Revista Brasileira de Milho e Sorgo
Brazilian Journal of Maize and Sorghum

ISSN 1980 - 6477

Journal homepage:
www.abms.org.br/site/paginas

GROWTH PROMOTION IN MAIZE INOCULATED WITH *Trichoderma harzianum*

Tassiano Botesine de Araújo
Faculdade Educacional de Medianeira, Medianeira, PR, Brazil
E-mail: tassianobotesine@hotmail.com
ID: https://orcid.org/0009-0006-7219-1725

Adilson Ricken Schuelter
Programa de Pós-gradução em Engenharia Agrícola (PGEAGRI), Universidade Estadual do Oeste do Paraná, Cascavel, PR, Brazil
E-mail: adilson_schuelter@yahoo.com.br
ID: https://orcid.org/0000-0002-5545-7601

Isabel Regina Prazeres de Souza*
Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil
E-mail: isabel.prazeres@embrapa.br
ID: https://orcid.org/0000-0003-2684-4300
***Corresponding author**

Silvia Renata Machado Coelho
Universidade Estadual do Oeste do Paraná, Cascavel, PR, Brazil
E-mail: silvia.coelho@unioeste.br
ID: https://orcid.org/0000-0002-1614-8021

Divair Christ
Universidade Estadual do Oeste do Paraná, Cascavel, PR, Brazil
E-mail: divair.christ@unioeste.br
ID: https://orcid.org/0000-0001-7179-4336

ABSTRACT – The current research evaluated the effectiveness of *Trichoderma harzianum* in promoting the vegetative growth of maize in a medium containing nutrient solution from seed inoculation with different bio-input doses. The experiment was conducted in an agricultural greenhouse containing 8 liters of polyethylene recipients containing sand, using a completely randomized design with five treatments and six replications. Seeds of the maize hybrid MSG1001 were inoculated with different doses of the commercial product based on *T. harzianum* for every 100 kg of seeds. The treatments were: T1: 0 ml (control), T2: 200 ml, T3: 400 ml, T4: 600 ml, and T5: 800 ml. Sowing was performed in recipients containing fine-washed sand and capillarity irrigation with nutrient solution until the end of the experiment. At 28 DAP, the following variables were assessed: shoot length (SL), root length (RL), total plant length (TPL), shoot dry mass (SDM), root dry mass (RDM), total dry mass (TDM), total chlorophyll V3 (TCV3), total chlorophyll V4 (TCV4) and stem diameter (SD). The gradual increase in the doses of bio-input based on *Trichoderma harzianum*, up to 800 ml 100 kg^{-1} of seeds, promoted an increase of 27%, 81%, 40%, 34%, 64%, and 42% in the variables SDM, RDM, TDM, SD, SL, and TPL, respectively. For RL, TCV3, and TCV4, there was an increase up to doses of 619, 321.6, and 435.6 ml 100 kg^{-1} of seeds, respectively. Furthermore, a positive correlation was obtained between bio-input dose and the potassium content and expressive differences in the nutrient content of the control concerning the other treatments, which were obtained in the multivariate analysis. Finally, it is concluded that *T. harzianum* influences the vegetative growth in maize. However, depending on the dose of the bio-input, it can result in different plant responses.

Keywords: *Zea mays* L., seeds, bio-input, growth, biomass, nutrients.

PROMOÇÃO DE CRESCIMENTO EM MILHO INOCULADO COM *Trichoderma harzianum*

RESUMO - O presente trabalho avaliou a efetividade de *T. harzianum* na promoção de crescimento vegetativo de plantas de milho em meio contendo solução nutritiva, oriundas da inoculação de sementes com diferentes doses do bioinsumo. O experimento foi conduzido em estufa agrícola em vasos de polietileno de 8l contendo areia, empregando-se delineamento inteiramente casualizado com 5 tratamentos e 6 repetições. Sementes do milho híbrido MSG1001 foram inoculadas com as doses de produto comercial a base de *T. harzianum* para cada 100 kg de sementes. Os tratamentos foram: T1: 0 ml (testemunha), T2: 200 ml, T3: 400 ml, T4: 600 ml e T5: 800 ml. A semeadura foi realizada em vasos contendo areia lavada de granulometria fina e irrigação por capilaridade com solução nutritiva até o término do período de condução do experimento. Aos 28 DAP foram avaliadas as variáveis: comprimento da parte aérea (SL), comprimento de raiz (RL), comprimento total da planta (TPL), massa seca de parte aérea (SDM), massa seca radicular (RDM), massa seca total (TDM), Clorofila total V3 (TCV3), clorofila total V4 (TCV4) e diâmetro do caule (SD). O aumento gradativo das doses do bioinsumo a base de *Trichoderma harzianum* até 800 ml 100 kg^{-1} de sementes promoveu incremento de 27%, 81%, 40%, 34%, 64% e 42% nas variáveis SDM, RDM, TDM, SD, SL e TPL, respectivamente. Para RL, TCV3 e TCV4, houve incremento até as doses de 619, 321,6 e 435,6 ml 100 kg^{-1} de sementes, respectivamente. Correlação positiva foi obtida entre dose de bioinsumo e teor de potássio, e diferenças expressivas em teor de nutrientes do controle em relação aos demais tratamentos foram obtidas na análise multivariada. Enfim, conclui-se que *T. harzianum* tem influência sobre o crescimento vegetativo em milho, mas que dependendo da dose do bioinsumo pode resultar em diferentes respostas das plantas.

Palavras-chave: *Zea mays* L., sementes, bioinsumo, crescimento, biomassa, nutrientes.

How to cite

ARAÚJO, T. B.; SCHUELTER, A. R.; SOUZA, I. R. P.; COELHO, S. R. M.; CHRIST, D. (2023). Growth promotion in maize inoculated with *Trichoderma harzianum*. **Revista Brasileira de Milho e Sorgo**, v. 22, e1269.

DOI: https://doi.org/10.18512/rbms2023v22e1269

Maize crops have contributed substantially to the Brazilian economy, being the cereal of the highest importance for the national production chain, mainly in agriculture, as a base component of feeding for poultry and swine (Contini et al., 2019; Godoi et al., 2008). In this context, in the 2022/23 crop, the export expectation is 25.7%, behind only the United States, which leads with a percentage of 29,8% of total exports (Fiesp, 2022).

Despite the increasing production in recent years, Fao's (2017) projections for 2050 indicate that the demand for grains for food and power generation needs an increase of 60% and 50%, respectively. Thus, it demonstrates the great challenge of meeting this future demand, considering the environmental impacts that will be generated and the increase in the natural resources used, which are increasingly scarce (Zulauf, 2000). Therefore, the search for alternatives that contribute to increasing crop performance, mainly maize, has grown due to its tremendous economic and social importance.

Achieving the yield potential of culture is challenging, especially in maize, a culture highly responsive to biotic and abiotic factors; there is a need to adapt the cultivation system for the greater use of the genetic potential of cultivars. In Brazil, maize is predominantly cultivated as the second crop in a monoculture system, with an increase of 12% compared to the 2021/22 agricultural year (Conab, 2022), but with environmental costs due to the excessive use of pesticides (Sarkar et al., 2021; De Vries & Wallenstein, 2017). In this context, agricultural soils that employ this production system have been more intensively investigated in recent years for the structural profile of microbial communities (Dlamini et al., 2023; Akinola et al., 2021; Fadiji et al., 2021). Work carried out by Dlamini et al. (2023) found that microbial diversity influences the effectiveness of the rhizosphere microbiome in modulating biological functions in managing and maintaining maize plant health.

Chemical treatment of seeds established itself as a practice adopted by farmers, enabling control and prevention of pests and early-cycle diseases (Vazquez et al., 2014). However, they can bring contamination risks to the environment and the people who carry out these activities (Waichman, 2012). Among the alternatives, bioproducts based on beneficial microorganisms have been made available for use in agriculture, including some genera of microorganisms capable of acting in the protection of seeds and the promotion of plant growth, through different mechanisms of action (Gomes et al., 2016).

Studies using fungi of the genus *Trichoderma* have shown effective results in protecting against phytopathogens (Natsiopoulos et al., 2022; Degani & Dor, 2021; Elshahawy & El-Sayed, 2018; Saravanakumar et al., 2017) due to their ability to colonize on the roots (Scudeler & Venegas, 2012). In addition, compounds produced by the fungus induce the formation of a greater volume of rootlets and participate in the decomposition of organic matter, increasing nutrient availability to plants (Gomes et al., 2016; Vergara et al., 2019). Recently, Hang et al. (2022) verified that these fungi promote the modification of soil fungal communities, and as a consequence, plant growth is promoted.

