *Crotalaria* species.

Material and Methods

The experiment was conducted in a greenhouse at the Embrapa Rice and Beans research center, Santo Antônio de Goiás, Goiás state (GO), Brazil from February to May 2019. The soil used was from the arable layer (0-0.20 m) of a clay-textured Oxisol (485, 182 and 333 g kg^{-1} of clay, silt and sand, repectively). The chemical characteristics of the soil were determined according to Donagema et al. (2011), with the following results: pH (H_2O) = 5.5; Ca^{2+} = 37.9 $mmol_c$ dm^{-3}; Mg^{2+} = 16.5 $mmol_c$ dm^{-3}; Al^{3+} = 1 $mmol_c$ dm^{-3}; $H^+ + Al^{3+}$ = 24 $mmol_c$ dm^{-3}; P = 30.6 mg dm^{-3}; K^+ = 185 mg dm^{-3}; Cu^{2+} = 1.3 mg dm^{-3}; Zn^{2+} = 1.9 mg dm^{-3}; Fe^{3+} = 34.3 mg dm^{-3}; Mn^{2+} = 30.6 mg dm^{-3}; organic matter (OM) = 29.3 g kg^{-1}; C_{total} = 1.47% and N_{total} = 0.12%. One week before planting, 10 L pots were filled with 8.0 kg of soil fertilized with 10 g of NPK (5-30-15). At planting, 10 mL of diluted liquid inoculant (*Bradyrhizobium japonicum*), under the commercial name "GRAP NOD" (5 mL diluted in 900 mL of water), was sprayed into the sowing furrow. Soil moisture was maintained near field capacity throughout the experiment.

A completely randomized design was used, with four repetitions for each of the three *Crotalaria* species: *C. juncea*, *C. spectabilis* and *C. ochroleuca* in individual experiments. Treatments consisted of six rhizobacteria isolates (*Bacillus* spp. (BRM 32109 and BRM 32110), *Pseudomonas* spp. (BRM32111), *Pseudomona fluorescens* (BRM 32112), *Burkholderia pyrrocinia* (BRM 32113), *Serratia* spp. (BRM32114)) (Table 1), one fungal isolate (*Trichoderma* spp. (T-26)) (biochemical characterization and taxonomic classification underway) and a control. The microorganisms were selected from upland rice fields and are currently stored and preserved in the Multifunction Microorganisms and Fungi Collection of Embrapa Rice and Beans. The control treatment consisted solely of water, with no microorganisms applied. The microorganisms were applied at three moments: (1) seed microbiolization, (2) soil drenched with microbial suspension 10 days after sowing (DAS) and (3) plants sprayed with microbial suspension at 21 DAS.

The rhizoacterial isolates were grown on solid medium 523 (Kado & Heskett, 1970), at 28 °C, for 24 hours. The concentration was set to A540 = 0.5 (10^8 CFU, colony-forming units), in a spectrophotometer. *Trichoderma* spp. was grown in a Petri dish containing potato dextrose agar (PDA) for 5 days and suspensions were prepared and bioformulated as described by Silva et al. (2012). The concentration of the biological suspension was 10^8 conidia mL^{-1}.

For microbiolization, *Crotalaria* seeds were immersed in each microorganism suspensions, and control seeds in water, for 24 hours under constant agitation at 25 °C.

For soil drenching, 100 mL of the suspension of each treatment and water (control treatment) were applied to the soil at 10 DAS.

For plant spraying, 30 mL of the suspension of each treatment and water (control treatment) were sprayed onto leaves at a constant pressure, using a CO_2 pressurized manual backpack sprayer equipped with a hollow-cone spray nozzle (TX-VS2), was performed at 21 DAS.

