Hindawi
Applied and Environmental Soil Science
Volume 2022, Article ID 3228594, 13 pages
https://doi.org/10.1155/2022/3228594

Research Article

Co-Inoculation of *Trichoderma asperellum* with *Bacillus subtilis* to Promote Growth and Nutrient Absorption in Marandu Grass

Sidney Daniel Araújo da Costa,[1] **Aline Figueiredo Cardoso**,[2] **Gledson Luiz Salgado de Castro**,[1] **Dalton Dias da Silva Júnior**,[3] **Thiago Carvalho da Silva**,[1] **and Gisele Barata da Silva**[1]

[1]*Federal Rural University of Amazonia (UFRA), Belém, Brazil*
[2]*Vale Institute of Technology (ITV), Belém, Brazil*
[3]*Federal University of Amazonas (UFAM), Manaus, Brazil*

Correspondence should be addressed to Gisele Barata da Silva; giselebaratasilva@gmail.com

Received 1 February 2022; Revised 5 November 2022; Accepted 2 December 2022; Published 17 December 2022

Academic Editor: Karan Singh

The authors have investigated the effects of different doses of mineral fertilizers combined with co-inoculation of *Bacillus subtilis* and *Trichoderma asperellum* to promote plant growth and use efficient nutrients in a plant *Urochloa* (Syn. *Brachiaria*) *brizantha* cv. Marandu. Individual experiments with doses of the nutrients nitrogen (N), phosphorus (P), and potassium (K) were carried out. The experiment was conducted in a completely randomized design, factorial 3×5. With the treatments: control non-inoculation, inoculation with Bacillus subtilisUFRA-92, and coinoculation (MIX) with *T. asperellum* (UFRA-06, UFRA-09, UFRA-12, and UFRA-52) + *B. subtilis* x nutrient omissions (0%, 25%, 50%, 75%, and 100%). Each treatment had five replications. Biometric parameters, nutrient content, and nutrient use efficiency were evaluated. The results showed that the inoculants promoted growth in Marandu grass. The use of inoculants promoted growth and increased N, P, and K uptake by Marandu grass. Co-inoculation changed leaf area, shoot length, elongation rate, and leaf appearance for N and K and root dry mass for P. In addition, the co-inoculation combined with doses of 75% of N, 50% of P, and 50% of K increased the nutritional content of the leaves by 256% of N, 280% of P, and 29% of K and provided greater agronomic efficiency, with increments of 462% of N, 544% of P, and 177% of K, compared to the control treatment. We present the potential of co-inoculation of *B. subtilis* and *T. asperellum* to promote the growth of *Urochloa* under reduced fertilizer application. There was an improvement in plant growth and nutrient use efficiency.

1. Introduction

Meeting global food demand requires efficient agricultural management. The projections demand greater land use and an increase in the need for mineral fertilizers, it is estimated that in 2050, 52million tons of fertilizers will be consumed [1]. Currently, Brazil ranks fourth in the world in terms of fertilizer consumption, and this implies a high cost and possibly greater impacts on the environment [2]. Activities such as agriculture have their share of contribution in this scenario. Although historically, fertilizers were used infrequently in the preparation of pastures, currently there is greater use [3]. In the Brazilian Amazon, there are at least 56.6 million hectares of pastures and the main problems are the impoverishment and degradation of soils [4].

The intensive use of land demonstrates the need to develop alternative technologies to efficiently use nutrients and do less damage to the environment. Thus, the use of growth-promoting microorganisms can contribute as a potent tool. Studies with the use of microorganisms in

different cultures show that they have different modes of action that favor plant growth, mainly due to the better use of nutrients. Growth-promoting microorganisms are responsible for nitrogen mineralization, phosphorus, and potassium solubilization [5]. In addition, they can alter the synthesis of plant hormones, such as auxin and gibberellin [6].

Bioinoculation in the grass is a technique used and has shown increases in the growth of the genera (*Azospirillum brasilense*, *Cynodon*, and *Megathyrsus*). Inoculation of *A. brasilense* associated with nitrogen fertilization in *Cynodon dactylon* promoted positive effects on shoot dry mass [7]. The use of biopromoter in *Megathyrsus* (Syn. *Panicum*) promoted significant increases in the accumulation of shoot and root biomass and higher concentrations of macro and micronutrients [8]. The effect of *Trichoderma* on grass development needs to be elucidated, to verify if it promotes increases, as shown in other crops of the *Poaceae* family [9–11]. Inoculation of *Trichoderma* increased the rate of twinning and growth in *Lolium multiflorum* compared to the noninoculated treatment [12]. *Bacillus* inoculation in *Lolium multiflorum* promoted a significant increase in shoot biomass [13]. Previous results also report that *Bacillus* sp. and *Trichoderma* sp. are growth-promoting agents, which contribute to the better development of several crops of the *Poaceae* family, such as rice, corn, and wheat [14–17].

The genera applied in this study, with characteristics that promote growth in other cultures, need to be studied as inoculants in the grass, aiming to increase growth and reduce mineral fertilization. Thus, the objective was to study the effects of different doses of mineral fertilizers combined with the co-inoculation of *Bacillus subtilis* and *Trichoderma asperellum* on growth promotion and nutrient use efficiency in *Urochloa* (Syn. *Brachiaria*) *brizantha* cv. Marandu.

2. Materials and Methods

2.1. Characterization of the Experimental Area. The experiment was carried out in the Seedling Production Unit of the Federal Rural University of Amazonia, Belém, State of Pará, Brazil. The temperature was 32°C and the humidity was 75%, and the regional climate according to the Köppen classification is Afi [18].

2.2. Microbial Isolates. The microbial isolates are stored and preserved in the collection of microorganisms owned by the Plant Protection Laboratory (PPL) of the Federal Rural University of Amazonia. *Bacillus subtilis* UFRA-92 (GenBank MN175193) from açaí palms was isolated and tested for plant growth promotion [19]. In addition, four isolates of *Trichoderma asperellum* (UFRA-06, UFRA-09, UFRA-12, and UFRA-52) were isolated from rhizosphere soils of reforested and native forest areas in the Amazon and tested for plant growth promotion [20].

2.3. Microbial Preparation and Inoculation. The*Bacillus subtilis*were grown in 523 solid medium for 24 h at 28°C. This was the culture medium in which the microorganism was grown. Then, bacterial suspensions were prepared with sterilized water. The concentration was adjusted in a spectrophotometer to 550 nm, corresponding to 10^8 colony-forming units (CFUs) [21, 22]. The isolates of *T. asperellum* were grown on PDA culture medium at ±28°C, with 12 h photoperiod for seven days. Then, the suspensions were prepared with sterilized water, and the concentration was adjusted to 6×10^8 conidia mL^{-1} [20]. The irrigation was carried out 14 days after seedling emergence, and each vase of polyethylene received 5 mL of microbial suspension [23]. When co-inoculated, 2.5 mL of *B. subtilis* and 2.5 mL of *T. asperellum* were used.

2.4. Experimental Design. Simultaneous and independent experiments were carried out to evaluate the effect of fertilization when doses of nitrogen (Exp. 1), phosphorus (Exp. 2), and potassium (Exp. 3) were reduced and associated with the inoculation of microorganisms. The experiment was conducted in a completely randomized design factorial3 × 5. The treatments were control without inoculation, inoculation with *Bacillus subtilis* UFRA-92, and coinoculation (MIX) with *T. asperellum* (UFRA-06, UFRA-09, UFRA-12, and UFRA-52) + *B. subtilis* x nutrient omissions (0%, 25%, 50%, 75%, and 100%) and each treatment with five replicates.

2.5. Substrate and Sowing. The soil used was collected at the Institute of Agricultural Sciences (UFRA) in 0 to 20 cm layer and classified as a dystrophic yellow latosol with a sandy loam texture [24]. Soil chemical analysis was performed according to [25] and is presented in Table S1. Initially, ten seeds of *Urochloa* (Syn. *Brachiaria*) *brizantha* cv. Marandu in polyethylene pots (20 cm × 30 cm × 0.05 cm), and after 14 days of sowing was performed, leaving one plant per pot.

2.6. Mineral Fertilization. Mineral fertilization was carried out seven days before sowing. The doses used for fertilization were 0, 25, 50, 75, and 100% according to the recommendation for the crop [26]. And the sources used were urea (N), simple superphosphate-P_2O_5 (P), and potassium chloride-K_2O (K). In experiment 1, N doses were tested and the recommendation was 50 $kg{\cdot}ha^{-1}$, equivalent to 0.208 g/vase. Regardless of the N dose, recommended fertilization of P (80 $kg{\cdot}ha^{-1}$) and K (40 $kg{\cdot}ha^{-1}$) was performed. In experiment 2, P doses were tested with a recommendation of 80 $kg{\cdot}ha^{-1}$, equivalent to 0.834 g/vase. Regardless of the P dose, N (50 $kg{\cdot}ha^{-1}$) and K (40 $kg{\cdot}ha^{-1}$) fertilization was performed. In experiment 3, K doses were tested and the recommendation was 40 $kg{\cdot}ha^{-1}$, equivalent to 0.125 g/vase. Regardless of the K dose, N (50 $kg{\cdot}ha^{-1}$) and P (80 $kg{\cdot}ha^{-1}$) fertilization was performed.

