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GROWTH PROMOTION OF SORGHUM BY MICRO-ORGANISMS

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Technologists at Biofuels

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This project combines scientific innovation with sustainable agriculture by using microorganisms to recover soils and promote sorghum growth. It has social, economic and environmental impacts; strengthens the production chain; and contributes to academic training and regional development.

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Luana Beatriz Gonçalves, born in Jaboticabal, São Paulo, on April 26, 1994. Daughter of Jacinta Martins Braga and Sebastião Mendes Gonçalves. Graduated in Biofuels Technology from the Faculty of Technology of Jaboticabal “Nilo De Stéfani” (2022). In March 2023, he started the MASTER course at the Graduate Program in AGRICULTURAL MICROBIOLOGY, at the Faculty of Agricultural and Veterinary Sciences - UNESP Campus de Jaboticabal, under the guidance of Prof. Dr. Everlon Cid Rigobelo, developing the project entitled “Growth promotion of sorghum by microorganisms” formalized in the present document.

“Questioning the way you think is the first step toward improving your choices.”

Profª. Dra. Larissa Albunio Silva

DEDICATION

I dedicate this work to my grandmother Conceição Ferreira de Jesus, my queen. I love you grandma!

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SUMMARY

GROWTH PROMOTION OF SORGHUM BY MICRO-ORGANISMS

ABSTRACT – Sorghum (*Sorghum bicolor* L. Moench) is a crop of great agricultural importance because of its adaptability to different soil and climate conditions and versatility of use. However, its high nutritional demand makes intensive use of chemical fertilizers necessary, which negatively impacts the environment. In this context, the inoculation of plant growth-promoting microorganisms (PGPMs) appears to be a sustainable alternative that promotes biological nitrogen fixation, phosphorus solubilization and the production of phytohormones, reducing the need for synthetic inputs. of the bacteria *Bacillus subtilis, Bacillus pumilus* and *Bacillus licheniformis*, in addition to the fungi *Purpureocillium lilacinum* and *Trichoderma harzianum*, in soils with a 20% reduction in mineral fertilization. The experiment was conducted in a greenhouse via a completely randomized design, with five replicates per treatment. Parameters such as plant height, shoot and root dry matter, and nitrogen and phosphorus contents were analyzed. The results indicated that the reduction in mineral fertilization by 20% did not significantly affect the development of the inoculated plants compared with the control with 100% fertilization. However, the treatments with *B. subtilis* and *T. harzianum* presented the lowest values for the soil phosphorus content, whereas *B. subtilis* presented the lowest height and shoot dry matter. These findings suggest that microbial inoculation may contribute to reducing the use of chemical fertilizers without significantly damaging sorghum growth. Despite the absence of statistically significant differences in most of the parameters evaluated, this study reinforces the importance of continuous research on microbial inoculants in sorghum culture. The application of PGPMs may be a promising strategy for mitigating the environmental impacts of agriculture, promoting more sustainable production systems.

Keywords: Biofertilization, Plant growth-promoting microorganisms, *Bacillus* spp.*, Trichoderma harzianum,* Agricultural sustainability

CHAPTER 1 - General Considerations

1. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is a plant that originated in Africa and plays a crucial role in food security in several regions of the world, especially in semiarid areas. With a historical importance that dates back thousands of years, sorghum was already cultivated in ancient civilizations as a food grain and fodder. Its botanical and physiological characteristics, such as its ability to adapt to different climatic and soil conditions, make it an extremely versatile and adaptable crop, which justifies its prominence in modern agriculture (Pereira et al., 2023; Oliveira, 2024; Santos, 2022).

The botanical and physiological characteristics of sorghum make it an extremely important crop in modern agriculture. Its ability to adapt to different climatic conditions and soil types is remarkable, making it a viable option in areas with water restrictions. In addition, sorghum has great genetic diversity, which allows the selection of varieties adapted to different environments. Its physiological characteristics, such as water use efficiency and resistance to stress, contribute to its relevance as an agricultural crop (Bosisio, 2024; Fagundes, 2023).

For sorghum cultivation, it is ideal that the soil and climate conditions are favorable, with well-drained, deep and fertile soil, preferably of medium texture. In terms of climate, sorghum adapts well to high temperatures and is resistant to drought, but for good development, it is important that there is rainfall and sunlight. Planting and management practices include the appropriate choice of planting area, the use of high-quality seeds, efficient weed and pest control, and adequate irrigation management to ensure productive and high-quality crops (Silva et al. al., 2021; Souza, 2024; Francisco et al., 2023).

Sorghum has specific nutritional needs and is demanding nutrients such as nitrogen, phosphorus, potassium, calcium and magnesium. The balance between these nutrients is essential for the healthy development of crops. In addition, the phenological stages of sorghum require different amounts of nutrients, which requires special attention during the crop cycle (Silva, 2024; COSTA, 2023).

To meet the nutritional needs of sorghum, it is important to adopt fertilization strategies that consider the correction and replacement of nutrients in the soil. The choice of fertilizers and the dose applied should be determined on the basis of soil analyses and specific technical recommendations for sorghum. In addition, the division of fertilization at strategic times throughout the crop cycle is essential to ensure an adequate supply of nutrients, contributing to better development and productivity of the crop (Reis et al., 2021; Telles et al., 2023).

The use of microorganisms as an alternative to the use of chemical fertilizers involves the implementation of practices that promote soil fertility and plant health in a sustainable manner. This may include the addition of beneficial microorganisms such as bacteria and fungi, which aid in nitrogen fixation, phosphorus solubilization and decomposition of organic matter. In addition, it is essential to choose plant varieties that are more adapted and resistant, as well as the use of crop rotations, which favor biodiversity in the soil. The regular monitoring of soil and plant conditions, together with biological control by means of microorganisms antagonistic to pathogens, is an effective practice. Thus, by adopting these approaches, it is possible to decrease the dependence on chemical fertilizers, promoting a more balanced and healthy agroecosystem (Lima, 2024; Gonçalves et al., 2023; Anas et al., 2024).

Despite the benefits of microbial inoculants in various crops, their application in sorghum has not been well studied, especially in systems with low dependence on chemical fertilizers. In this context, the present study aimed to evaluate the impact of inoculation with growth-promoting microorganisms in sorghum, emphasizing the reduction in the use of chemical fertilizers and their effects on the physiological and agronomic attributes of the plant, such as the nitrogen concentration. and phosphorus, height, diameter and plant dry mass.

2. LITERATURE REVIEW

2.1 Sorghum (*Sorghum bicolor* L. Moench)

Sorghum stands out as a crop of high agricultural importance, especially in semiarid regions, owing to its remarkable adaptability to adverse environmental conditions, such as water stress and soil salinity. Among the various sorghum varieties, sorghum is widely cultivated for the production of forage for animal feed, with agronomic characteristics that make it a viable option in livestock production systems (Tabosa et al., 2021; Oliveira, 2024).

Sorghum varieties are classified on the basis of their purpose, with five categories standing out. Sorghum is cultivated mainly for the production of grains intended for human and animal consumption. Forage sorghum is used in the production of forage for animal feed and can be used in the form of grazing, hay or silage. Sweet sorghum is characterized by a high sugar content in the stalks and is used in the production of ethanol and other biofuels. Sorghum broom is used in the manufacture of brooms and other household items because of its long and resistant fibers. Biomass sorghum is cultivated for energy production, with a calorific value similar to that of sugarcane, eucalyptus and elephant grass (Tavazoh et al., 2024).

The appropriate choice of forage sorghum cultivar, together with management practices that consider the local soil and climate conditions, is essential to maximize the production of high-quality forage in semiarid regions. The continuous evaluation and development of cultivars adapted to abiotic stresses, such as drought and salinity, are essential for the sustainability of forage production and, consequently, for the economic viability of livestock in these regions.

In addition, the salinity of irrigation water is a critical factor that can affect the development and productivity of sorghum. Studies have shown that certain sorghum varieties, such as Ponta Negra, have greater tolerance to salinity, maintaining satisfactory yields even when irrigated with water, with an average electrical conductivity of 4.19 dS m^{-1} (Guimarães et al., 2022).

2.2 Use of Chemical Fertilizers in Agriculture: Benefits and Challenges

The use of chemical fertilizers in agriculture has been widely adopted to meet the nutritional needs of crops and ensure high yields. These inputs provide essential nutrients such as nitrogen, phosphorus and potassium, which are essential for plant development. However, inadequate management of these fertilizers can cause serious environmental and human health consequences (Bastos et al., 2021).

Excessive or incorrect application of chemical fertilizers can lead to contamination of water bodies through surface runoff and leaching, resulting in water eutrophication. This phenomenon compromises the quality of aquatic ecosystems, promoting excessive algal proliferation and reducing the levels of dissolved oxygen, which negatively impacts aquatic fauna. In addition, the accumulation of these compounds in the soil can change its physical and chemical properties, reducing its long-term fertility and compromising future agricultural productivity (Mendes et al., 2010).

Studies indicate that the indiscriminate use of chemical fertilizers is also associated with negative impacts on the health of farmers, including acute and chronic poisoning. Prolonged exposure to these compounds can lead to the development of respiratory, dermatological and neurological diseases, in addition to increasing the risk of cancer (Maas et al., 2020).

In view of these challenges, sustainable alternatives have been developed to mitigate environmental impacts and promote more balanced agriculture. The use of organomineral fertilizers and biofertilizers has shown promise because they combine mineral nutrients with organic matter, resulting in the gradual release of nutrients and improving soil quality. In addition, practices such as crop rotation and the use of organic waste as fertilizer reduce the dependence on chemical fertilizers, promoting the sustainability of agricultural production (Amazon AgroSciences, 2025).