Gas exchange was measured by using a portable gas exchange analyzer in the infrared region (LCpro+, ADC BioScientific, Hoddesdon, England). Photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), internal CO_2

Table 1. Isolate code, origin, biochemical characteristics and taxonomic classification of six rhizobacterial isolates

Isolate code[a]	Origin[b]	Color[c]	Biochemical characteristics					Taxonomic class
			AIA[d]	Cellulase[e]	Phosph.[f]	Sider.[g]	Biofilm[h]	
BRM 32109	GO/Brazil	White		+	+			*Bacillus* spp.
BRM 32110	PA/Brazil	White		+	+	+		*Bacillus* spp.
BRM 32111	PA/Brazil	Yellow		+	+		+	*Pseudomanas* spp.
BRM 32112	GO/Brazil	Yellow		+	+	+	+	*Pseudomanas* spp.
BRM 32113	PA/Brazil	Pink	+	+		+		*Burkolderia* spp.
BRM 32114	PA/Brazil	Pink	+	+	+	+		*Serratia* spp.

[a]Isolate code of the rhizobacterial and fungal isolates in the Multifunctional Microorganism and Fungi Collection of Embrapa Rice and Beans collection; [b]Geographical origin of each isolate; [c]Colony color, biochemical characterization and taxonomics classification of each isolate; [d]Indol acetic acid producer; [e]Cellulase producer; [f]Phosphatase producer; [g]Sideropher producer; [h]Exopolysaccharides producer

concentration (Ci, µmol mol^{-1}) and leaf temperature (Tleaf, ºC) were obtained. Readings were taken between 08:00 and 10:00 a.m., at 65 days after emergence (DAE). Samples were collected from the middle third of the youngest fully expanded leaves on the main stem. The equipment was set to use concentrations of 370-400 mol mol^{-1} CO_2 in the air, which is the reference condition used in the IRGA phothosynthesis chamber. The photon flux density photosynthetic active (PPFD) used was 1200 µmol [quanta] m^{-2} s^{-1}. The minimum equilibration time set for performing the reading was 2 min.

Shoot and root dry weight for each plot (one plant per pot) were determined in the full flowering stage. The shoots and roots were washed in water, dried in a forced-air circulation oven at 65 ºC for 72 hours and, then, weighed. After weighing, the dried shoot and root samples were ground and P, K, Ca, Mg, S, Cu, Fe, Mn, Zn and Mo concentrations determined as described by Donagema et al. (2011). Fe was only quantified in the shoots.

Most of the data showed normal distribution, with exception of shoot Mn (*C. juncea*), Fe and Mo (*C. spectabilis)*) and Fe (*C. ochroleuca*) concentration and root Cu, Mn and Zn (*C. juncea*), K, Ca, Mg, S and Mn (*C. spectabilis)*) and P, Ca, Mn, Zn and Mo (*C. ochroleuca*) concentration. These data were log-transformed (Log10 Y) for statistical analysis. The transformed and non-transformed data were submitted to analysis of variance (ANOVA). Tukey's test was performed for each *Crotalaria* species, at $p \leq 0.05$. Sisvar software 5.1 was used for statistical analyses (Ferreira, 2011).

Results and Discussion

No significant gas exchange effects caused by microorganisms were observed in *Crotalaria* plants under the experimental conditions. The respective values obtained for A, E, gs (number and activity of stomata) and Ci ranged from 10.34 to 18.99 µmol CO_2 m^{-2} s^{-1}, 1.61 to 2.14 mmol H_2O m^{-2} s^{-1}, 0.11 to 0.25 mol H_2O m^{-2} s^{-1} and 156 to 242 µmol mol^{-1} for *C. juncea,* 15.74 to 21.46 µmol CO_2 m^{-2} s^{-1}, 2.82 to 4.58 mmol H_2O m^{-2} s^{-1}, 0.19 to 0.26 mol H_2O m^{-2} s^{-1} and 185 to 235 µmol mol^{-1} for *C. spectabilis,* and 13.07 to 25.69 µmol CO_2 m^{-2} s^{-1}, 2.67 to 5.27 mmol H_2O m^{-2} s^{-1}, 0.15 to 0.31 mol H_2O m^{-2} s^{-1} and 179 to 214 µmol mol^{-1} for *C. ochroleuca*. Leaf temperature ranged from 28.0 to 35.3 ºC during assessment of the three *Crotalaria* species. According to Lang et al. (2015), it is essential to determine the influence of multifunctional microorganisms on plant physiology. This can be achieved by monitoring plant health and increased photoassimilate, biomass and grain production. The effect of multifunctional microorganisms on plant physiology is particularly evident under stressed conditions (Ahemad & Kilbret, 2014).