2.7. Morphogenetic and Biometric Analysis. At 35 days after emergence, biometric parameters were evaluated and calculated according to Gomide and Gomide [27]: leaf appearance rate (LAR- leaves. $Tiller^{-1}$. day^{-1}) was found by the number of leaves that appeared divided by the number of

days cycle evaluation, leaf elongation rate (LER- cm. tiller^{-1}. day^{-1}) was found by the difference between the final and initial lengths of leaf blades divided by the number of days of the experiment. Plant height, leaf, and root dry mass were also measured, and leaf area was calculated using equation ($Y = 0.7480 \times (\text{length} \times \text{width})$) [28] For biomass evaluation, five plants by treatment were collected and separated into shoots and roots. The plant material was dried in an oven (60°C) until reaching constant mass.

2.8. Nutritional Analysis. The leaves were collected and placed to dry in an oven with forced air circulation, at an average temperature of 60°C until reaching constant weight. Afterward, milling was carried out in model Willey , ensuring the homogenization of the sample. Subsequently, the decomposition of the plant tissue was carried out, to determine the levels of nutrients (N, P, and K) with perchloric nitric solubilization, using the wet method [25].

2.9. Nutrient Use Efficiency (NUE). The efficiency of nutritional use was calculated from the agronomic efficiency (AE), physiological efficiency (PE), apparent recovery efficiency (ARE), and nutrient response efficiency (NRE) according to Choudhary et al. [29], using the following formulas:

$$\text{AE (kg kg)}: \frac{\text{Biomass inoculated pots (kg)} - \text{Biomass uninoculated pots (kg)}}{\text{Applied nutrient content (kg)}},$$
$$\text{PE (kg kg)}: \frac{\text{PBVI (kg)} - \text{PBVNI (kg)}}{\text{NAVA} - \text{NAVNA (kg)}}, \quad (1)$$

where PBVI = production of biomass from inoculated pots, PBVNI = production of biomass from uninoculated pots, NAVI = nutrient absorbed from inoculated pots, and NAVNI = nutrient absorbed from uninoculated pots.

$$\text{ARE (\%)}: \frac{\text{NAVI (kg)} - \text{NAVNI (kg)}}{\text{Applied nutrient content (kg)}} \text{ X } 100,$$
$$\text{NRE (kg kg)}: \frac{\text{Production (kg)}}{\text{Available nutrient content (kg)}}. \quad (2)$$

2.10. Statistical Analysis. Data were evaluated for normality of residuals by the Kolmogorov–Smirnov test. Then, they were subjected to analysis of variance, and when F values were significant, the SNK test was performed with 5% probability for the isolated effect of microorganisms. When there was an isolated effect of the doses and interaction between the factors, a regression analysis was performed (Table S2). Nutritional analysis data were subjected to the t-test ($p < 0.05$).

3. Results

Biometric variables showed an interaction between dose and microorganism factors ($p < 0.05$). For N, the factor doses and microorganisms were significant for the variables height, leaf area, and leaf appearance rate. Plant height presented adjustment to the quadratic regression model with maximum efficiency point at 79%, 53%, and 87% of N using MIX, UFRA-92, and control, respectively (Figure 1(a)). The leaf area as a function of N doses increased linearly for the control and UFRA-92, while it showed a quadratic adjustment for the MIX, with a maximum point at 46% (Figure 1(b)). Leaf appearance rate increased linearly as a function of doses for the three treatments (Figure 1(c)).

There was a significant interaction ($p < 0.05$ for phosphorus doses for root dry mass. It presented fit to the quadratic model for MIX and UFRA-92 with maximum efficiency point at 43% and 64%, respectively, while the control had linear growth (Figure 2).

MIX and UFRA-92 in height presented adjustment to the quadratic model, with maximum efficiency points at 61% and 65%, respectively, while the control showed a positive linear increase for potassium doses (Figure 3(a)). The leaf area presented adjustment to the quadratic model in the control and UFRA-92, with maximum efficiency points at 43% and 50%, respectively, while the MIX presented adjustment to the linear model (Figure 3(b)). The leaf elongation rate had a quadratic behavior for the control with a maximum efficiency point at 82%, while the MIX and UFRA-92 had an adjustment to the linear model for the doses (Figure 3(c)).

The isolated action of the nutrient had a significant effect at ($p < 0.05$) on the biometric variables. For nitrogen, the factor acts in a dependent manner for shoot dry mass, root dry mass, root length, and leaf elongation rate (Figure 4), and the average behavior of the variables had linear growth as a function of nitrogen doses.

For phosphorus, regardless of the microorganisms, all variables had quadratic growth, with maximum efficiency point at 65% for height (Figure 5(a)), 53% for shoot dry mass (Figure 5(b)), 67% for the length of root (Figure 5(c)), and 65% for leaf area (Figure 5(d)), while leaf elongation rate (Figure 5(e)) and leaf appearance (Figure 5(f)) had linear behavior.

For potassium doses, the isolated action of the doses promoted a quadratic behavior with a maximum efficiency point in 65% in height (Figure 6(a)), 66% in root Dry mass (Figure 6(b)), and 62% in root length (Figure 6(c)) and showed a linear increase for leaf appearance rate (Figure 6(d)).

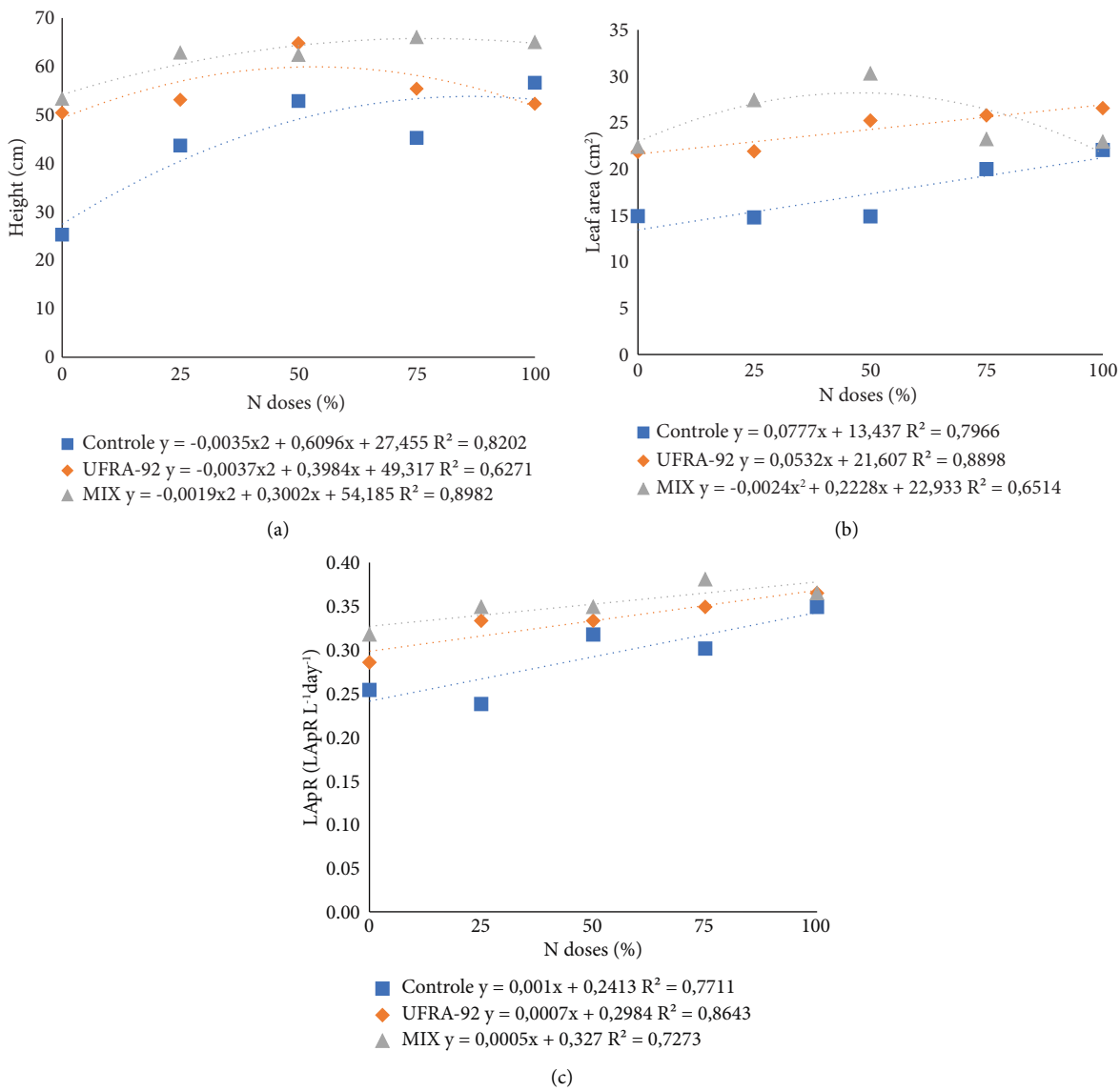

Figure 1: Effect of inoculation with *Bacillus subtilis* (UFRA-92) and *Trichoderma asperellum* + UFRA-92 (MIX) as a function of nitrogen doses on height (a), leaf appearance rate (b), and leaf area (c) of *Urochloa brizantha* cv. Marandu.