It is essential that farmers receive appropriate technical guidance for the efficient management of fertilizers, aiming to maximize nutrient uptake by plants and minimize environmental impacts and human health. The adoption of good agricultural practices, together with the constant monitoring of soil and crop conditions, is essential to ensure sustainable and safe agricultural production (Bastos et al., 2021).

Although chemical fertilizers play a crucial role in modern agriculture, it is imperative to balance their use with sustainable practices that preserve the environment and human health. The transition to more ecological production systems requires joint efforts by farmers, researchers and policymakers to ensure food security and the conservation of natural resources for future generations.

2.3 Plant Growth-Promoting Microorganisms (PPPMs)

The use of plant growth-promoting microorganisms (CPPMs) has emerged as a promising and ecologically correct alternative to chemical fertilizers in agriculture. These microorganisms, including beneficial bacteria and fungi, act through several mechanisms, such as biological nitrogen fixation, phosphate solubilization and phytohormone production, contributing to the healthy development of plants and the sustainability of agricultural systems (Spolaor et al., 2016).

2.3.1 Biological Nitrogen Fixation in Ecosystems

Biological nitrogen fixation is one of the main mechanisms of action of plant growth-promoting microorganisms. The ability of some bacteria, such as those of the genus *Rhizobium*, to fix atmospheric nitrogen in forms usable by plants, such as ammonia, has a significant effect on crop growth and development. This symbiosis allows plants to access essential nutrients, such as nitrogen, more efficiently, reducing the need for nitrogen fertilization and contributing to the sustainability of agricultural systems. In addition, nitrogen fixation can promote a reduction in greenhouse gas emissions resulting from a decrease in the application of synthetic fertilizers (Almeida, 2024; Oliveira et al., 2023).

2.3.2 Solubilization of Phosphates

Phosphate solubilization is an important mechanism performed by plant growth-promoting microorganisms, which transform insoluble phosphorus into a form that is usable by plants. This process contributes to the supply of one of the essential nutrients for plant growth, improving the availability of phosphorus in the soil. Microorganisms that promote plant growth can secrete organic acids and phosphatase enzymes, which act in the solubilization of phosphate. In addition, the solubilization of phosphate by these microorganisms may help reduce the use of phosphate fertilizers, contributing to the sustainability of agriculture (Souza, 2024; Goulart, 2024).

2.3.3 Production of Phytohormones

The production of plant hormones by PGPMs is one of the key mechanisms that contributes to the healthy development of plants. These microorganisms can synthesize a variety of plant hormones, such as auxins, cytokinins and gibberellins, which play key roles in plant growth and development. The production of these hormones by microorganisms is essential for the promotion of root growth, increase in biomass and improvement of plant resistance to biotic and abiotic stresses. In addition, the plant hormones produced by plant growth-promoting microorganisms are important for the regulation of plant development, which makes them valuable components in promoting plant health and productivity (Parrales et al., 2022; Rezende et al., 2021; Magalhães, 2023).

2.4 Types of plant growth-promoting microorganisms

Bacteria are among the most common types of PGPMs and function in nitrogen fixation, the production of plant hormones and the inhibition of pathogens. Fungi also play crucial roles, especially in the solubilization of phosphate in the soil, facilitating its uptake by plants. Actinomycetes have been increasingly studied because of their ability to produce bioactive substances that stimulate plant growth and health, making them essential for sustainable and organic agriculture (Queiroz and Oliveira, 2023; Gommides, 2022; Santos, 2022; Santos, 2022). 2024; Cavalcante et al., 2022).

2.4.1 Bacteria

Plant growth-promoting bacteria are beneficial microorganisms that can be found in symbiosis with plant roots, assisting in the absorption of nutrients and increasing resistance to stress. Among the bacteria of the genus *Azospirillum*, the ability to fix nitrogen stands out, contributing to the supply of nutrients essential for plant development. In addition, bacteria of the genus *Bacillus* have the ability to produce metabolites that promote plant growth, such as the production of plant hormones and phosphate solubilization, providing significant benefits for sustainable and organic agriculture (Lima, 2024; Queiroz and Oliveira, 2023).

2.4.2 Fungi

Fungi are PGPMs that play a key role in promoting plant health. They can form symbioses with plant roots, helping them absorb water and essential nutrients. In addition, fungi can solubilize phosphate, increasing the availability of this nutrient to plants. Some species of fungi also produce substances that stimulate plant growth, such as auxins and cytokinins. Therefore, the role of fungi as PGPMs is highly important in sustainable agriculture and in promoting plant development (Cruz, 2024; Santos et al., 2024; Tigre et al., 2024; Cortat et al., 2022).

2.4.3 Actinomycetes

Actinomycetes are soil microorganisms known for their ability to produce a wide range of bioactive compounds, including substances that promote plant growth. Among the actinomycete genera associated with these activities, *Streptomyces* is the most commonly studied. Studies have shown that actinomycetes promote plant growth through phosphate solubilization, the synthesis of plant hormones and the production of antibiotics that decrease competition for nutrients. In addition, they play crucial roles in protecting plants against pathogens, contributing to the health and productivity of agricultural crops (Martins et al., 2022; Candido, 2023; Santos, 2024).

2.5 Microbial Species Used as Plant Growth Promoters

2.5.1 *Bacillus subtilis*

Bacillus subtilis is widely recognized for its beneficial characteristics, which favor plant growth. Among its general properties, it can solubilize phosphates in the soil, increasing the availability of this essential nutrient for plants, as described by Jha et al. (2014). In addition, *Bacillus subtilis* has the ability to produce plant hormones, such as auxins and gibberellins, which are fundamental for the healthy development of plants (Verma et al., 2018). Morphologically, *Bacillus subtilis* is a gram-positive bacterium that forms spores under adverse conditions, which results in significant resistance to hostile environments, such as variations in temperature and humidity (Berg et al., 2014). This ability to form endospores allows the bacterium to persist in the soil for long periods, which is crucial for its functionality in agricultural ecosystems.

The beneficial mechanisms of action of *Bacillus subtilis* include the inhibition of pathogens through the production of antibiotic and phytochemical substances, in addition to promoting systemic resistance in plants, strengthening them against diseases (Liu et al., 2019). The colonization of plant roots is important because it creates a protective barrier that reduces the entry of pathogens and, consequently, improves the vigor of the root system (Guan et al., 2021). With respect to soil recovery, *Bacillus subtilis* plays an important role as a bioremediation agent, contributing to the restoration of fertility and microbial biodiversity in degraded soils (Kumar et al., 2020). Its interaction with different crops, such as sorghum, increases the resistance of plants to environmental stresses, which results in improved productivity (Prabhu et al., 2018).

Thus, *Bacillus subtilis* is an essential microorganism in sustainable agriculture, offering viable and ecological solutions for the improvement of agricultural practices and for the promotion of healthier ecosystems (Sousa, 2022; Silva Júnior, 2022; Lobo, 2023; Granha and Delú, 2024).

2.5.2 *Bacillus pumilus*

Bacillus pumilus is a bacterial species widely found in soil and stands out for its beneficial characteristics for the environment and agriculture (SILVA et al., 2020). This bacterium has a morphology typical of bacilli, presents itself as a gram-positive organism and often forms spores, which makes it resistant to adverse conditions (Costa, 2019). The mechanisms of action that contribute to its positive effects include the production of enzymes that promote the solubilization of phosphates, increasing the availability of this essential nutrient for plants (Almeida et al., 2021).

In addition, *Bacillus pumilus* has the ability to fix atmospheric nitrogen, which results in a significant improvement in plant nutrition, favoring its growth and development (Lopes, 2022). These mechanisms make it a promising agent for the recovery of degraded soils, as it can help restore fertility, promoting a healthier environment for plant growth (Martins, 2020).

Research indicates that the application of *Bacillus pumilus* can lead to significant increases in the productivity of agricultural crops, confirming its importance in sustainable and organic agriculture (Freitas et al., 2021; Aguiar, 2024; Rocha, 2022; Rodrigues, 2024; Silva and Dourado, 2022; Pereira et al., 2021).

2.5.3 *Bacillus licheniformis*

Bacillus licheniformis is a soil bacterium widely recognized for its diverse contributions to plant growth. This species shows a remarkable ability to solubilize phosphate, which is essential for optimizing phosphorus uptake by plants, resulting in more robust and healthy development, as described by Barra et al. (2020).

In addition, studies indicate that *Bacillus licheniformis* produces antimicrobial substances that play crucial roles in the protection of plants against pathogens, resulting in an increase in crop yield, according to Silva et al. (2021). More recent studies have investigated its efficacy as a disease biocontrol agent and its role as an inducer of resistance in plants, emphasizing its importance not only in promoting growth but also in effectively protecting crops, as discussed by Ferreira and Mendes (2022). Thus, *B. licheniformis* is a valuable tool for sustainable agriculture, contributing to both productivity and plant health (Vale and Oliveira, 2024; Soares, 2024; Souza, 2020; Pereira, 2024).

2.5.4 *Purpureocillium lilacinum*

Formerly known as *Paecilomyces lilacinus*, *Purpureocillium lilacinum* is a filamentous fungus found in diverse habitats, including cultivated and uncultivated soils, forests, grasslands, deserts and estuarine sediments. It stands out for its ability to parasitize eggs of phytopathogenic nematodes, especially those of the genus *Meloidogyne*, known as root-knot nematodes (Paz Filho, 2019; Ruy, 2018).

Owing to this characteristic, it has been used as a biocontrol agent to manage nematode populations that affect agricultural crops. Studies have shown that the application of *P. lilacinum* can significantly reduce nematode populations in host plants, contributing to the sustainable management of agricultural pests (Costa, 2022).