In addition, there was no significant effect on shoot and root phytomass accumulation in *C. juncea*, *C. spectabilis* and *C. ochroleuca* compared to their respective controls (Tables 2 and 3). However, *C. spectabilis* and *C. ochroleuca* differed between microorganism treatments. *C. spectabilis* treated with BRM 32113 exhibited higher SDMB (28.79 g) than that of

Table 2. Shoot dry matter biomass (SDMB) and shoot macro/micronutrients concentration of *C. juncea*, *C. spectabilis* and *C. ochroleuca*, grown in soil containing multifunctional microorganisms

Treatment	SDMB (g plant^{-1})	Macronutrients					Micronutriens				
		P	K	Ca	Mg	S	Cu	Fe	Mn	Zn	Mo
		(g kg^{-1})					(mg kg^{-1})				(µg kg^{-1})
					C. juncea						
32109	24.66 a	2.37 a	15.82 a	9.76 a	2.74 a	2.61 a	4.07 a	841.26 b	117.90 a	48.74 a	142.33 a
32110	24.73 a	2.07 a	15.06 a	7.78 a	2.35 a	1.86 a	3.41 a	281.95 c	72.88 a	43.09 a	172.40 a
32111	19.24 a	2.26 a	15.07 a	9.48 a	2.90 a	2.68 a	4.33 a	484.46 ab	135.73 a	37.90 a	136.99 a
32112	24.30 a	2.06 a	15.19 a	7.38 a	2.37 a	1.81 a	3.29 a	484.69 ab	74.63 a	42.08 a	155.79 a
32113	23.52 a	2.38 a	13.16 a	8.67 a	2.46 a	2.52 a	4.42 a	1388.13 a	94.46 a	45.57 a	140.66 a
32114	24.00 a	2.07 a	15.17 a	7.31 a	2.46 a	1.83 a	4.39 a	530.18 ab	75.76 a	35.17 a	116.45 a
T-26	22.32 a	2.23 a	14.62 a	8.49 a	2.68 a	2.03 a	4.28 a	451.15 ab	75.41 a	44.35 a	178.25 a
Control	23.33 a	2.17 a	14.58 a	8.65 a	2.61 a	2.20 a	4.04 a	573.12 ab	78.01 a	45.49 a	149.33 a
CV (%)	13.8	10.0	11.0	21.7	14.3	25.2	14.5	31.7	6.5	17.9	38.4
					C. spectabilis						
32109	25.73 ab	2.34 ab	14.09 a	13.77 ab	2.18 a	3.33 a	5.87 a	600.03 a	80.30 a	65.72 a	260.77 a
32110	14.00 c	2.77 a	13.35 a	13.94 ab	2.68 a	3.46 a	6.76 a	221.96 a	86.05 a	62.16 a	293.97 a
32111	17.88 bc	2.33 ab	11.84 a	14.31 a	2.62 a	3.60 a	6.56 a	230.95 a	96.43 a	62.62 a	176.27 a
32112	22.12 abc	2.45 ab	11.67 a	13.94 ab	2.48 a	3.42 a	5.81 a	143.96 a	74.53 a	58.89 a	221.16 a
32113	28.70 a	2.72 a	13.17 a	14.56 a	2.30 a	3.30 a	6.88 a	426.63 a	76.40 a	67.53 a	340.27 a
32114	17.21 bc	2.33 ab	15.00 a	13.77 ab	2.47 a	3.30 a	6.12 a	280.09 a	116.09 a	67.79 a	174.01 a
T-26	18.85 bc	2.08 b	12.32 a	11.35 ab	2.35 a	3.08 a	5.49 a	298.57 a	83.29 a	56.33 a	187.27 a
Control	24.69 ab	2.15 ab	11.42 a	11.03 b	2.23 a	2.73 b	6.60 a	204.56 a	79.79 a	57.31 a	306.59 a
CV (%)	20.4	11.1	12.5	10.6	10.7	9.7	11.9	11.7	29.1	19.1	9.1
					C. ochroleuca						
32109	24.91 cd	2.08 abc	14.57 a	6.68 a	3.03 a	2.91 ab	5.59 ab	411.56 a	77.76 a	65.34 a	251.21 ab
32110	10.80 e	1.72 bc	13.23 a	5.76 a	2.30 a	2.96 ab	3.17 b	129.56 a	78.10 a	52.60 a	93.19 b
32111	40.16 a	1.54 c	16.16 a	5.39 a	2.94 a	2.32 ab	4.27 ab	298.46 a	76.76 a	55.71 a	257.90 ab
32112	28.28 bcd	1.83 bc	14.48 a	6.89 a	3.00 a	3.06 ab	4.07 ab	267.76 a	61.81 a	49.05 a	316.17 a
32113	22.51 cd	2.64 a	14.98 a	6.74 a	3.07 a	3.78 a	6.20 a	222.23 a	75.90 a	58.61 a	311.28 a
32114	17.73 de	1.93 abc	15.12 a	8.62 a	3.14 a	3.63 a	4.48 ab	311.98 a	64.42 a	61.65 a	178.08 ab
T-26	32.32 abc	2.43 ab	15.50 a	8.19 a	3.36 a	3.50 a	5.46 ab	199.31 a	81.71 a	77.58 a	290.23 ab
Control	37.41 ab	1.39 c	14.23 a	5.94 a	2.87 a	2.73 ab	3.25 b	113.48 a	55.06 a	67.71 a	206.27 ab
CV (%)	14.9	12.8	11.3	18.0	12.7	11.9	19.4	10.6	18.2	21.1	28.0

