DISCIPLINARUM SCIENTIA

Disciplinarum Scientia. Série: Naturais e Tecnológicas
Santa Maria, v. 27, n. 2, p. 205-220, 2026

Recebido em: 09.03.2026. Aprovado em: 06.05.2026.
ISSN 2176-462X | DOI: doi.org/10.37779/nt.v27i2.5743

INFLUENCE OF pH ON THE GROWTH AND SPORULATION OF MICROBIAL BIO-INPUT AGENTS[1]

INFLUÊNCIA DO pH SOBRE O CRESCIMENTO E A ESPORULAÇÃO DE AGENTES DE BIOINSUMOS MICROBIANOS

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ABSTRACT

The use of microbial bio-inputs has become an increasingly widespread practice in agricultural systems. Considering the high variability of soil conditions and the fact that the success of microbial agents depends not only on their intrinsic efficiency but also on their ability to adapt to the environment into which they are introduced, it is essential to investigate the relationships between soil parameters and microorganisms. This study aimed to determine the influence of medium pH on the establishment, growth, and sporulation of three fungal species of the genus *Trichoderma* and the bacterium *Azospirillum brasilense*. In vitro assays were conducted to evaluate the effect of five pH levels on: (1) the sporulation of *T. asperelloides*, *T. virens*, *T. harzianum*, and *A. brasilense*; and (2) the mycelial growth rate and dry biomass production of *T. asperelloides, T. virens*, and *T. harzianum*. The experiments were performed under controlled temperature (25 ± 1 °C) and photoperiod (12 h) conditions in a completely randomized design arranged in a factorial scheme consisting of five pH levels (4, 5, 6, 7, and 8) and four microbial species. Medium pH significantly affected the growth and sporulation of the fungal isolates of the genus *Trichoderma* and the bacterium *A. brasilense*, demonstrating that each microorganism has a specific optimal pH range. The optimal pH range for sporulation of *T. asperelloides, T. harzianum*, and *A. brasilense* was between 4 and 5, whereas *T. virens* exhibited optimal sporulation under slightly higher pH conditions (5-6). Significant differences were also observed in the mycelial growth rate of the three *Trichoderma* species across different pH levels. These results demonstrate that medium pH is a determining factor for the functional performance of microorganisms used in bio-inputs and highlight the need to integrate edaphic and ecophysiological compatibility into strain selection strategies to maximize agronomic efficiency in agricultural systems.

Keywords: Acidity, *Azospirillum brasilense*, Biological inputs, *Trichoderma* sp.

1 Scientific research paper.

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RESUMO

O uso de bioinsumos é uma prática crescente em ambientes agrícolas. Considerando a complexa variabilidade dos solos e que o sucesso dos agentes microbianos depende não somente da sua eficiência, mas de sua capacidade de adaptação ao meio onde serão inseridos, é fundamental investigar as relações entre parâmetros do solo e microrganismos. Este trabalho objetivou determinar a influência do pH do meio sobre o estabelecimento, o crescimento e a esporulação de três espécies fúngicas do gênero Trichoderma e da bactéria Azospirillum brasilense. Ensaios in vitro foram conduzidos para determinar o efeito de cinco níveis de pH sobre: 1. a esporulação de T. asperelloides, T. virens, T. harzianum e A. brasilense; e 2. a velocidade de crescimento e a biomassa seca micelial de T. asperelloides, T. virens e T. harzianum. Os ensaios in vitro foram conduzidos em ambiente com controle de temperatura (25±1 °C) e luminosidade (fotoperíodo de 12h) em delineamento experimental inteiramente casualizado em esquema bifatorial correspondente a cinco valores de pH (4, 5, 6, 7 e 8) e quatro espécies de microrganismos. O nível de pH do meio influenciou significativamente o crescimento e a esporulação dos isolados fúngicos do gênero Trichoderma e da bactéria A. brasilense, demonstrando que cada microrganismo possui uma faixa ótima de pH. A faixa de pH ideal para a esporulação de T. asperelloides, T. harzianum e A. brasilense situa-se entre 4 e 5, enquanto a espécie T. virens apresenta condições ótimas de esporulação em faixas mais elevadas de pH (5 a 6). Diferenças significativas foram observadas quanto à velocidade de crescimento micelial das três espécies de Trichoderma sp. em meios com diferentes níveis de pH. Esses resultados demonstram que o pH do meio constitui um fator determinante para o desempenho funcional de microrganismos utilizados em bioinsumos e destacam a necessidade de integrar a compatibilidade edáfica e ecofisiológica na seleção de estirpes para maximizar a eficiência agronômica em sistemas agrícolas.