2.5.5 *Trichoderma harzianum*

Trichoderma harzianum is a fungus widely distributed in soil and is known for its ability to act as a biocontrol agent against a variety of phytopathogens. Its mechanism of action includes direct parasitism by other fungi, the production of antimicrobial compounds and the induction of systemic resistance in plants. Owing to these properties, *T. harzianum* has been used to control plant diseases caused by phytopathogenic fungi, contributing to reducing the use of chemical fungicides and promoting more sustainable agricultural practices (Ruy, 2018).

In addition to its role in biocontrol, *T. harzianum* has been studied for its ability to promote plant growth, possibly through the production of plant hormones and improvement of nutrient availability in the soil (Maia and Lazaretti, 2024). valuable tool in modern agriculture, aiming to increase productivity in a sustainable way.

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CHAPTER 2 – Microbial Inoculants and Fertilizer Reduction in Sorghum Cultivation: Implications for Sustainable Agriculture 1

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Abstract:

Sorghum (*Sorghum bicolor* L. Moench) is an important cereal crop that serves as a staple food source, animal feed, and feedstock in various industrial applications. This study aimed to evaluate the effects of microbial inoculation on the growth and nutrient uptake of sorghum. The treatments included the application of *Bacillus subtilis*, *B. pumilus*, *B. licheniformis*, *Purpureocillum lilacinum*, and *Trichoderma harzianum* were tested under two soil fertility levels: 100% and 80% fertilization. The experiment was conducted in a greenhouse using a completely randomized design, with five replicates per treatment. Plant growth parameters, such as height, shoot and root dry matter, and nitrogen and phosphorus content in the shoots and roots, were assessed. The results showed no statistically significant differences between the treatments for most of the evaluated parameters, except for plant height and shoot dry matter, where the *B. subtilis* treatment exhibited the lowest values. The *B. subtilis*, and *T. harzianum* treatments also resulted in the lowest phosphorus content in the soil. Interestingly, the treatments that received 80% of the recommended fertilizer dose did not differ significantly from those that received 100%, suggesting the potential for microbial inoculants to reduce fertilizer usage. Although the microbial treatments did not significantly enhance sorghum growth in this study, evaluating their effects remains important for developing eco-friendly alternatives to chemical fertilizers. Further research is needed to optimize the application of microbial inoculants and to understand their impact on soil health and agricultural productivity.

Keywords: Sustainable production; microbial inoculum; plant growth; sorghum

1. Introduction

Sorghum *(Sorghum bicolor* L. Moench) is one of the most important cereal crops and ranks as the fifth most significant grain crop worldwide (Castro-Jácome & Tovar-Pérez, 2023). It serves as a staple food source for over 300 million people in Africa and Asia, and is cultivated for various purposes, including human food, animal

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feed, renewable energy, and industrial applications (Kazungu et al., 2023). The United States, Nigeria, Mexico, India, Sudan, China, Ethiopia, Argentina, Australia, and Burkina Faso are the top ten sorghum producers in the world (Djanaguiraman et al., 2018). Specifically, the United States leads with 8.4 million tons, followed by Nigeria (6.5 million tons), Mexico (6.0 million tons), India and Sudan (4.5 million tons each), China (3.8 million tons), and Ethiopia (3.7 million tons) (Djanaguiraman et al., 2018).

Interestingly, while Africa produces 40% of the global sorghum crop, with West Africa accounting for just over half of Africa's production, the Americas contribute approximately 37%, and Asia and Oceania produce 20% (Djanaguiraman et al., 2018). It is worth noting that India, despite being a major producer, has seen a decline in production due to competition for resources and socioeconomic factors (Djanaguiraman et al., 2018).

Interestingly, the importance of sorghum extends beyond its traditional use. It is increasingly recognized as a promising feedstock for the production of bioactive compounds, particularly the kafirin protein fraction, which can generate biologically active peptides with antioxidant, anticancer, antimicrobial, and anti-inflammatory properties (Castro-Jácome & Tovar-Pérez, 2023). In addition, sorghum is gaining popularity as a gluten-free alternative to wheat, making it suitable for patients with celiac disease (Mcginnis & Painter, 2020). The global importance of sorghum stems from its versatility, nutritional value, and adaptability to harsh environments. Its ability to produce reasonable yields in poor soils and limited rainfall conditions makes it a crucial crop for food security in arid and semi-arid regions (Mccuistion et al., 2018). Furthermore, the potential of sorghum as a second-generation biofuel crop, offering ethanol from grain, stem, and biomass, adds to its significance in addressing global energy challenges (Arumugam et al., 2021).

Sorghum is gaining importance as a valuable feed grain for livestock, particularly in regions facing challenging environmental conditions. Its versatility and nutritional properties make it an attractive option for animal-feed production. Sorghum can be utilized by both non-ruminant and ruminant production systems as a source of energy and protein (Mccuistion et al., 2018). When processed correctly and in balance with other feed ingredients, sorghum can serve as the primary grain source in animal diets. The feeding value of sorghum for livestock is generally 95% or more of the feeding value of yellow dent maize (Rooney et al., 2016). This makes it a suitable alternative to corn for animal-feed preparation. Interestingly, while early studies have indicated that sorghum-based diets are inferior to corn-based diets in terms of animal performance, recent studies have shown no significant difference between the two. This improvement is attributed to the development of low-tannin sorghum varieties (Ronda et al., 2018; Yousuf et al., 2023). Additionally, sorghum has a lower incidence of mycotoxins than corn, further enhancing its suitability as animal feed.

Sorghum production faces several challenges in various regions. Insect damage is a significant issue, with at least 150 insect species being capable of infesting sorghum varieties worldwide. These pests can complete multiple generations within a growing season, targeting various parts of the plant at different developmental stages and causing substantial biomass loss (Guo et al., 2011). Another challenge to produce sorghum is related to climate change that poses a severe threat to sorghum production, particularly in regions, such as Somalia. The long-term impacts of increased temperatures and rainfall variability have been shown to significantly hamper sorghum yields (Warsame et al., 2022). Drought, resulting from changes in temperature and rainfall patterns, causes significant yield losses in sorghum crops (Birhanu & Negussie, 2024). In Ethiopia, biotic, socioeconomic, and abiotic factors can limit sorghum production and productivity. Specific challenges include drought, Striga (a parasitic weed), disease, and insect pests (Werkissa & Temesgen, 2022). Additionally, soil acidity is a concern in some regions, such as the Great Plains of the United States, where low soil pH can reduce grain sorghum yields (Butchee et al., 2012; (Warsame et al., 2022; Onono, 2018).

Plant growth-promoting microorganisms (PGPM), including bacteria and fungi, can significantly improve sorghum production through various mechanisms. PGPM, such as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), enhance nutrient uptake, promote plant growth, and increase crop yield (Ansari et al., 2024). These microorganisms can improve sorghum biomass production by synthesizing hormones, fixing nitrogen, and solubilizing phosphate and potassium (Ansari et al., 2024). For instance, certain bacterial strains can increase grain starch content, whereas AMF can enhance the protein content in grains, leading to improved nutritional value of sorghum (Fasusi et al., 2023). Interestingly, the combination of PGPR and AMF can have additive effects on grain composition, potentially fulfilling both consumer and industrial requirements (Berta et al., 2013).

Moreover, PGPM can help sorghum plants withstand biotic and abiotic stresses, reducing the need for agrochemicals and promoting sustainable agriculture (Sreedevi et al., 2022)

Harnessing the potential of PGPM can lead to increased sorghum productivity while maintaining soil health. The use of these microorganisms as biofertilizers and biopesticides offers an eco-friendly approach for improving crop production (Sreedevi et al., 2022). However, it's important to note that the effectiveness of microbial inoculants can be limited by competition with indigenous strains and environmental factors, necessitating further research to optimize their application in sorghum cultivation (Liu-Xu et al., 2024).

The objective of this study was to verify whether the inoculation of the microorganisms *Bacillus subtilis*, *B. pumilus*, *B. licheniformis*, *Purpureocillum lilacinum*, and *Trichoderma harzianum* were tested under two soil fertility levels: 100% and 80% fertilization

2. Materials and Methods

All the microorganisms in this study originated from the collection of the Agricultural Microbiology Laboratory at the UNESP campus in Jaboticabal, Brazil. These bacteria, initially isolated from a corn plant, was identified through sequencing, with its sequence available under GenBank accession. Concurrently, the *P. lilacinum* and *T. harzianum* fungi was isolated by Dr. Noemi Carla Baron Consentino during her doctoral research. These strains were sourced from the soil on a rural property in Taquaritinga, São Paulo, Brazil. For inoculum development, *B. subtilis*, *B. pumilus*, *B. licheniformis* were cultured in nutrient broth for 48 h at 28°C, whereas *P. lilacinum* and *T. harzianum* were cultured in potato dextrose broth for 14 d at 28°C. After the incubation period, the concentration was evaluated using the serial dilution method and standardized to 1 × 10^9 CFU mL^{-1}. The microorganisms were subjected to two fertilizer conditions: the first utilizing 100% of the fertilizer, as determined by soil fertility analysis, and the second employing 80% of the fertilizer quantity indicated by soil fertility assessment. The treatments were as follows: T1, control treatment without inoculation microbial, applied at 100%; T2*, Bacillus subtilis* applied at 100%; T3, *Bacillus pumilus* applied at 100%; T4*, Bacillus licheniformis* applied at 100%; T5, *P. lilacinum applied* at 100%; T6, *T. harzianum* applied at 100%; T7, control treatment without microbial inoculation, applied at 80%; T8, *Bacillus subtilis* applied at 80%; T9, *Bacillus pumilus* applied at 80%; T10, *Bacillus licheniformis* applied at 80%; T11, *P. lilacinum* applied at 80%; T12*, T. harzianum* applied at 80%.