Different letters in columns indicate significant diferences at $p \leq 0.05$ by the Tukey test; Shoot data for Mn (*C. juncea*), Fe and Mo (*C. spectabilis*)) and Fe (*C. ochroleuca*) were log-transformed (Log10 Y) for statistical analysis

Table 3. Root dry matter biomass (RDMB) and root macro/micronutrients concentration of *C. juncea*, *C. spectabilis* and *C. ochroleuca*, grown in soil containing multifunctional microorganisms

Treatment	RDMB (g plant^{-1})	Macronutrients					Micronutriens			
		P	K	Ca	Mg	S	Cu	Mn	Zn	Mo
		(g kg^{-1})					(mg kg^{-1})			(μg kg^{-1})
C. juncea										
32109	5.11 a	1.98 a	18.10 a	10.29 a	2.87 a	6.12 a	41.64 a	243.04 a	156.19 ab	494.04 a
32110	7.29 a	2.09 a	19.25 a	11.41 a	2.64 a	5.82 ab	48.53 a	288.29 a	290.77 a	727.36 a
32111	8.06 a	1.70 a	12.49 a	5.25 a	1.94 a	3.86 ab	30.23 a	416.81 a	112.80 ab	796.78 a
32112	5.18 a	2.01 a	18.17 a	8.28 a	2.79 a	5.82 ab	42.28 a	317.07 a	231.20 ab	767.70 a
32113	6.08 a	1.93 a	15.21 a	8.50 a	2.24 a	4.93 ab	29.42 a	234.18 a	187.59 ab	729.79 a
32114	7.84 a	1.87 a	15.23 a	5.46 a	2.16 a	4.22 ab	19.72 a	209.19 a	101.31 b	513.46 a
T-26	5.30 a	1.97 a	17.03 a	9.17 a	2.77 a	5.61 ab	24.44 a	249.70 a	177.22 ab	699.89 a
Control	8.32 a	1.40 a	11.20 a	6.01 a	1.73 a	3.09 b	27.39 a	228.28 a	123.64 ab	532.10 a
CV (%)	22.0	20.3	30.2	32.9	23.6	25.8	11.7	7.8	8.1	20.6
C. spectabilis										
32109	16.78 a	1.93 a	8.64 a	9.80 a	2.42 a	4.06 a	36.89 a	261.98 a	165.31 a	470.59 a
32110	11.39 a	1.75 a	12.11 a	4.31 a	2.26 a	4.70 a	24.47 a	321.20 a	103.95 ab	693.42 a
32111	10.88 a	1.68 a	10.10 a	5.33 a	2.12 a	4.35 a	24.38 a	285.79 a	89.49 b	578.35 a
32112	15.36 a	1.59 a	7.87 a	7.37 a	2.35 a	3.76 a	30.98 a	230.22 a	101.06 ab	440.50 a
32113	16.96 a	1.79 a	8.06 a	4.47 a	2.18 a	3.11 a	29.55 a	252.70 a	103.61 ab	607.53 a
32114	11.43 a	1.93 a	10.08 a	5.36 a	2.27 a	3.98 a	29.96 a	324.13 a	116.57 ab	504.06 a
T-26	9.58 a	1.81 a	6.82 a	6.66 a	2.71 a	3.76 a	28.21 a	210.12 a	102.17 ab	444.45 a
Control	17.65 a	1.94 a	9.19 a	6.04 a	2.52 a	4.18 a	30.15 a	215.33 a	130.57 ab	623.59 a
CV (%)	25.3	23.0	22.1	26.6	30.2	23.3	24.6	6.4	25.0	19.9
C. ochroleuca										
32109	12.87 bcde	2.04 a	15.44 a	4.72 a	2.21 a	6.15 a	31.23 ab	255.69 a	129.90 ab	967.30 a
32110	5.79 de	1.77 a	6.36 b	9.86 a	2.26 a	3.12 a	42.24 a	427.85 a	138.62 ab	818.60 a
