



USE OF TRICHODERMA AND BACILLUS IN SUSTAINABLE BIOLOGICAL MANAGEMENT FOR SUNFLOWER (*Helianthus annuus* L.) IN THE AMAZON

Lucas Moreira da Silva¹
Daniel da Silva Parente²
João Marcos Santos Coelho³
Sebastiana Cledilene Rocha da Silva⁴
Jean Paulo Dias Paiva⁵
Bruna Sayuri Fujiyama Valente⁶
Josue Valente Lima Fujiyama⁷
Adriano Pereira de Barros⁸
Robson José Lopes Abreu⁹
Larissa Almeida Alves¹⁰

ABSTRACT

Objective: This study aimed to evaluate the effects of the combined application of the microorganisms *Trichoderma asperellum* and *Bacillus subtilis* on the development of sunflower (*Helianthus annuus* L.), aiming at sustainable alternatives to the use of chemical fertilizers.

Theoretical Framework: The research is based on the potential of plant growth-promoting microorganisms, such as *Trichoderma* and *Bacillus*, which act in nutrient solubilization, phytohormone production, and biological control of phytopathogens. These microorganisms contribute to agricultural sustainability by reducing dependence on chemical inputs.

Method: The experiment was conducted in a randomized block design with four treatments and three repetitions in Tomé-Açu (PA), from May to August 2023. Seeds were treated with commercial microorganisms and evaluated for plant height, stem diameter, leaf area, and fresh mass of stem, leaf, and capitulum.

Results and Discussion: The co-inoculation of *Trichoderma* + *Bacillus* showed better results in variables such as stem diameter, leaf area, and fresh mass of vegetative and reproductive parts. The control treatment stood out in height during the early stages, but the microbial combination outperformed the others at the end of the cycle, demonstrating synergy between the bioinoculants.

Research Implications: The joint application of these microorganisms shows promise for sustainable sunflower management, with potential for increased productivity and reduced use of chemical fertilizers.

Originality/Value: The study contributes to the literature by demonstrating the positive impact of co-inoculation of bioinoculants in Amazonian conditions, promoting gains in productivity and sustainability in sunflower cultivation.

Keywords: Agriculture, Biofuel, Bioinoculants, Oilseed, Productivity and Technology.

¹ Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil. E-mail: lucasmoreiradasilva00@gmail.com

² Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil. E-mail: danielparente602@gmail.com

³ Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil. E-mail: joao.12show@gmail.com

⁴ Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil. E-mail: cleide.rafa2011@gmail.com

⁵ Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil. E-mail: paivamodel@gmail.com

⁶ Federal Rural University of Amazonia, Paragominas, Pará, Brazil. E-mail: bruna.sayuri@ufra.edu.br

⁷ Federal Rural University of Amazonia, Paragominas, Pará, Brazil. E-mail: josue.valente@yahoo.com.br

⁸ Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil.

E-mail: adriano.barros@discente.ufra.edu.br

⁹ Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil. E-mail: robsonjoserjla007@gmail.com

¹⁰ Universidade Federal Rural da Amazônia, Tomé-Açu, Pará, Brazil. E-mail: larissaalmeida27alves@gmail.com



USO DE TRICHODERMA E BACILLUS NO MANEJO BIOLÓGICO SUSTENTÁVEL PARA A PRODUÇÃO DE GIRASSOL (*Helianthus annuus* L.) NA AMAZÔNIA

RESUMO

Objetivo: Este estudo teve como objetivo avaliar os efeitos da aplicação combinada dos microrganismos *Trichoderma asperellum* e *Bacillus subtilis* no desenvolvimento do girassol (*Helianthus annuus* L.), visando alternativas sustentáveis ao uso de fertilizantes químicos.

Referencial Teórico: A pesquisa baseia-se no potencial dos microrganismos promotores de crescimento vegetal, como *Trichoderma* e *Bacillus*, que atuam na solubilização de nutrientes, produção de fitohormônios e no controle biológico de fitopatógenos. Esses microrganismos contribuem para a sustentabilidade agrícola ao reduzir a dependência de insumos químicos.

Método: O experimento foi conduzido em blocos casualizados com quatro tratamentos e três repetições, em Tomé-Açu (PA), no período de maio a agosto de 2023. As sementes foram tratadas com os microrganismos comerciais e avaliadas quanto à altura de planta, diâmetro do caule, área foliar e massas frescas do caule, folha e capítulo.

Resultados e Discussão: A coinoculação de *Trichoderma* + *Bacillus* apresentou melhores resultados em variáveis como diâmetro do caule, área foliar e massa fresca das partes vegetativas e reprodutivas. O tratamento controle se destacou em altura nos estágios iniciais, mas a combinação microbiana superou os demais ao final do ciclo, mostrando sinergismo entre os bioinoculantes.

Implicações da Pesquisa: A aplicação conjunta desses microrganismos se mostra promissora para o manejo sustentável do girassol, com potencial de incremento produtivo e redução no uso de fertilizantes químicos.

Originalidade/Valor: O estudo contribui para a literatura ao demonstrar o impacto positivo da coinoculação de bioinoculantes em condições amazônicas, promovendo ganhos em produtividade e sustentabilidade na cultura do girassol.

Palavras-chave: Agricultura, Biocombustível, Bioinoculantes, Oleaginosa, Produtividade e Tecnologia.

USO DE TRICHODERMA Y BACILLUS EN LA GESTIÓN BIOLÓGICA SOSTENIBLE PARA LA PRODUCCIÓN DE GIRASOL (*Helianthus annuus* L.) EN LA AMAZONÍA

RESUMEN

Objetivo: Este estudio tuvo como objetivo evaluar los efectos de la aplicación combinada de los microorganismos *Trichoderma asperellum* y *Bacillus subtilis* en el desarrollo del girasol (*Helianthus annuus* L.), buscando alternativas sostenibles al uso de fertilizantes químicos.

Marco Teórico: La investigación se basa en el potencial de los microorganismos promotores del crecimiento vegetal, como *Trichoderma* y *Bacillus*, que actúan en la solubilización de nutrientes, producción de fitohormonas y control biológico de fitopatógenos. Estos microorganismos contribuyen a la sostenibilidad agrícola al reducir la dependencia de insumos químicos.