The experimental setup was a completely randomized design within a greenhouse located in Jaboticabal, SP, Brazil (21° 15’ 17″ S, 48° 19’ 20″ W).

The microorganisms were applied via soil, the inoculum at a concentration of 1×10^9 CFU mL^{-1} was applied once directly to the soil in a volume of 10 mL per pot. During the 30-day study period, each treatment group received five weekly microbial reinoculations through foliar application, consisting of the same concentrations and volumes as the initial inoculation.

Three plants of sorghum from Embrapa were initially planted in each 5-liter pot, which were subsequently thinned to two plants per pot after initial growth. The pots were filled up to 90% of their capacity with eutrophic red latosol soil, characterized by its chemical properties, including a pH of 6.9, 10% organic matter, 23 mg/dm^3 available phosphorus, 0.7 $mmolc/dm^3$ available potassium, 79 $mmolc/dm^3$ calcium, 13 $mmolc/dm^3$ magnesium, and 11 $mmolc/dm^3$ hydrogen. Controlled environmental conditions were maintained in the greenhouse at a temperature of 24 ± 2°C, 50 ± 2% relative humidity, and a light cycle of 16:8 h light to dark, fitting the region’s Aw climate classification by Köppen and Geiger. In this study, soybean plants were assessed for several growth parameters. The plant height was measured from the apex to the base of the plant. The biomass was then processed by splitting the shoots from the roots, which were dried in a forced ventilation oven at 65°C for 72–96 h and subsequently weighed on a semi-analytical scale. The total dry mass of the plants was calculated by summing the weights of dried shoots and roots.

2.1 Determination of nitrogen and phosphorus in shoots and roots

Five hundred micrograms of dried and ground plant samples were weighed and placed into 50 mL digestion tubes, which were left to decouple at room temperature for 1.5 hours. The tubes were then positioned in a digestion block and heated to 80°C for 20 min before the temperature was increased to 160°C. The tubes were monitored and removed once the material ascended the tube walls and most of the HNO_3 evaporated, leaving a clear solution. After cooling, 1.3 mL concentrated $HClO_4$ was added to each tube. The tubes were returned to the block, the temperature was increased to 210°C, and digestion was deemed complete when the solution turned colorless with dense white vapors of $HClO_4$ and H_2O formed above the dissolved material. The tubes were cooled and the contents were diluted to 25 mL with water in a snap-cap glass (Wan et al., 2020). For phosphorus analysis, 1 mL of the digested sample was transferred to a test tube to which 4 mL of water and 2 mL of reagent mix (comprising equal parts of 5% ammonium molybdate and 0.25% vanadate) were added. The mixture was allowed to rest for 15 min before measuring absorbance at 420 nm using a UV spectrophotometer (Falchini et al., 2020).

2.2 Statistical analysis

Statistical analyses were conducted to evaluate the effects of different treatments on plant growth parameters and nitrogen and phosphorus content. Analysis of variance (ANOVA) was performed using an F-test within the AgroEstat program (Barbosa and Maldonado Junior, 2015). Where significant differences were detected, mean comparisons were conducted using the Scott-Knott test at the 5% probability level. Additionally, to associate the plant growth attributes and their nitrogen and phosphorus contents with the treatments, Principal Component Analysis (PCA) was performed on the standardized values (Hahn, 1985).

3. Results

Treatment T2 (*Bacillus subtilis* applied at 100%) had the lowest plant height compared to the others, but there was no significant difference (Figure 1).

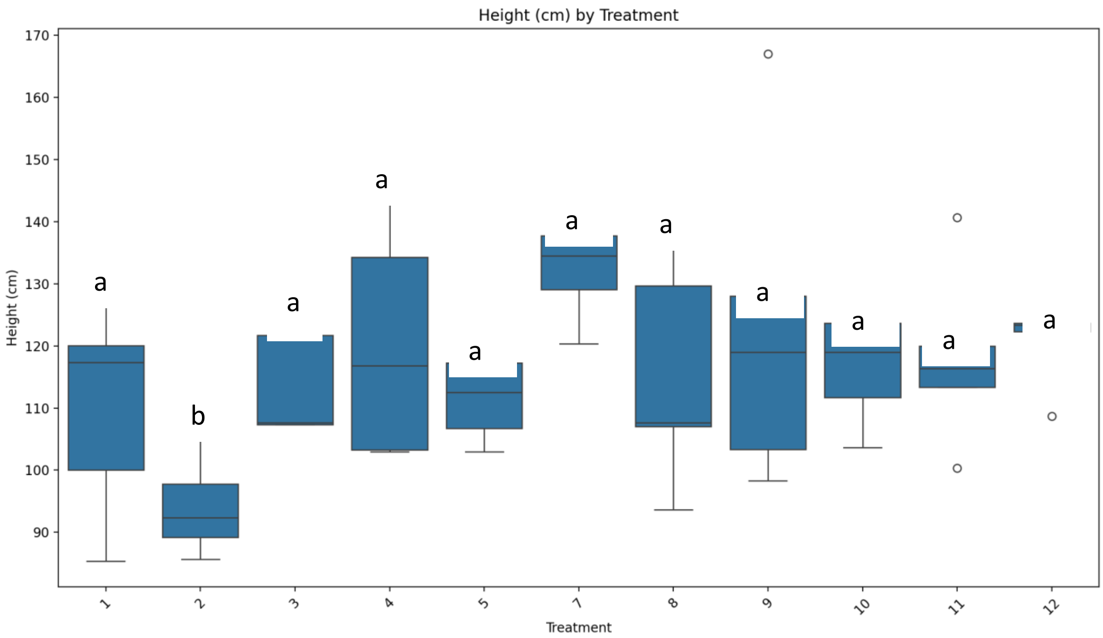


Figure 1. Average sorghum plant heights under different treatments.

One of the main results was that there was no significant difference in the diameters of the sorghum plants among the treatments.

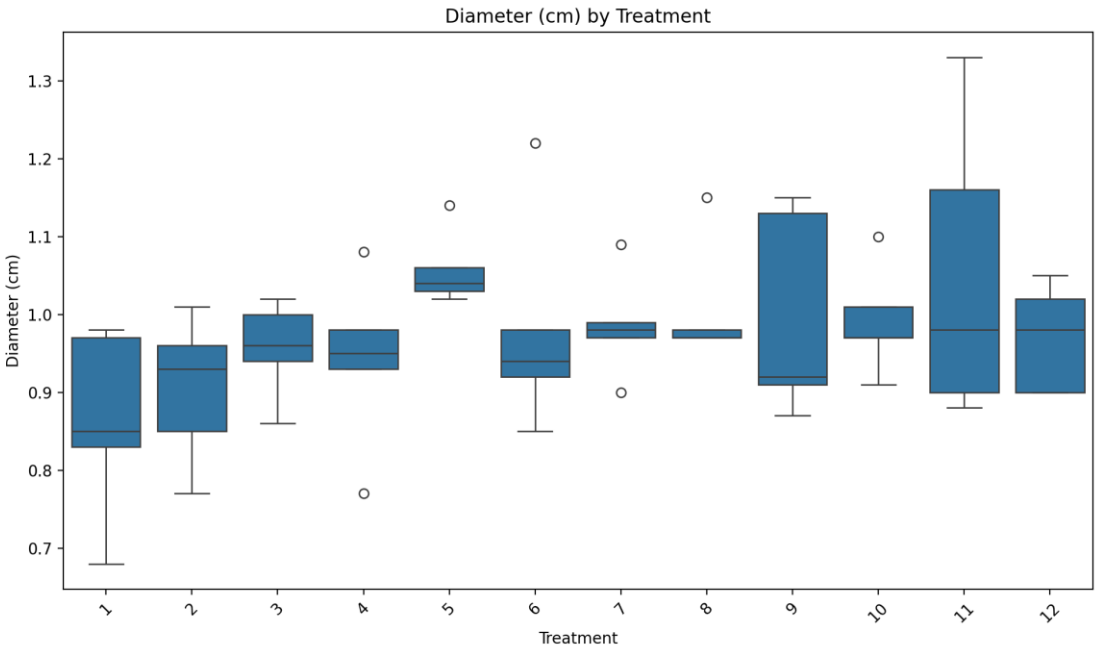


Figure 2. Average of sorghum plant diameter under various treatments.

Another significant finding was that Treatment T2 (*Bacillus subtilis* applied at 100%) had the lowest SDM value, with no significant difference observed among the other treatments (Figure 3).