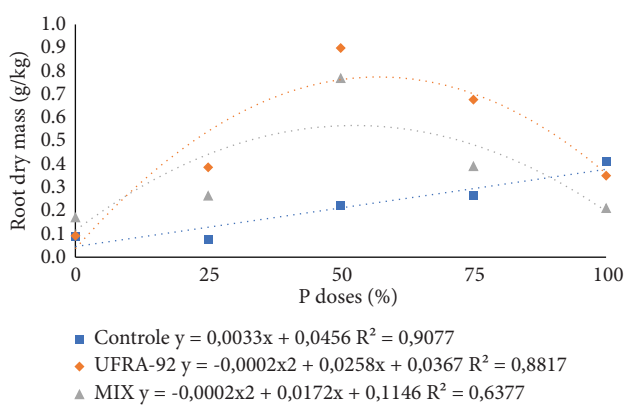

Figure 2: Effect of inoculation with *Bacillus subtilis* (UFRA-92) and *Trichoderma asperellum* + UFRA-92 (MIX) as a function of phosphorus doses on the dry mass root of *Urochloa brizantha* cv. Marandu.

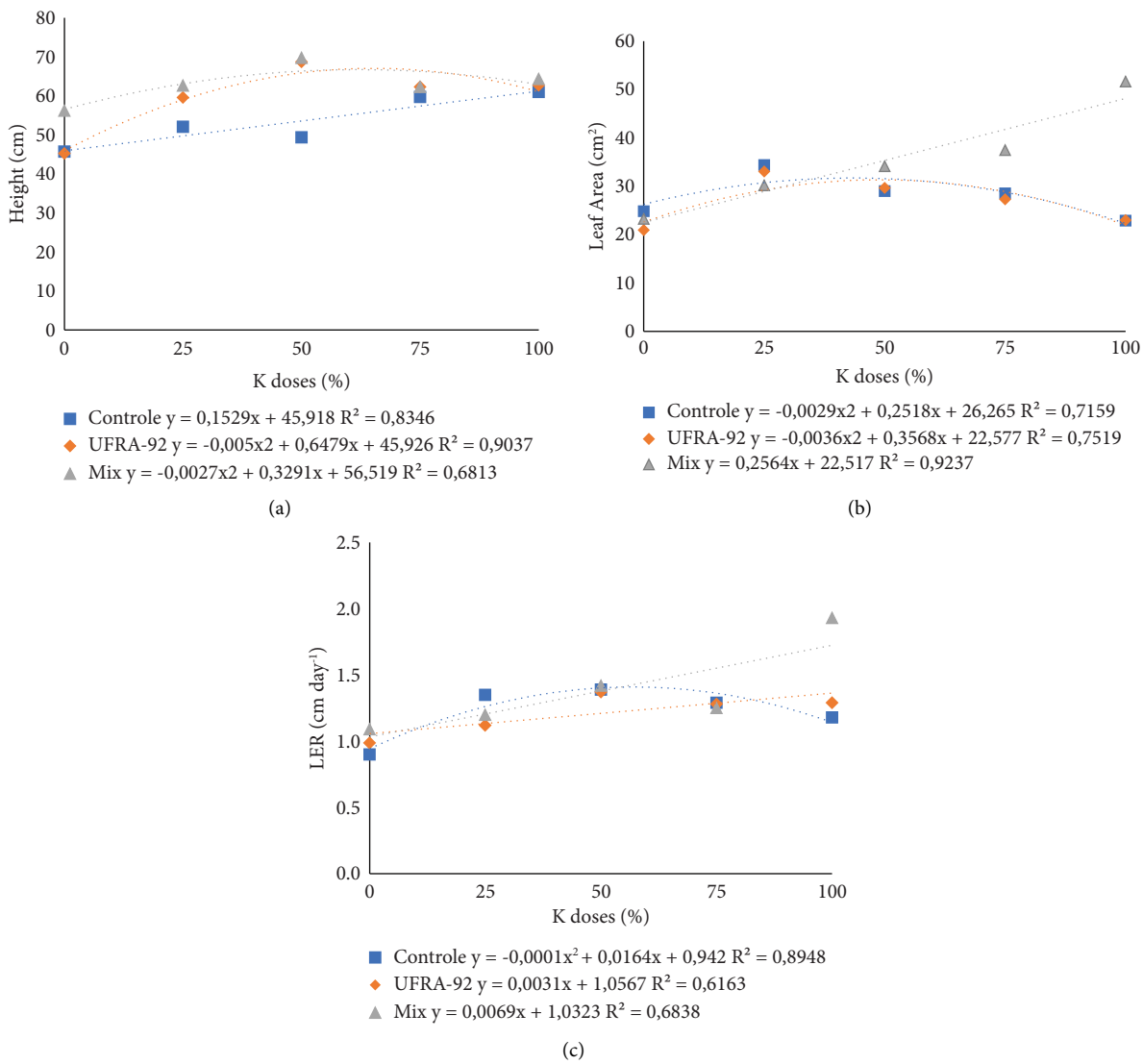

Figure 3: Effect of inoculation with *Bacillus subtilis* (UFRA-92) and *Trichoderma asperellum* + UFRA-92 (MIX) as a function of potassium doses on height (a), leaf area (b), and leaf elongation rate (c) of *Urochloa brizantha* cv. Marandu.

The effect of inoculation regardless of nutrient doses was significant at ($p < 0.05$) for biometric variables, compared to the control (Table 1). Considering a dose of 75% N, the microorganisms promoted an increase in the growth of *U. brizantha*, up to 104% in root dry matter, 194% in shoot matter, 22% in root length, and 11% in the rate of leaf elongation, concerning the control.

Inoculation with the microorganisms combined with 50% P increased the growth of Marandu grass. The application of UFRA-92 promoted an increase of 156% in shoot dry mass, 51% in shoot length, 20% in root length, and 9.3% in leaf elongation rate about the control (100% P) (Table 1). Only for the variable leaf area (31%), the MIX provided a greater increase concerning UFRA-92 (Table 1).

For the dose of 50% K, the MIX provided greater gains than the isolated application of UFRA-92, with an increase of 117% in root dry mass, 34% in root length, and 20% in the rate of leaf appearance, concerning control. The exception was for the variable shoot dry mass (47%) increment in plants that received only UFRA-92 (Table 1).

3.1. Nutritional Content. From the results found, the best doses were selected as a function of root and aerial biomass to carry out the nutritional analysis. Thus, material collections were carried out at doses of 75% of N, 50% of P, and 50% of K from inoculated plants compared to the 100% control.

The nutritional content of shoots increased as a function of microorganisms. The MIX associated with a 75% reduction of N, 50% of P, and 50% of K had an increase of 256%, 280%, and 29%, compared to the control (100%), respectively. Inoculation with UFRA-92 provided increases of 35% in N, 75% in P, and 11% in K when compared to the control (100%) (Figure 7).

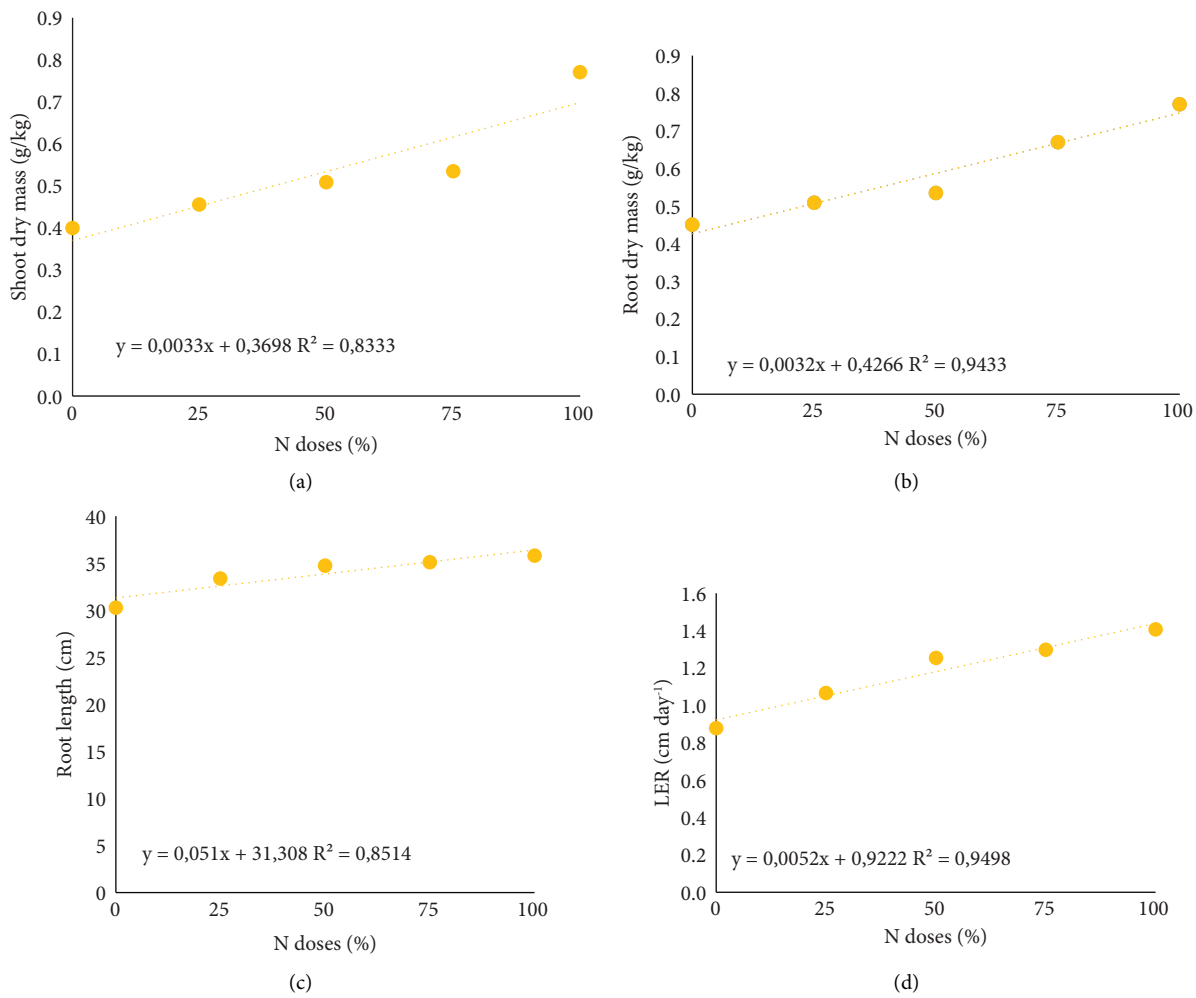

Figure 4: Isolated effect of reduced nitrogen doses on shoot dry mass (a), root dry mass (b), root length (c), and leaf elongation rate (d) of *Urochloa brizantha* cv. Marandu.