Método: El experimento se llevó a cabo en un diseño de bloques al azar con cuatro tratamientos y tres repeticiones en Tomé-Açu (PA), de mayo a agosto de 2023. Las semillas fueron tratadas con microorganismos comerciales y evaluadas en cuanto a altura de la planta, diámetro del tallo, área foliar y masa fresca del tallo, la hoja y el capítulo.

Resultados y Discusión: La coinoculación de *Trichoderma* + *Bacillus* mostró mejores resultados en variables como el diámetro del tallo, el área foliar y la masa fresca de las partes vegetativas y reproductivas. El tratamiento de control destacó en altura en las etapas iniciales, pero la combinación microbiana superó a los demás al final del ciclo, demostrando sinergia entre los bioinoculantes.

Implicaciones de la investigación: La aplicación conjunta de estos microorganismos se presenta como prometedora para el manejo sostenible del girasol, con potencial para aumentar la productividad y reducir el uso de fertilizantes químicos.



Originalidad/Valor: El estudio contribuye a la literatura al demostrar el impacto positivo de la coinoculación de bioinoculantes en condiciones amazónicas, promoviendo aumentos en productividad y sostenibilidad en el cultivo de girasol.

Palabras clave: Agricultura, Biocombustible, Bioinoculantes, Oleaginosa, Productividad y Tecnología.

RGSA adopts the Creative Commons CC BY Attribution License (<https://creativecommons.org/licenses/by/4.0/>).



1 INTRODUCTION

Sunflower (*Helianthus annuus* Linnaeus) is a plant that has revealed an increase in cultivation areas in Brazil. This culture has been used for the manufacture of edible oil, biofuel, decoration, animal feed, among other applications (Souza *et al.*, 2015).

The sunflower has as main application human nutrition, highlighting the high quality oil, good oxidation stability and nutritional and functional properties beneficial to the diet. In the animal diet, it is used in the form of bran, silage and also as food for birds, using its achenes, thus representing a significant source of protein. In addition, its flower attracts bees, contributing to the integrated production of honey (Freire, 2016).

According to FAO data (2016), the largest producers of the cultivar are Ukraine, Russia and Argentina, with almost 70% of all world production that in 2023 reached 35.95 million tons. The Brazilian territory obtained a notorious increase in sunflower production in 2012, with 123.6 thousand tons, with a jump to 158.6 thousand tons in 2014.

The continuous search for increased productivity, coupled with the great diversity of climatic, soil and management conditions in Brazil, makes it challenging to adapt a single cultivar to multiple regions (Porto *et al.*, 2009). In addition, another challenge faced by producers is crop health, since pests and diseases can significantly compromise productivity.

Despite the challenges posed by climate and soil variability, Brazil is among the largest agricultural producers in the world. This scenario is the result not only of favorable conditions present in some regions, but also of technological advancement and modernization of the agricultural sector. However, despite the high technification, the losses caused by pests and diseases are still significant, leading to excessive use of chemical pesticides, which can have negative impacts on both human health and the environment (Praça *et al.*, 2009).

In this context, it is essential to adopt sustainable technologies that not only contribute to the conservation of the environment, but also help to reduce costs with chemical pesticides and mineral fertilizer, as highlighted by Dias-Filho (2011). In this sense, Machado *et al.* (2012)



state that the use of plant growth promoting microorganisms emerges as one of the most relevant strategies for current agriculture.

The application of microorganisms as bioinoculants enhances the absorption of nutrients by plants, reducing production costs and minimizing environmental impacts resulting from excessive use of fertilizers (Pedraza *et al.*, 2010). Thus, the use of microorganisms not only favors the increase of agricultural production, but also adds competitiveness and value to the product, in addition to reducing costs for the producer (Braga Junior *et al.*, 2018; Diaz *et al.*, 2019).

The fungi of the genus *Trichoderma* are widely studied due to their versatility and benefits for agriculture and industry. They act in the biological control of pathogens, promote plant growth and assist in the availability of essential nutrients to plants (Silva *et al.*, 2019).

This interaction between microorganisms and plants occurs through biochemical reactions, in which fungi release secondary metabolites that are recognized by plants. Among these compounds, indole-3-acetic acid (IAA) stands out as a regulator of root development and plant growth (Martínez-Medina *et al.*, 2014).

The genus *Bacillus* comprises a group of Gram-positive bacteria, with about 360 species, which are distinguished by their adaptability to the rhizosphere of plants. These bacteria colonize the roots and promote a significant increase in crop productivity, according to Paiva *et al.* (2020).

Among the species of this genus, *Bacillus subtilis* stands out for its ability to synthesize phytohormones that stimulate the growth of the root system. Among these compounds, indole-3-butanoic acid (IBA) is one of the main ones, promoting the increase of root hair and, consequently, improving the absorption of water and nutrients (Buchelt *et al.*, 2019).

Thus, the objective of this study was to evaluate the effects of the combined application of the microorganisms *Trichoderma asperellum* and *Bacillus subtilis* on sunflower development. In addition, we sought to investigate the potential of these bioinoculants as a sustainable alternative to reduce the use of chemical fertilizers, improve crop productivity and contribute to a more efficient and environmentally responsible agriculture.



2 THEORETICAL FRAMEWORK

2.1 SUNFLOWER AND ITS AGRICULTURAL IMPORTANCE

Sunflower is a plant that produces an oil of high quality and nutritional value, suitable both for human consumption and for the nutrition of pigs and poultry. In addition, its parts can be used as silage, an excellent forage option, and in practices such as green manure, medicinal and ornamental use, according to Souza *et al.* (2004).

The plant also has multiple functions in addition to oil production, with its parts being used in various ways, which further expands its importance in the agricultural context. Due to its wide adaptation to different climates and regions, sunflower has attracted increasing worldwide interest, especially as a viable alternative for the production of raw material for biofuel production (Souza *et al.*, 2004).

Another great advantage of sunflower is its contribution to the sustainability of agriculture. Studies indicate that the rotation of sunflower crops in areas of corn and soybeans provides significant gains in productivity. Specifically, in regions that adopt sunflower rotation, there is an increase of 15 to 20% in corn productivity and 10% in soybean productivity (Embrapa, 2012). These data highlight the importance of sunflower not only as a commercial crop, but also as an effective strategy for sustainable agricultural management.