Inoculation of maize seeds with *Trichoderma* spp aims to control phytopathogens (Ferrigo et al., 2014; Coninck et al., 2020; Zin & Badaluddin, 2020). However, there needs to be more information about the effect of the inoculant dose and its relationship with the stimulation of plant growth in the early stages of development. Furthermore, this plant-microorganism interaction can promote the formation of chloroplast pigments and the nutritional status of plants, which needs to be verified.

This study aimed to evaluate the effect of maize seed treatment with *T. harzianum* on the initial development of plants grown in sand using a nutrient solution in a capillary irrigation system.

Material and Methods

Experimental structure

The experiment was conducted in an agricultural greenhouse on a farm in the municipality of Matelândia (PR) in western Paraná from September to October 2020. Plant analyses were performed at the Chemistry Laboratory of the UDC Medianeira, Paraná, Brazil. A "chapel-type" agricultural greenhouse was used, totaling 5 m length x 5 m width x 3 m height, with a transparent plastic top cover, 100 microns thick, of the crystal type and the sides covered with black shading fabric (70%).

Preparation of pots and characterization of the irrigation system

The vessels with a capacity of 8 liters were drilled on the side of the base to facilitate the flow of water and nutrient solution. Fine-grained sand (0.05 to 0.2 mm) was used as soil material for filling the pots, previously washed to eliminate impurities with remains of organic matter.

After filling the recipients, they were irrigated by adding potable water from artesian wells on the surface until full soil saturation. The recipients were left to drain excess water through the holes in the base. Each recipient was then placed in the structure that allowed to maintain a water level 2 to 3 cm above the holes on the base's side (Figures 1A and B).

For installation of the experiment in the recipients, so that they could receive individualized capillarity irrigation, a wooden platform with a height of 1.5 m from the ground surface was built, divided into individualized compartments for recipients with 24 cm of diameter on the top edge, and 20.5 cm in the lower base, the height of 21 cm and capacity of 8 L. Each compartment was lined with plastic tarpaulin, before placement of recipients, with a distance of 5 cm between the perimeter of the recipient and the structure, to allow individualized irrigation and avoid any contamination (Figures 1).

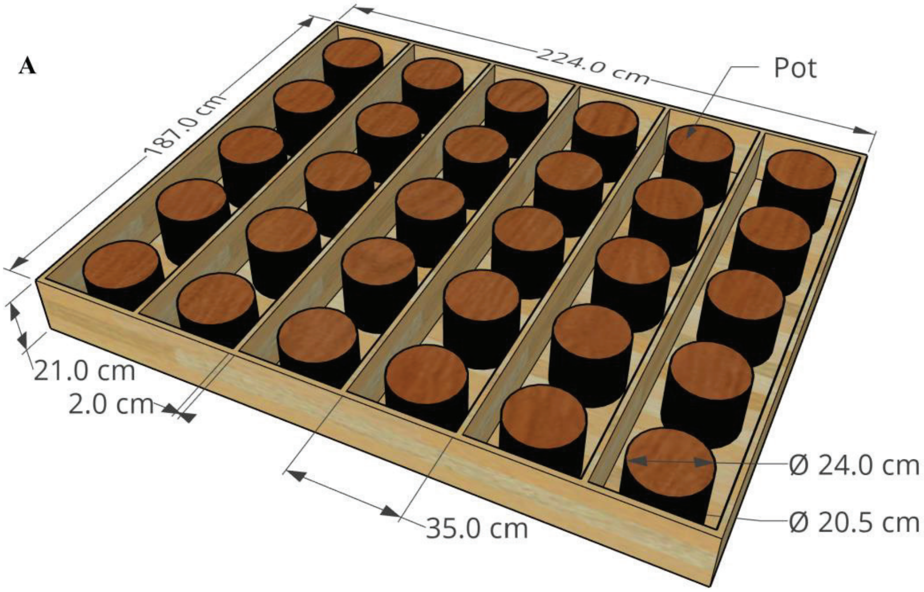

Figure 1. Schematic representation of the platform built for installation of the experiment with capillarity irrigation.

Experimental Planning

Seeds of the simple hybrid MSG1001 (MaisGenes Sementes LTDA, Toledo, Paraná) were inoculated with the fungus *T. harzianum*, employing the commercial product StimuControl containing the strain CCT 7589 (Simbiose Indústria e Comércio de Fertilizantes e Insumos Microbiológicos LTDA). The commercial product is guaranteed 1x109 UFC L^{-1} of the fungus. The treatments used the following volumes of the commercial product: T1 : 0 ml (control), T2: 200 ml, T3: 400 ml, T4: 600 ml, and T5: 800ml, having as reference the maximum product spray volume of 400 ml 100 kg^{-1} of seeds.

The experimental design was completely randomized with five treatments and six replications, totaling 30 experimental units, each represented by a recipient containing two plants.

Inoculation was carried out under agitation and homogenization of the fungal inoculum on the surface of the seeds inside a plastic bag for 30 seconds. Then the seeds were immersed in the solution and left to dry at room temperature (25°C) for 15 min. Immediately after, planting is made using four seeds/recipient, at a depth of 3 cm, with later thinning to 2 seedlings per recipient. At this stage, the recipients with moisture in the field capacity were arranged in the wooden structure in the agricultural greenhouse.

The first irrigation was required five days after sowing, adding the nutrient solution on the outside of the recipients until the level reached 2 to 3 cm above the holes at its base. At this stage, seed reserve germination and digestion have already occurred. Therefore, water availability was monitored daily at 8:00 a.m., 12:30 p.m., and 5:30 p.m. Whenever the solution level outside the recipients dropped below the holes on the base sides, new irrigation with the nutrient solution was applied. Irrigation was necessary once a day until the stage from V3 (three leaves) to V4 (four leaves). Then, due to the increase in the evapotranspirometric demand of plants from high temperatures, irrigations with the nutrient solution were given twice a day. During experimentation, minimum and maximum temperatures were recorded inside the agricultural greenhouse (Figure 2).

The nutrient solution was prepared as described by Hoagland & Arnon (1950) for a volume of 500 liters with potable water from an artesian well, adjusting the pH and electrical conductivity (EC) to 6.0 and 1.5 ms cm-1, respectively. The nutrients used in the formulation were calcium nitrate (Ca(N03), potassium nitrate (KN03), potassium sulfate (K2S04), potassium chloride (KCl), ammonium nitrate (NH4N03), magnesium sulfate (MgS04), manganese sulfate (MnSO4), boric acid (H3B03), zinc sulfate (ZnS04), copper sulfate (CuS04), sodium molybdate (Na2MoO4), iron chelate (6%) (Fe-EDTA), monopotassium phosphate (KH2PO4) and monoammonium phosphate (NH4H2PO4). For the plant's supply of nutrients, the application of nutrient solution started at (V1) until the period of the experiment uninstallation, stages V4-V5. Initially, 2 liters of solution were applied per recipient, and the replacement was performed when the solution level reached below the holes on the side of the recipient base.

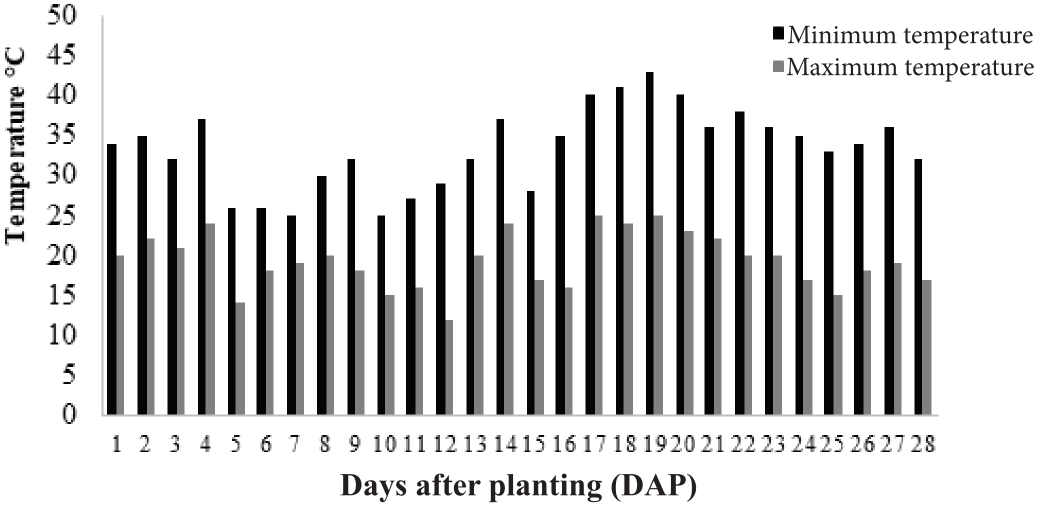

Figure 2. Measures of minimum and maximum temperatures in the greenhouse during the experiment.