Palavras-chave: *Acidez, Azospirillum brasilense, Insumos biológicos, Trichoderma sp.*

1 INTRODUCTION

Microbial agricultural inputs are widely known as biological agents capable of controlling a broad range of phytopathogens, promoting plant growth, and inducing resistance to environmental stresses, especially water deficit and salinity stress. Abiotic stresses are potentially harmful to plant development and, consequently, to crop productivity (Islam *et al*., 2016; Tiwari *et al*., 2017; Shahwar *et al*., 2023; Reis *et al*., 2024; Ferioun *et al*., 2025). These stresses can be mitigated using bio-inputs by promoting the establishment of beneficial microbial associations within the plant root system.

Plant growth promotion by certain groups of microorganisms is directly related to the production of various organic compounds and phytohormones that, upon interacting with plants, enhance development from the root system to the aerial parts, thereby positively influencing plant physiology and crop yield efficiency (Rouphael; Colla, 2020; Hasan *et al*., 2024; Giri; Virk, 2025). Studies indicate that the benefits of using bio-inputs extend beyond quantitative yield increases, acting especially on qualitative crop parameters and enhancing the quality of fruits, fibers, grains, and plant-derived products (Dini *et al*., 2020; Steffen *et al*., 2021; Gonçalves *et al*., 2025; Rosales-Castillo *et al*., 2025).

Among the microbial agents used in the production of bio-inputs, species of the genus *Trichoderma* account for approximately 50-60% of fungal microbiological agents (Rush *et al*., 2021). Currently, nearly 80 *Trichoderma*-based biofungicides are available on the global market, including seven approved by the European Commission for use within European Union Member States. The *Trichoderma* species most employed in agriculture include *T. asperellum*, *T. asperelloides*, *T. atroviride*, *T. harzianum*, *T. virens*, *T. viride*, *T. hamatum*, and *T. gamsii* (Thambugala *et al*., 2020; Zin; Badaluddin, 2020; Sharma; Salwan; Sharma, 2022; Tyśkiewicz *et al*., 2022; Kumar *et al*., 2023).

Among bacterial agents, strains of the genus *Azospirillum* are widely used in agriculture to enhance nutrient availability - particularly nitrogen - for various agricultural crops (Cruz-Hernández *et al*., 2022). According to Pii *et al*. (2015) and Giri *et al*. (2025), bacteria of the genus *Azospirillum* represent the most widely used free-living bacteria due to their benefits in promoting plant growth.

Physiological plasticity refers to the ability of microorganisms to respond to environmental fluctuations through metabolic and physiological adjustments, representing a key trait for survival and functional performance in heterogeneous environments such as agricultural soils. Among abiotic factors, medium pH plays a central role in regulating microbial activity, directly influencing vegetative growth, enzymatic processes, and reproductive capacity. In the context of microbial bio-inputs, physiological plasticity governs the capacity of microorganisms, including species of the genus *Trichoderma* and the bacterium *Azospirillum brasilense*, to establish, grow, and sporulate under distinct acidity conditions, reflecting their ecophysiological adaptability and agronomic efficiency. Therefore, evaluating microbial responses across different pH levels provides essential insights into the limits and range of physiological plasticity, supporting the selection of strains better adapted to specific edaphic conditions and enhancing the effectiveness of bio-input technologies in sustainable agricultural systems (Díaz-Rodríguez *et al*., 2025; Steffen *et al*., 2026).

Although microorganisms from the genera *Trichoderma* and *Azospirillum* have been known, studied, and used in agriculture for several decades, further research is required to define the physiological plasticity and the optimal environmental, nutritional, and physiological conditions to maximize the efficiency of different species and commercial isolates in crop systems. Given the recognized influence of soil conditions on the performance of microorganisms used as bio-inputs, it was hypothesized that medium pH exerts a direct and differential effect on the establishment, vegetative growth, and sporulation of fungal species of the genus *Trichoderma* and of the bacterium *Azospirillum brasilense*. Specifically, it was expected that slightly acidic pH ranges would favor the physiological and reproductive activity of these microorganisms, albeit with species- and strain-specific responses reflecting different levels of ecophysiological adaptation and suitability for agricultural environments with varying acidity levels.

In this context, the objective of this study was to determine the influence of medium pH on the establishment, growth, and sporulation of three fungal species of the genus *Trichoderma* and of the bacterium *Azospirillum brasilense*.