In addition to its agronomic benefits, sunflower has attracted attention as a viable alternative in biofuel production. With its wide adaptation to different climatic conditions, this plant can be grown in several regions, being an excellent source of raw material for the production of biodiesel. The increased demand for biofuels, combined with its agronomic and environmental advantages, places sunflower as a key crop in modern agriculture, aligned with the sustainability and innovation needs of the agricultural sector (Souza *et al.*, 2004).

2.2 GROWTH PROMOTING MICROORGANISMS

Plant Growth Promoting Bacteria (PGPBs) are essential microorganisms in plant development, promoting healthy plant growth through direct and indirect mechanisms. They produce bioactive compounds such as phytohormones, in addition to solubilizing phosphorus, fixing nitrogen and facilitating the absorption of nutrients by the roots, which makes these bacteria fundamental for the nutrition and vigor of plants, according to O'Callaghan *et al.*, (2022).



In addition to promoting growth, BPCPs increase the resistance of plants to abiotic stresses, such as drought and salinity, and offer protection against phytopathogens. This occurs by mechanisms such as competition for space and nutrients, antibiotic production and induction of systemic resistance, which makes BPCPs a sustainable alternative in agriculture, reducing dependence on chemical inputs (O'Callaghan *et al.*, 2022).

Among the strains of BPCPs, the Phosphate Solubilizing Microorganisms (PSMs), especially of the genus *Bacillus*, are highlighted. These bacteria are versatile, promoting plant growth by solubilizing nutrients such as phosphorus and potassium. In addition, *Bacillus* also protects plants against phytopathogens and insect pests, through the production of antimicrobial compounds and induction of systemic resistance, making it an effective and sustainable strategy for the biological management of crops (Silva *et al.*, 2023; Pereira *et al.*, 2020; Ferreira *et al.*, 2019).

Another relevant microorganism is the fungus *Trichoderma*, which, in addition to promoting plant growth, acts as a biocontrol of phytopathogens. *Trichoderma* colonizes plant roots, competing with pathogens for space and nutrients, and produces bioactive metabolites that stimulate growth and increase stress resistance. The combination of *Bacillus* and *Trichoderma* as MSPs offers a robust strategy for sustainable agriculture, promoting soil health, reducing chemical inputs and increasing agricultural productivity (Harman *et al.*, 2004).

2.3 SUSTAINABILITY IN AGRICULTURAL MANAGEMENT

The use of organic products in agriculture has been increasingly highlighted due to the benefits they provide for the sustainability of the sector. In this context, research institutions and companies have invested heavily in the development of new technologies to integrate biological control products (PBC) into agricultural production systems. PBCs, which are part of bioinputs, play a key role in accelerating the growth of the sector, mainly because of their ability to promote more sustainable agricultural practices and less dependent on conventional chemicals (Kraemer, 2022).

The adoption of management strategies based on scientific knowledge has been crucial for the transition to a more sustainable agriculture. Innovative technologies, such as bioinputs and biological control agents, have optimized the use of natural resources and promoted more efficient and environmentally responsible practices, representing an advance in agriculture 4.0. These technologies are being widely adopted, both in organic and conventional agriculture,



contributing to the integrated management of diseases and pests and reducing dependence on chemical inputs (Raymaekers *et al.*, 2020).

Biological control has been consolidated as an effective approach, especially where traditional methods have limitations. It complements integrated pest and disease management, bringing benefits to producers, consumers and the environment. Among the advantages, we highlight the reduction of the use of chemical pesticides, the reduction of pest resistance, the lower concentration of residues in food, greater specificity in combating target pests and the restoration of beneficial organisms in the agroecosystem (Romeiro, 2007).

The indiscriminate use of synthetic fertilizers, widely used in the nutritional management of large agricultural crops, has caused significant environmental impacts. Among the negative effects are the aggravation of the greenhouse effect, contamination of groundwater and surface water, degradation of water quality and imbalance in soil microbiota. In addition, these inputs can pose direct risks to human health, which reinforces the urgent need to adopt more sustainable and less harmful agricultural practices to the environment (Rana *et al.*, 2020).

In this scenario, the adoption of biological products and biological control emerges as strategic alternatives for agriculture. These practices not only increase productivity, but also promote soil health, reduce environmental impacts, and promote the well-being of agricultural communities. These advances highlight the importance of integrating innovative solutions that meet the needs of modern agriculture in a responsible and sustainable manner.

3 METHODOLOGY

The experiment was carried out at the Acará Valley Agricultural Association (AAVA), by the Agricultural Dynamic Extension Program of the Federal Rural University of Amazonia, located in the municipality of Tomé-açu, northeast region of Pará. The geographical coordinates of the site are: 2° 24' 53" S 48° 8' 60" W, carried out from May to August 2023.

According to Santos *et al.* (2020) and according to the Köppen classification, the climate of the region is characterized as mesothermal and humid, of the Am type, presenting an average annual temperature of 26 °C and relative humidity of approximately 85%.

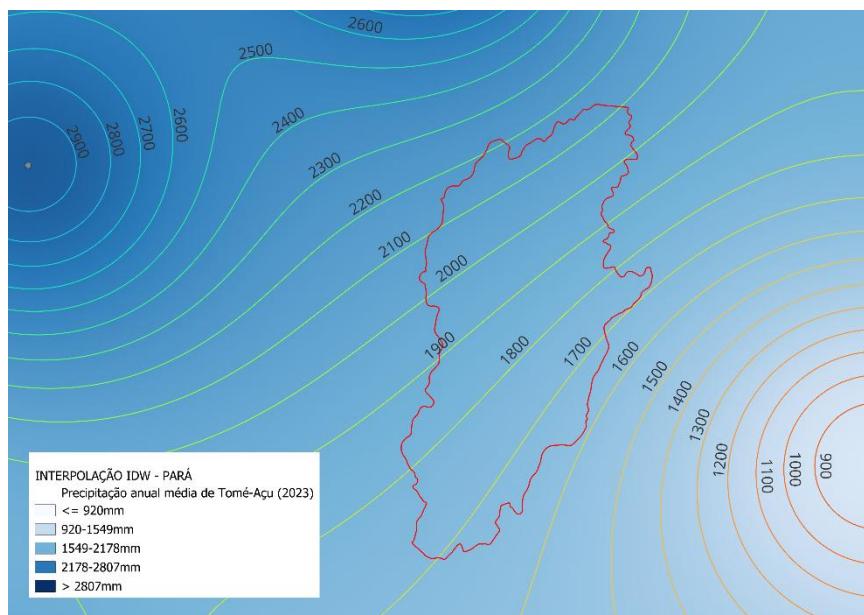
The rainfall analysis of Tomé-Açu was made based on data collected in rainfall stations and using the IDW interpolation method, which estimates values based on points with known values in the vicinity of the object of study. This information was obtained from the National Institute of Meteorology (INMET) and refers to the period from January 2023 to December



2023. The evaluation results indicated an average annual rainfall of 1,900 mm, with some seasonal variations. The rainy season is distributed from January to April.