Data Collect

At 28 days after planting (DAP), plants in the stages V4-V5 were evaluated for the following variables: shoot height (SH), measured from the ground to the apex of the plant; root length (RL), measured from the bottom of the sheath to the end of the root system; total plant length (TPL), obtained by the sum between SH and RL; stem diameter (SD) measured at 1 cm from ground level with the aid of a digital caliper; root dry mass (RDM), shoot dry mass (SDM) and total dry mass (TDM) obtained from the use of a drying oven at 65oC.

Chlorophyll contents were determined using fully extended leaf discs from the third (V3) and fourth leaves (V4). For the determination of chlorophyll contents “a” (CLORA) and chlorophyll “b” (CLORB), it was collected for each experimental unit four leaf discs (0.2827 cm2 disc-1). Immediately after collection, the discs were immersed in 4 ml of dimethylsulfoxide (DMSO) for three hours in a water bath at 65oC. Next, spectrophotometer reading was performed for absorbances at 649 nm and 665 nm, followed by the use of equations 1 and 2 developed by Lichtenthaler (1987), Equation 1º - Chl “a” = (12.19.A665) – (3.45.A649) and Equation 2º - Chl “b” = (21.99.A649 – (5.32.A665). Finally, obtaining the levels of DMSO in µg ml^{-1} and area for the two leaf discs per experimental unit of 0.5654 cm2 was carried out with the results conversion in µg.cm2 for each pigment.

To determine macro and micronutrients, the leaf tissue deprived of the midrib was submitted to drying in an oven at 60 oC with forced ventilation per 48 h, according to the recommendation described by Silva (1999). Then, they were analyzed in the Laboratory LabAgro (Serranópolis do Iguaçu, PR, Brazil) for the contents of Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulfur (S) Iron (Fe), Copper (Cu) Boron (B) and Manganese (Mn), in the leaf tissue according to the methodology described by Carmo et al. (2000).

Statistical analysis

The data obtained were initially checked for normality using the Shapiro-Wilk test and homoscedasticity using the Bartlett test. Then, the analysis of variance (ANOVA) was performed for each of the variables noted in the experiment.

For the variables showing significance for the bio-input dose effect, data were submitted to regression analysis, directing to adjust a mathematical model using a linear or quadratic equation that best adapted the results.

Pearson’s correlation test was used at 5% probability to verify the effect of doses on the accumulation of macro and micronutrients analyzed. The principal component analysis (PCA) was used to evaluate macro jointly and micronutrients quantified in leaf tissue.

All statistical tests were performed using the significance level of 5% and the programs

SISVAR (Ferreira, 2011) and GENES (Cruz, 2013).

Results and Discussion

Based on the results obtained for shoot length (SL) as a function of the bio-input dose (Figure 3), a positive linear behavior was detected (Figure 4A). The increase in the bio-input dose ($1x10^6$ UFC of *T. harzianum* per ml) applied to the seeds up to the maximum dose evaluated resulted in an estimated increase of 0.0317 cm $plant^{-1}$ ml^{-1}. Considering the maximum dose evaluated, the estimated gain accumulation was 25.36 cm $plant^{-1}$, is 64% higher concerning T1 (control).

Positive results of *T. harzianum* inoculation on shoot growth of maize plants associated with antagonism to Fusarium at 30 DAP, under greenhouse conditions, were verified by Saravanakumar et al. (2017). Furthermore, a proteomic analysis of maize seedling roots inoculated with *T. harzianum*, performed by Shoresh & Harman (2008), found increased activation of metabolic processes related to carbohydrates and photosynthesis, providing the growing plant with more energy and carbon sources. In another study, maize seeds inoculated with *T. harzianum* also increased in the plant's aerial part, associated with the increase in the content of nucleic acids, chlorophyll pigments, total protein, and total protein and phytohormones during the maize cycle (Akladious & Abbas, 2014). In addition, Contreras-Cornejo et al. (2009) and Vergara et al. (2019) suggest that plant growth promotion by *Trichoderma* spp. may be related to the increased capacity of the roots to absorb nutrients, resulting from the release of compounds from this fungus, which induce the formation of radicels.

Regarding the variable root length (Figure 4B), an increase was observed up to the dosage of 619 ml 100 kg^{-1} (47.8 cm $plant^{-1}$), being that this value was obtained with the estimative of the function maximum point based on the derivation of Y (root length) concerning X (bio-input) (Banzatto & Kronka, 2013). However, when applying 800 ml 100 kg^{-1} of seeds, considering the adjusted quadratic function, the value of 46.8 cm $plant^{-1}$, specifically, a reduction of approximately 2% in root length concerning the highest efficiency dose, 619 mL 100 kg^{-1}. Therefore, considering the conditions of the experiment, for this variable, the most appropriate response model was the non-linear one and doses above 619 ml 100 kg^{-1} of bio-input applied to seeds did not promote an increase in root length under the evaluated conditions.

The total length of the maize plants (TLP) showed a linear increase when applying increasing doses of the bio-input based on *T. harzianum* up to the maximum dose of 800 ml 100 kg^{-1} seed (Figure 4C). The estimated increase concerning the control was 0.0427 cm per milliliter of the applied product. At the highest dose, the observed increase of 34.1 cm represented 42% in gain for total plant length compared to the control.

DOI: https://doi.org/10.18512/rbms2023v22e1269

For stem diameter (SD), a linear increase was detected with an increasing dose of bio-input (Figure 4D). The estimated gain was 0.0053 mm plant^{-1} for each ml of the product applied, reaching 4.2 mm plant^{-1} of stem diameter increment at the dosage of 800 ml 100 kg^{-1} of seed, representing 35% more concerning the control. The increase in the diameter of the stem promoted by the *T. harzianum* was also verified in tomato seedlings (Santana Baños et al., 2016) and onion bulbs (Coskuntuna & **Özer**, 2008). According to Egamberdieva et al. (2017), establishing microbial symbiosis for growth promotion and nutrient obtention is related to phytohormones' biosynthesis. In this context, indole-acetic-acid (AIA), gibberellins (GAs), abscisic acid (ABA), salicylic acid (AS), and cytokinins (CKs) are described in *Trichoderma* spp. (Illescas et al., 2021), whereas AIA and GAs are phytohormones involved in signaling events related to biomass accumulation in shoots and roots (Chowdappa et al., 2013).

By evaluating the accumulation of root (RDM), shoot (SDM), and total biomass (TDM) as a function of increasing doses of bio-input (Figures 5A, 5B, and 5C), it was detected for both linear and positive increments. With the application of each milliliter of bio-input to maize seeds, it is estimated that an increase of 0.0009 g for the variables RDM and SDM corresponds to an increase of 27% and 81% concerning the control, respectively. For the TDM variable, the estimated dry mass gain was 0.0018 g for each milliliter of bio-input applied to seeds, reaching an increment of 1.44 g in the maximum dosage, representing 40% more concerning the control. In this context, different studies show the influence of *Trichoderma* spp inoculation, modifying the root architecture associated with increased biomass and root hairs in species such as maize (Harman et al., 2004; Contrejas-Cornejo et al., 2009; Chagas et al., 2017).

The promotion of plant growth by *Trichoderma* spp. has been verified in axenic systems and soil under different environmental conditions (Kumar et al., 2017; Contreras-Cornejo et al., 2009; Adams et al., 2007). In a study carried out by Contreras-Cornejo et al. (2009) involving the cultivation of Arabidopsis thaliana, the authors detected a pronounced increase in biomass and the number of secondary roots of mutant seedlings for genes related to transport or signaling by auxins in co-culture with T. virens, concerning the control without the fungus. Furthermore, they detected compounds synthesized by this fungus that are related to indole-3-acetic acid, indole-3-acetaldehyde, and indole-3-ethanol, which may suggest the contribution of these auxinic compounds in promoting the seedling's growth.

The increase in biomass and plants root hairs by *Trichoderma* spp (Harman et al., 2004; Contrejas-Cornejo et al., 2009; Chagas et al., 2017) can result in the improvement of the carrying capacity for the shoots, in addition to providing an increase in the efficiency of water use and nutrient absorption by plants (Contrejas-Cornejo et al., 2009). In maize, Fu et al. (2020)

Figure 3. Maize plants of the hybrid MSG1001 at the 28 DAP from seeds inoculated with different volumes of the bio-input based on *T. harzianum* (T1: 0.0 ml (control); T2: 200 ml; T3: 400 ml; T4: 600 ml and T5: 800 ml). In each of the different treatments, there are two plants per photo.

