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ORIGINAL ARTICLE

Microorganisms as growth promoters of *Acmella oleracea* grown under different cultivation systems[1]

Microrganismos como promotores de crescimento de *Acmella oleracea* cultivada em diferentes sistemas de cultivo

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HIGHLIGHTS:

The fresh and dry mass production of Acmella oleracea *is increased by entomopathogenic microorganisms and* Trichoderma asperellum.
The protected cultivation system for A. oleracea *allows the microorganisms to perform better.*
Microorganism inoculation could become a more sustainable alternative for growing A. oleracea.

ABSTRACT: A great challenge to overcome is how to maintain and increase the productivity of vegetables, such as jambu (*Acmella oleracea*), by using natural processes and living organisms that stimulate plant production and release fewer toxic residues into the environment. The objective of this study was to evaluate the growth of *A. oleracea*, based on biometric, and physiological responses, following the application of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* and the growth promoter *Trichoderma asperellum*, under protected and unprotected planting systems, in the rainy and dry seasons of the Amazon. Two trials were conducted simultaneously, in randomized blocks, in a commercial area of peri-urban agriculture in the municipality of Ananindeua, Pará state, Brazil, in protected and unprotected hanging beds. *Metarhizium anisopliae*, *B. bassiana*, and *T. asperellum* isolates promoted growth in jambu in protected and unprotected plantation systems, in both crop cycles. Of note, *M. anisopliae* matched the results obtained with the growth promoter *T. asperellum* and stood out for favoring greater performance in all of the evaluated growth variables, especially in the protected cultivation system and in rainy season. In addition, *A. oleracea* grew better in all treatments in a protected planting system and in both evaluated periods. Therefore, *A. oleracea* treated with *M. anisopliae*, *B. bassiana*, or *T. asperellum* presented better growth, produced more biomass, and exhibited superior gas exchange.

Key words: biomass, gas exchange, cultivation system, jambu

RESUMO: Um dos grandes desafios a serem superados é manter e aumentar a produtividade de hortaliças, como o jambu (*Acmella oleracea*), utilizando processos naturais e organismos vivos que estimulem a produção vegetal, com menos resíduos tóxicos no meio ambiente. O objetivo deste estudo foi avaliar o crescimento de plantas de *A.oleracea*, por meio de respostas biométricas e fisiológicas, após a aplicação dos fungos entomopatogênicos *Beauveria bassiana* e *Metarhizium anisopliae* e do promotor de crescimento *Trichoderma asperellum*, sob sistemas de plantio protegido e não protegido, nas estações chuvosa e seca da Amazônia. Dois ensaios foram conduzidos simultaneamente, em blocos casualizados, em uma área comercial de agricultura periurbana no município de Ananindeua, Pará, Brasil, em canteiros suspensos protegidos e não protegidos. Os isolados fúngicos de *M. anisopliae, B. bassiana* e *T. asperellum* promoveram o crescimento em plantações de jambu, em sistemas de plantio protegido e desprotegido, em ambos os ciclos de cultivo. No entanto, *M. anisopliae* se igualou aos resultados obtidos com o promotor de crescimento *T. asperellum* e se destacou por favorecer maior desempenho em todas as variáveis de crescimento avaliadas, principalmente no sistema de cultivo protegido e no período chuvoso amazônico. Além disso, as plantas de *A. oleracea* apresentaram melhor crescimento em todos os tratamentos, em sistema de plantio protegido, nos dois períodos avaliados. Portanto, plantas de *A. oleracea* tratadas com fungos como *M. ansiopliae, B. bassiana* e *T. asperellum* apresentaram maior crescimento, biomassa e trocas gasosas.

Palavras-chave: biomassa, trocas gasosas, sistema de cultivo, jambu

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Introduction

Jambu (*Acmella oleracea* - Asteraceae) is an unconventional food plant (UFP) of the Amazon region, with significant economic potential (Silva et al., 2020). This UFP is widely consumed in regional dishes and alcoholic beverages. In addition, jambu contains around 0.7% essential oil, which is of pharmaceutical interest (Lorenzi & Matos, 2002).

In recent years, the global agriculture industry has been reevaluating its direction due to growing concerns about the potential environmental impacts of pesticide use (Bennekou, 2019). The use of microorganisms to promote plant growth is a potentially effective way to reduce the amount of chemical fertilizers used in agricultural production while simultaneously increasing their effectiveness (Spolaor et al., 2016).

Entomopathogenic fungi are defined as biopesticides because they parasitize insects, but they also allow plants to grow and increase crop production (Islam et al., 2021). The benefits of using *Trichoderma* spp. as a growth promoter have been observed from germination to management in the first years of life in the field (Jaroszuk-Ściseł et al., 2019). Although *Trichoderma* spp. can promote growth and have been widely studied in various crops (Chagas Júnior et al., 2021, 2022), there are no reports on its use in jambu cultivation. Likewise, there are no studies on the colonization of jambu crops with entomopathogenic fungi to promote growth. In this context, the present study aimed to evaluate the growth promotion of jambu, based on biometric and physiological responses, after the application of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* and the growth promoter *Trichoderma asperellum*, under protected and unprotected planting systems, and in the rainy and dry seasons of the Amazon.