Figure 1

Average annual rainfall of Tomé-Açu.



Before the installation of the experiment, soil was collected at 0-20 cm and sent to the laboratory Solos & Plantas for physical and chemical analyzes, macro and micronutrients. According to Embrapa (1999), the soil of the experimental area is classified as Argissolo Amarelo Distrófico Concrecionário A moderate texture medium/clay, with the following chemical characteristics: pH CaCl₂ 4.9; H₂O 5.7; Ca 4.9 cmolc/dm³; Mg 1.0 cmolc/dm³; Ca+Mg 5.0 cmolc/dm³; Al 0.0 cmolc/dm³; H+Al 6.6 cmolc/dm³; CTC 8.0 cmolc/dm³; P (Mel) 45 cmolc/dm³; K 1.2 cmolc/dm³; Na 0.0 mg/dm³; K 69 mg/dm³; S 3.2 mg/dm³; B 0.3 mg/dm³; Cu 1.2 mg/dm³; Fe 249 mg/dm³; Mn 15.8 mg/dm³; Zn 7 mg/dm³; M.O 3.9%.

The experiment was conducted in an area of 7.5 x 9.5 m (71.25 m²). For the installation of the experiment, the area was cleaned and the vegetation was removed, aiming at the demarcation of the experimental plots. These presented, individually, dimensions of 2 x 2 m (4 m²), with spacing of 0.50 m between rows and 0.40 m between plants.

Planting fertilization was performed according to soil chemical analysis, correlating and adapting with the recommendation of Lira *et al.* (as cited in Moura *et al.*, 2020, p. 2) for sunflower cultivation. The sources used were urea (N), potassium chloride (K) and Simple



Superphosphate (P). The application was distributed among the seeding lines, using 24 g of urea, 14.5 g of KCl and 19 g of SFS.

The experimental design was a randomized block design with four treatments: T1, control (without application of microorganisms); T2, *Trichoderma asperellum* (*Trichoplus JCO*); T3, *Bacillus subtilis* (*BS10*); and T4, *Trichoderma asperellum* (*Trichoplus JCO*) + *Bacillus subtilis* (*BS10*), each with three replicates.

For dosage of both products, followed the manufacturer's recommendation. For *Trichoderma asperellum* (*Trichoplus JCO*) the recommended dosage was 5 g/kg of seeds. For *Bacillus subtilis* (*BS10*) the recommended dosage was 5 ml/kg of seeds. The seeds were treated with the corresponding commercial products at the time of sowing, and three seeds were placed per pit with 0.05 m depth.

After seed emergence, straw was introduced as vegetation cover, serving as protection and to maintain soil moisture. As a biological pest control strategy, the commercial product *BioMatch* (*JCO Bioproducts*) and Neem Extract were used. For *BioMatch*, the proportion was 200 g/ha and for Neem Extract, 10 ml/liter of water was diluted.

Plant height (AP), stem diameter (DC) and leaf area (AF) were analyzed at 30, 45 and 60 days after sowing (DAS), using a tape measure and a graduated caliper. The diameter of the chapter (DCAP) was analyzed at 60 and 94 DAS. The DC was determined by the average of three measurements, being made at the bottom, midpoint and upper part of the stem. The DCAP was determined by averaging two measurements made perpendicularly in the area of existence of developing achenes (Freitas *et al.*, 2020).

The fresh masses of the chapter (MFCAP), leaves (MFF) and stem (MFC) were determined at 94 DAS. The collected material was taken to the laboratory of Ecology, Morphology and Systematics of Arthropods of the Federal Rural University of Amazonia, campus of Tomé-Açu. For the determination of MFCAP, MFF and MFC, the plants were divided into chapter, leaf + petiole and stem. Subsequently, they were packed in paper bags previously identified with the treatments and determined on a digital precision scale, model SF-400 (Freitas *et al.*, 2020).

The analyzes were performed using the R Studio software and it was verified if there were significant differences between the treatments by the F of ANOVA ($p < 0.05$), after the comparison of multiple means by the *Duncan* test at a significance level of 5% (Chagas, 2017).



4 RESULTSS AND DISCUSSÕES

In Table 1, the results indicated that the control treatment presented superior performance in plant height at 30 days after sowing (DAS), standing out with 30.15% increase in relation to the combination of commercial products. Although the control treatment provided higher plants, Sandini *et al.* (2024) points out that plant height is a very significant variable, however, in the case of sunflower (*Helianthus annuus* Linnaeus) culture, it is directly associated with a greater susceptibility to lodging.

For the stem diameter, a statistical difference was observed, with *Trichoderma asperellum* obtaining the best performance, surpassing the control treatment by 22% at 30 DAS. Santos (2020), obtained similar results in the production of grafted seedlings of dwarf cashew 'BRS 226'. Inoculation during sowing, followed by re-inoculation after grafting, resulted in a significant increase in the stem diameter of the seedlings compared to the control treatment.

Santos *et al.* (2020), when studying *Handroanthus serratifolius* seedlings grown in nursery, observed that the application of *Trichoderma asperellum* in the substrate after planting promoted a significant increase in stem diameter, compared to the control treatment, with evaluations carried out 180 days after planting.

Stems with larger diameters have greater potential to present a high number of leaf primordia (Rose & Hasse; Ritchie & Landis as cited in Santos, 2020, p. 45). In this context, the use of growth-promoting microorganisms stands out as an effective strategy to promote the structural development of plants, directly reflecting on important variables such as stem diameter.

Table 1

Result of plant height (AP), stem diameter (DC) and leaf area (AF) treated with Trichoderma asperellum (TrichoPlus JCO) and Bacillus subtilis (BS10) in 30 days after sowing (DAS).