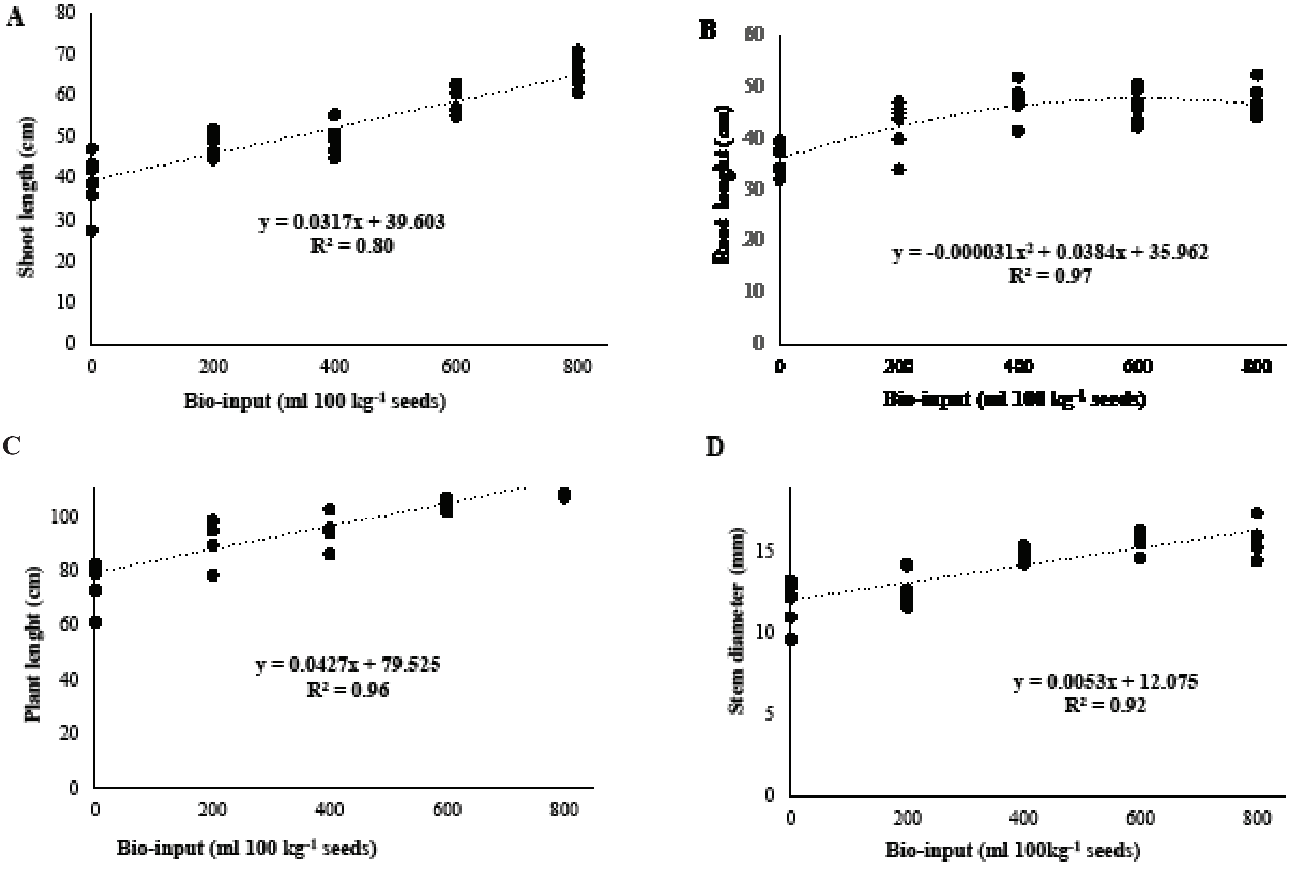

Figure 4. Shoot length (A), root length (B), total length (C), and stem diameter (D) of maize hybrid MSG1001 plants at 28 days after planting (DAP) as a function of bio-input volume based on *T. harzianum*.

DOI: https://doi.org/10.18512/rbms2023v22e1269

found that the inoculation of T. asperellum promoted modifications in the soil microbiome, closely related to soil nutrients and the increment of bio-input doses. Regarding foliar nutrients, in this study, a positive correlation was detected between doses of the bio-input and nutrient content only for potassium, r = 0.70 (p-value = 0.002), which according to Vieira (2011), is classified as moderate.

The technique of multivariate analysis by principal components (PCA), used for variables of different types, which includes studies of plant mineral nutrition (Dube et al., 2019), it was possible to condense 80.09% of the variation for the macro and micronutrients levels of the treatments in PC1 and PC2. Furthermore, by the graphical distribution of scores for treatments and factor loadings (Figure 6), there is a wide dispersion from T2 to T5 concerning T1 (control), being possible to verify the differential behavior of the plants when submitted to different doses of *T. harzianum*. Concerning the control, plants from treatments with at least 400 ml kg^{-1} seed of bio-input (T3 to T5) showed increased foliar contents for K, N, Fe, Zn, and Cu.

The use of 200 ml kg^{-1} seed of bio-input (T2) resulted in an intermediate behavior concerning nutrient accumulation in leaves; it can be suggested that the amount of T. *harzianum* may have been insufficient to promote changes in the plant to modify processes of absorption and accumulation of nutrients.

In maize, Fu et al. (2020) detected that seed inoculation with T. *asperellum* promoted an increase in the availability of soil nutrients, which was not quantified in plant tissue but led to an increase in grain production between 4.87 and 20.26%. However, the results of this study involving the inoculation of maize and T. *harzianum*, and in other species of plants, reveal the modification in the accumulation of mineral elements (Marra et al., 2021; Hoyos-Carvajal et al., 2015; Li et al., 2015). Hoyos-Carvajal et al. (2015) verified that bean plants in the flowering stage, with seeds inoculated with different strains of *Trichoderma* spp, promoted an increase in foliar nutrient contents according to soil type, demonstrating the importance of the agricultural substrate in the solubilization of these minerals by this fungus. In tomato plants, Azarmi et al. (2011) demonstrated an increase in the levels of Ca, Mg, P, and K associated with reduced seedling growth inoculated with an isolate of *T. harzianum*. This result was attributed to the induction of the transport of nutrients from the root system to the shoot and the reduction in the accumulation of biomass may have come from an excessive concentration of compounds synthesized by the fungus. In this context, Vinale & Sivasithamparam (2020) highlight that several strains of *Trichoderma* are effective for stimulating plant development by activating an auxin-dependent mechanism and activating AIA. However, the plant growth is closely related to the concentration of compounds synthesized by this fungus.

Concerning the total chlorophyll content A+B, a growing increase was found up to the