Material and Methods

The bioassays were performed in a commercial area belonging to the Associação dos Produtores e Hortifrutigranjeiros da Gleba do Guajará (APHA), located in the urban and peri-urban area of the municipality of Ananindeua, Pará state, Brazil (1° 19' 28.441" S, 48° 23' 17.318" W). Two trials were carried out simultaneously in two growing seasons, the first in March 2021, during the rainy season (a period of intense rain), and the second in September 2021, during the dry season (a period of lower rain intensity), under field conditions, in protected and unprotected hanging beds.

The experiment was conducted using a randomized block design with a 4 × 2 factorial arrangement, corresponding to the three fungi, namely *B. bassiana*, *M. anisopliae*, and *T. asperellum* (pool of *T. asperellum* isolates UFRA T06, UFRA T09, UFRA T12, and UFRA T52), and the control with water; and two growing systems, namely protected and unprotected. There were five replicates of 16 plants each, with two plants per replicate evaluated considering the effect of the border.

Figures 1-4 provide details on the climate in the area where the experiment was conducted, namely Ananindeua, Pará state, Brazil. The graphs present the average air humidity; the minimum, average, and maximum temperatures; and the average daily rainfall in March 2021 (Figures 1 and 2) and September 2021 (Figures 3 and 4).

The *B. bassiana*, *M. anisopliae*, and *T. asperellum* isolates are native to the Brazilian Amazon and belong to the Mycoteca of the Laboratório de Produção Vegetal of the Universidade Federal da Amazônia (UFRA), where they are stored at 26 °C in Castelanni. The entomopathogenic isolates were selected through toxicological screening bioassays that showed promising results in terms of pathogenicity in the endophytic colonization of the *Tenebrio molitor* beetle in the laboratory. *Trichoderma asperellum* has previously been reported as a

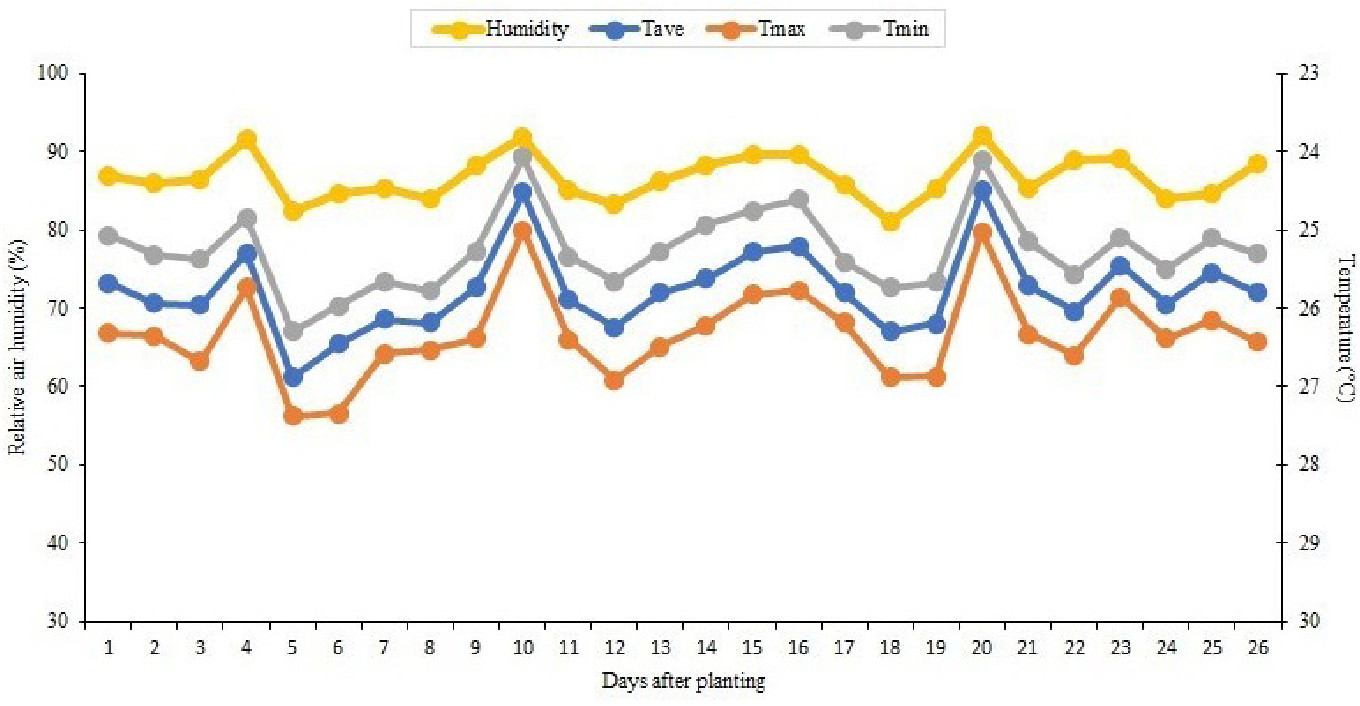


Figure 1. Average relative air humidity and minimum (Tmin), average (Tave), and maximum (Tmax) temperatures measured during the experiment in the rainy season, March 2021 (municipality of Ananindeua, Pará state, Brazil)