3.2. Nutrient Use Efficiency (NUE). From the best doses evaluated in the nutritional analysis, 75% of N, 50% of P, and 50% of K, calculations of efficient use were performed, compared to the control (100%). The results showed significant values of ($p < 0.05$) for all indicators of efficient use. Inoculation with MIX and UFRA-92 showed greater agronomic efficiency, generating increments of 462% and 144% for N, 544% and 103% for P, and 177% and 122% for K, respectively, when compared to the control (100%) (Table 2).

The inoculation of MIX and UFRA-92, on physiological efficiency, provided increases of 113% and 100% for N, 376% and 13% for P, and 14% and 7% for K, respectively, concerning the control (100%). The apparent recovery efficiency presented increments of 672% and 144% for N, 570% and 188% for P, and 217% and 136% for K in the MIX and UFRA-92 treatments, respectively. The increments found in the nutrient recovery efficiency were 369% and 128% for N, 312% and 200% for P, and 132% and 109% for K in the MIX and UFRA-92 treatments, respectively, when compared to 100% control (Table 2).

4. Discussion

Grass inoculation with growth-promoting microorganisms has been studied and has shown significant results in the growth and reduction of mineral fertilizer [30–32]. The genera applied in this study, with growth-promoting characteristics in other crops, need to be studied as inoculants in the grass, aiming to increase growth and reduce mineral fertilization.

In the present study, from experiments under greenhouse conditions, the synergistic effect of the co-inoculation of *B. subtilis* with *T. asperellum* in improving growth, biomass production, and N, P, and K uptake in Marandu grass was demonstrated, resulting in better nutrient use efficiency. The synergistic interactions that favored plant growth may have occurred from the formation of a biofilm MIX between *Bacillus* and *Trichoderma*, due to increased intracellular growth or colonization of the bacteria in the fungal hypha.

These mechanisms allow wide colonization of microorganisms along the roots and even penetration into the plant tissue [33], a condition that may contribute to

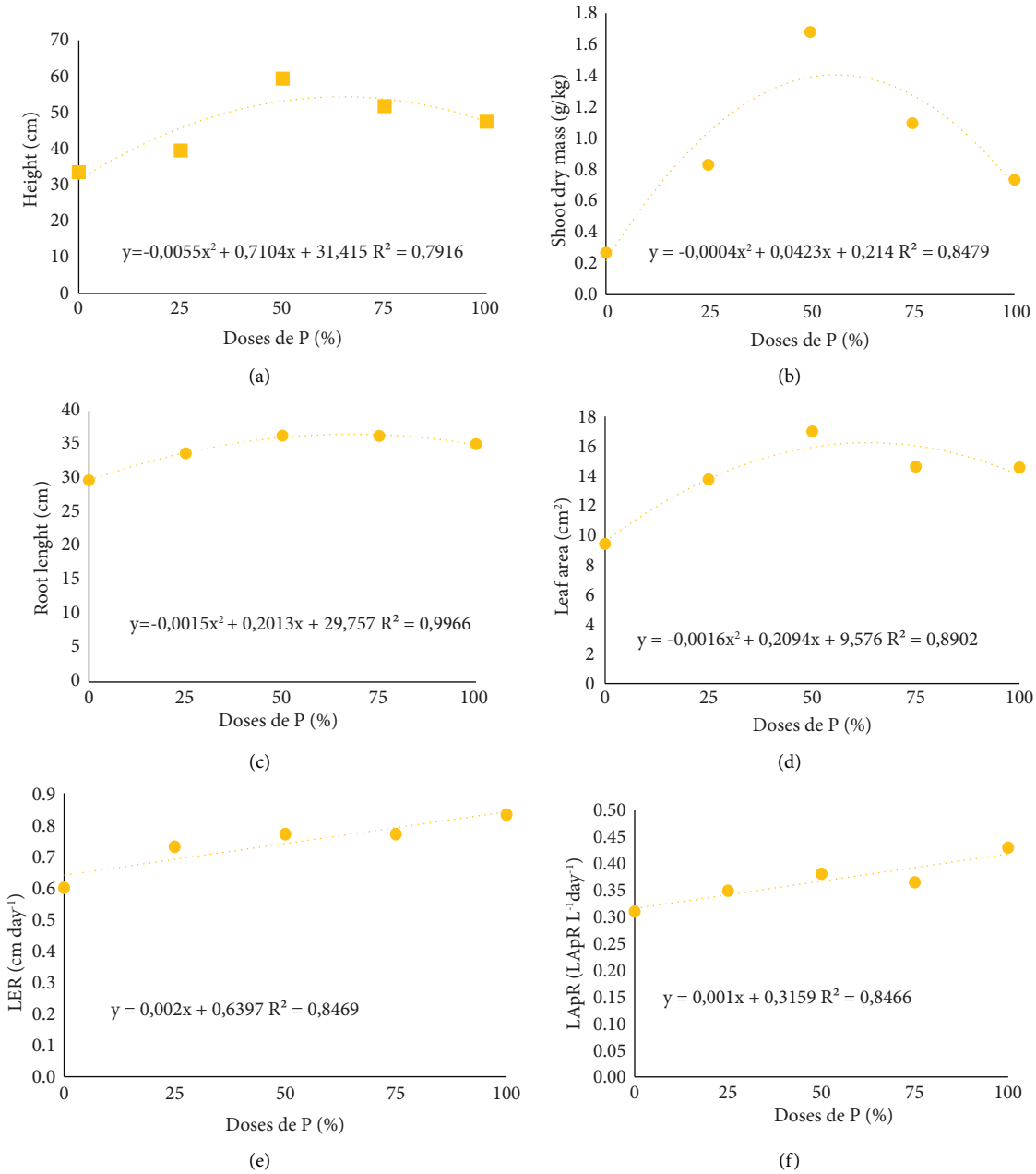

Figure 5: Isolated effect of reduced phosphorus doses on height (a), shoot dry mass (b), root length (c), leaf area (d), leaf elongation rate (e), and leaf appearance rate (f) of *Urochloa brizantha* cv. Marandu.

explaining the expressive increase in all variables of the growth of Marandu grass, when inoculated with UFRA-92 and *Trichoderma* (MIX), independent of the nutrient, concerning the isolated inoculation of UFRA-92.

The co-inoculation promoted a greater increase in height, leaf area, and leaf appearance rate (Figure 1). The results found to support the hypothesis that plants inoculated with biostimulants favor grass growth in reduced doses of mineral fertilizers. The reduction in N can be attributed to the mineralization of organic N, the acquisition and assimilation of mineral N, and/or the reduction in volatilization and leaching, leading to an increase in grass growth.

The greater development of inoculated plants may also be associated with greater production of hormones linked to plant growth (auxin, gibberellin, and cytokinins) and hormones linked to physiological processes that promote greater plant growth. As noted by Paungfoo-Lonhienne et al. [34], *Pennisetum clandestinum* inoculated with *Paraburkholderia* sp. and seeded with 50% organic fertilizers and 50% conventional N produced yields similar to those obtained with the conventional 100% N fertilizer. *Urochloa* sp. inoculated with PGPR at reduced N doses also showed significant increases in the rate of appearance and leaf elongation, possibly due to the greater production of plant hormones [35].