Treatments	PA (cm)	DC (mm)	PA (cm ²)
30 DAS ⁽¹⁾			
T1 (Control)	22.75 a ⁽²⁾	5.24 b	12.81 a
T2 (Trichoderma)	18.00 ab	6.75 a	15.35 a
T3 (Bacillus)	18.61 ab	6.24 ab	14.50 a
T4 (Trichoderma + bacillus)	15.89 b	6.30 ab	14.15 a
VC (%)	13.57	10.24	10.91

⁽¹⁾ DAS = days after sowing. ⁽²⁾ Averages followed by the same lowercase letter in the columns do not differ from each other by the Duncan test at 5%. CV: Coefficient of Variation.

According to Table 2, the control treatment was superior in relation to the combination of *Trichoderma* + *Bacillus*, with an increase of 23.78% in plant height at 45 DAS. These results



may be related to the period evaluated, as pointed out by Diaz (2018), which suggests that the positive influence on height growth promoted by the isolated or combined application of the microorganisms tends to manifest itself more clearly in longer periods.

However, treatment with *Trichoderma* + *Bacillus*, in turn, showed the best results for leaf area, with an increase of 4% in 45 DAS in relation to the control treatment. In addition, the isolated treatment with *Trichoderma* presented a performance similar to the combination of the two commercial products (*Trichoderma* + *Bacillus*).

According to Lima (2023), this is a variable of great importance, since its influence is directly linked to processes such as gas exchange and interception of sunlight, processes that are vital for plants.

According to Alves (2021), the photosynthetic rate of a crop is directly affected by the decrease in leaf area. In this way, the leaf area index favors growth, since the interception of light and photosynthesis ends up increasing, in contrast, it also increases crop transpiration.

Table 2

Result of plant height (AP), stem diameter (DC) and leaf area (AF) treated with Trichoderma asperellum (TrichoPlus JCO) and Bacillus subtilis (BS10) in 45 days after sowing (DAS).

Treatments	PA (cm)	DC (mm)	PA (cm ²)
45 DAS ⁽¹⁾			
T1 (Control)	79.16 a ⁽²⁾	13.27 a	20.57 b
T2 (Trichoderma)	84.43 a	12.86 a	25.53 ab
T3 (Bacillus)	80.65 a	11.49 a	21.58 b
T4 (Trichoderma + Bacillus)	60.33 b	13.85 a	26.69 a
VC (%)	6.61	17.51	10.37

⁽¹⁾ DAS = days after sowing. ⁽²⁾ Averages followed by the same lowercase letter in the columns do not differ from each other by the Duncan test at 5%. CV: Coefficient of Variation.

For plant height, the control treatment and the combination of *Trichoderma* + *Bacillus* presented statistically equal results. On average, 18% and 8.7% increase were obtained when compared to the isolated use of *Bacillus* and *Trichoderma*, respectively. However, the isolated treatments obtained different results from each other at 60 DAS.

For leaf area, the treatment with *Trichoderma* + *Bacillus* showed the best results, an increase of 19.51% in 60 DAS in relation to the control treatment. These results corroborate those of Costa (2022), who reported similar benefits with the co-inoculation of *Trichoderma asperellum* and *Bacillus subtilis* in Mandaru grass (*Urochloa brizantha*), showing an increase in plant height and leaf area.

According to Costa (2022), the synergistic effect of co-inoculation of *B. subtilis* with *T. asperellum* resulted in higher vegetative growth, increased biomass production and better



absorption of nutrients such as Nitrogen (N), Phosphorus (P) and Potassium (K). Consistent with these data, the combination of the commercial products applied in the present work provided greater efficiency in the growth in height and leaf area of the sunflower crop at 60 DAS, highlighting the potential of coinoculation in improving crop performance.

Diaz (2018) points out that the growth promotion provided by *Bacillus subtilis* results from the increase of nitrogen fixation, the solubilization of essential nutrients, the synthesis of phytohormones and the improvement of the chemical and physical properties of the soil, fundamental factors for the healthy development of plants.

One study highlights that fungi, especially species of the genus *Trichoderma*, have high capacity to solubilize phosphates and micronutrients, contributing significantly to plant nutrition and, consequently, to plant growth and productivity (Azarmi *et al.*, 2011).

According to Castro and Oliveira (2005), the sunflower intensely translocates nitrogen (N), phosphorus (P) and potassium (K) from the leaves to the reproductive organs, especially during the flowering phases (R5 and R6) and at the beginning of the filling of achenes (R7). These phases require a greater amount of nutrients to ensure the proper development of inflorescence and formation of healthy achenes, which can directly impact the size and quality of the chapter.

This process of nutrient translocation is fundamental for the development of CAPD, as evidenced by the 22.14% increase observed with the combination of *Trichoderma* + *Bacillus* at 60 DAS, in relation to the control treatment (Table 3). This combination may have contributed to the efficiency in the transfer of nutrients to the chapter, favoring its growth.

In addition, the largest leaf area of the plants results in a larger photosynthetic area, as pointed out by Costa *et al.* (2012). With the increase in the leaf area, the capacity of the plant to absorb light and to direct nutrients to the reproductive organs, like the chapter, is also potentiated. Thus, the combination of commercial products may be a key explanation for the observed increase in sunflower DCAP.

Table 3

Result of plant height (AP), stem diameter (DC), leaf area (AF) and chapter diameter (DCAP) treated with *Trichoderma asperellum* (TrichoPlus JCO) and *Bacillus subtilis* (BS10) in 60 days after sowing (DAS).

Treatments	PA (cm)	DC (mm)	PA (cm ²)	BED (cm)
60 DAS ⁽¹⁾				
T1 (Control)	113.60 a ⁽²⁾	10.94 a	20.75 b	34.78b
T2 (Trichoderma)	103.00 b	11.92 a	24.78 ab	38.89 ab



T3 (<i>Bacillus</i>)	92.50 c	11.22 a	21.00 b	37.11 ab
T4 (<i>Trichoderma</i> + <i>bacillus</i>)	112.00 to	14.27 a	25.78 a	44.66 a
VC (%)	3.75	22.24	8.85	11.86

⁽¹⁾DAS = days after sowing. ⁽²⁾Averages followed by the same lowercase letter in the columns do not differ from each other by the Duncan test at 5%. CV: Coefficient of Variation.

The results presented in Table 4, for fresh leaf mass (FFM), the combination of commercial products (*Trichoderma* + *Bacillus*) obtained growth of 59.65% at 94 DAS, surpassing the control treatment. In addition, the isolated use of *Bacillus* was 39.4% higher than the treatment without the use of microorganisms (control).