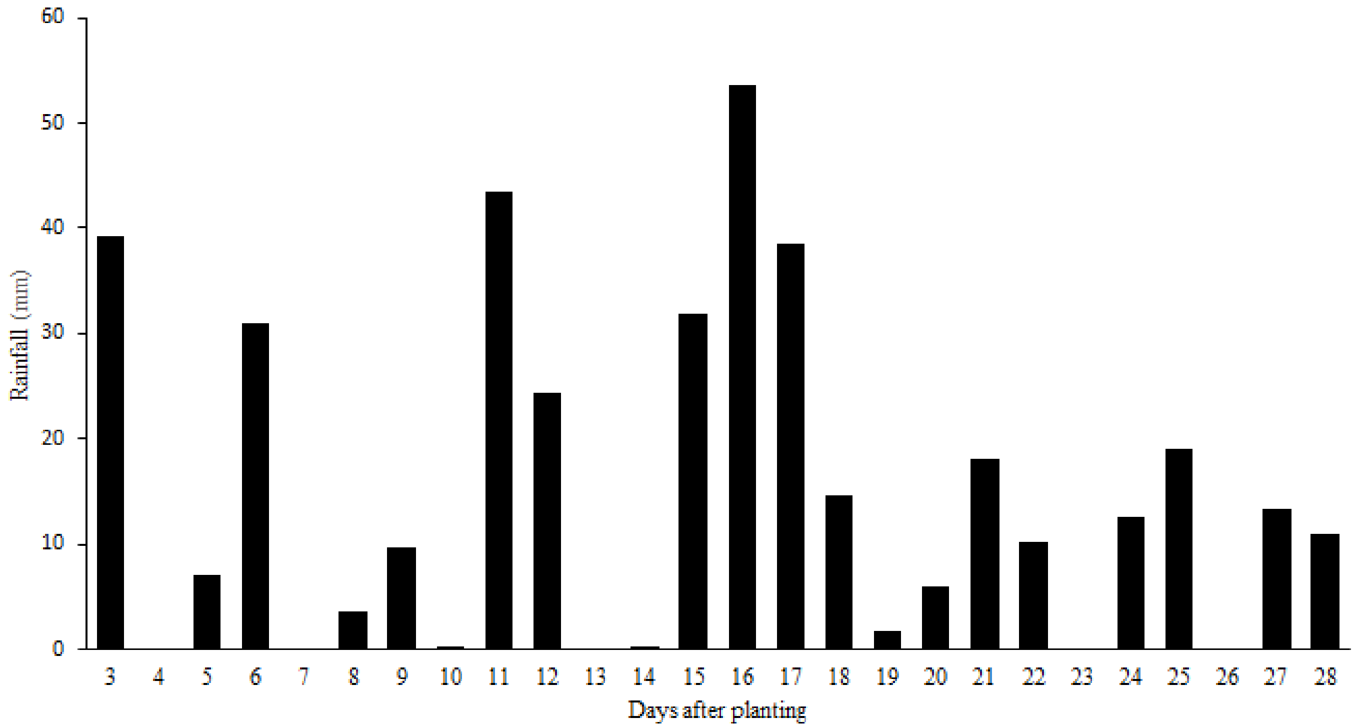


Figure 2. Average daily rainfall measured during the experiment in the rainy season, March 2021 (municipality of Ananindeua, Pará state, Brazil)