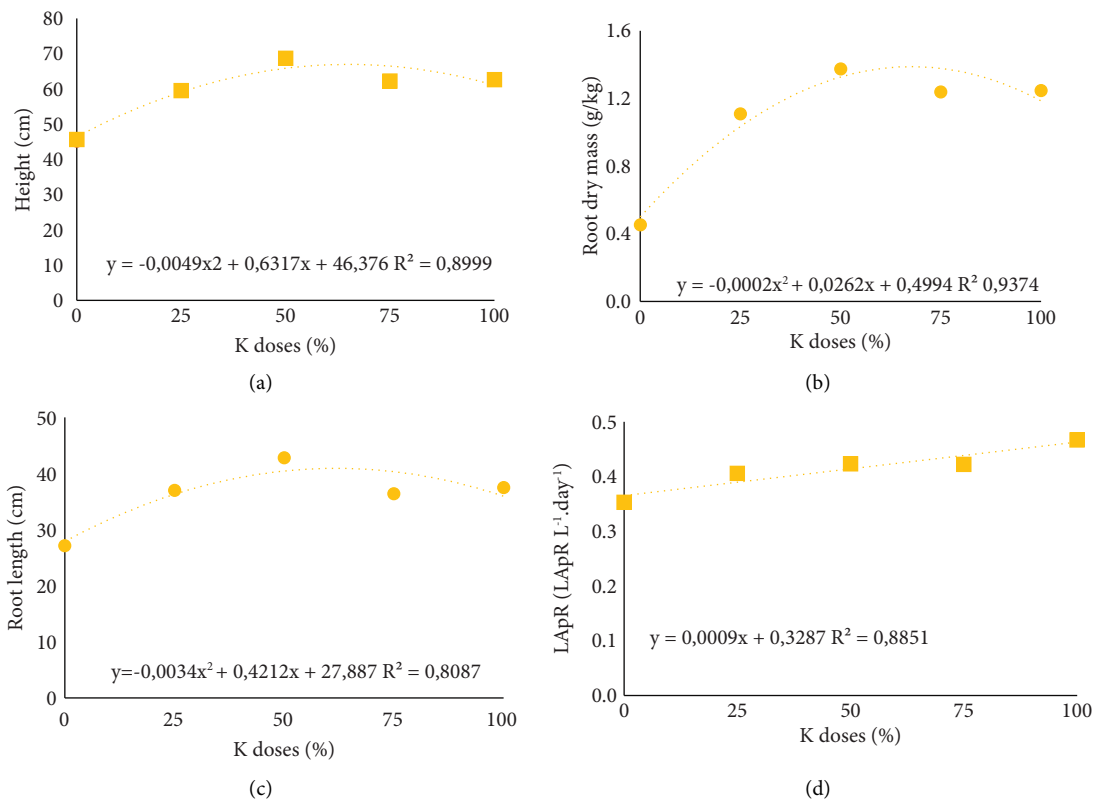

Figure 6: Isolated effect of reduced potassium doses on height (a), root dry mass (b), root length (c), and leaf appearance rate (d) of *Urochloa brizantha* cv. Marandu.

Table 1: Effect of inoculation with *Bacillus subtilis* (UFRA-92) and *Trichoderma asperellum* + UFRA-92 (MIX), associated with 75% N, 50% P_2O_5, and 50% K_2O, on biometric parameters of *Urochloa brizantha* cv. Marandu.

Treatments	RDM g/kg	SDM g/kg	RL cm	Height cm	LA cm^2	LER cm^2	LApR cm^2
Control (N)	0.50*c*	0.37*c*	34.6*b*	—		1.2*b*	—
UFRA-92	0.67*b*	0.53*b*	33.8*b*	—	—	1.4*a*	—
MIX	1.02*a*	1.09*a*	42.3*a*	—	—	1.3*a*	—
Control (P)	—	0.73*b*	33.70*b*	40.53*b*	15.02*b*	0.7*b*	0.35*b*
UFRA-92	—	1.87*a*	40.33*a*	61.10*a*	16.99*b*	0.8*a*	0.38*a*
MIX	—	1.68*a*	36.33*b*	59.40*a*	20.45*a*	0.7*b*	0.35*b*
Control (K)	0.66*c*	1.76*b*	35.30*c*	—	—	—	0.35*b*
UFRA-92	1.38*b*	2.58*a*	42.83*b*	—	—	—	0.37*b*
MIX	1.83*a*	2.48*a*	47.20*a*	—	—	—	0.42*a*

Nitrogen is responsible for the vegetative growth of the plant; in this sense, the greater absorption and translocation of N stimulated by the microorganisms MIX and UFRA-92 promoted greater shoot growth and greater leaf expansion. The increase in leaf area from bacterial co-inoculation in *U. brizantha* was verified by Lopes et al. [23], with an increase of 700% and 108%, when compared to unfertilized plants and fertilized plants, respectively. The increases found in reduced doses of N, associated with microorganisms, can also be related to the greater activity of enzymes responsible for the mineralization of this nutrient, while high doses of inorganic fertilizers can inhibit their action [36].

Root development is an important characteristic of the plant, as it allows the establishment and exploitation of water and nutrients, essential for its growth. In the present study, co-inoculation associated with a 50% reduction in the recommended dose of P provided greater root dry mass (Figure 2). It is possible to infer that the mechanism of phosphorus solubilization by microorganisms resulted in a high accumulation of P in the plant fertilized with 50% of the dose when compared to fertilization only with mineral fertilizer.

The greatest root development found can be related to the participation of phosphorus in plant development, as it acts in physiological processes, root growth, tiller size, and

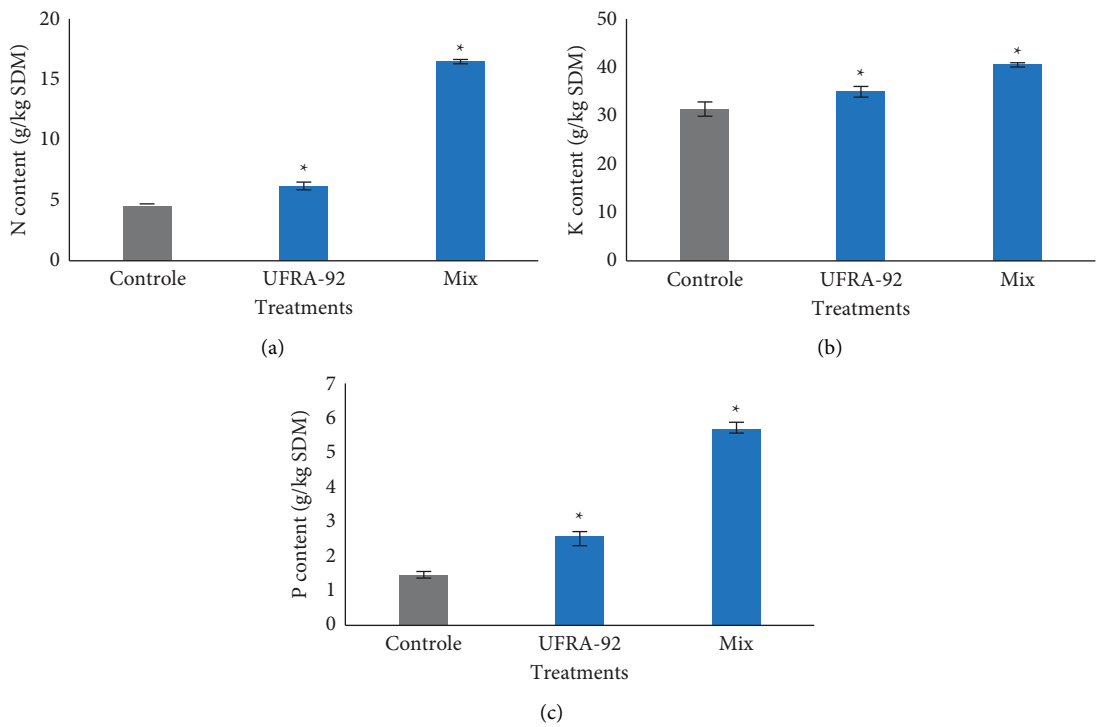

Figure 7: Effect of inoculation with *Bacillus subtilis* (UFRA-92) and *Trichoderma asperellum* + UFRA-92 on the shoot dry mass nutritional content of *Urochloa brizantha* cv. Marandu by the t test ($p < 0.05$).

Table 2: Effect of inoculation with *Bacillus subtilis* (UFRA-92) and *Trichoderma asperellum* + UFRA-92 (MIX), associated with 75% N, 50% P_2O_5, and 50% K_2O, on agronomic efficiency indices (EA), physiological efficiency (PE), apparent recovery efficiency (ARE), and nutrient response efficiency (NRE) of *Urochloa brizantha* cv. Marandu.

Treatments	AE kg/kg^{-1}	PE kg/kg^{-1}	ARE %	NRE kg/kg^{-1}
Control (N)	1.26*c*	29.87*c*	4.75*c*	6.05*c*
UFRA-92	3.08*b*	59.89*b*	11.59*b*	13.79*b*
MIX	7.08*a*	63.90*a*	36.71*a*	28.37*a*
Control (P)	2.68*c*	0.75*c*	1.34*c*	11.86*c*
UFRA-92	5.45*b*	0.85*b*	3.87*b*	35.62*b*
MIX	17.26*a*	3.57*a*	8.98*a*	48.85*a*
Control (K)	23.77*c*	48.20*b*	43.19*c*	58.05*c*
UFRA-92	52.90*b*	51.75*b*	102.22*b*	121.45*b*
MIX	66.00*a*	55.04*a*	136.93*a*	134.55*a*

tillering [37]. Inoculated plants showed greater root development, as microorganisms can stimulate root growth by synthesizing or altering hormonal metabolic pathways, such as IAA and cytokinins, which act to elongate hairs and multiply root meristem cells [38]. In previous studies, with *Tanzania* grass (*Panicum maximum*) inoculated with rhizobacteria, an increase in root biomass was observed, which was associated with an increase in IAA concentration and a reduction in ethylene [39].