2 MATERIALS AND METHODS

2.1 ISOLATION OF MICROORGANISMS

Commercial strains of *Trichoderma harzianum* Rifai and *Azospirillum brasilense* were obtained through direct isolation from commercial products registered with MAPA on potato dextrose agar (PDA) medium (extract from 200 g of peeled potatoes, 20 g of dextrose, 15 g of agar, and distilled water to a final volume of 1000 mL).

The liquid formulation of the commercial product Trichodermil® (Koppert) was used, containing *T. harzianum* (strain ESALQ-1306) at a minimum concentration of 2×10^9 viable conidia/mL. As inoculum for the bacterium *A. brasilense*, a liquid formulation of the commercial product SIGLO AZP® was used, containing the strains AbV5 and AbV6 at a concentration of 200 million cells per mL of product (2×10^8 CFU/mL). The non-commercial strains belonging to the species *Trichoderma asperelloides* (strain T4) and *Trichoderma virens* (strain T10) are part of the beneficial fungi collection of the State Center for Forest Diagnosis and Research (DDPA/SEAPI).

The three *Trichoderma* species were initially multiplied in Petri dishes containing PDA medium and maintained in a growth chamber under controlled temperature (25 ± 1 °C) and light (12-hour photoperiod). After seven days of incubation, pure cultures of each isolate were used as inoculum for the in vitro growth and sporulation assays.

All in vitro bioassays for sporulation and mycelial growth were carried out with five treatments, corresponding to culture medium with pH adjusted to values of 4, 5, 6, 7, and 8. Adjustment of the medium pH was performed by adding hydrochloric acid solution (1 M) or sodium hydroxide (1 M) to the culture medium, using a bench pH meter. Subsequently, the culture media were sterilized in an autoclave for 20 minutes at 121 °C and 1 atm.

2.2 MICROBIAL SPORULATION BIOASSAY

The sporulation bioassay for the three *Trichoderma* sp. strains was carried out in 9-cm Petri dishes containing PDA medium corresponding to each treatment. The experimental design was completely randomized in a bifactorial scheme (5 pH values × 3 *Trichoderma* species), with five replicates, totaling 75 experimental units.

After solidification of the medium, 9-mm-diameter PDA discs containing mycelium from pure cultures of each of the three *Trichoderma* strains were transferred to the center of 90-mm-diameter Petri dishes. The experimental units were maintained in an environment with controlled temperature (25 ± 1 °C) and light (12-hour photoperiod) for seven days.

For spore quantification, 10 mL of sterile distilled water was added to each Petri dish. The spores were dispersed in the solution using a Drigalski spatula. Subsequently, the suspension was transferred to a beaker, and spore concentration was determined using a Neubauer chamber under a light microscope by counting the number of spores present in 100 µL of suspension. Counts were performed in duplicate. Final spore concentration was expressed as spores mL^{-1}.

The sporulation bioassay for *A. brasilense* was conducted in 125-mL Erlenmeyer flasks containing 50 mL of liquid potato-dextrose culture medium adjusted to the respective pH levels. The experimental design was completely randomized, consisting of five pH treatments and five replicates, totaling 25 experimental units. Each flask received 1 mL of *A. brasilense* inoculum from the commercial formulation.

The flasks, sealed with aluminum foil and plastic film, were maintained in an environment with controlled temperature (25 ± 1 °C) and light (12-hour photoperiod) for seven days. The contents were manually shaken daily for 1 minute. After incubation, spores were counted under a microscope using a Neubauer chamber.

2.3 MYCELIAL GROWTH RATE BIOASSAY

Five pH levels (4, 5, 6, 7, and 8) were evaluated for in vitro cultivation of *T. asperelloides*, *T. virens*, and *T. harzianum* on PDA medium.

Mycelial discs (9 mm in diameter) from pure cultures were placed at the center of 90-mm-diameter Petri dishes containing PDA adjusted to the respective pH levels. The plates were maintained in a growth chamber with controlled temperature (25 ± 1 °C) and light (12-hour photoperiod) for seven days.

Colony diameter was measured daily along two perpendicular axes using a digital caliper. The mycelial growth rate index (MGRI), expressed in mm day^{-1}, was calculated according to the formula:

$$MGRI = \Sigma (D - Da) / N$$

where D = mean colony diameter on the current day; Da = mean colony diameter on the previous day; and N = number of days after inoculation.

The experimental design used was completely randomized in a bifactorial scheme (5 pH values × 3 *Trichoderma* species), with five replicates, totaling 75 experimental units.