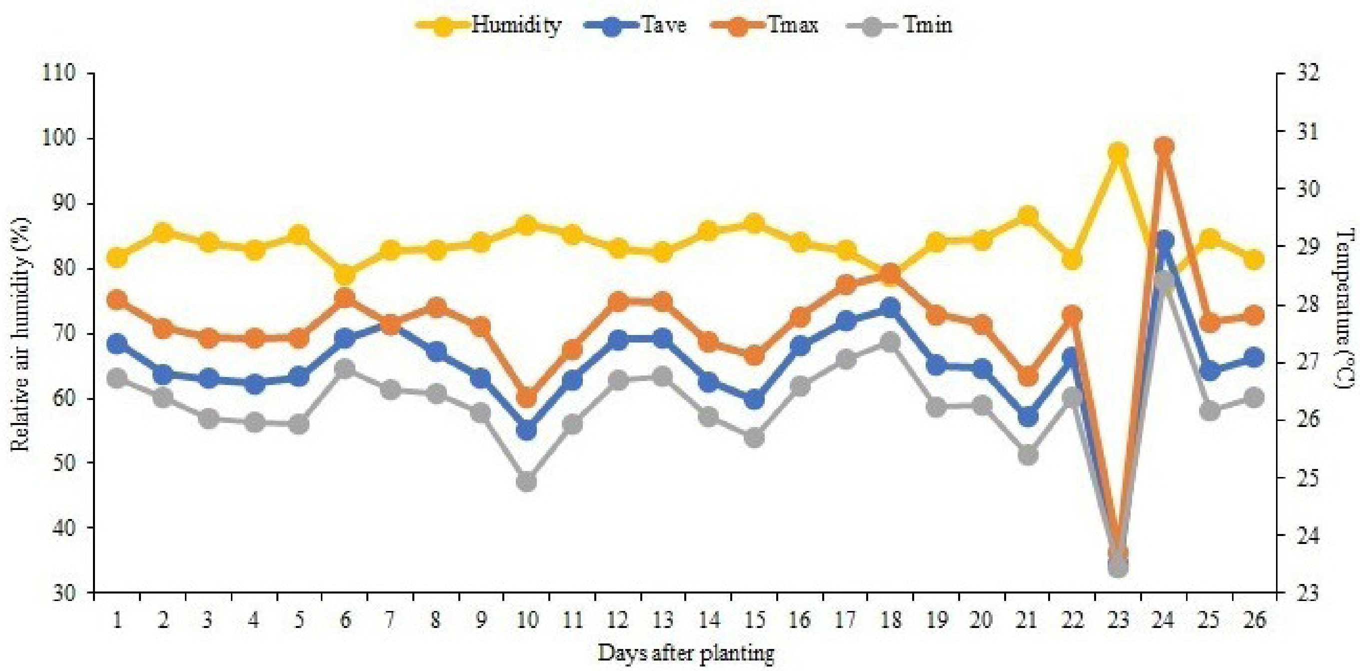


Figure 3. Average relative air humidity and minimum (Tmin), average (Tave), and maximum (Tmax) temperatures measured during the experiment in the dry season, September 2021 (municipality of Ananindeua, Pará state, Brazil)

growth promoter for rice (Sousa et al., 2021) and banana (Maués et al., 2022). Therefore, this study highlights represents its first use as a growth promoter for jambu in the Brazilian Amazon.

The three fungal isolates were prepared from discs stored in Castelanni and placed in Petri dishes containing potato dextrose agar (PDA) for the growth of pure colonies. Conidia were picked with a sterile spatula and suspended in sterile distilled water. Each suspension was adjusted to 1×10^8 conidia mL^{-1} using a Neubauer chamber, and rice was inoculated with suspensions at the adjusted concentration for storage.

The seeds of the yellow-flowered variety of *A. oleracea* were obtained from the active seed bank of APHA. The seeds were sown in 128-cell expanded polystyrene trays, using a substrate based on sieved organic compost of crushed *Euterpe oleracea* stones and poultry litter in a 2:1 ratio (v/v). Ten seeds were sown per cell to ensure two seedlings per cell at the time of transplanting, which required thinning two weeks after germination to obtain the desired number of seedlings.