The increments found, with 50% of K^+, in height, leaf area, and leaf elongation rate can be attributed to differentiated absorption by the inoculated plants (Figure 3). The association with microorganisms causes changes in the architecture of the root system, such as density, length, and amount of root hairs that directly interfere in the exploration and acquisition of the nutrient in the soil, as well as may have provided a lower rate of K^+ leaching due to root biomass per soil volume.

Results found by Archana et al. [40] showed that the inoculation of *Bacillus* spp. promoted an increase in corn growth and yield and nutrient accumulation, when compared to plants not inoculated with mineral fertilization. Forage sowed with substrate containing mica (K^+ source) and co-inoculated with *Bacillus* and *Azotobacter* resulted in biomass accumulation (70%) and higher nutritional content of N (118%) and K (165%), when compared to the uninoculated control [41].

The isolated effect of nitrogen provided an increase in shoot and root dry mass, root length, and leaf elongation rate (Figure 4). For P, the influenced variables were height, shoot

dry mass, root length, leaf area, elongation rate, and leaf appearance (Figure 5), while for K, the variables were height, dry mass, root length, and leaf appearance rate (Figure 6).

The increase observed in these variables may be indirectly associated with changes in plant architecture, as root length, influenced by the three experiments, allows for greater exploitation of water and nutrients. N and K in the soil have a high rate of leaching and volatilization; in this sense, a greater number of root hairs may have allowed greater absorption and, as a consequence, greater production of root and aerial biomass, as well as the rate of appearance and leaf elongation. For P, we can infer that produced organic acids contributed to its greater availability. Co-inoculation of microorganisms promotes cumulative effects on the host by increasing the availability of nutrients, from solubilization to the production of hormones linked to plant growth [42].

The action of microorganisms regardless of N, P, and K doses was positive on all biometric variables compared to the control (100%). Banayo et al. [43], evaluating the effect of biofertilizers at different rates of inorganic fertilizers on corn yield, found that there was no interaction between the factors, but inoculated treatments showed higher yields at reduced doses of inorganic fertilization, while the isolated use of inorganic fertilizers presents a positive linear behavior, depending on the increase in doses, the same behavior can be verified in this study.

In the present study, inoculation with MIX increased the absorption rate and accumulation of macronutrients (Figure 7). It is reported that the genus of microorganisms used in this work has effective mechanisms that contribute to nutrient availability [44, 45]. The bacterial isolate is one of the genera reported in the literature with the ability to fix atmospheric nitrogen [46]. The *Trichoderma* used presents reports of improvements in the nutritional quality of other cultures of the *Poaceae* family [47].

Nitrogen fixation by this microorganism happens because this genus has a group of Nif genes, which is responsible for encoding nitrogenase, the enzyme responsible for nitrogen fixation [48]. A study carried out by Ding et al. [49] based on the isolation of bacteria from the rhizosphere, in the region of China, found that seven isolates, including the genus *Bacillus*, had the Nif gene.

Phosphate fertilizers, when applied to soil, can be rapidly mobilized into insoluble compounds with Al^{3+} and Fe^{3+} in acidic soils and Ca^{2+} in neutral to alkaline soils [50]. On the other hand, the availability of P can occur through the release of organic acids and the enzymes phytases and phospholipases, produced by microorganisms [51]. Acidification of the rhizosphere through the release of H+ causes this proton to compete for the phosphorus adsorption sites bound to iron or aluminum, causing the transformation into orthophosphate $(H_2PO_4)^{-2}$, a soluble formula found in acidic soils [52, 53].

The solubilization of rock phosphate was recorded in rice inoculated with *Trichoderma*, which resulted in greater growth and P content [54]. Another mechanism is the production of siderophores, which are low molecular weight binders with high affinity for Fe^{3+} and Al^{3+} ions, causing the solubilization of P bound to these ions [55]. The microorganisms that make up the MIX evaluated in this study are producers of siderophores [56] and may have contributed to the high absorption and accumulation of P in *U. brizantha* inoculated and fertilized with 50% P.

Bacillus inoculation resulted in gains in shoot and root, due to greater solubilization of this nutrient [57]. These results demonstrate that the applied microorganisms contributed to nutrient solubilization, even at low doses, favoring their absorption and reducing fixation losses. Rice and wheat inoculated with PGPR and combined with 75% of N, P, and K showed an increase in productivity compared to just complete chemical fertilization (100%), reaching a reduction of 25% of N [58, 59].

The study showed that plants inoculated with MIX and UFRA-92 made it possible to reduce 25% of nitrogen, 50% of phosphorus, and 50% of potassium, providing greater efficiency in the use of nutrients, when compared to the control (100%). These results demonstrate that the applied microorganisms contributed to the fixation and solubilization of nutrients, even at low doses, favoring their absorption and reducing losses due to volatilization, leaching, and fixation, and the results of these benefits were reflected in plant growth.

The results presented support the hypothesis that inoculation with microorganisms increases the efficient use of nutrients. The greater efficiency found is due to the reduced use of mineral fertilizer in conjunction with inoculation, considering that excessive use can harm the action of microorganisms [60]. The importance of efficient use of nutrients in modern agriculture is of great relevance due to the economic and environmental impacts that can be controlled over the years of production [61].

According to Goldstein [62], rhizosphere colonizing microorganisms can replace 25% to 50% of mineral fertilizer, and studies have shown that it is possible to employ the best combinations between microorganisms to obtain better results [63]. The co-inoculation of microorganisms with synergistic effects was verified by Gohil et al. and Vaid et al. [64, 65] in rice plants, which promoted an increase in the yield and absorption of nutrients when compared to noninoculated.

An experiment carried out with co-inoculation in grass [41] found that the use of microorganisms promoted an increase in K^+ absorption and N fixation when compared to the control treatment, and this behavior is related to the production of organic acids and phytohormones involved in root growth, which increase the area explored by the roots.

The efficient use of N, P, and K was also reported by Duarah et al. [66], and the authors evaluated the benefits of the association of a solubilizing microorganism in rice. It was verified that in reduced doses of NPK (50%), the nutritional content and the evaluated growth parameters had better performance when there was inoculation of biopromoters. Elekhtyar [67] found that the yield efficiency of rice plants co-inoculated under doses of NPK showed greater agronomic efficiency in the treatment inoculated with 75% of inorganic fertilizer when compared to the treatment with the recommended dose.

The benefits of inoculation with biopromoters are reported by Zeffa et al. [68] in maize plants inoculated with *Azospirillum* sp., inferring that inoculated plants have higher NUE and reduced damage caused by the reduction of N content in the soil. Similar results are also reported by Yaghoubi Khanghahi et al. [69], who found higher nutritional content (N, P) in rice plants when there was an association of microorganisms versus reduced doses of K, and the evaluated rates of efficient use were significantly higher when compared to 100% of the dose.

The application of phosphate-solubilizing bacteria associated with inorganic NPK increased the content of NPK and NUE and reduced the leaching rate of nutrients in grass [66]. Grass inoculation associated with inorganic fertilization with PK promoted higher NUE, representing an increase of 11% in N, 30% in P, and 17% in K when compared to plants fertilized only with inorganic fertilizer [70].

New trials from reduced dose macronutrient formulations combined with co-inoculation with *B. subtilis* + *T. asperellum* in *Urochloa (*Syn. *Brachiaria) brizantha* cv. Marandu should be carried out to investigate the physiological and biochemical mechanisms altered by biostimulation. The co-inoculation of *Bacillus subtilis* + *Trichoderma asperellu*m in *Urochloa brizantha* cv. Marandu induced greater growth by changing the absorption behavior using a lower dose of mineral fertilizer in 25% N, 50% P, and 50% K, resulting in increased nutritional efficiency.

5. Conclusion

The results of this study showed that the co-inoculation of *Trichoderma asperellum* with *Bacillus subtilis* promoted the growth of Marandu grass under different doses of mineral fertilizer. In addition, the reduction in mineral fertilization of 25% N, 50% P, and 50% K, when associated with microorganisms, favored efficiency in the use of nutrients by more than 100%. This study is an important contribution to new research, and it contributes to agriculture with less environmental impact and to the sustainable use of non-renewable resources.

Data Availability

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, B. subtilis - MN175193. UFRA-T06 - MK086063, UFRA-T09 - MK086064, UFRA-T12 - MK086065, and UFRA- T52 - MK086066.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to thank the Amazon Foundation for the Support of Studies and Research (FAPESPA/Brazil) for granting a scholarship to the first author. The authors would also like to thank the Plant Protection Laboratory (LPP) for providing the facilities. This study was financially supported by the Federal University of Amazonia (UFRA) and the Graduate Program in Agronomy (PgAgro).

Supplementary Materials

Table 1: composite soil properties before fertilization at a depth of 0–0.2 m. Table 2: the influence of different factors/treatments on plant parameters. (*Supplementary Materials*)

References

[1] "FAO-Food and Agriculture Organization of the United Nations," 2021, https://www.fao.org/3/ap106e/ap106e.

[2] "International Fertilizer Industry Association," 2021, http://www.fertilizer.org/.

[3] S. I. A. Pereira, D. Abreu, H. Moreira, A. Vega, and P. M. L. Castro, "Plant growth-promoting rhizobacteria (PGPR) improve the growth and nutrient use efficiency in maize (Zea mays L.) under water deficit conditions," *Heliyon*, vol. 6, no. 10, Article ID e05106, 2020.

[4] "MapBiomas," 2022, https://mapbiomas.org/.