32111	26.66 a	1.34 a	8.47 ab	3.51 a	1.71 a	3.15 a	26.13 ab	198.75 a	81.52 b	633.65 a
32112	16.06 bc	3.14 a	13.85 ab	8.27 a	2.05 a	5.39 a	31.56 ab	900.43 a	106.44 b	689.15 a
32113	8.62 cde	1.82 a	10.68 ab	6.31 a	2.06 a	4.41 a	29.49 ab	319.09 a	91.25 b	1048.77 a
32114	4.48 e	2.17 a	11.41 ab	8.76 a	2.81 a	6.66 a	35.39 ab	420.16 a	240.20 a	829.21 a
T-26	14.26 bcd	1.87 a	13.48 ab	4.81 a	3.16 a	6.93 a	29.58 ab	280.37 a	175.92 ab	667.21 a
Control	18.83 ab	1.29 a	8.71 ab	5.24 a	2.70 a	3.86 a	18.68 b	207.04 a	102.29 b	456.34 a
CV (%)	26.2	51.8	24.9	34.2	25.8	26.5	25.6	12.1	5.8	4.8

Different letters in columns indicate significant diferences at $p \leq 0.05$ by the Tukey test; Root data for Cu, Mn and Zn (*C. juncea*), K, Ca, Mg, S and Mn (*C. spectabilis*) and Ca, Mn, Zn and Mo (*C. ochroleuca*) were log-transformed (Log10 Y) for statistical analysis

plants treated with BRM 32110 (14.00 g), BRM 32111 (17.88 g), BRM 32114 (17.21 g) and T-26 (18.85 g) (Table 2), whereas the highest SDMB value for. *C. ochroleuca* was observed in the BRM 32111 treatment (40.16 g), with values of 24.91, 10.80, 28.28, 22.51 and 17.73 g for BRM 32109, BRM 32110, BRM 32112, BRM 32113 and BRM 32114, respectively. *C. ochroleuca* treated with BRM 32111 exhibited higher RDMB (26.66 g) than that obtained for the other treatments (Tables 2 and 3). Additionally, BRM 32110, BRM 32113 and BRM 32114 displayed reduced root biomass (5.70, 8.62 and 4.48, respectively), differing from the control treatment. Root and shoot biomass are important in cover crops because they represent better protection against soil erosion, nutrient cycling, breaking compacted soil layers and lower soil temperature when compared to conventional tillage (Nascente et al., 2013). With respect to shoot macro and micronutrient concentration, *C. spectabilis* plants treated with multifunctional microorganisms exhibited increased S concentration (23%), while those inoculated with BRM 32111 (*Pseudomonas* spp.) and BRM 32113 (*Burkholderia* spp.) showed a higher Ca concentration (31%) in relation to the control (Table 2). In *C. ochroleuca*, shoot concentration of P rose by 83% after inoculation with BRM 32113 and T-26 (*Trichoderma* spp.) and Cu concentration by 91% when compared to the control treatment. Shoot macro and micronutrient concentrations were similar between treatments for *C. juncea*.