Santos (2020) reported a significant increase in leaf area in ‘BRS 226’ dwarf cashew grafted seedlings when using *Trichoderma asperellum* strains, applied both at sowing and after grafting. Similarly, Guerra *et al.* (2024) observed increases in leaf area in maize, highlighting the use of *Bacillus subtilis* in seed treatment, which showed better results than the control treatment.

These increases in leaf area may be related to the production of growth-promoting hormones, such as indole-3-acetic acid (IAA) and indole-3-butanoic acid (IBA), synthesized by beneficial microorganisms (Guerra *et al.*, 2024). Thus, the combined use of *Trichoderma* + *Bacillus* has the potential to promote a more vigorous vegetative development, resulting in plants with greater leaf area and, consequently, greater leaf fresh mass (FFM) compared to control.

For the fresh stem mass (MFC), the isolated treatment of *Bacillus* and the combination of commercial products (*Trichoderma* + *Bacillus*), obtained statistically equal results. However, they showed difference in relation to the other treatments. On average, they showed an increase of 42.9% and 33.8% compared to the control treatment and the isolated use of *Trichoderma*, respectively.

According to Ortas *et al.* (2017 as cited by Costa, 2022), the joint action of microorganisms, such as fungi and bacteria, enhances the effects on plant growth. This synergism between *Trichoderma* and *Bacillus* favors nutrient absorption, root and aerial development, as well as protecting the plant against adverse conditions (Elhaissoufi *et al.*, 2020).

In this context, the observed results reinforce that the combined use of microorganisms promotes a significant increase in stem growth, overcoming the control treatment and highlighting the synergistic effect between *Trichoderma* and *Bacillus* in improving the vegetative characteristics of the plant.

For the fresh mass of the chapter, the isolated treatment with *Bacillus* and the combined use (*Trichoderma* + *Bacillus*) do not differ from each other. Despite this, they presented



superior results in relation to the other treatments. On average, increases of 48.6% and 26.2% were obtained at 94 DAS, comparing the control treatment and the isolated use of *Trichoderma*, respectively.

Chagas Junior *et al.* (2022) points out that the use of *Trichoderma asperellum* promotes germination, growth and productivity of plants. Paiva *et al.* (2020) demonstrated that *Bacillus subtilis* has a significant impact on corn grain yield in several experiments.

When combined, *Trichoderma* + *Bacillus* presented superior results for the fresh mass of the chapter in relation to the control treatment, reinforcing the synergistic effect between the two microorganisms. Plants with larger chapters are directly related to greater productivity in the sunflower, that is, the combination potentiated the development of the plants.

Table 4

Result of leaf fresh mass (FFM), stem fresh mass (MFCL) and fresh mass of the chapter (FCM) treated with *Trichoderma asperellum* (TrichoPlus JCO) and *Bacillus subtilis* (BS10) in 94 days after sowing (DAS).

Treatments	MFF (g)	MFCL (g)	MFCA (g)
94 DAS ⁽¹⁾			
T1 (Control)	70.00 c ⁽²⁾	226.50 b	386.00 c
T2 (Trichoderma)	92.00 bc	262.50 b	553.50 b
T3 (Bacillus)	115.50 b	381.00 a	736.00 to
T4 (Trichoderma + bacillus)	173.50 a	412.50 a	765.00 a
VC (%)	11.67	7.71	7.83

⁽¹⁾ DAS = days after sowing. ⁽²⁾ Averages followed by the same lowercase letter in the columns do not differ from each other by the Duncan test at 5%. CV: Coefficient of Variation.

5 CONCLUSION

Thus, the results show that the combined application of *Trichoderma asperillum* and *Bacillus subtilis* promoted significant improvements in sunflower development, especially for variables related to productivity. In addition, the use of these bioinoculants has demonstrated potential as a sustainable alternative to reduce dependence on chemical fertilizers, contributing to a more efficient and environmentally responsible agriculture.



ACKNOWLEDGMENTS

The authors express their gratitude to the Universidade Federal Rural da Amazônia, campus of Tomé-Açu for the institutional support. They also thank JCO Bioprodutos, in the person of the Research and Development coordinator, Josué Valente Lima Fujiyama, for supplying the essential commercial products for the experiment. To the Group of Studies in Soils of the Amazon (GESAM), for the commitment and dedication in the activities developed. Finally, a special thanks to our advisor, Dr. Bruna Sayuri Fujiyama Valente, for her guidance, teachings and fundamental contribution to the development of this work.

REFERENCES

- Alves, P. B. (2021). *Relação entre atividades de copa e cambial de clones de Eucalyptus spp. em diferentes densidades de plantio* (Dissertação de mestrado, Universidade de São Paulo). <https://repositorio.usp.br/item/003049525>
- Azarmi, R.; Hajieghrari, B.; Giglou, A. (2011). Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. *African Journal Of Biotechnology*, 10 (31), p. 5850-5855. Academic Journals. <http://dx.doi.org/10.5897/ajb10.1600>.
- Braga Junior, G.M.; Chagas, L.F.B.; Amaral, L.R.O.; Miller, L.O.; Chagas Junior, A.F. (2018). Efficiency of inoculation by *Bacillus subtilis* on soybean biomass and productivity. *Revista Brasileira de Ciências Agrárias - Brazilian Journal Of Agricultural Sciences*, Recife, 13 (4), p. 1-6. *Revista Brasileira de Ciencias Agrarias*. <http://dx.doi.org/10.5039/agraria.v13i4a5571>.
- Buchelt, A. C.; Metzler, C. R.; Castiglioni, J. L.; Dassoller, T. F.; Lubian, M. S. (2019). Aplicação De Bioestimulantes E *Bacillus Subtilis* Na Germinação E Desenvolvimento Inicial Da Cultura Do Milho. *Revista de Agricultura Neotropical*, Cassilândia-Ms, 6 (4), p. 69-74. *Revista de Agricultura Neotropical*. <http://dx.doi.org/10.32404/rean.v6i4.2762>.
- Castro, C. de, & Oliveira, F. A. de. (2005). Nutrição e adubação do girassol. In F. de C. C. Leite, J. L. B. D. Leite, & C. L. de Almeida (Ed.), *Girassol no Brasil* (Cap. 13, pp. 317–373). Embrapa
- Chagas Junior, A. F.; Luz, L. L.; Martins, A. L. L.; Queiroz, A. S. B.; Chagas, L. F. B.; Giongo, M.; Portella, A. C. F.; Scheidt, G. (2022). Alterações morfofisiológicas na cultura do arroz e milho ocasionados pelo *Trichoderma asperellum*. *Journal Of Biotechnology And Biodiversity*, 10 (4), p. 287-296. Universidade Federal do Tocantins. <http://dx.doi.org/10.20873/jbb.uft.cemaf.v10n4.chagasjunior>.
- Chagas, L. F. B., Martins, A. L. L., Carvalho Filho, M. R., Miller, L. O., Oliveira, J. C., & Chagas Junior, A. F. (2017). *Bacillus subtilis* e *Trichoderma* sp. no incremento da biomassa em plantas de soja, feijão-caupi, milho e arroz. *Revista Agri-Environmental Sciences*, 2(3), p. 10–18. <https://revista.unitins.br/index.php/agri-environmental-sciences/index>