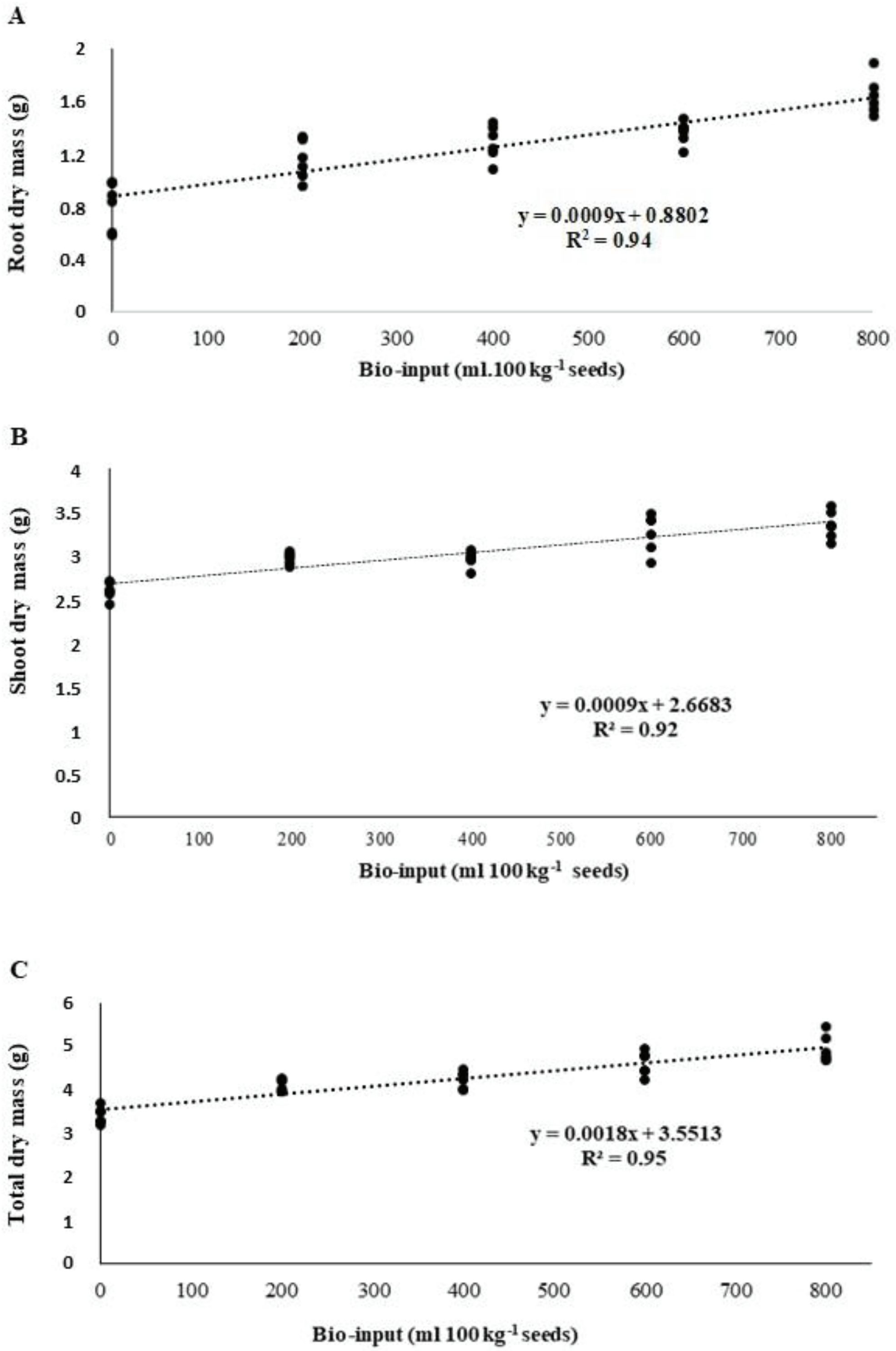

Figure 5. Effect of inoculant dose based on *T. harzianum* on the root dry mass (A), shoot dry mass (B), and total dry mass (C) of the hybrid MSG1001 at 28 DAP.

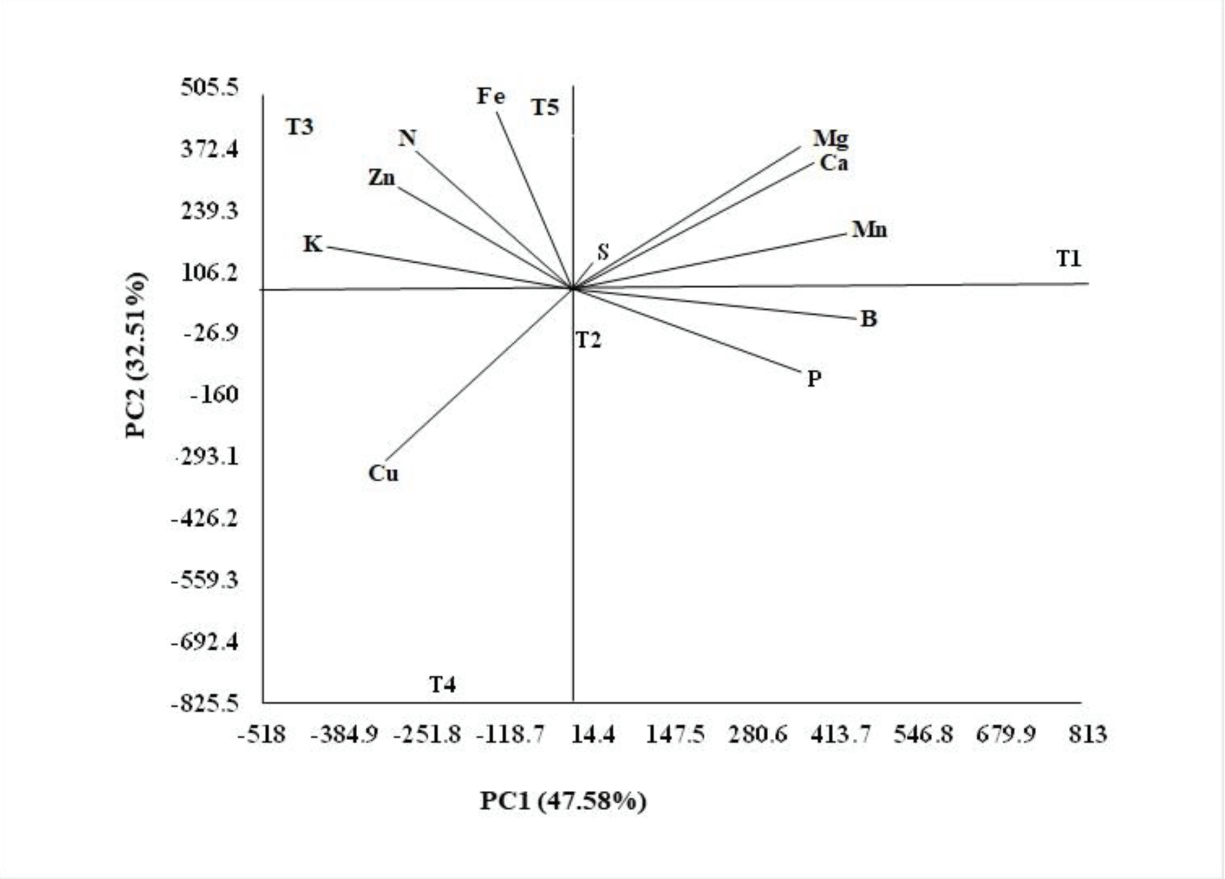

Figure 6. Biplot graphic dispersion of treatments based on factorial scores and loads for the variables studied, using the analysis of main components: principal components 1 (PC1) and 2 (PC2), and the respective percentages of the variation explanation.

dose of around 400 ml 100 kg^{-1} of seed for both the third (V3) and the fourth (V4) fully expanded leaves (Figure 7). In V3 for the adjusted equation model, an estimated value of 40.9 µg cm-2 for total chlorophyll at the dose of 400 ml 100 kg^{-1} of seed was obtained. However, at the maximum point of the function, the dose of 321.6 mL kg^{-1} for a total chlorophyll content of 41.52 µg cm-2 suggests that it is possible to increase the total chlorophyll levels with a dose of bio-input lower than 400 ml 100 kg^{-1} of seed. In V4, at the maximum point of the function, the dose of 435.6 ml 100 kg^{-1} of seed reached 42.5 µg.cm-2 of total chlorophyll content, similar to those obtained in V3. Through the analysis of the main components, it appears that T3 (400 ml 100 kg^{-1}) is positively correlated with the increase in nitrogen (Figure 6), which according to several studies, reveals that this nutrient is directly related to photosynthetic activity (Field & Mooney, 1986; Argenta et al., 2004; Schlemmer et al., 2013; Matos Pereira et al., 2015).

Following Harman et al. (2012), the interaction between *Trichoderma*-plant causes changes in the architecture of the root system, enabling the increase of its surface area, associated with increased water and nutrient absorption capacity, that leads to beneficial physiological changes to plants such as resistance to pathogens and photosynthetic efficiency. In this context, this study found substantial changes in the root system, where doses higher than T3 showed stagnation of root growth in length (Figure 4B), without compromising the accumulation of plant biomass in the initial stages (Figure 5), despite the substantial modification of the nutrient content.

Nitrogen, absorbed almost exclusively by mass flow, is the most required macronutrient and the one that promotes more significant limitation to the accumulation of biomass in maize (Sheoran et al., 2021; Asibi et al., 2019; Malavolta et al., 1997), which is positively correlated with chlorophyll content. Furthermore, Piekielek et al. (1995) mention that chlorophyll content can be used to predict the plant status for this nutrient (N), influenced by the genetic constitution of the cultivar and the cultivation environment. Concerning the results of this research, in the dose of 600 ml 100 kg^{-1} of seed (T4), the estimated value of total chlorophyll was 40.21 μg cm-2, representing a 5.3% reduction concerning the highest efficiency dose (T3). This decrease shows that some factors may have negatively influenced the content of photosynthetic pigments, which may be associated with the reduction of the nutrient content, among which the nitrogen (Figure 7).

The reduction observed in the total chlorophyll content on the third and fourth leaves may be due to some unknown factor interfering harmfully in the plant at higher doses of the product. Alonso–Ramírez et al. (2014) verified the parasitism of the fungus strain T. *harzianum* in mutant plants of Arabidopsis as a result of lower production of defense compounds, such as salicylic acid, and that without the contribution of this compound. Also, there being no barriers that prevent the arrival of the fungus to the vascular cambium, it disseminates systemically throughout the entirety of the plant, bringing it to collapse. Hjejord & Tronsmo (2003) suggested that high concentrations of the bio-input containing T. atroviride can result in self-inhibition, i.e., an environment of high competition for space and nutrients by the fungus. This situation may result in a reduction in viable conidia, with a consequent substantial reduction in the fungus population and the beneficial effects of the biological agent.