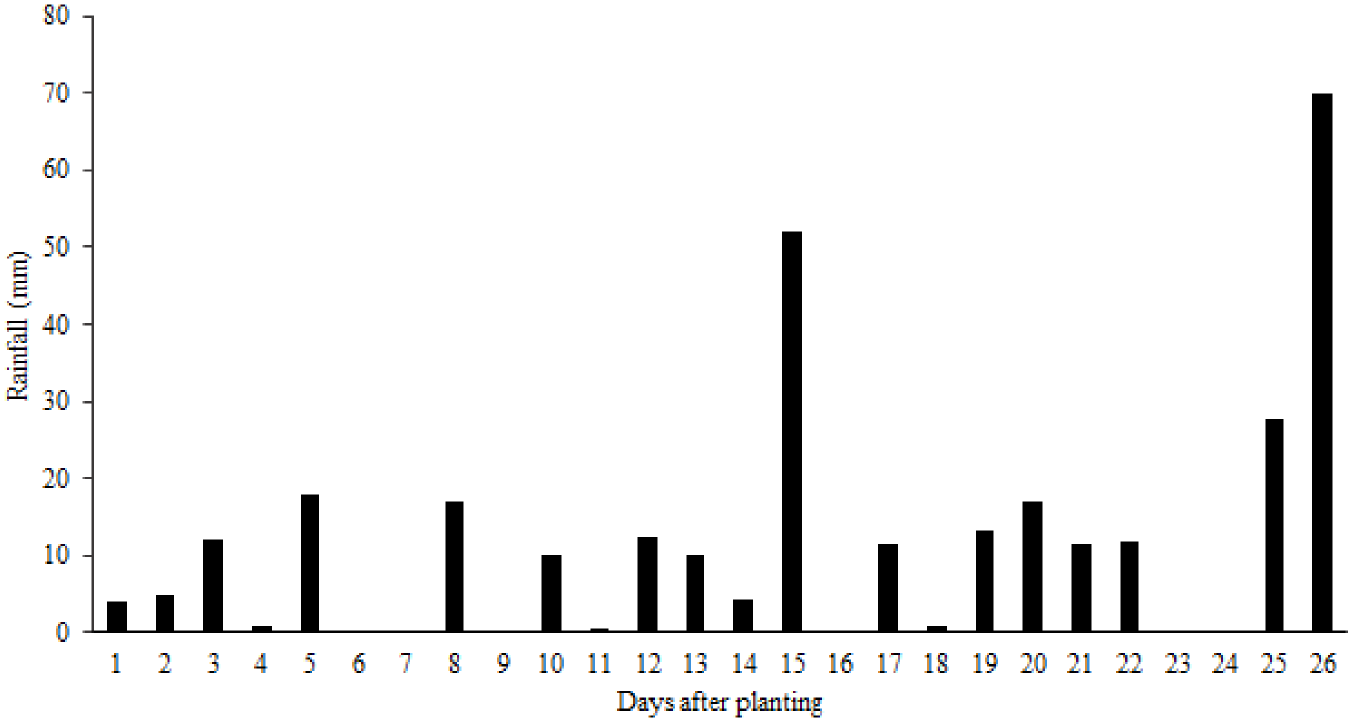


Figure 4. Average daily rainfall measured during the experiment in the dry season, September 2021 (municipality of Ananindeua, Pará state, Brazil)

The tray containing the seedlings was placed in a protected environment with 20% reduced irradiance and manually watered twice a day until the substrate reached its field capacity, according to EMBRAPA (1979). The plants with four leaves were transplanted 20 days after sowing, with two seedlings per hole at a distance of 5 × 10 cm.

The study involved the use of both protected and unprotected hanging beds in wooden "arched roof" greenhouses shaped like an inverted parabola with a flat arrow. The beds were 80 cm high, 1.40 m wide, and 20 m long, while the greenhouses were 25 m long, 7 m wide, and 3 m high. The protected system was covered with 100-μm diffusion plastic film with ultraviolet (UV) protection. In addition, both systems received one-time fertilization of 3 kg m^{-2} organic compost. The compost was incorporated into the top 10 cm of the soil. The seedlings were irrigated with a micro-sprinkler at a flow rate of 90 L h^{-1} twice a day for 20 min each time. Weed control was performed manually.

Two applications of each treatment were made: once on the day the seedlings were transplanted, and then 15 days after transplanting. The fungi were inoculated by applying 20 mL of the conidial suspension directly to the substrate and watering each plant with 20 mL of the suspension (1×10^8 conidial mL^{-1}).

Jambu was harvested 25 days after transplanting, when it reached commercial size. The following biometric variables were analyzed: (a) plant height (cm), determined with a tape measure, measuring the plant from the neck to the apex of the shoot (inflorescence); (b) collar diameter, measured with a digital caliper (precision of 0.02 mm); (c) fresh and shoot dry mass (g), determined after drying in a forced-air oven at 65 °C until the material reached a constant mass, using a digital balance (precision of 0.01 g); (d) the robustness index, calculated as the ratio of plant height to collar diameter; and (e) the chlorophyll index, obtained using a portable SPAD meter, with five readings made on the second leaf, physiologically mature and fully expanded, from the top of the main stem, according to the recommendations of Sampaio et al. (2021).

Gas exchange was assessed in the morning, between 9:00 and 11:00 a.m. The reading was taken on the second leaf from the apex of the main stem under an internal carbon dioxide (CO_2) concentration of 400 μmol mol^{-1} and artificial photosynthetically active radiation (PAR) of 1,200 μmol photons m^{-2} s^{-1}. The measurement interval was adjusted according to the results obtained from the diurnal gas exchange curve for the species (Sampaio et al., 2021). The net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (C_i, μmol CO_2 mol^{-1} air), and transpiration rate (E, mmol H_2O m^{-2} s^{-1}) were evaluated with a portable infrared gas analyzer (IRGA, model LI 6400XT, LICOR®). The transpiration rate data did not yield coherent results. The instantaneous carboxylation efficiency was assessed with the A/Ci ratio.

Prior to statistical analysis, the presence of discrepant data (Grubbs, 1969) and the normality of errors (Shapiro & Wilk, 1965) were checked. The data were subjected to analysis of variance using the F test ($p \leq 0.05$), and the means were compared using the Tukey test ($p \leq 0.05$) using R version 4.1.0 (R Core Team, 2020).