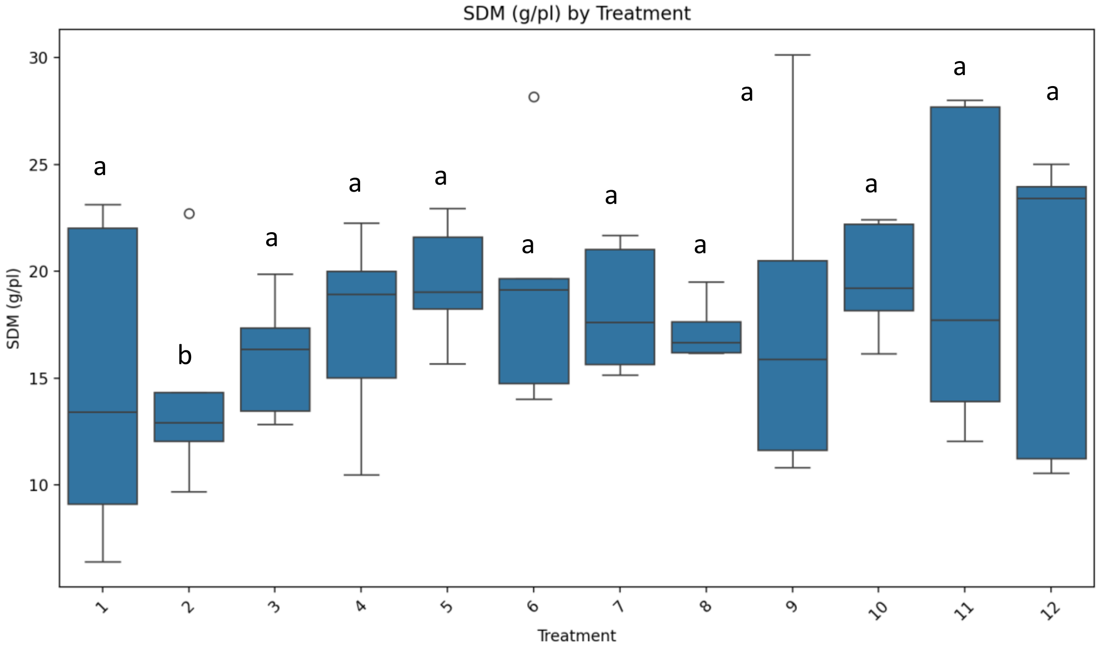


Figure 3. The average shoot dry matter of a sorghum plant is under different treatments.

Confidence in the experiment's results is bolstered by the lack of statistical difference in RDM between the treatments (Figure 4).

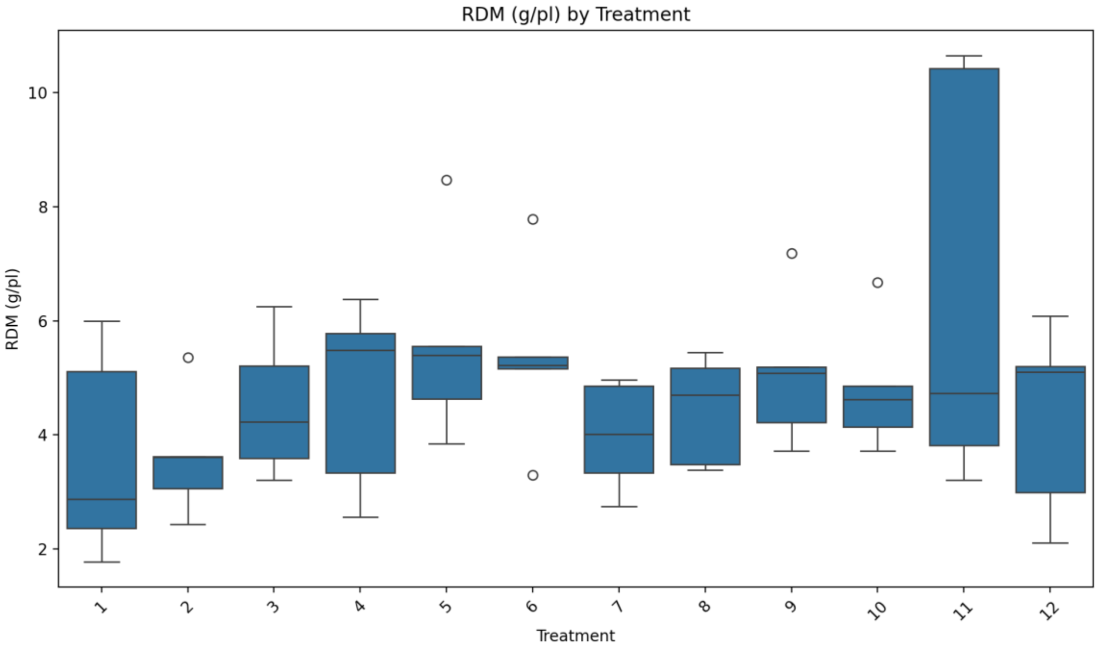


Figure 4. Average of the root dry matter of the sorghum plant under different treatments.

There was no statistical difference among the treatments regarding the phosphorous content from the SDM (Figure 5).

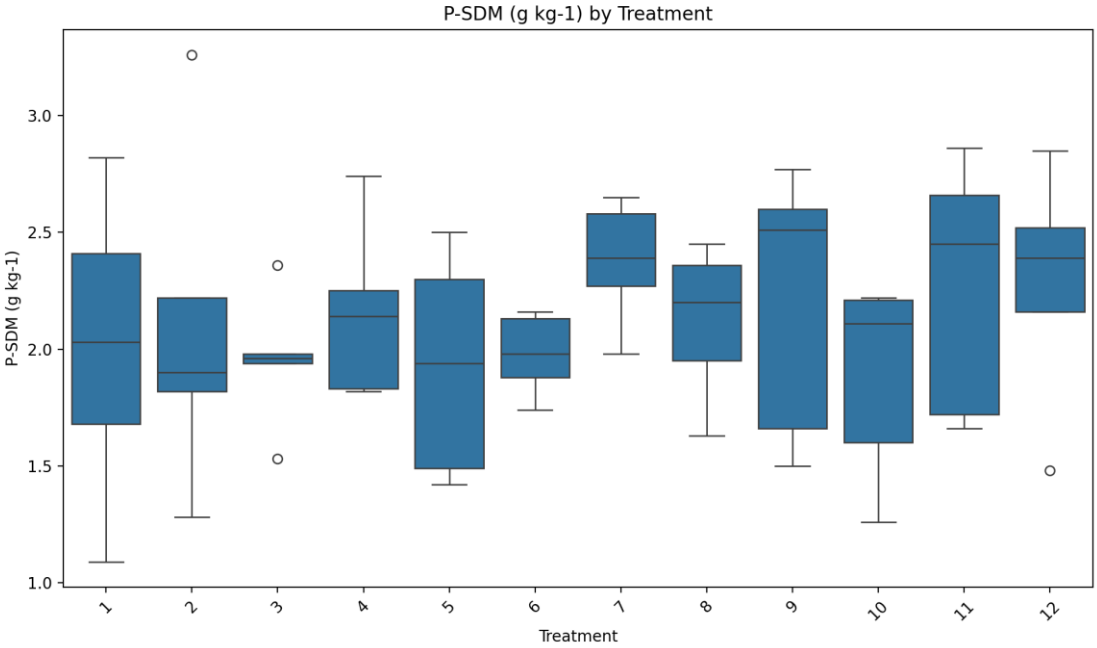


Figure 5. Average nitrogen content in the shoot dry matter under sorghum crop under different treatments.

Regarding the phosphorus content from RDM, there was no statistical difference between the treatments (Figure 6).

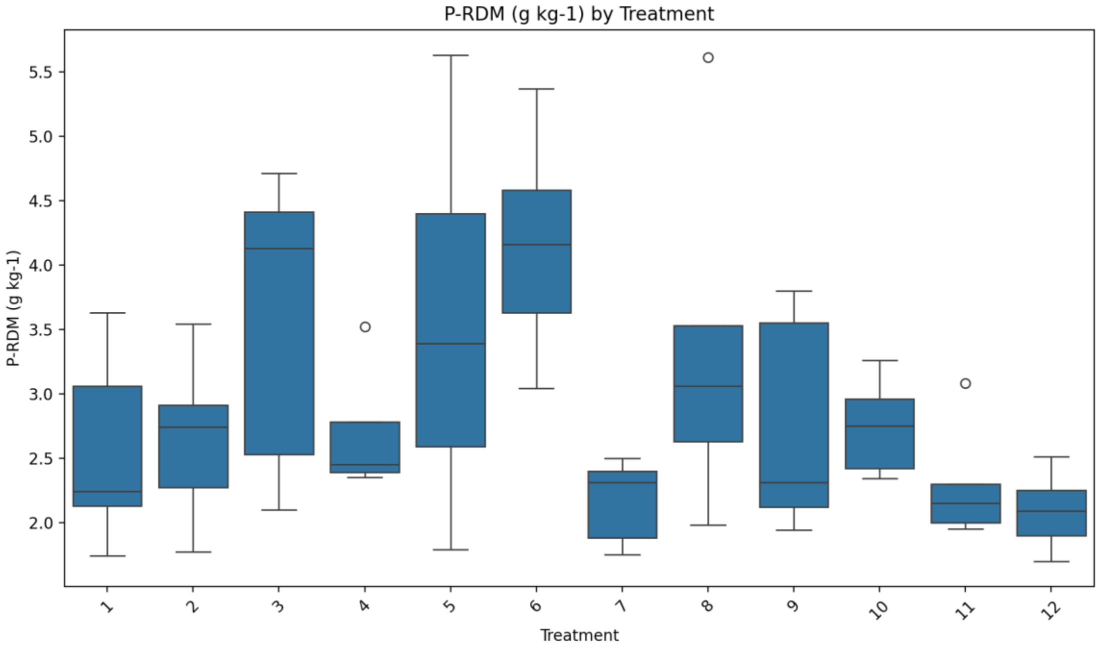


Figure 6. Under different treatments, the average phosphorous content in the root dry matter is under sorghum crop.

Regarding the phosphorous content in the soil, treatments T2 (*Bacillus subtilis* applied at 100%) and T3 (*Bacillus pumilus* applied at 100%) had the lowest values, whereas the other treatments did not differ (Figure 7).