2.4 FUNGAL MYCELIAL BIOMASS BIOASSAY

The same five pH treatments were evaluated in liquid potato-dextrose culture medium. A 9-mm-diameter mycelial disc from each *Trichoderma* strain (*T. asperelloides*, *T. virens*, and *T. harzianum*) was added to 50 mL of the liquid medium corresponding to each treatment, contained in a 125-mL Erlenmeyer flask.

After inoculation, the flasks were maintained in an environment with controlled temperature (25 ± 1 °C) and light (12-hour photoperiod) for seven days. Fungal fresh mass (mg) was determined using an analytical balance after removing the mycelial with tweezers and blotting it on absorbent paper to remove excess culture medium.

After fresh mass determination, the fungal samples were dried in a forced-air circulation oven at 65 °C until constant weight was reached. Following this step, dry mass (mg) was determined.

The experimental design was completely randomized in a bifactorial scheme (5 pH values × 3 *Trichoderma* species), with five replicates, totaling 75 experimental units.

2.5 STATISTICAL ANALYSES

Data was subjected to analysis of variance (ANOVA) using the F test, and when significant, the means were compared using Tukey's test at the 5% probability level. Statistical analyses were performed using the SISVAR statistical software (Ferreira, 2019).

3 RESULTS AND DISCUSSION

The pH levels of the culture medium significantly influenced the growth, sporulation, and activity of the fungal isolates of the genus *Trichoderma* and of the bacterium *Azospirillum brasilense*, demonstrating that each microorganism has an optimal pH range. Given the increasing adoption of microbial bio-inputs in agriculture and the high variability of soil chemical conditions, understanding the physiological responses of microorganisms to environmental factors such as pH is essential to ensure their effectiveness under field conditions. Identifying optimal growth and sporulation ranges for widely used microorganisms, such as species of the genus *Trichoderma* and the bacterium *Azospirillum brasilense*, provides critical information for improving strain selection and recommendation strategies. Such knowledge supports the development of more efficient bio-input technologies and contributes to enhancing agronomic performance in sustainable agricultural systems (Díaz-Rodríguez *et al*., 2025; Steffen *et al*., 2026).