The results of the present study confirm the beneficial relationship of the T. *harzianum* with the plant, revealed by the promotion of growth, particularly of the root system. As stated by Zin and Badaluddin (2020), plants naturally exert a connection with the organisms of the rhizosphere, which is necessary for plant development and nutrient assimilation. In the experiment, which was performed on washed sand, young plants showed changes in nutrient content and morphometric characters, regardless of the occurrence of a colloidal environment. In general, plants are living beings with a high capacity to recognize organisms and their secondary

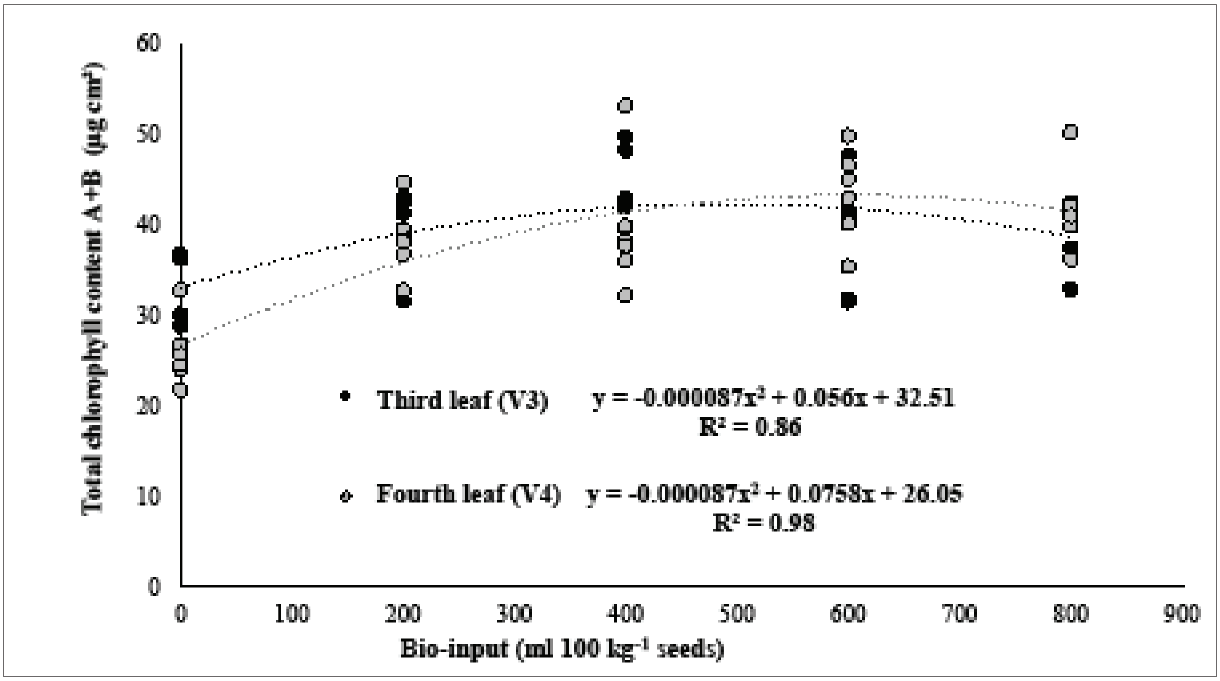

Figure 7. Total chlorophyll content of the third and fourth leaf, wholly expanded, from the hybrid MSG1001 at 28 DAP as a function of inoculant doses.

metabolites, such as phytohormones and other volatile compounds (Zin & Badaluddin, 2020). However, the synergistic mechanisms between these organisms still need to be elucidated.

The success in establishing maize in the cultivation area is closely related to the expression of productive capacity determined by biotic and abiotic factors. In this context, guaranteeing environmental conditions in which plants are stimulated to grow through the use of *Trichoderma* is of fundamental importance since it helps in the control of soil fungi (Elad et al., 1980; Ferrigo et al., 2014; Zin & Badaluddin, 2020) and promotes plant growth (Gomes et al., 2016; Vergara et al., 2019), thus ensuring a uniform stand. However, the concentration of the bio-input must be considered, which depending on its population, may cause adverse consequences to plant growth. Therefore, it is suggested the development of additional studies that can elucidate positive and negative effects using a more significant number of corn hybrids and application of different doses of the fungus *T. harzianum* regarding crop productivity under field conditions.

Conclusions

In a sand capillary irrigation system, *T. harzianum* strain CCT 7589 shows a positive effect in promoting the growth of the maize hybrid MSG1001, up to the dose of 800 ml 100 kg^{-1} of seed of the commercial product, with expressive increments on the variables SDM, RDM, TDM, SD, SL and TPL, of approximately 27%, 81%, 40%, 35%, 64%, and 42%, respectively, concerning the control.

For the RL, TCV3, and TCV4, this bio-input promotes significant gains of 33%, 27%, and 63% concerning the control for 619 ml $100kg^{-1}$ seeds, 321.6 ml doses $100kg^{-1}$ seeds, and 435.6 ml $100kg^{-1}$ seeds, respectively.

Maize seeds treated with different doses of the bio-input result in the formation of young plants with variation in nutrient accumulation in the leaf tissue, indicating a positive correlation between potassium content and concentration of *T. harzianum*. In addition, in doses between 400 to 800 ml 100 kg^{-1} seed of bio-input (T3 to T5), especially in T3, revealed by the multivariate analysis, there is an increase in the foliar contents of K, N, Fe, Zn, and Cu.

References

ACOMPANHAMENTO da Safra Brasileira [de] Grãos, v. 10 - safra 2022/23: segundo levantamento: novembro 2022. Brasília, DF: Conab, 2022. Available at: https://www.conab.gov.br/component/k2/item/download/45077_bf66ebb079ea1dafbb9fcdbb0b884d0e. Access on: 4 Dec. 2022.

ADAMS, P.; DE-LEIJ, F. A.; LYNCH, J. M. *Trichoderma harzianum* Rifai 1295-22 mediates growth promotion of crack willow (*Salix fragilis*) saplings in both clean and metal-contaminated soil. **Microbial Ecology**, v. 54, n. 2, p. 306-313, 2007.
DOI: https://doi.org/10.1007/s00248-006-9203-0

AKINOLA, S. A.; AYANGBENRO, A. S.; BABALOLA, O. O. Metagenomic insight into the community structure of maize-rhizosphere bacteria as predicted by different environmental factors and their functioning within plant proximity. **Microorganisms**, v. 9, n. 7, 1419, 2021.
DOI: https://doi.org/10.3390/microorganisms9071419

AKLADIOUS, S. A.; ABBAS, S. M. Application of *Trichoderma harzianum* T22 as a biofertilizer potential in maize growth. **Journal of Plant Nutrition**, v. 37, n. 1, p. 30-49, 2014.
DOI: https://doi.org/10.1080/01904167.2013.829100

ALONSO-RAMÍREZ, A.; POVEDA, J.; MARTÍN, I.; HERMOSA, R.; MONTE, E.; NICOLÁS, C. Salicylic acid prevents *Trichoderma harzianum* from entering the vascular system of roots. **Molecular Plant Pathology**, v. 15, n. 8, p. 823-831, 2014.
DOI: https://doi.org/10.1111/mpp.12141

ARGENTA, G.; SILVA, P. R. F. D.; SANGOI, L. Leaf relative chlorophyll content as an indicator parameter to predict nitrogen fertilization in maize. **Ciência Rural**, v. 34, n. 5, p. 1379-1387, 2004.
DOI: https://doi.org/10.1590/S0103-84782004000500009

ASIBI, A. E.; CHAI, Q.; COULTER, J. A. Mechanisms of nitrogen use in maize. **Agronomy**, v. 9, n. 12, article 775, 2019. DOI: https://doi.org/10.3390/agronomy9120775

AZARMI, R.; HAJIEGHRARI, B.; GIGLOU, A. Effect of Trichoderma isolates on tomato seedling growth response and nutrient uptake. **African Journal of Biotechnology**, v. 10, n. 31, p. 5850-5855, 2011.
DOI: https://doi.org/10.5897/AJB10.1600

BANZATTO, D. A; KRONKA, S. N. **Experimentação agrícola**. 4. ed. Jaboticabal: Funep, 2013. 237 p.

CARMO, C. A. F. de S. do; ARAUJO, W. S. de; BERNARDI, A. C. de C.; SALDANHA, M. F. C. **Métodos de análise de tecidos vegetais utilizados na Embrapa Solos**. Rio de Janeiro: Embrapa Solos, 2000. 41 p. (Embrapa Solos. Circular Técnica, 6). Available at:

http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/337672. Access on: 20 Oct. 2021.