[5] G. Santoyo, G. Moreno-Hagelsieb, M. del Carmen Orozco-Mosqueda, and B. R. Glick, "Plant growth-promoting bacterial endophytes," *Microbiological Research*, vol. 183, pp. 92–99, 2016.

[6] J. Fukami, C. de la Osa, F. J. Ollero, M. Megías, and M. Hungria, "Co-inoculation of maize with *Azospirillum brasilense* and Rhizobium tropici as a strategy to mitigate salinity stress," *Functional Plant Biology*, vol. 45, no. 3, pp. 328–339, 2018.

[7] P. F. Aguirre, C. J. Olivo, P. F. Rodrigues, D. R. Falk, C. B. Adams, and H. P. Schiafino, "Forage yield of Coastcross-1 pastures inoculated with Azospirillum brasilense," *Acta Scientiarum. Animal Sciences*, vol. 40, Article ID 36392, 2017.

[8] G. C. R. Sá, C. L. M. Carvalho, A. Moreira et al., "Biomass yield, nitrogen accumulation and nutritive value of Mavuno grass inoculated with plant growth-promoting bacteria," *Communications in Soil Science and Plant Analysis*, vol. 50, no. 15, pp. 1931–1942, 2019.

[9] V. Karuppiah, J. Sun, T. Li, M. Vallikkannu, and J. Chen, "Co-cultivation of *Trichoderma asperellum* GDFS1009 and *Bacillus* amyloliquefaciens 1841 causes differential gene expression and improvement in the wheat growth and biocontrol activity," *Frontiers in Microbiology*, vol. 10, p. 1068, 2019.

[10] T. P. d. Sousa, A. A. Chaibub, G. B. d. Silva, and M. C. C. d. Filippi, "Trichoderma asperellum modulates defense genes and potentiates gas exchanges in upland rice plants," *Physiological and Molecular Plant Pathology*, vol. 112, Article ID 101561, 2020.

[11] C. Lagos, J. Larsen, A. Fuentes et al., "Inoculation of Triticum aestivum L.(*Poaceae*) with plant-growth-promoting fungi alleviates plant oxidative stress and enhances phenanthrene dissipation in soil," *Agronomy*, vol. 11, no. 3, p. 411, 2021.

[12] N. Banjac, R. Stanisavljević, I. Dimkić, N. Velijević, M. Soković, and A. Ćirić, "*Trichoderma harzianum* IS005-12 promotes germination, seedling growth and seedborne fungi suppression in Italian ryegrass forage," *Plant Soil and Environment*, vol. 67, no. 3, pp. 130–136, 2021.

[13] T. Ke, J. Zhang, Y. Tao et al., "Individual and combined application of Cu-tolerant *Bacillus* spp. enhance the Cu

phytoextraction efficiency of perennial ryegrass," *Chemosphere*, vol. 263, Article ID 127952, 2021.

[14] S. Li, L. Cui, J. Cheng, T. Chen, Y. Fu, and J. Xie, "Assessment of two *Trichoderma harzianum* strains for biocontrol against rice sheath blight and growth promotion of rice," *Acta Phytopathologica Sinica*, vol. 48, no. 1, pp. 98–107, 2018.

[15] M. Ikram, N. Ali, G. Jan et al., "*Trichoderma reesei* improved the nutrition status of wheat crop under salt stress," *Journal of Plant Interactions*, vol. 14, no. 1, pp. 590–602, 2019.

[16] K. L. Rana, D. Kour, T. Kaur, R. Devi, A. Yadav, and A. N. Yadav, "Bioprospecting of endophytic bacteria from the Indian Himalayas and their role in plant growth promotion of maize (Zea mays L.)," *Journal of Applied Biology & Biotechnology*, vol. 9, no. 3, pp. 41–50, 2021.

[17] O. Degani, O. Rabinovitz, P. Becher, A. Gordani, and A. Chen, "Trichoderma longibrachiatum and Trichoderma asperellum confer growth promotion and protection against late wilt disease in the field," *Journal of Fungi*, vol. 7, no. 6, p. 444, 2021.

[18] W. V. D. Silvestre, P. A. Silva, L. F. Palheta et al., "Differential tolerance to water deficit in two açaí (Euterpe oleracea Mart.) plant materials," *Acta Physiologiae Plantarum*, vol. 39, no. 1, pp. 4–10, 2017.

[19] G. L. S. de Castro, M. C. F. Rêgo, W. V. D. Silvestre, T. F. V. Batista, and G. B. da Silva, "Açaí palm seedling growth promotion by rhizobacteria inoculation," *Brazilian Journal of Microbiology*, vol. 51, no. 1, pp. 205–216, 2020.

[20] J. C. Silva, D. B. Torres, D. C. Lustosa, M. C. C Filippi, and G. B. Silva, "Biocontrol of sheath blight on rice and growth promotion by Trichoderma isolates from the Amazon," *Amazon Journal of Agricultural and Environmental Sciences*, vol. 55, no. 4, pp. 243–250, 2012.

[21] C. I. Kado and M. G. Heskett, "Selective media for isolation of agrobacterium, corynebacterium, erwinia, *Pseudomonas* and *xanthomonas*," *Phytopathology*, vol. 60, no. 6, pp. 969–976, 1970.

[22] M. C. C. Filippi, G. B. Da Silva, V. L. Silva-Lobo, M. V. C. Côrtes, A. J. G. Moraes, and A. S. Prabhu, "Leaf blast (Magnaporthe oryzae) suppression and growth promotion by rhizobacteria on aerobic rice in Brazil," *Biological Control*, vol. 58, no. 2, pp. 160–166, 2011.

[23] M. J. S. Lopes, M. B. Dias-Filho, T. H. R. Castro, and G. B. Silva, "Light and plant growth-promoting rhizobacteria effects on *Brachiaria brizantha* growth and phenotypic plasticity to shade," *Grass and Forage Science*, vol. 73, no. 2, pp. 493–499, 2018.

[24] S. B. Silva and S. I. A. N. Silva, *Concepts and stages of soil analysis*, Technical Education and University Extension, p. 49.

[25] C. D. S. Carmo, W. S. de Araujo, A. D. C. Bernardi, and M. F. C. Saldanha, *Métodos de análise de tecidos vegetais utilizados na Embrapa Solos*, Embrapa Solos, Rio de Janeiro, Brazil, 2000.

[26] M. E. R. Santos and D. D. Fonseca, *Adubação de pastagens em sistemas de produção animal*, Editora UFV, viçosa do ceará, Brazil, 2016.

[27] C. A. M. Gomide and J. A. Gomide, "Morfogênese de cultivares de Panicum maximum Jacq," *Revista Brasileira de Zootecnia*, vol. 29, no. 2, pp. 341–348, 2000.

[28] S. Bianco, R. A. Brendolan, P. L. Alves, and R. A. Pitelli, "Estimativa da área foliar de plantas daninhas: *Brachiaria* decumbens Stapf. e *Brachiaria brizantha* (Hochst.) Stapf," *Planta Daninha*, vol. 18, no. 1, pp. 79–83, 2000.

[29] M. Choudhary, V. S. Meena, R. P. Yadav et al., "Does PGPR and mycorrhizae enhance nutrient use efficiency and efficacy in relation to crop productivity," in *Field Crops: Sustainable Management by PGPR*, pp. 45–68, Springer, Berlin, Germany, 2019.

[30] D. R. Kidd, M. H. Ryan, R. E. Haling et al., "Rhizosphere carboxylates and morphological root traits in pasture legumes and grasses," *Plant and Soil*, vol. 402, no. 1-2, pp. 77–89, 2016.

[31] M. E. Griffin, R. B. Muntifering, M. K. Mullenix, D. W. Held, and S. L. Dillard, "Evaluation of plant growth-promoting rhizobacteria on stockpiled bermudagrass," *Crop, Forage & Turfgrass Management*, vol. 6, no. 1, Article ID e20028, 2020.

[32] S. Tshewang, Z. Rengel, K. H. Siddique, and Z. M. Solaiman, "Microbial consortium inoculant increases pasture grasses yield in low-phosphorus soil by influencing root morphology, rhizosphere carboxylate exudation and mycorrhizal colonisation," *Journal of the Science of Food and Agriculture*, vol. 102, no. 2, pp. 540–549, 2021.

[33] J. A. Warmink, R. Nazir, B. Corten, and J. D. Van Elsas, "Hitchhikers on the fungal highway: the helper effect for bacterial migration via fungal hyphae," *Soil Biology and Biochemistry*, vol. 43, no. 4, pp. 760–765, 2011.

[34] C. Paungfoo-Lonhienne, M. Redding, C. Pratt, and W. Wang, "Plant growth promoting rhizobacteria increase the efficiency of fertilisers while reducing nitrogen loss," *Journal of Environmental Management*, vol. 233, pp. 337–341, 2019.

[35] C. F. D. Duarte, U. Cecato, M. Hungria et al., "Morphogenetic and structural characteristics of *Urochloa* species under inoculation with plant-growth-promoting bacteria and nitrogen fertilisation," *Crop & Pasture Science*, vol. 71, no. 1, pp. 82–89, 2020.

[36] A. P. D. d. Silveira, V. M. R. Sala, E. J. B. N. Cardoso, E. G. Labanca, and M. A. P. Cipriano, "Nitrogen metabolism and growth of wheat plant under diazotrophic endophytic bacteria inoculation," *Applied Soil Ecology*, vol. 107, pp. 313–319, 2016.