According to Baldotto et al. (2010), fresh and dry matter of the root and shoot systems increased in pineapple plantlets inoculated with *Burkholderia*, resulting in 115, 112 and 69% higher N, P and K concentrations, respectively, than those obtained in controls. The genus *Burhholderia* includes phytopathogenic bacteria (Burkholder, 1950), endophytic diazotrophic bacteria (Perin et al., 2006) and symbiotic strains of the beta-rhizobia group that induce the formation of nitrogen-fixing root nodules in host plants (Rasolomampianina et al., 2005). The *Burkholderia* spp. used in the present study exhibited cellulase activity, in addition to producing IAA (índole-3-acetic acid) and siderophores (Table 1). Nevertheless, other studies have reported additional biochemical characteristics for *Burkholderia,* including phosphate solubilization (Ghosh et al., 2016), ACC deaminase activation (Onofre-Lemus et al., 2009) and biocontrol (Esmaeel et al., 2020).

Based on these results, microorganisms seem to stimulate greater nutrient availability in the soil solution, leading to accumulation of these nutrientes in the shoots of *Crotalaria* plants (Pérez-Garcia et al., 2011; Zhang et al., 2011). Teodoro et al. (2011) reported that different herbaceous leguminous species, including *Crotalaria* spp., demonstrated potential for nutrients recycling and N input (approximately 19.94 kg ha^{-1} of N) in crop production systems in the Brazilian Cerrado.

With regard to root macro and micronutrient concentration, S concentration rose by 98% and Zn by 135% in *C. juncea*

inoculated with BRM 32109 and BRM 32110, respectively, when compared to the control treatment (Table 3). Concentrations of Cu and Zn rose by 126 and 136% in *C. ochroleuca* treated with BRM 32110 and BRM 32114, respectively, in relation to the control. In *C. spectabilis* plants, root macro and micronutrient concentrations were similar between treatments.

Soil with low fertility predominates in tropical areas, making soil fertility management essential in maintaining an economically and environmentally sustainable farming system. The use of green manures and crop residues exerts different conditioning effects on the soil; however, the main objectives of this practice in low-fertility tropical soils are to improve the cation exchange capacity (CEC) and provide nutrients such as N, P, and S. Additionally, the decomposition of residues and OM releases nutrients such as Ca, Mg, K and trace elements (Valadares et al., 2016).

Overall, the three *Crotalaria* species treated with multifunctional microorganisms, showed no significant differences in agronomic performance, except for increasead shoot and root nutrient accumulation. This is essential to ensure greater nutrient availability for subsequent crops in soil that typically exhibits low fertility.

Conclusions

1. Multifunctional microorganisms, selected from upland rice fields, improved the nutritional status of *Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*.

2. In shoots, Ca concentration increased in *C. spectabilis* inoculated with BRM 32111 and BRM 32113, and those treated with multifunctional microorganisms generally showed higher S concentrations. *C. ochoroleuca* treated with BRM 32113 and T-26 exhibited higher P concentration and higher Cu concentration when inoculated with BRM 32113.

3. In roots, S and Zn concentrations were higher in *C. juncea* treated with BRM 32109 and BRM 32110, respectively; while *C. ochroleuca* inoculated with BRM 32110 and BRM 32114 showed greater Cu and Zn concentrations, respectively.

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