- Costa, A. C. P. R. da, Costa, N. V. da, Pereira, M. R. R., & Martins, D. (2012). Efeito da deriva simulada de glyphosate em diferentes partes da planta de *Eucalyptus grandis*. *Semina: Ciências Agrárias*, 33(5), p. 1663–1672.
<https://ojs.uel.br/revistas/uel/index.php/semagrarias/article/view/7730>
- Costa, S. D. A. da. (2022). *Bacillus subtilis* associado a *Trichoderma asperellum* e seus efeitos na morfometria, uso dos nutrientes e fisiologia (Tese de doutorado, Universidade Federal Rural da Amazônia). Repositório Institucional UFRA.
<https://repositorio.ufra.edu.br/jspui/handle/123456789/1842>
- Dias-Filho, M. B. (2011). *Degradação de pastagens: processos, causas e estratégias de recuperação* (4ª ed., rev., atual. e ampl.). Edição do Autor.
- Diaz, P. A. E. (2018). *Bacillus spp. como promotores de crescimento na cultura do algodão* (Dissertação de mestrado, Universidade Estadual Paulista “Júlio de Mesquita Filho”).
<https://repositorio.unesp.br/server/api/core/bitstreams/6d3ed2f0-8e4f-4389-85c8-a01ddfdb8b1/content>
- Diaz, P. A. E.; Baron, N. C.; Rigobelo, E. C. (2019). *Bacillus spp. as plant growth-promoting bacteria in cotton under greenhouse conditions. Australian Journal Of Crop Science*, Jaboticabal, 13(12), p. 2003-2014. Southern Cross Publishing.
<http://dx.doi.org/10.21475/ajcs.19.13.12.p2003>.
- Elhaisoufi, W.; Khourchi, S.; Ibnyasser, A.; Ghoulam, C.; Rchiad, Z.; Zeroual, Y.; Lyamlouli, K.; Bargaz, A. (2020). As rizobactérias solubilizadoras de fosfato podem ter uma influência mais forte nas características da raiz do trigo e na fisiologia aérea do que a solubilização da rizosfera P. *Frontiers In Plant Science*, 11, p. 1-15. Frontiers Media SA.
<http://dx.doi.org/10.3389/fpls.2020.00979>.
- Embrapa. (1999). *Geoprocessamento e plotagem* (A. Guilherme S. Campos, Ed.). Sudam Superintendência do Desenvolvimento da Amazônia Secretaria Especial de Políticas Regionais.
- Embrapa. (2012). *Tecnologias de produção de soja – Região Central do Brasil – 2012 e 2013*. Embrapa Soja.
- Ferreira, M. H., et al. (2019). *Gêneros bacterianos promissores para práticas agrícolas: uma visão sobre propriedades promotoras de crescimento vegetal e aspectos de segurança microbiana. Science of the Total Environment*, 682, p. 779–799.
- Food and Agricultural Organization (FAO). (2014). FAOSTAT – Statistics database.
<https://www.fao.org/>
- Freire, J. de O. (2016). *Cultivo do girassol irrigado sob diferentes lâminas de água e doses de nitrogênio* (Tese de doutorado, Universidade Federal Rural do Semi-Árido).
- Freitas, G. Q.; Cabral Filho, F. R.; Teixeira, M. B.; Alves, D. K. M.; Cunha, F. N.; Soares, J. A. B.; Gomes, L. F. (2020). Crescimento e desenvolvimento do girassol irrigado e adubado com organomineral. *Research, Society And Development*, Goiás, 9 (8), p. 1-17. Research, Society and Development. <http://dx.doi.org/10.33448/rsd-v9i8.5627>.