CHAGAS, L. F. B.; CHAGAS JUNIOR, A. F.; SOARES, L. P.; FIDELIS, R. R. *Trichoderma* na promoção do crescimento vegetal. **Revista de Agricultura Neotropical**, v. 4, n. 3, p. 97-102, 2017.
DOI: https://doi.org/10.32404/rean.v4i3.1529

CHOWDAPPA, P.; KUMAR, S. M.; LAKSHMI, M. J.; UPRETI, K. K. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. **Biological Control**, v. 65, n. 1, p. 109-117, 2013.
DOI: https://doi.org/10.1016/j.biocontrol.2012.11.009

CONINCK, E.; SCAUFLAIRE, J.; GOLLIER, M.; LIÉNARD, C.; FOUCART, G.; MANSSENS, G.; MANAUT, F.; LEGRÈVE, A. *Trichoderma atroviride* as a promising biocontrol agent in seed coating for reducing Fusarium damping-off on maize. **Journal of Applied Microbiology**, v. 129, n. 3, p. 637-651, 2020.
DOI: https://doi.org/10.1111/jam.14641

CONTINI, E.; MOTA, M. M.; MARRA, R.; BORGHI, E.; MIRANDA, R. A.; SIILVA, D. D.; MACHADO, J. R. A.; COTA, L. V.; COSTA, R. V.; MENDES, S. M. **Milho**: caracterização e desafios tecnológicos. Brasília, DF: Embrapa; Sete Lagoas: Embrapa Milho e Sorgo, 2019. (Desafios do Agronegócio Brasileiro, 2). Nota técnica. Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/195075/1/Milho-caracterizacao.pdf.
Access on: 20 Oct. 2020.

CONTRERAS-CORNEJO, H. A.; MACÍAS-RODRÍGUEZ, L.; CORTÉS-PENAGOS, C.; LÓPEZ-BUCIO, J. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in Arabidopsis. **Plant Physiology**, v. 149, n. 3, p. 1579-1592, 2009.
DOI: https://doi.org/10.1104/pp.108.130369

COŞKUNTUNA, A.; ÖZER, N. Biological control of onion basal rot disease using *Trichoderma harzianum* and induction of antifungal compounds in onion set following seed treatment. **Crop Protection**, v. 27, n. 3/5, p. 330-336, 2008. DOI: https://doi.org/10.1016/j.cropro.2007.06.002
CRUZ, C. D. Genes: a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum. Agronomy**, v. 35, n. 3, p. 271-276, 2013.
DOI: https://doi.org/10.4025/actasciagron.v35i3.21251

DEGANI, O.; DOR, S. Trichoderma biological control to protect sensitive maize hybrids against late wilt disease in the field. **Journal of Fungi**, v. 7, n. 4, 315, 2021.
DOI: https://doi.org/10.3390/jof7040315

DE VRIES, F. T.; WALLENSTEIN, M. D. Below-ground connections underlying above-ground food production: a framework for optimising ecological connections in the rhizosphere. **Journal of Ecology**, v. 105, n. 4, p. 913-920, 2017.
DOI: https://doi.org/10.1111/1365-2745.12783

DLAMINI, S. P.; AKANMU, A. O.; FADIJI, A. E.; BABALOLA, O. O. Maize rhizosphere modulates the microbiome diversity and community structure to enhance plant health. **Saudi Journal of Biological Sciences**, v. 30, n. 1, 103499, 2023.
DOI: https://doi.org/10.1016/j.sjbs.2022.103499

DUBE, S. P.; MARAIS, D.; MAVENGAHAMA, S.; VAN JAARSVELD, C. M.; GERRANO, A. S. Variability in leaf mineral content of *Corchorus accessions* in South Africa. **Research on Crops**, v. 20, n. 1, p. 121-128, 2019.
DOI: https://doi.org/10.31830/2348-7542.2019.017

EGAMBERDIEVA, D.; WIRTH, S. J.; ALQARAWI, A. A.; ABD-ALLAH, E. F.; HASHEM, A. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. **Frontiers in Microbiology**, v. 8, article 2104, 2017.
DOI: https://doi.org/10.3389/fmicb.2017.02104

ELAD, Y.; CHET, I.; KATAN, J. *Trichoderma harzianum*: a biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. **Phytopathology**, v. 70, n. 2, p. 119-121, 1980.

DOI: http://dx.doi.org/10.1094/Phyto-70-119

ELSHAHAWY, I. E.; EL-SAYED, A. E. B. Maximizing the efficacy of Trichoderma to control *Cephalosporium maydis*, causing maize late wilt disease, using freshwater microalgae extracts. **Egyptian Journal of Biological Pest Control**, v. 28, n. 1, article 48, 2018.
DOI: https://doi.org/10.1186/s41938-018-0052-1

FAO. **The future of food and agriculture**: trends and challenges. Rome, 2017. Available at: https://www.fao.org/3/i6583e/i6583e.pdf. Access on: 20 Oct. 2021.

FADIJI, A. E.; KANU, J. O.; BABALOLA, O. O. Metagenomic profiling of rhizosphere microbial community structure and diversity associated with maize plant as affected by cropping systems. **International Microbiology**, v. 24, n. 3, p. 325-335, 2021.
DOI: https://doi.org/10.1007/s10123-021-00169-x

FEDERAÇÃO DAS INDÚSTRIAS DO ESTADO DE SÃO PAULO. **Safra mundial de milho 2022/23**: 6º levantamento do USDA. São Paulo, 2022. Available at: https://sitefiespstorage.blob.core.windows.net/uploads/2022/10/file-20221013220312-boletimmilhooutubro2022.pdf. Access on: 4 Dec. 2022.

FERREIRA, D. F. SISVAR: a computer statistical analysis system. **Ciência e Agrotecnologia**, v. 35, n. 6, p. 1039-1042, 2011.
DOI: https://doi.org/10.1590/S1413-70542011000600001

FERRIGO, D.; RAIOLA, A.; RASERA, R.; CAUSIN, R. *Trichoderma harzianum* seed treatment controls *Fusarium verticillioides* colonization and fumonisin contamination in maize under field conditions. **Crop Protection**, v. 65, p. 51-56, 2014.
DOI: https://doi.org/10.1016/j.cropro.2014.06.018

FIELD, C.; MOONEY, H. A. The photosynthesis-nitrogen relationship in wild plants. In GIVNISH, T. (ed.). **On the economy of plant form and function**. Cambridge: Cambridge University Press, 1986. p. 25-55.

FU, J.; XIAO, Y.; LIU, Z.; ZHANG, Y.; WANG, Y.; YANG, K. *Trichoderma asperellum* improves soil microenvironment in different growth stages and yield of maize in saline-alkaline soil of the Songnen Plain. **Plant, Soil and Environment**, v. 66, n. 12, p. 639-647, 2020.
DOI: https://doi.org/10.17221/456/2020-PSE

GODOI, M. J. D. S.; ALBINO, L. F. T.; ROSTAGNO, H. S.; GOMES, P. C.; BARRETO, S. L. D. T.; VARGAS JUNIOR, J. G. D. Utilização de aditivos em rações formuladas com milho normal e de baixa qualidade para frangos de corte. **Revista Brasileira de Zootecnia**, v. 37, n. 6, p. 1005-1011, 2008. DOI: https://doi.org/10.1590/S1516-35982008000600008

GOMES, E. A.; SILVA, U. de C.; OLIVEIRA-PAIVA, C. A.; LANA, U. G. de P.; MARRIEL, I. E.; SANTOS, V. L. dos. **Microrganismos promotores do crescimento de plantas**. Sete Lagoas: Embrapa Milho e Sorgo, 2016. 51 p. (Embrapa Milho e Sorgo. Documentos, 208). Available at: http://www.infoteca.cnptia.embrapa.br/infoteca/handle/doc/1063799. Access on: 21 Oct. 2021.