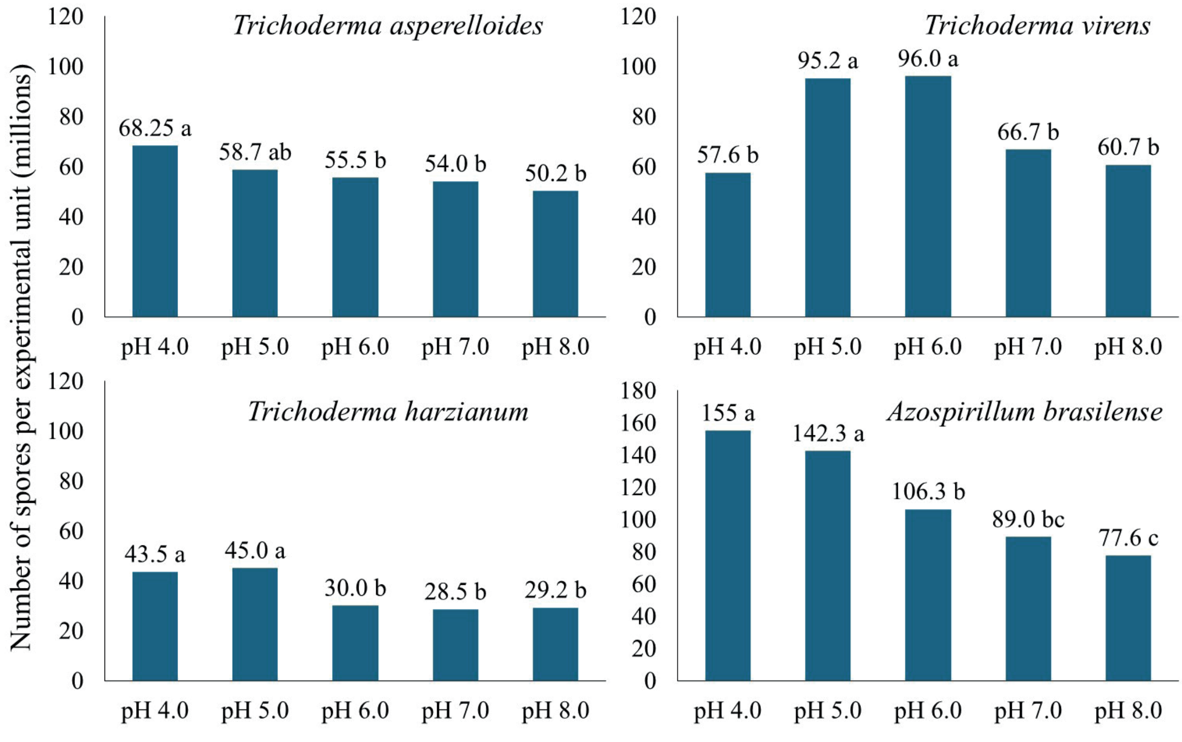
3.1 MICROBIAL SPORULATION BIOASSAY

Sporulation of microorganisms in the culture medium is an important variable, especially for fungal and bacterial genera used in the formulation of bio-inputs. This process indicates that the microorganism can adapt to the environmental conditions of the medium in which it was inoculated, successfully completing its reproductive cycle through spore production.

In this study, medium pH significantly affected the sporulation capacity of both the bacterium *A. brasilense* and the three fungal species of the genus *Trichoderma* (Figure 1). These findings are consistent with Martinez *et al*. (2023), Díaz-Rodríguez *et al*. (2025), and Sravanthi; Kumar; Jaiswal (2026), who point out that the medium acidity is a critical variable influencing the activity of microorganisms used in formulations and may consequently affect the efficiency of microorganisms inoculated under field conditions.

The strains belonging to the three *Trichoderma* species showed differences in sporulation intensity across the evaluated pH levels. Two species exhibited a progressive decrease in sporulation as medium pH increased, with the highest spore concentrations observed at pH 4.0 and 5.0 (Figure 1). A different pattern was observed for the species *T. virens*, which showed a peak of sporulation at pH 5.0 and 6.0 (Figure 1).

Figure 1 - Mean number of spores of *Trichoderma asperelloides*, *Trichoderma virens*, *Trichoderma harzianum*, and *Azospirillum brasilense* in culture medium with varying pH values. Means of five replicates.



Source: Authors' own work.

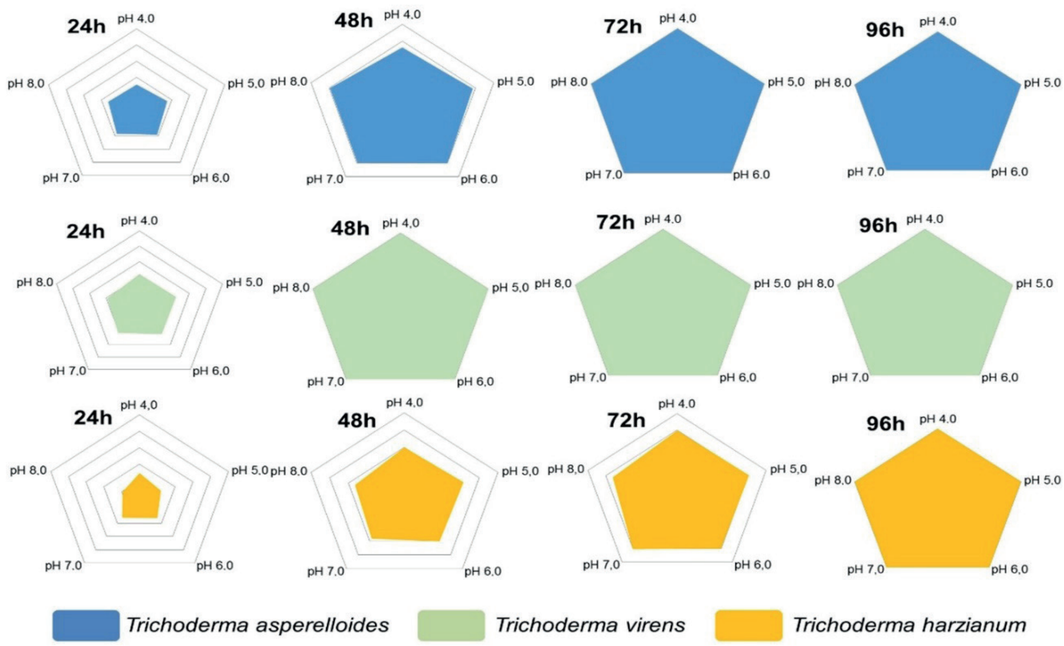
Considering the variability of agricultural soils in relation to pH and the frequent need to adjust soil acidity to meet crop requirements, these results highlight the importance of understanding the optimal pH ranges of bio-input agents prior to their introduction into agricultural environments. Such knowledge may enhance microbial establishment and activity, thereby optimize agronomic benefits and improve the return on agricultural inputs for producers.