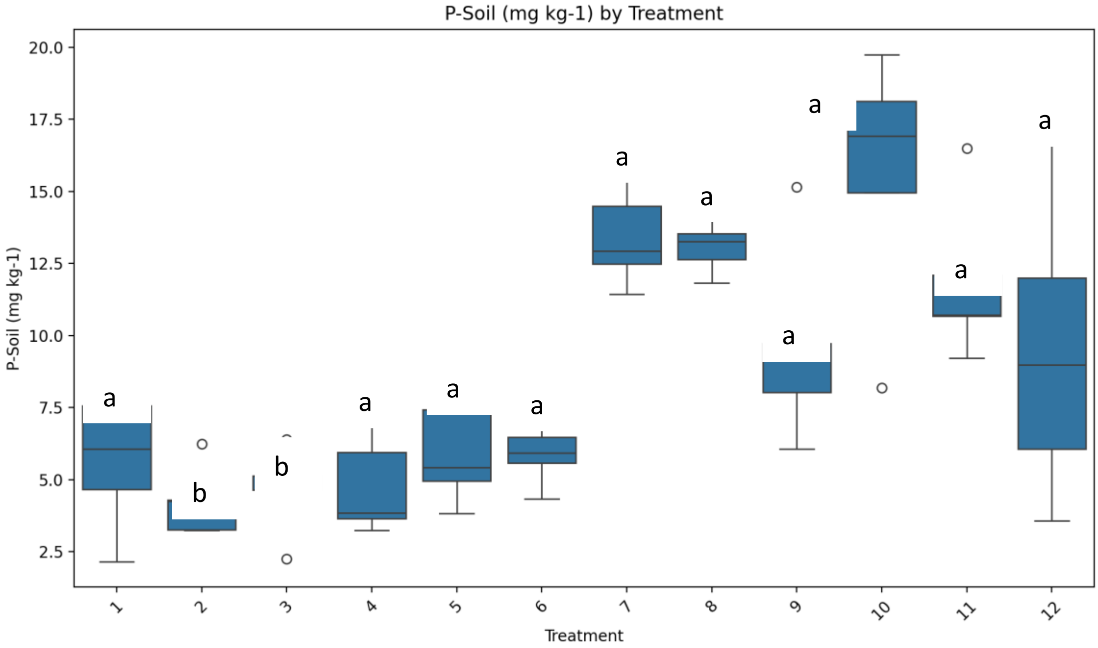


Figure 7. The average phosphorous content in the soil under sorghum crops is under different treatments.

Regarding the nitrogen content from SDM, there was no statistical difference between the treatments (Figure 8).

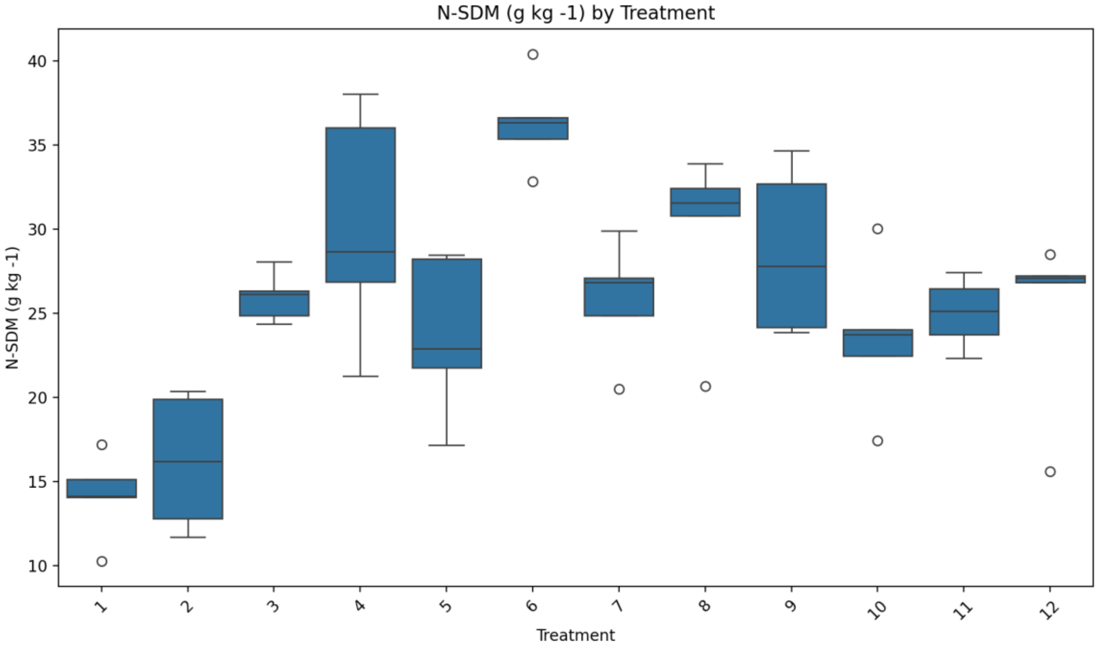


Figure 8. Average nitrogen content from the shoot dry matter under sorghum crop under different treatments.

Regarding the nitrogen content from RDM, there was no statistical difference between the treatments (Figure 9).

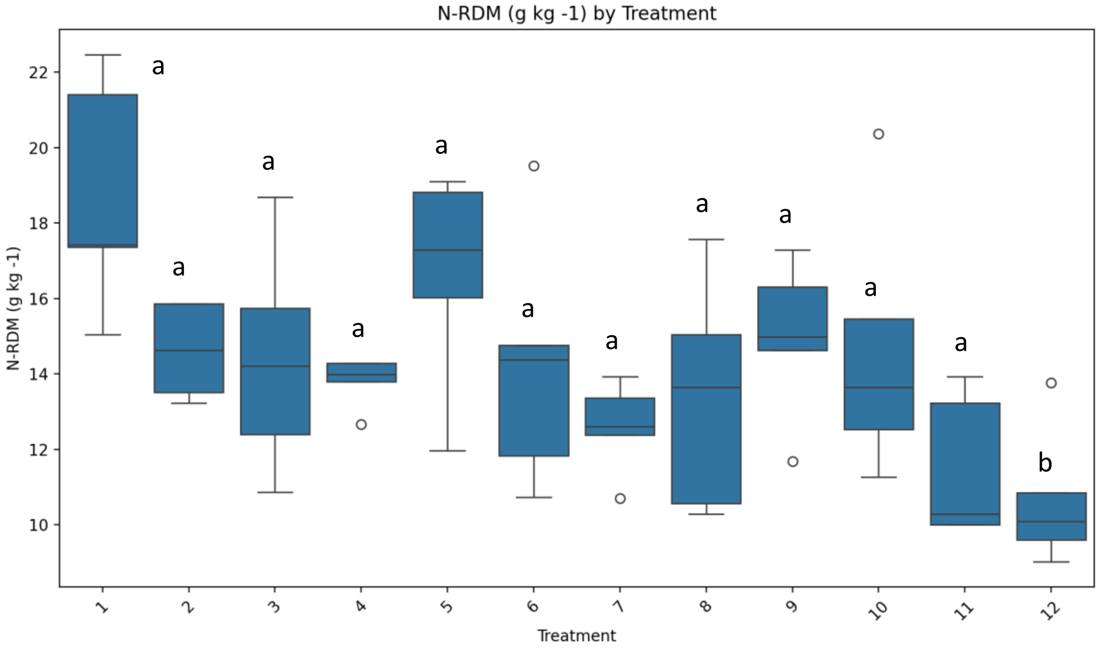


Figure 9. Average nitrogen content from root dry matter under sorghum crop under different treatments.

Regarding the soil's ammonium content, there was no statistical difference among the treatments (Figure 10).