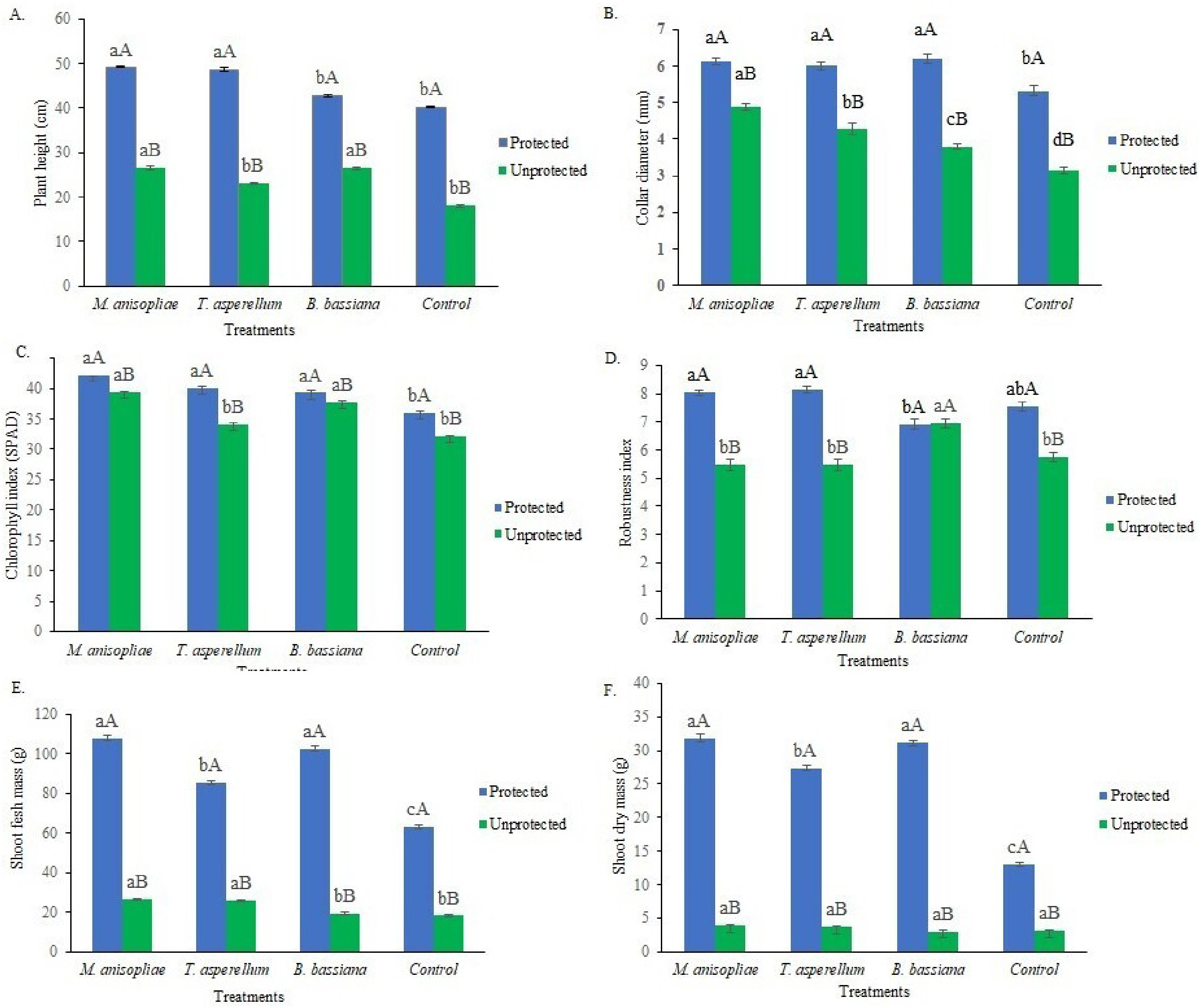
Results and Discussion

During the rainy season, entomopathogenic microorganisms were selected as growth promoters for jambu in both protected and unprotected growing systems. Twenty-five days after transplanting, there was a significant difference in almost all the growth variables between the two planting systems. The only nonsignificant differences was for the robustness index in the *B. bassiana* treatment. It is worth noting that the plants in the protected growing system, treated with different fungi, showed an increase in all analyzed variables (Figures 5A-D). Compared with the unprotected system, plants grown in the protected system showed a 90% increase in shoot growth, a 47% increase in collar diameter, a 10% increase in the chlorophyll index, and a 30% increase in the robustness index. The robustness index, which considers plant development and biomass distribution, is an indicator of plant quality (Lima Filho et al., 2019). Overall, treatment with *M. anisopliae* and *T. asperellum* showed stronger growth promotion in jambu compared with the control treatment (Figures 5A-D).

The direct benefits of interaction with endophytic fungi include an increase in the acquisition of nutrients and the amount of phytohormones in the plant. These benefits are directly related to an increase in biomass production, root system development, plant height, weight, reproduction, and productivity. Due to these benefits, they can be called biofertilizers (Bamisile et al., 2018), or growth-promoting bioinputs.

Acmella oleracea cultivation necessitates warm and humid climatic conditions, with temperatures exceeding 25 ºC, well-drained soils, and adequate organic matter content (MAPA, 2010). The impact of climatic conditions on jambu cultivation may explain the greater development of plants associated with fungi during the rainy season (Santos & Gentil, 2015). As reported by Zhang et al. (2016), the use of beneficial microorganisms can increase chlorophyll levels and thus favor plant growth. Under the trial's conditions, the constant rainfall during the rainy season reduced light and led to longer periods of cloudiness. The temperatures ranged from 23 to 28 °C during this period, and the total rainfall was 480 mm. Moreover, the average temperature of 25.5 °C was the lowest throughout the year, which created ideal conditions for fungal growth (INMET,2021).