The successful use of bio-inputs depends on the adaptation of microbial strains to the inoculation environment. Investing in the inoculation of even the most efficient commercial microbial strain for plant growth promotion or for biological pest control has limited value if the microorganism is unable to survive and adapt to the environmental conditions of the site of application (Papin *et al*., 2024; Pei *et al*., 2026). Regardless of the form in which the microorganism is present in the bio-input - whether as a reproductive structure (spores) or in vegetative form (mycelium) - the first requirement is adaptation, followed by growth and reproduction. Only microorganisms that successfully establish and proliferate in the target environment will be able to fully express their agronomic potential and generate benefits for crops.

3.2 MYCELIAL GROWTH RATE BIOASSAY

Significant differences were observed in the mycelial growth rate of the three species of the genus *Trichoderma* in media with different pH levels, indicating variation in the establishment dynamics of strains of this fungal genus under distinct acidity conditions (Figure 2, Table 1).

Figure 2 - In vitro mycelial growth of *Trichoderma asperelloides*, *Trichoderma virens*, and *Trichoderma harzianum* in culture medium with varying pH values over 96 hours in a controlled environment. Average of five replicates.



Source: Authors' own work.

Although rapid mycelial growth is a well-documented characteristic of fungi belonging to the genus *Trichoderma*, particularly when compared with other agriculturally important fungal genera, the data observed in this study showed a clear advantage for the strain of *T. virens* over *T. asperelloides* and *T. harzianum* (Figure 2, Table 1).

The *T. virens* showed greater radial mycelial growth, reaching the edges of the 9-cm-diameter Petri dish within the first 48 hours of incubation at all pH levels of the culture medium. This result demonstrates the rapid capacity of the strain to adapt and establish itself under varying pH conditions (Figure 2, Table 1).

In comparison with other two *Trichoderma* species, *T. asperelloides* displayed a higher initial mycelial growth rate than the commercial strain *T. harzianum*, which was the last to completely occupy the surface of the culture medium, requiring up to 96 hours of incubation (Figure 2, Table 1).

Initial growth parameters of biological agents are essential to ensuring the establishment and survival of microorganisms under field conditions. In the case of fungi of the genus *Trichoderma*, commonly used as biological control agents and plant growth promoters, rapid establishment resulting from high mycelial growth rate is directly related to biological efficiency (Cavalcante *et al*., 2025; Marques *et al*., 2025). This relationship is explained by the fact that competition for water, nutrients, oxygen and space constitutes one of the primary mechanisms underlying biological control strategies employed by these agents. Therefore, a higher initial mycelial growth rate enhances the microorganism's competitive ability in the environment, thereby increasing its potential for successful establishment and biocontrol activity.

Table 1 - Average diameter (cm) of colonies of *Trichoderma asperelloides*, *Trichoderma virens* and *Trichoderma harzianum* grown in culture medium with varying pH values over 96 hours in a controlled environment. Average values from five replicates.

pH 4.0	***T. asperelloides***	***T. virens***	***T. harzianum***	**CV (%)**
24h	2.80 b*	3.93 a	2.60 b	4.96
48h	6.55 b	9.00 a	5.40 c	3.78
72h	9.00 a	9.00 a	7.17 b	3.17
96h	9.00 a	9.00 a	9.00 a	0
pH 5.0	***T. asperelloides***	***T. virens***	***T. harzianum***	**CV (%)**
24h	3.12 b	3.97 a	2.15 c	2.86
48h	6.95 b	9.00 a	5.67 c	4.02
72h	9.00 a	9.00 a	7.25 b	2.85
96h	9.00 a	9.00 a	9.0 a	0
pH 6.0	***T. asperelloides***	***T. virens***	***T. harzianum***	**CV (%)**
24h	3.40 ab	3.90 a	2.90 b	5.69
48h	7.20 b	9.00 a	5.45 c	5.02
72h	9.00 a	9.00 a	7.15 b	4.53
96h	9.00 a	9.00 a	9.00 a	0
pH 7.0	***T. asperelloides***	***T. virens***	***T. harzianum***	**CV (%)**
24h	3.27 ab	3.72 a	2.82 b	4.99
48h	7.15 b	9.00 a	5.10 c	5.18
72h	9.00 a	9.00 a	7.22 b	2.94
96h	9.00 a	9.00 a	9.00 a	0
pH 8.0	***T. asperelloides***	***T. virens***	***T. harzianum***	**CV (%)**
24h	2.90 b	3.47 a	1.72 c	2.06
48h	7.12 b	9.00 a	4.75 c	3.43
72h	9.00 a	9.00 a	6.50 b	4.71
96h	9.00 a	9.00 a	9.00 a	0

*Numbers followed by the same lowercase letter in rows do not differ from each other by Tukey's test at a 5% probability level.