[37] M. Rasul, S. Yasmin, M. Suleman et al., "Glucose dehydrogenase gene containing phosphobacteria for biofortification of Phosphorus with growth promotion of rice," *Microbiological Research*, vol. 223-225, pp. 1–12, 2019.

[38] E. Khare and N. K. Arora, "Biosurfactant based formulation of *Pseudomonas guariconensis* LE3 with multifarious plant growth promoting traits controls charcoal rot disease in Helianthus annus," *World Journal of Microbiology and Biotechnology*, vol. 37, no. 4, pp. 55–14, 2021.

[39] R. G. Machado, E. L. S. de Sá, M. Bruxel, A. Giongo, N. da Silva Santos, and A. S. Nunes, "Indoleacetic acid producing rhizobia promote growth of Tanzania grass (Panicum maximum) and pensacola grass (paspalum saurae)," *International Journal of Agriculture and Biology*, vol. 15, no. 5, 2013.

[40] D. S. Archana, M. S. Nandish, V. P. Savalagi, and A. R. Alagawadi, "Characterization of potassium solubilizing bacteria (KSB) from rhizosphere soil," *Bioinfolet-A Quarterly Journal of Life Sciences*, vol. 10, no. 1, pp. 248–257, 2013.

[41] B. B. Basak and D. R. Biswas, "Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promotion and nutrient acquisition by a forage crop," *Biology and Fertility of Soils*, vol. 46, no. 6, pp. 641–648, 2010.

[42] M. Ahmad, L. Pataczek, T. H. Hilger et al., "Perspectives of microbial inoculation for sustainable development and environmental management," *Frontiers in Microbiology*, vol. 9, p. 2992, 2018.

[43] N. P. M. Banayo, P. C. S. Cruz, E. A. Aguilar, R. B. Badayos, and S. M. Haefele, "Evaluation of biofertilizers in irrigated

rice: effects on grain yield at different fertilizer rates," *Agriculture*, vol. 2, no. 1, pp. 73–86, 2012.

[44] V. S. Meena, B. R. Maurya, and J. P. Verma, "Does a rhizospheric microorganism enhance K+ availability in agricultural soils?" *Microbiological Research*, vol. 169, no. 5-6, pp. 337–347, 2014.

[45] R. Abafita Abawari, F. Asefa Tuji, and D. Muleta Yadete, "Phosphate solubilizing bio-fertilizers and their role in bioavailable P nutrient: an overview," *International Journal of Applied Agricultural Sciences*, vol. 6, no. 6, p. 162, 2020.

[46] F. A. A. Zakry, Z. H. Shamsuddin, K. Abdul Rahim, Z. Zawawi Zakaria, and A. Abdul Rahim, "Inoculation of *Bacillus sphaericus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the 15N isotope dilution technique," *Microbes and Environments*, vol. 27, no. 3, pp. 257–262, 2012.

[47] S. Silletti, E. Di Stasio, M. J. Van Oosten et al., "Biostimulant activity of azotobacter chroococcum and *Trichoderma harzianum* in durum wheat under water and nitrogen deficiency," *Agronomy*, vol. 11, no. 2, p. 380, 2021.

[48] D. Goswami, J. N. Thakker, and P. C. Dhandhukia, "Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review," *Cogent Food & Agriculture*, vol. 2, no. 1, 2016.

[49] Y. Ding, J. Wang, Y. Liu, and S. Chen, "Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region," *Journal of Applied Microbiology*, vol. 99, no. 5, pp. 1271–1281, 2005.

[50] M. Billah, M. Khan, A. Bano, T. U. Hassan, A. Munir, and A. R. Gurmani, "Phosphorus and phosphate solubilizing bacteria: keys for sustainable agriculture," *Geomicrobiology Journal*, vol. 36, no. 10, pp. 904–916, 2019.

[51] K. P. Ingle and D. A. Padole, "Phosphate solubilizing microbes: an overview," *International Journal of Current Microbiology and Applied Sciences*, vol. 6, no. 1, pp. 844–852, 2017.

[52] J. Zhu, M. Li, and M. Whelan, "Phosphorus activators contribute to legacy phosphorus availability in agricultural soils: a review," *Science of the Total Environment*, vol. 612, pp. 522–537, 2018.

[53] Y. Zhang, *Soil Bypass P for Enhanced Availability in Acid Soils*, Norwegian University of Life Sciences, Elizabeth Stephansens, Norway, 2021.

[54] L. Borges Chagas, A. F. Chagas Junior, M. Rodrigues de Carvalho, L. de Oliveira Miller, and B. Orozco Colonia, "Evaluation of the phosphate solubilization potential of *Trichoderma* strains (*Trichoplus* JCO) and effects on rice biomass," *Journal of Soil Science and Plant Nutrition*, vol. 15, no. 3, pp. 794–804, 2015.

[55] P. Ghosh, B. Rathinasabapathi, and L. Q. Ma, "Phosphorus solubilization and plant growth enhancement by arsenic-resistant bacteria," *Chemosphere*, vol. 134, pp. 1–6, 2015.

[56] A. Palyzová, K. Svobodová, L. Sokolová, J. Novák, and Č. Novotný, "Metabolic profiling of Fusarium oxysporum f. sp. conglutinans race 2 in dual cultures with biocontrol agents *Bacillus amyloliquefaciens, Pseudomonas aeruginosa*, and *Trichoderma harzianum*," *Folia Microbiologica*, vol. 64, no. 6, pp. 779–787, 2019.

[57] M. Turan, N. Ataoglu, and F. Sahin, "Effects of *Bacillus* FS-3 on growth of tomato (Lycopersicon esculentum L.) plants and availability of phosphorus in soil," *Plant Soil and Environment*, vol. 53, no. 2, pp. 58–64, 2008.

[58] B. Shaharoona, M. Naveed, M. Arshad, and Z. A. Zahir, "Fertilizer-dependent efficiency of *Pseudomonas* for improving growth, yield, and nutrient use efficiency of wheat (Triticum aestivum L.)," *Applied Microbiology and Biotechnology*, vol. 79, no. 1, pp. 147–155, 2008.

[59] M. T. Rose, T. L. Phuong, D. K. Nhan, P. T. Cong, N. T. Hien, and I. R. Kennedy, "Up to 52% N fertilizer replaced by biofertilizer in lowland rice via farmer participatory research," *Agronomy for Sustainable Development*, vol. 34, no. 4, pp. 857–868, 2014.

[60] G. Nahed and A. El-Aziz, "Stimulatory effect of NPK fertilizer AND Benzyladenine on growth and chemical constituents of Codiaeumvariegatum L. plants," *American-Eurasian Journal of Agricultural & Environmental Sciences*, vol. 2, no. 6, pp. 711–719, 2007.

[61] A. O. Adesemoye and J. W. Kloepper, "Plant–microbes interactions in enhanced fertilizer-use efficiency," *Applied Microbiology and Biotechnology*, vol. 85, no. 1, pp. 1–12, 2009.

[62] A. H. Goldstein, "Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria," *Biological Agriculture & Horticulture*, vol. 12, no. 2, pp. 185–193, 1995.

[63] G. S. Kang, V. Beri, B. S. Sidhu, and O. P. Rupela, "A new index to assess soil quality and sustainability of wheat based cropping systems," *Biology and Fertility of Soils*, vol. 41, no. 6, pp. 389–398, 2005.

[64] N. Gohil, V. P. Ramani, D. G. Chaudhary, A. S. Bhanvadiya, and D. Kumar, "Effects of PGPR on micronutrients availability in soil and its translocation in rice under loamy sand textured soil," *International Journal of Current Microbiology and Applied Sciences*, vol. 8, no. 7, 2019.

[65] S. K. Vaid, B. Kumar, A. Sharma, A. K. Shukla, and P. C. Srivastava, "Effect of zn solubilizing bacteria on growth promotion and zn nutrition of rice," *Journal of Soil Science and Plant Nutrition*, vol. 14, no. ahead, pp. 889–910, 2014.

[66] I. Duarah, M. Deka, N. Saikia, and H. P. Deka Boruah, "Phosphate solubilizers enhance NPK fertilizer use efficiency in rice and legume cultivation," *Biotech*, vol. 1, no. 4, pp. 227–238, 2011.

[67] N. M. Elekhtyar, "Impact of three strains of *Bacillus* as bio NPK fertilizers and three levels of mineral NPK fertilizers on growth, chemical compositions and yield of Sakha 106 rice cultivar," *International Journal of ChemTech Research*, vol. 8, no. 4, pp. 2150–2156, 2015.

[68] D. M. Zeffa, L. J. Perini, M. B. Silva et al., "*Azospirillum brasilense* promotes increases in growth and nitrogen use efficiency of maize genotypes," *PLoS One*, vol. 14, no. 4, Article ID e0215332, 2019.

[69] M. Yaghoubi Khanghahi, H. Pirdashti, H. Rahimian, G. A. Nematzadeh, and M. Ghajar Sepanlou, "Nutrient use efficiency and nutrient uptake promoting of rice by potassium solubilizing bacteria (KSB," *Cereal Research Communications*, vol. 46, no. 4, pp. 739–750, 2018.

[70] M. A. Baldotto, L. E. B. Baldotto, R. B. Santana, and C. R. Marciano, "Initial performance of maize in response to NPK fertilization combined with Herbaspirillum seropedicae," *Revista Ceres*, vol. 59, no. 6, pp. 841–849, 2012.