There were significant differences when comparing the growing systems in terms of their biomass increase. The fresh



Lowercase letters compare means within the same growing system and uppercase letters compare means between the growing systems by Tukey's test ($p \leq 0.05$)

Figure 5. Plant height (A), collar diameter (B), the chlorophyll index (C), the robustness index (D), shoot fresh mass (E), and shoot dry mass (F) of *Acmella oleracea* treated with an entomopathogenic fungus (*Beauveria bassiana* or *Metarhizium anisopliae*) or a growth promoter (*Trichoderma asperellum*) in protected and unprotected systems during the rainy season

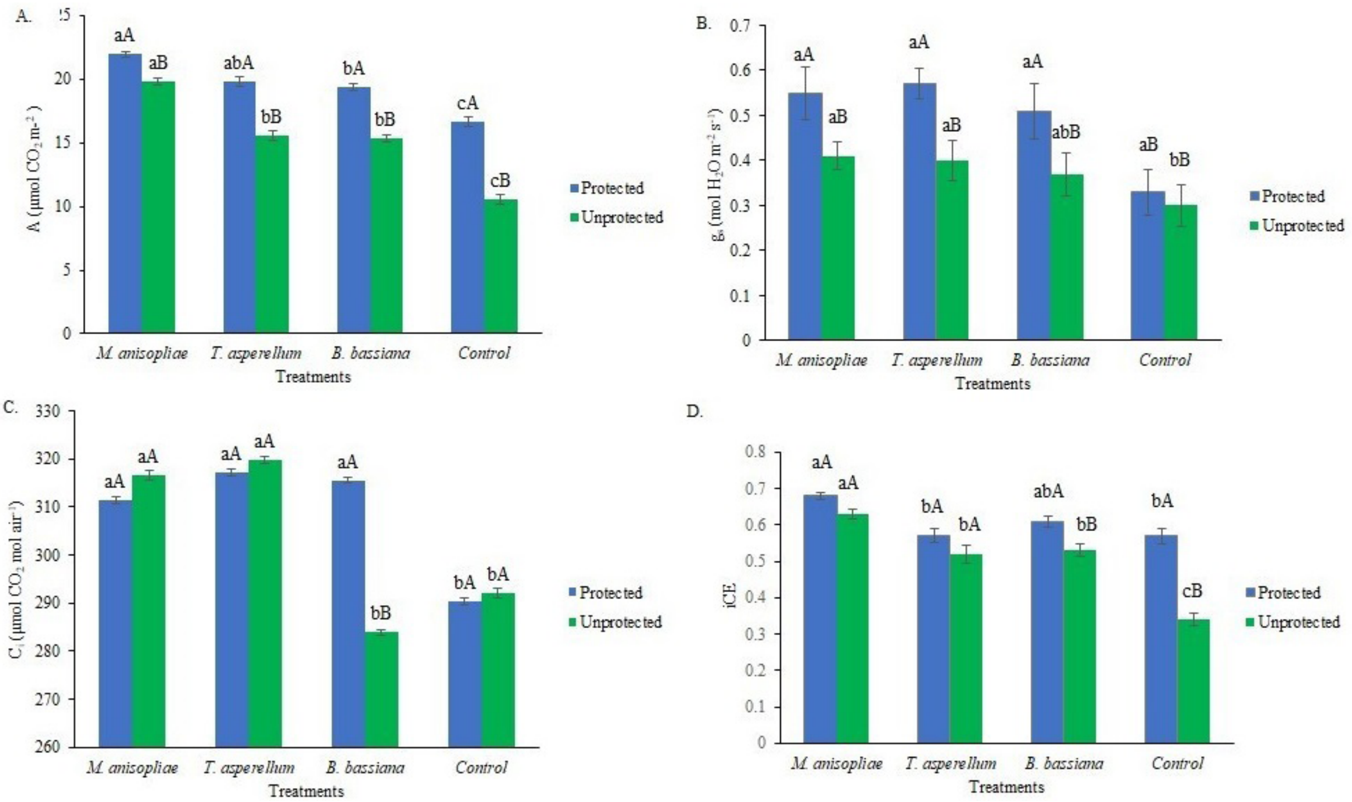
and dry mass of the shoot increased significantly more in the protected system, 299 and 650%, respectively (Figures 5E and F). In the protected system, treatment with *M. anisopliae* and *B. bassiana* resulted in a 71 and 35% increase in fresh plant mass, respectively, compared with the control treatment. However, there was no significant difference between the two treatments. There were significant differences in the fresh shoot mass in the unprotected system for the *M. anisopliae* and *T. asperellum* treatments compared with the control, namely 45 and 41%, respectively (Figure 5E).

Similarly to the findings of the present study, Farias et al. (2018) investigated the inoculation of *Purpeorocillium lilacinum* in conjunction with four other fungi, namely *B. bassiana*, *M. anisopliae*, *Pochonia chlamydosporia*, and *T. asperellum*. They observed positive results in terms of growth promotion variables in soybean and maize. However, the consortium test made it difficult to determine which microorganism was the primary contributor to plant growth. The entomopathogenic fungi *B. bassiana* and *Metarhizium* spp. have been reported as plant inoculants that promote plant growth in crops such as tomatoes, beans, and corn (Jaber & Enkerli, 2017; Tall & Meyling, 2018), resulting in higher yields (Jaber et al., 2018).