Source: Authors' own work.

Comparing the three species of the genus *Trichoderma* evaluated in this study, the species *T. virens* again demonstrated superior performance, highlighting its potential suitability for inclusion in bio-inputs intended for plant production, especially for crops that require soil acidity correction, such as olive (*Olea europaea* L.), fig (*Ficus carica* L.), and aromatic species such as lavender (*Lavandula* sp.) and rosemary (*Salvia rosmarinus* Spenn.).

The effect of pH on mycelial growth and spore production in certain *Trichoderma* species, as well as on the proliferation of spore-forming bacteria, may constitute a determining factor for the field adaptation of these microbial agents (Onilude; Seyi-Amole, 2018). According to these authors, enhanced microbial adaptation and greater sporulation efficiency occur within the pH range of 4.0 to 5.5. This pattern was observed for three of the microorganisms evaluated in this study: the bacterium *A. brasilense* and the fungi *T. asperelloides* and *T. harzianum* (Figures 1 and 2). However, *T. virens* exhibited a distinct response pattern, characterized by higher sporulation across a broader pH range extending up to pH 6.0.

The differential growth and sporulation patterns observed among the evaluated microorganisms can be closely associated with pH-dependent enzymatic regulation, a fundamental mechanism underlying microbial adaptation to chemically variable environments. Changes in medium pH directly influence enzyme structure and catalytic efficiency, thereby affecting substrate utilization and overall metabolic performance. In species of the genus *Trichoderma*, the activity of extracellular hydrolytic enzymes, including chitinases, glucanases, and proteases, is strongly modulated by environmental pH, influencing nutrient acquisition and fungal development (Sharma *et al*., 2022; Martinez *et al*., 2023). Thus, the pH ranges that promoted higher growth and sporulation in the present study likely correspond to conditions that enhanced enzymatic efficiency, supporting faster nutrient assimilation and accelerating physiological processes required for microbial establishment and reproduction.

Beyond primary enzymatic regulation, the responses observed in sporulation and growth may also be linked to the modulation of secondary metabolite production, which is highly sensitive to environmental pH. In microorganisms used as bio-inputs, particularly species of *Trichoderma*, the synthesis of antibiotics, siderophores, and volatile bioactive compounds plays a critical role in plant growth promotion and biological control of phytopathogens (Rush *et al*., 2021; Martinez *et al*., 2023). The variation in sporulation intensity across pH levels suggests that specific acidity conditions may activate metabolic pathways associated with survival and ecological fitness. This indicates that pH acts not only as a physicochemical constraint but also as a regulatory signal capable of modulating gene expression involved in secondary metabolism, thereby enhancing the functional performance and ecological adaptability of microbial inoculants.

The significant differences in mycelial growth rates observed among the evaluated species highlight the strategic role of radial expansion in microbial competition, a key ecological mechanism in resource-limited environments. Rapid mycelial growth, particularly under specific pH conditions, promotes early substrate colonization and enhances competitive ability for nutrients, water, oxygen, and physical space. In *Trichoderma* spp., fast colonization is widely recognized as a major determinant of successful biological control, as it enables efficient exclusion of competing microorganisms and pathogens (Rush *et al*., 2021; Sharma *et al*., 2022). Therefore, the enhanced growth performance observed under favorable pH conditions in the present study likely reflects an increased competitive capacity, reinforcing the importance of selecting strains capable of rapid establishment to ensure functional persistence and agronomic effectiveness in field environments.

3.3 FUNGAL MYCELIAL BIOMASS BIOASSAY

Significant differences were observed in fungal mycelial biomass production among the three *Trichoderma* cultivated in media with different pH levels (Table 2).

Table 2 - Dry biomass of mycelium (mg) of *Trichoderma asperelloides*, *Trichoderma virens* and *Trichoderma harzianum* in culture media with different pH values. Average values of five replicates.

Treatment	pH values					CV (%)
	4.0	5.0	6.0	7.0	8.0	
T. asperelloides	224.7 b AB*	258.8 a A	247.5 ab A	224.0 a AB	177.6 a B	7.45
T. virens	196.2 b A	169.5 b B	170.2 b AB	149.7 b B	124.8 b B	8.76
T. harzianum	274.4 a A	287.2 a A	274.1 a A	209.2 ab B	197.4 a B	6.17
CV (%)	12.87	14.51	16.18	11.45	14.87	

*Numbers followed by the same lowercase letter in columns and uppercase in rows do not differ from each other by Tukey's test at a 5% probability level.
Source: Authors' own work.