- Guerra, A. M. N. de M., Gomes, T. M., Dias, M. dos S., & Santos, A. S. dos. (2024). Formas de aplicação de *Bacillus methylothrophicus* e *Bacillus subtilis* no crescimento e produção de milho. *Recital - Revista de Educação, Ciência e Tecnologia de Almenara/MG*, 6(1), 94–106. <https://doi.org/10.46636/recital.v6i1.420>
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species – Opportunistic, avirulent plant symbionts. *Nature Reviews Microbiology*, 2(1), 43–56. <https://doi.org/10.1038/nrmicro818>
- Kraemer, A. P. N. (2022). *Avaliação do potencial de isolados de Trichoderma spp. no biocontrole de doenças da soja e no tratamento de sementes* (Dissertação de Mestrado). Instituto Federal Goiano – Campus Urutaí, Urutaí, Goiás.
- Lima, A. H. F. de. (2023). *Modelagem para estimativa não-destrutiva de área foliar do feijão-caupi [Vigna unguiculata (L.) Walp.] variedade Paulistinha* (TCC de graduação, Universidade Federal de Campina Grande). <https://dspace.sti.ufcg.edu.br:8080/xmlui/handle/riufcg/29190>
- Machado, D. F. M.; Parzianello, F. R.; Silva, A. C. F.; Antonioli, Z. I. (2018). *Trichoderma* no Brasil: o fungo e o bioagente. *Revista de Ciências Agrárias*, Santa Maria, Rs, 35(1), p. 274-288. *Revista de Ciências Agrárias*. <http://dx.doi.org/10.19084/RCA.16182>.
- Martínez-Medina, A.; Alguacil, M. M.; Pascual, J. A.; Van Wees, S. C. M. (2014). Phytohormone Profiles Induced by *Trichoderma* Isolates Correspond with Their Biocontrol and Plant Growth-Promoting Activity on Melon Plants. *Journal Of Chemical Ecology*, 40 (7), p. 804-815. Springer Science and Business Media LLC. <http://dx.doi.org/10.1007/s10886-014-0478-1>.
- Moura, M. da C. F., Dutra, A. S., Mesquita, R. O., Silva, M. L. dos S., & Lima, E. N. (2020). Respostas fisiológicas e bioquímicas de cultivares de girassol submetido à adubação com resíduo orgânico. *Nativa*, 8 (3), p. 308–313. <https://doi.org/10.31413/nativa.v8i3.9340>
- O’Callaghan, M., Ballard, R. A., & Wright, D. (2022). *Soil microbial inoculants for sustainable agriculture: Limitations and opportunities*. *Soil Use and Management*, 38 (3), p. 1340–1369. <https://doi.org/10.1111/sum.12811>
- Paiva, C. A. O.; Marriel, I. E.; Gomes, E. A.; Cota, L. V.; Santos, F. C.; Tinoco, S. M. S.; Lana, U. G. P.; Oliveira, M. C.; Mattos, B. B.; Alves, V. M. C.; Ribeiro, V. P.; Junior, R. V. Recomendação agrônômica de cepas de *Bacillus subtilis* (CNPMS B2084) e *Bacillus megaterium* (CNPMS B119) na cultura do milho. Sete Lagoas: Embrapa Milho e Sorgo, 2020. 18 p. Disponível em: <https://www.infoteca.cnptia.embrapa.br/handle/doc/1120362>. Acesso em: 28 abr 2025.
- Pedraza, R. O.; Teixeira, K. R.; Fernández Scavino, A.; García De Salamone, I.; Baca, B. E.; Azcón, R.; Baldani, V. L.; Bonilla, R (2010). Microorganismos que mejoran el crecimiento de las plantas y la calidad de los suelos. *Revisión. Corpoica. Ciencia y Tecnología Agropecuaria*, 11 (2). <https://doi.org/10.21930/rcta.vol11-num2>.



- Pereira, N. C. M., et al. (2020). *A produtividade do milho e a eficiência do uso do fósforo respondem a doses de fósforo associadas a bactérias promotoras de crescimento vegetal*. *Frontiers in Environmental Science*, 8. <https://doi.org/10.3389/fenvs.2020.00040>
- Porto, W. S., Carvalho, C. G. P. de, Pinto, R. J. B., Oliveira, M. F. de, & Oliveira, A. C. B. de. (2009). Adaptabilidade e estabilidade de genótipos de girassol para a região subtropical do Brasil. *Ciência Rural*, 39 (9), p. 2452–2459. <https://www.scielo.br/j/cr/a/msW33sDzkKh5SWXg9sjKGNt/>
- Praça, L. B.; Morinaga, C.; Medeiros, P. T.; Melatti, V. M.; Martins, E. S.; Dumas, V. F.; Falcão, R.; Monnerat, R. G. (2009). Isolamento e caracterização de estirpes de *Bacillus thuringiensis* coletadas em solos do oeste baiano. *Universitas: Ciências da Saúde*, Brasília, 7(2), p. 1-18. Disponível em: <https://www.alice.cnptia.embrapa.br/alice/handle/doc/884041>. Acesso em: 29 abr. 2025.
- Rana, K. L., et al. (2020). Endophytic microbes: Biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie van Leeuwenhoek*, 113 (8), p. 1075-1107. <https://doi.org/10.1007/s10482-020-01391-1>
- Raymaekers, K., Ponet, L., Holtapples, D., & Berckmans, B. (2020). *Screening for novel biocontrol agents applicable in plant disease management – A review*. Biological Control, Academic Press Inc.
- Romeiro, A. R. (2007). Perspectivas para políticas agroambientais. In P. Ramos (Ed.), *Dimensões do agronegócio brasileiro: Políticas, instituições e perspectivas* (p. 283-312). MDA.
- Sandini, A. H., Guimarães, A. G., Botelho, G. R., Torres Junior, C. da C., Santos, E. A. F. dos, & Belen, G. D. (2024). Crescimento vegetativo de genótipos de milho com diferentes inoculantes e adubações nitrogenadas. *Scientia Agraria*, 20 (2), p. 1–5. <https://doi.org/10.5380/rsa.v20i2.95536>
- Santos, J. M. R. dos. (2020). *Trichoderma como bioestimulante do crescimento de mudas de cajueiro-anão* (Dissertação de mestrado, Universidade Federal do Ceará). Repositório Institucional UFC. <http://www.repositorio.ufc.br/handle/riufc/56236>
- Santos, M. F.; Santos, L. E.; Costa, D. L.; Vieira, T. A.; Lustosa, D. C. (2020). *Trichoderma* spp. on treatment of *Handroanthus serratifolius* seeds: effect on seedling germination and development. *Heliyon*, 6 (6), p. 1-8. Elsevier BV. <http://dx.doi.org/10.1016/j.heliyon.2020.e04044>.
- Santos, L. S., Nahum, J. S., Santos, C. B., & Silva Junior, O. M. da. (2020). Paisagem rural da microrregião de Tomé-Açu sob a ótica bertrandiana. *Revista Brasileira de Geografia Física*, 12 (7), p. 2694–2715. <https://doi.org/10.26848/rbgf.v12.7.p2694-2715>
- Silva, C. F. B., Brígida, A. I. S., Taniguchi, C. A. K., & Pinto, G. A. S. (2019). Uso do *Trichoderma* na cultura da banana. In M. C. Meyer, S. M. Mazaro, & J. C. Silva (Ed.), *Trichoderma: uso na agricultura* (pp. 433–444). Embrapa. <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1120485/uso-do-trichoderma-na-cultura-da-banana>



Silva, L. I., et al. (2023). *Microrganismos solubilizadores de fósforo: uma chave para uma agricultura sustentável*. *Agricultura*, 13, p. 1–30.

Souza, A., Oliveira, M. F., & Castiglioni, V. B. R. O. (2004). O boro na cultura do girassol. *Semina: Ciências Agrárias*, 25 (1), p. 27-34.

Souza, F. R. de, et al. (2015). Características agronômicas do cultivo de girassol consorciado com *Brachiaria ruziziensis*. *Revista Ciência Agronômica*, 46(1), p. 110–116.