HANG, X.; MENG, L.; OU, Y.; SHAO, C.; XIONG, W.; ZHANG, N.; LIU, H.; LI, R.; SHEN, O.; KOWALCHUK, G. A. Trichoderma-amended biofertilizer stimulates soil resident Aspergillus population for joint plant growth promotion. **npj Biofilms and Microbiomes**, v. 8, n. 1, article 57, 2022.
DOI: https://doi.org/10.1038/s41522-022-00321-z

HARMAN, G. E.; HERRERA-ESTRELLA, A. H.; HORWITZ, B. A.; LORITO, M. Trichoderma-from basic biology to biotechnology. **Microbiology**, v. 158, n. 1, p. 1-2, 2012.
DOI: https://doi.org/10.1099/mic.0.056424-0

HARMAN, G. E.; PETZOLDT, R.; COMIS, A.; CHEN, J. Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17 and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. **Phytopathology**, v. 94, n. 2, p. 147-153, 2004.
DOI: https://doi.org/10.1094/PHYTO.2004.94.2.147

HJELJORD, L. G.; TRONSMO, A. Effect of germination initiation on competitive capacity of *Trichoderma atroviride* P1 conidia. **Phytopathology,** v. 93, n. 12, p. 1593-1598, 2003.
DOI: https://doi.org/10.1094/PHYTO.2003.93.12.1593

HOAGLAND, D. R.; ARNON, D. I. **The water-culture method for growing plants without soil**. Berkeley: University of California, 1950. 32 p. (Circular, 347).
HOYOS-CARVAJAL, L.; CARDONA, A.; OSORIO, W.; ORDUZ, S. Efecto de diversos aislamientos de *Trichoderma* spp. en la absorción de nutrientes en fríjol (*Phaseolus vulgaris*) en dos tipos de suelo. **Revista Colombiana de Ciencias Hortícolas**, v. 9, n. 2, p. 268-278, 2015.
DOI: http://dx.doi.org/10.17584/rcch.2015v9i2.4183

ILLESCAS, M.; PEDRERO-MÉNDEZ, A.; PITORINI-BOVOLINI, M.; HERMOSA, R.; MONTE, E. Phytohormone production profiles in Trichoderma species and their relationship to wheat plant responses to water stress. **Pathogens**, v. 10, n. 8, article 991, 2021.
DOI: https://doi.org/10.3390/pathogens10080991

KUMAR, K.; MANIGUNDAN, K.; AMARESAN, N. Influence of salt tolerant Trichoderma spp. on growth of maize (*Zea mays*) under different salinity conditions. **Journal of Basic Microbiology**, v. 57, n. 2, p. 141-150, 2017.
DOI: https://doi.org/10.1002/jobm.201600369

LI, R. X.; CAI, F.; PANG, G.; SHEN, Q. R.; LI, R.; CHEN, W. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. **PloS ONE**, v. 10, n. 6, e0130081, 2015.
DOI: https://doi.org/10.1371%2Fjournal.pone.0130081

LICHTENTHALER, H. K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. **Methods in Enzymology**, v. 148, p. 350-382, 1987. DOI: http://dx.doi.org/10.1016/0076-6879(87)48036-1

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. **Avaliação do estado nutricional das plantas**: princípios e aplicações. 2nd ed. Piracicaba: Potafos, 1997. 390 p.

MARRA, R.; LOMBARDI, N.; PICCOLO, A.; BAZGHALEH, N.; PRASHAR, P.; VANDENBERG, A.; WOO, S. Mineral biofortification and growth stimulation of lentil plants inoculated with Trichoderma strains and metabolites. **Microorganisms**, v. 10, n. 1, article 87, 2021.

DOI: https://doi.org/10.3390/microorganisms10010087
NATSIOPOULOS, D.; TZIOLIAS, A.; LAGOGIANNIS, I.; MANTZOUKAS, S.; ELIOPOULOS, P. A. Growth-promoting and protective effect of *Trichoderma atrobrunneum* and *T. simmonsii* on tomato against soil-borne fungal pathogens. **Crops**, v. 2, n. 3, p. 202-217, 2022.
DOI: https://doi.org/10.3390/crops2030015

PEREIRA, L. de M.; PEREIRA, E. de M.; REVOLTI, L. T. M.; ZINGARETTI, S. M.; MÔRO, G. V. Seed quality, chlorophyll content index and leaf nitrogen levels in maize inoculated with *Azospirillum brasilense*. **Revista Ciência Agronômica**, v. 46, n. 3, p. 630-637, 2015.
DOI: https://doi.org/10.5935/1806-6690.20150047

PIEKIELEK, W. P.; FOX, R. H.; TOTH, J. D.; MACNEAL, K. E. Use of a chlorophyll meter at the early dent stage of corn to evaluate nitrogen sufficiency. **Agronomy Journal**, v. 87, n. 3, p. 403-408, 1995. DOI: https://doi.org/10.2134/agronj1995.00021962008700030003x

SANTANA BAÑOS, Y.; DEL BUSTO CONCEPCIÓN, A.; GONZÁLEZ FUENTES, Y.; AGUIAR GONZÁLEZ, I.; CARRODEGUAS DÍAZ, S.; PÁEZ FERNÁNDEZ, P. L.; DÍAZ LUGO, G. Efecto de *Trichoderma harzianum* Rifai y FitoMas-E® como bioestimulantes de la germinación y crecimiento de plántulas de tomate. **Centro Agrícola**, v. 43, n. 3, p. 5-12, 2016. Available at: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0253-57852016000300001&lng=es&nrm=iso. Access on: 20 Oct. 2021.

SARAVANAKUMAR, K.; LI, Y.; YU, C.; WANG, Q. Q.; WANG, M.; SUN, J.; GAO, J.; CHEN, J. Effect of *Trichoderma harzianum* on maize rhizosphere microbiome and biocontrol of Fusarium Stalk rot. **Scientific Reports**, v. 7, n. 1, p.1-13, 2017.
DOI: https://doi.org/10.1038/s41598-017-01680-w

SARKAR, S.; GIL, J. D. B.; KEELEY, J.; JANSEN, K. **The use of pesticides in developing countries and their impact on health and the right to food.** Luxemburgo: European Union, 2021. Available at: https://www.europarl.europa.eu/cmsdata/219887/Pesticides%20health%20and%20food.pdf. Access on: 20 Nov. 2021.

SCHLEMMER, M.; GITELSON, A.; SCHEPERS, J.; FERGUSON, R.; PENG, Y.; SHANAHAN, J.; RUNDQUIST, D. Remote estimation of nitrogen and chlorophyll contents in maize at leaf and canopy levels. **International Journal of Applied Earth Observation and Geoinformation**, v. 25, p. 47-54, 2013.
DOI: https://doi.org/10.1016/j.jag.2013.04.003

SCUDELER, F.; VENEGAS, F. N. *Trichoderma harzianum* associado ou não a fungicidas em tratamento de sementes na cultura do milho (*Zea mays* L.). **Revista Ensaios e Ciência**, v. 16, n. 5, p. 9-19, 2012.

SHEORAN, S.; KUMAR, S.; KUMAR, P.; MEENA, R. S.; RAKSHIT, S. Nitrogen fixation in maize: breeding opportunities. **Theoretical and Applied Genetics**, v. 134, n. 5, p. 1263-1280, 2021.
DOI: https://doi.org/10.1007/s00122-021-03791-5

SHORESH, M.; HARMAN, G. E. The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. **Plant Physiology**, v. 147, n. 4, p. 2147-2163, 2008. DOI: https://doi.org/10.1080/01904167.2013.829100

SILVA, F. C. da (org.). **Manual de análises químicas de solos, plantas e fertilizantes**. Brasília, DF: Embrapa Comunicação para Transferência de Tecnologia; Rio de Janeiro: Embrapa Solos; Campinas: Embrapa Informática Agropecuária, 1999. 370 p.

VAZQUEZ, G. H.; CARDOSO, R. D.; PERES, A. R. Tratamento químico de sementes de milho e o teste de condutividade elétrica. **Bioscience Journal**, v. 30, n. 3, p. 773-781, 2014. Available at: https://seer.ufu.br/index.php/biosciencejournal/article/view/18081. Access on: 18 Dec. 2021.

VERGARA, C.; ARAUJO, K. E. C.; SOUZA, S. R. D.; SCHULTZ, N.; SAGGIN, O. J.; SPERANDIO, M. V. L.; ZILLI, J. E. Plant-mycorrhizal fungi interaction and response to inoculation with different growth-promoting fungi. **Pesquisa Agropecuária Brasileira**, v. 54, e25140, 2019. DOI: https://doi.org/10.1590/S1678-3921.pab2019.v54.25140

VIEIRA, S. **Introdução a bioestatisitíca**. 4. ed. Rio de Janeiro: Elsevier, 2011.

VINALE, F.; SIVASITHAMPARAM, K. Beneficial effects of Trichoderma secondary metabolites on crops. **Phytotherapy Research**, v. 34, n. 11, p. 2835-2842, 2020. DOI: https://doi.org/10.1002/ptr.6728

ZIN, N. A.; BADALUDDIN, N. A. Biological functions of Trichoderma spp. for agriculture applications. **Annals of Agricultural Sciences**, v. 65, n. 2, p. 168-178, 2020.
DOI: https://doi.org/10.1016/j.aoas.2020.09.003

ZULAUF, W. E. O meio ambiente e o futuro. **Estudos Avançados**, v. 14, n. 39, p. 85-100, 2000. Available at: https://www.scielo.br/pdf/ea/v14n39/v14a39a09.pdf. Access on: 31 Mar. 2020.

WAICHMAN, A. V. A problemática do uso de agrotóxicos no Brasil: a necessidade de construção de uma visão compartilhada por todos os atores sociais. **Revista Brasileira de Saúde Ocupacional**, v. 37, n. 125, p. 42-47, 2012.
DOI: https://doi.org/10.1590/S0303-76572012000100007