At pH 4.0, *T. harzianum* exhibited the greatest dry mycelial biomass production among the evaluated species, whereas the remaining species did not differ statistically from one another at this pH level. For the commercial strain *T. harzianum*, higher pH levels (7.0 and 8.0) significantly reduced dry mycelial biomass production (Table 2), suggesting that this strain may experience limitations in growth and establishment in soils with neutral or alkaline pH.

For *T. asperelloides*, culture media adjusted to pH 5.0 and 6.0 yielded the highest mean mycelial biomass. In contrast, *T. virens* showed a response pattern distinct from that observed for sporulation and radial mycelial growth rate. Higher values of dry mycelial biomass were obtained in culture media with pH 4.0 (196.2 mg) and 6.0 (170.2 mg), with biomass at pH 6.0 not differing statistically from those at pH 5.0 and 7.0. The lowest biomass production by *T. virens* occurred at pH 8.0 (Table 2).

The results obtained in this study are consistent with those reported by Steffen *et al.* (2026), which demonstrated the ability of these microorganisms to modulate medium pH and adjust their enzymatic activity according to environmental conditions. These findings reinforce the concept that the success of bio-inputs depends not only on an optimal pH range but also on the physiological and metabolic plasticity of the isolates. The differential response observed among species in terms of sporulation and growth suggests that selecting strains adapted to specific soil acidity conditions may enhance the agronomic efficiency of bio-inputs. This interpretation supports the discussion presented by Boeni *et al*. (2024), who emphasized the importance of compatibility between plant growth-promoting microorganisms and soil conditions to maximize the performance of crops such as olive - the species evaluated in their study.

In this context, the integration of pH adaptation, the capacity to modulate the medium, and microbial metabolic activity reinforces the need for inoculation strategies based on soil characteristics and microbial physiological criteria to optimize the use of bio-inputs in agricultural systems.

Although all three *Trichoderma* species evaluated in this study demonstrated the ability to establish, produce mycelium, and form spores across the tested pH levels, significant differences were observed in the vegetative and reproductive growth parameters of the fungal species at the different pH levels. These differences identified under laboratory conditions may translate into variations in field performance, potentially influencing microbial efficiency and agronomic response in agricultural crops. Therefore, determining and understanding the optimal growth and development conditions of microbial agents is essential to optimize the use of biological tools in the field and to select strains best adapted the specific soil and crop conditions in which they will be employed, thereby maximizing the return from technological investments in plant production.

While the present study provides controlled evidence on the influence of pH on microbial growth, sporulation, and biomass production, it is important to acknowledge that the experiments were conducted entirely under in vitro conditions, which do not fully reproduce the complexity of soil environments. Factors such as microbial interactions, nutrient heterogeneity, soil structure, and environmental fluctuations may significantly influence microbial performance under field conditions. Nevertheless, in vitro assays remain essential for identifying fundamental physiological responses and defining optimal growth ranges, representing a critical initial step for predicting microbial behavior and guiding the selection of strains with greater potential for successful establishment in agricultural systems. Therefore, future studies under field conditions are necessary to validate the ecological relevance and agronomic applicability of the patterns observed in this study.

CONCLUSIONS

The present study demonstrates that medium pH is a key determinant of the physiological performance and reproductive capacity of *Trichoderma* spp. and *Azospirillum brasilense*, significantly influencing sporulation, mycelial growth, and biomass production, with species-specific responses across the evaluated pH range. Slightly acidic conditions (pH 4.0-6.0) generally favored the development of *T. asperelloides*, *T. harzianum*, and *A. brasilense*, whereas *T. virens* exhibited greater tolerance and rapid establishment across different pH levels, indicating higher physiological plasticity.

These findings highlight that the agronomic efficiency of microbial bio-inputs depends on compatibility between soil conditions and the ecophysiological requirements of the inoculated microorganisms. Accordingly, selecting strains adapted to specific soil pH conditions and integrating soil chemical characterization into inoculation strategies are essential to maximize microbial establishment, metabolic activity, and functional performance in agricultural systems. Overall, these results highlight the critical nature of pH-microorganism interactions in the strategic deployment of microbial bio-inputs. Integrating environmental pH constraints into the selection and recommendation process is essential for ensuring the efficacy of sustainable agriculture technologies.

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