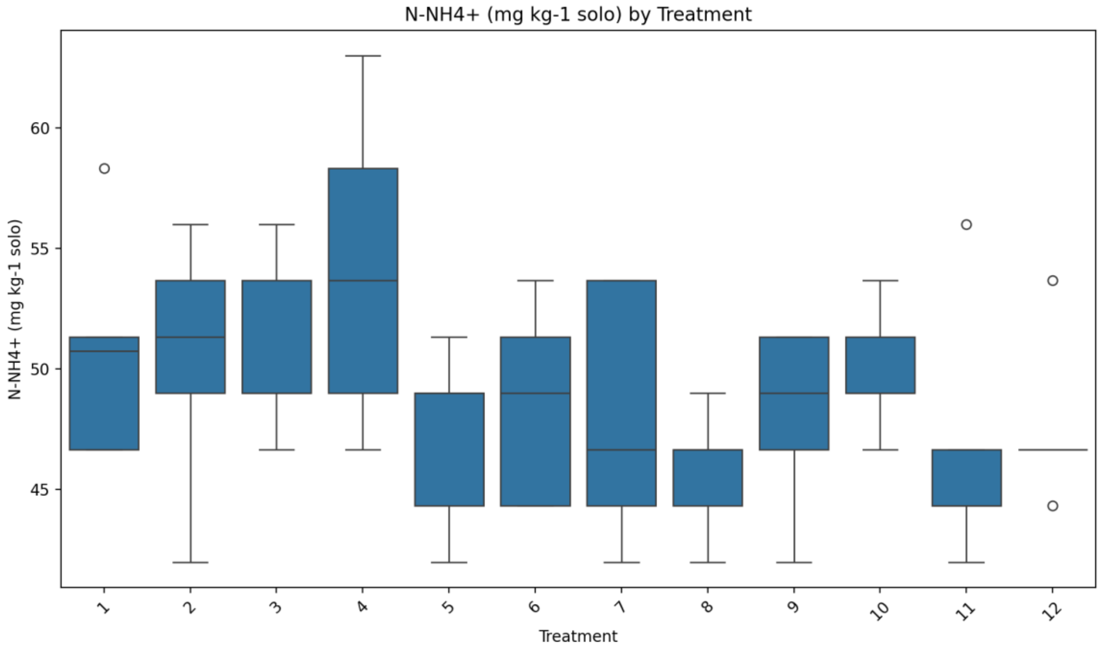
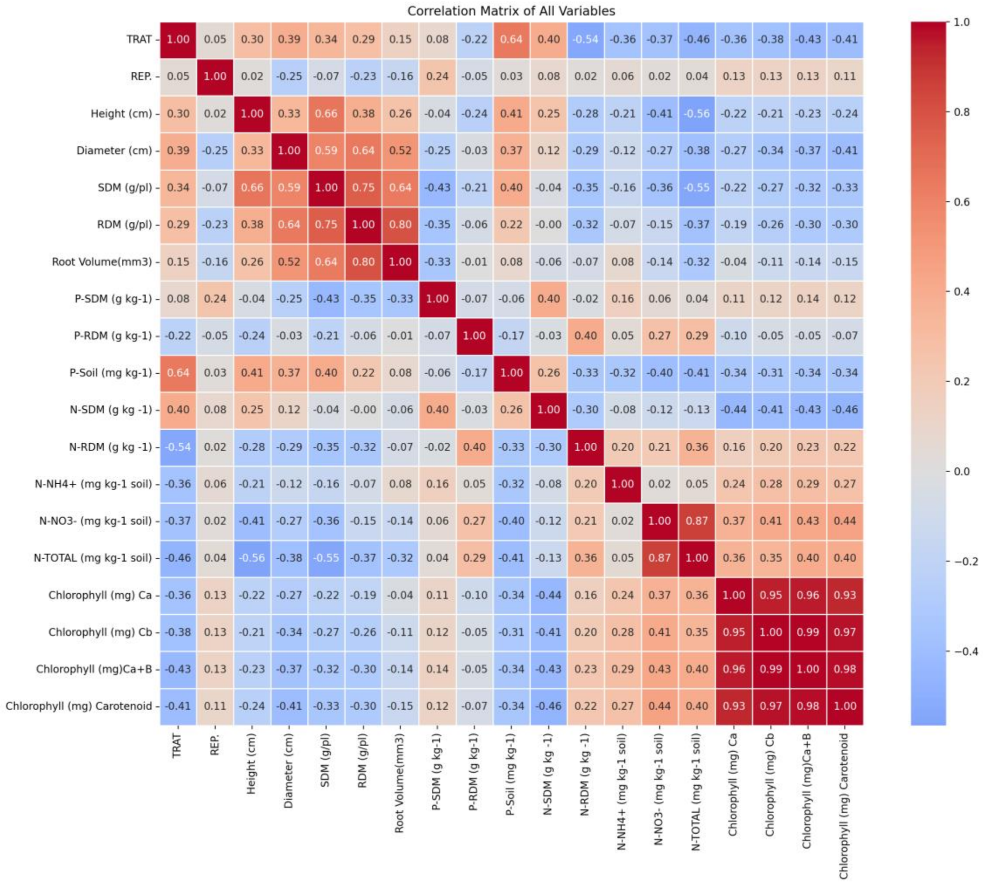


Figure 10. Average ammonium content in the soil under sorghum crop under different treatments.

The figure 11 is a correlation matrix, which shows the relationships between various variables in the dataset. Each cell in the matrix represents the correlation coefficient between two variables, with the color indicating the strength and direction of the relationship. P-Soil and N-SDM correlations. P-Soil (mg kg-1) shows a moderate positive correlation with several variables, particularly N-SDM (mg kg-1), indicating that higher phosphorus in soil is associated with higher nitrogen content in shoot dry matter On the other hand, P-Soil also has a positive correlation with height (cm) and Diameter (cm), suggesting that phosphorus availability in the soil could be related to plant growth metrics. Negative correlations with nitrate (N-NO_3-), N-NO_3- (mg kg-1 soil) shows a negative correlation with Chlorophyll a, b, and total chlorophyll, indicating that higher soil nitrate levels might be associated with lower chlorophyll levels. This could be due to an imbalance in nutrient availability affecting chlorophyll production.



	TRAT	REP.	Height (cm)	Diameter (cm)	SDM (g/pl)	RDM (g/pl)	Root Volume(mm3)	P-SDM (g kg-1)	P-RDM (g kg-1)	P-Soil (mg kg-1)	N-SDM (g kg -1)	N-RDM (g kg -1)	N-NH4+ (mg kg-1 soil)	N-NO3- (mg kg-1 soil)	N-TOTAL (mg kg-1 soil)	Chlorophyll (mg) Ca	Chlorophyll (mg) Cb	Chlorophyll (mg)Ca+B	Chlorophyll (mg) Carotenoid
TRAT	1.00	0.05	0.30	0.39	0.34	0.29	0.15	0.08	-0.22	0.64	0.40	-0.54	-0.36	-0.37	-0.46	-0.36	-0.38	-0.43	-0.41
REP.	0.05	1.00	0.02	-0.25	-0.07	-0.23	-0.16	0.24	-0.05	0.03	0.08	0.02	0.06	0.02	0.04	0.13	0.13	0.13	0.11
Height (cm)	0.30	0.02	1.00	0.33	0.66	0.38	0.26	-0.04	-0.24	0.41	0.25	-0.28	-0.21	-0.41	-0.56	-0.22	-0.21	-0.23	-0.24
Diameter (cm)	0.39	-0.25	0.33	1.00	0.59	0.64	0.52	-0.25	-0.03	0.37	0.12	-0.29	-0.12	-0.27	-0.38	-0.27	-0.34	-0.37	-0.41
SDM (g/pl)	0.34	-0.07	0.66	0.59	1.00	0.75	0.64	-0.43	-0.21	0.40	-0.04	-0.35	-0.16	-0.36	-0.55	-0.22	-0.27	-0.32	-0.33
RDM (g/pl)	0.29	-0.23	0.38	0.64	0.75	1.00	0.80	-0.35	-0.06	0.22	-0.00	-0.32	-0.07	-0.15	-0.37	-0.19	-0.26	-0.30	-0.30
Root Volume(mm3)	0.15	-0.16	0.26	0.52	0.64	0.80	1.00	-0.33	-0.01	0.08	-0.06	-0.07	0.08	-0.14	-0.32	-0.04	-0.11	-0.14	-0.15
P-SDM (g kg-1)	0.08	0.24	-0.04	-0.25	-0.43	-0.35	-0.33	1.00	-0.07	-0.06	0.40	-0.02	0.16	0.06	0.04	0.11	0.12	0.14	0.12
P-RDM (g kg-1)	-0.22	-0.05	-0.24	-0.03	-0.21	-0.06	-0.01	-0.07	1.00	-0.17	-0.03	0.40	0.05	0.27	0.29	-0.10	-0.05	-0.05	-0.07
P-Soil (mg kg-1)	0.64	0.03	0.41	0.37	0.40	0.22	0.08	-0.06	-0.17	1.00	0.26	-0.33	-0.32	-0.40	-0.41	-0.34	-0.31	-0.34	-0.34
N-SDM (g kg -1)	0.40	0.08	0.25	0.12	-0.04	-0.00	-0.06	0.40	-0.03	0.26	1.00	-0.30	-0.08	-0.12	-0.13	-0.44	-0.41	-0.43	-0.46
N-RDM (g kg -1)	-0.54	0.02	-0.28	-0.29	-0.35	-0.32	-0.07	-0.02	0.40	-0.33	-0.30	1.00	0.20	0.21	0.36	0.16	0.20	0.23	0.22
N-NH4+ (mg kg-1 soil)	-0.36	0.06	-0.21	-0.12	-0.16	-0.07	0.08	0.16	0.05	-0.32	-0.08	0.20	1.00	0.02	0.05	0.24	0.28	0.29	0.27
N-NO3- (mg kg-1 soil)	-0.37	0.02	-0.41	-0.27	-0.36	-0.15	-0.14	0.06	0.27	-0.40	-0.12	0.21	0.02	1.00	0.87	0.37	0.41	0.43	0.44
N-TOTAL (mg kg-1 soil)	-0.46	0.04	-0.56	-0.38	-0.55	-0.37	-0.32	0.04	0.29	-0.41	-0.13	0.36	0.05	0.87	1.00	0.36	0.35	0.40	0.40
Chlorophyll (mg) Ca	-0.36	0.13	-0.22	-0.27	-0.22	-0.19	-0.04	0.11	-0.10	-0.34	-0.44	0.16	0.24	0.37	0.36	1.00	0.95	0.96	0.93
Chlorophyll (mg) Cb	-0.38	0.13	-0.21	-0.34	-0.27	-0.26	-0.11	0.12	-0.05	-0.31	-0.41	0.20	0.28	0.41	0.35	0.95	1.00	0.99	0.97
Chlorophyll (mg)Ca+B	-0.43	0.13	-0.23	-0.37	-0.32	-0.30	-0.14	0.14	-0.05	-0.34	-0.43	0.23	0.29	0.43	0.40	0.96	0.99	1.00	0.98
Chlorophyll (mg) Carotenoid	-0.41	0.11	-0.24	-0.41	-0.33	-0.30	-0.15	0.12	-0.07	-0.34	-0.46	0.22	0.27	0.44	0.40	0.93	0.97	0.98	1.00

Figure 11. Correlation among the parameters analyzed from the sorghum plant under different treatments under vase conditions.

The figure is a PCA (Principal Component Analysis) biplot, which provides insight into the variance and relationships among different treatments and variables in a dataset. Here’s an interpretation of this plot: PC1 (Principal Component 1) and PC2 (Principal Component 2) are the primary axes, representing the two main dimensions that capture the most variance in the data: PC1 (34.40% explained variance): This axis captures the largest proportion of variability among the variables. PC2 (15.05% explained variance): This axis captures the second-largest proportion of variability. Together, PC1 and PC2 explain about 49.45% of the total variance in the dataset, giving a fairly good representation of the data's variability. This biplot highlights how different treatments influence plant growth and nutrient variables. Variables related to chlorophyll and growth appear to be positively correlated and are associated with treatments on the positive side of PC1.

Nutrient forms, especially nitrates and ammonium, show an inverse relationship with growth and chlorophyll, suggesting that higher nitrogen availability in certain forms may not directly correspond to increased chlorophyll content or growth. The plot allows for visual assessment of which treatments might lead to desired growth and nutrient outcomes based on their positioning relative to the variable vectors.

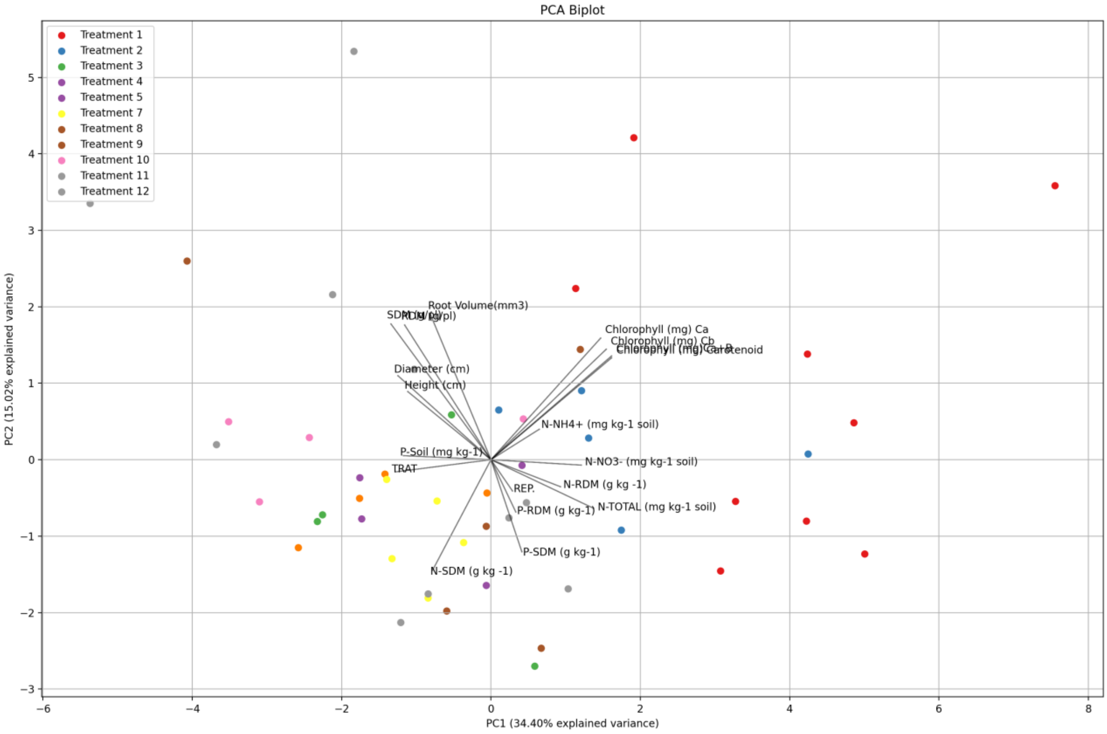


Figure 12. PCA (Principal Component Analysis) biplot, which provides insight into the variance and relationships among different treatments and variables in a dataset.

4. Discussion

In general, there was no statistically significant difference between the treatments with respect to the evaluated parameters, with the exception of height, where T2 (*Bacillus subtilis* applied at 100%) exhibited the lowest value, and shoot dry matter for the same treatment. Additionally, treatments T2 (*Bacillus subtilis* applied at 100%) and T3 (*Bacillus pumilus* applied at 100%) demonstrated the lowest values for phosphorus content in the soil, and treatment T2 (*Bacillus subtilis* applied at 100%) presented the lowest overall value. These results suggest that the application of different treatments had a minimal impact on most of the evaluated parameters. However, T2 had the most notable effects, particularly in terms of reduced plant height and shoot dry matter. These findings indicate that although the treatments generally did not produce significant differences, there may be specific aspects of treatment T2 (*Bacillus subtilis* applied at 100%) that warrant further investigation to understand its unique impacts on plant growth and soil nutrient content.

The microorganisms used in this study demonstrated several capabilities to promote plant growth. *Bacillus subtilis*, *B. pumilus*, and *B. licheniformis* can fix atmospheric nitrogen and provide this nutrient to plants, thereby enabling the reduction of nitrogen fertilizer application. *Bacillus pumilus* demonstrated nitrogen fixation capabilities in nitrogen-free medium, accumulating ammonium and enhancing the growth of *Chlorella vulgaris* (Hernandez et al., 2008). Similarly, *B. pumilus* inoculation improved tomato growth, nitrogen uptake, and soil nitrogenase activity, particularly when combined with nitrogen fertilization (Masood et al., 2020). *B. subtilis* and *B. lichenifomis* strains exhibit plant growth-promoting attributes in maize, including increased chlorophyll, carbohydrate, and protein contents (Dubey, 2021).

Interestingly, the effectiveness of these bacteria in promoting plant growth and nitrogen uptake may depend on the presence of chemical fertilizers. For instance, *Herbaspirillum seropedicae* and *Gluconacetobacter diazotrophicus* increased the nitrogen content in maize leaves and roots under both fertilized and unfertilized conditions, whereas *B. pumilus* and *B. amyloliquefaciens* enhanced the phosphorus content (Nascimento et al., 2020). This suggests that these bacteria can be used in conjunction with reduced chemical fertilization doses. Some studies have demonstrated the positive effects of *Bacillus* spp. on sorghum crops. *Bacillus subtilis* strains IPACC26 and IPACC30 were found to be particularly effective in promoting plant growth and nitrogen accumulation in sorghum, outperforming the other isolates (Aquino et al., 2019). Similarly, the *Bacillus* sp. PIB1B and PLB1B isolates have demonstrated increased effects on sorghum plant growth in greenhouse tests (De Fretes et al., 2021). These findings highlight the potential of *Bacillus* species as biofertilizer agents for sorghum cultivation.

There are no available studies on the fungi *P. lilacinum* in sorghum crops. *Purpureocillum lilacinum* has been studied in various commodities, including pineapple, maize, soybean, and legumes (Santos et al., 2023), but it has not yet been evaluated in sorghum crops. It has shown potential as a biocontrol agent against plant-parasitic nematodes and as a plant growth promoter in crops, such as cotton, peanut, and maize (Parajuli et al., 2014). This fungus has also been evaluated for its effectiveness against root-knot nematodes in eggplants (Khan & Tanaka, 2023).

Although sorghum is not specifically mentioned, studies on other crops suggest that *P. lilacinum* could potentially be beneficial for sorghum as well. This fungus has the ability to inhibit plant-pathogenic fungi and nematodes, produce siderophores and indole-3-acetic acid (IAA), and enhance plant development (Santos et al., 2023). These properties could be valuable for sorghum cultivation, but further research is needed to confirm their efficacy in this specific crop.

Although there was no increase in plant growth in the treatments that received 100% of the fertilizers along with microbial inoculants, it is important to note that there was no significant difference between the treatments that received 80% of the fertilizer dose and 100% of the fertilizer dose. This result indicates that these microorganisms can potentially be used to reduce the fertilizer dose. Despite the effect of the inoculation of microorganisms on sorghum growth, this kind of study is important because microbial inoculants, such as plant growth-promoting rhizobacteria (PGPR), have shown potential to enhance plant growth, nutrient uptake, and stress tolerance. However, their effectiveness can vary significantly under different biotic and abiotic conditions (Egamberdieva et al., 2017). Evaluating their performance helps to identify which strains are most effective in specific environments and for particular crops, ensuring optimal results in agricultural applications. Interestingly, some microbial inoculants have demonstrated secondary beneficial effects beyond their primary functions. For instance, plant growth-promoting microorganisms (PGPM) may also reduce disease, whereas biological control agents (BCA) can stimulate plant growth even in the absence of pathogens (Avis et al., 2008). This multifaceted nature of microbial inoculants highlights the importance of a comprehensive evaluation to fully understand and utilize their potential benefits.

The evaluation of microbial inoculants on the plants, including sorghum crop is essential for developing effective and eco-friendly alternatives to chemical fertilizers and pesticides (Dukare et al., 2021). This allows for the selection of appropriate strains, optimization of application methods, and understanding of their impact on soil microbial communities and overall ecosystem health (Li et al., 2023). This knowledge is crucial for improving agricultural productivity, while maintaining ecological stability and reducing the environmental impacts associated with conventional agrochemicals.

5. Conclusion

The present study evaluated the effects of microbial inoculation on sorghum growth and nutrient uptake. The results showed limited significant differences between treatments for most parameters, with the *B. subtilis* treatment exhibiting the lowest values for plant height and shoot dry matter. Interestingly, treatments that received 80% of the recommended fertilizer dose performed similarly to those that received 100%, suggesting the potential for reduced fertilizer usage with microbial inoculants. Although the microbial treatments did not significantly enhance sorghum growth in this study, such evaluations are crucial for developing eco-friendly alternatives to chemical fertilizers and pesticides. The effectiveness of microbial inoculants can vary under different conditions, highlighting the need for comprehensive assessments to optimize their application and to understand their impact on soil health and agricultural productivity.

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