There were significant differences in gas exchange when comparing the evaluated variables between the growing systems. Plants in the protected system showed a 27% increase in A compared with the unprotected system (Figure 6A). Similarly, there was a notable difference in g_s in plants from both growing systems (Figure 6B), with the protected system showing a 33% increase compared with the unprotected system. Regarding Ci, treatment with *B. bassiana* in the protected system resulted in 11% increase compared with the unprotected system, while the other treatments showed no significant differences (Figure 6C). In terms of iCE, there was a notable difference for the *B. bassiana* treatment (Figure 6D), with a 15% increase in the protected system compared with the unprotected system. A higher net rate of photosynthesis can support the high energy demand and significantly impact the photosynthetic yield and other variables, such as stomatal conductance (Centritto et al., 2009).

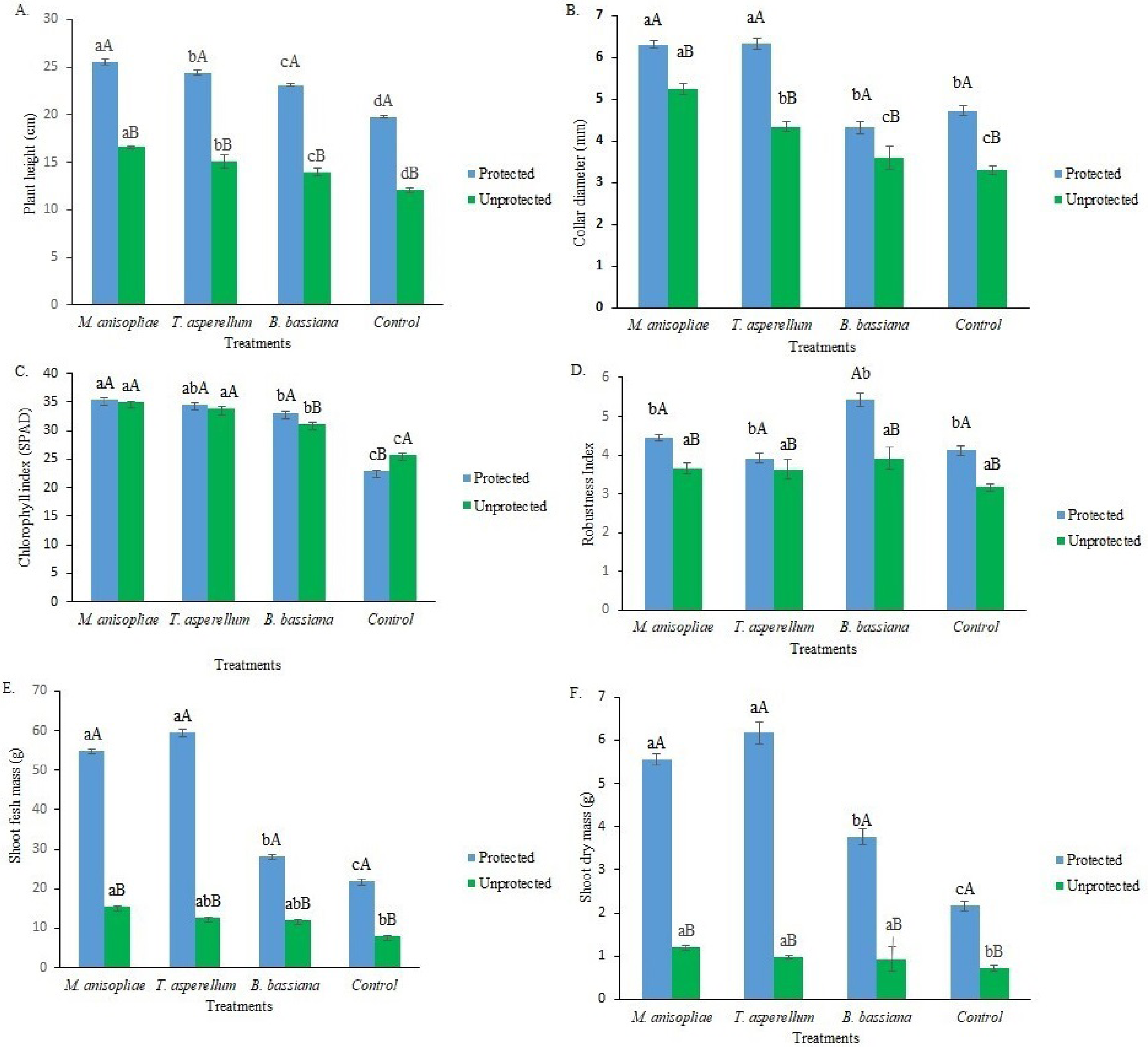
Hwang et al. (2011) reported similar results, indicating that plants treated with fungal culture filtrates (FCF) from endophytic fungi for growth promotion displayed improvements in A, E, iCE, and water use efficiency (WUE, A/E) compared with controls: 89, 27, 90, and 84%, respectively. These benefits were more significant in plants injected with FCF than in plants sprayed with FCF. It is worth noting that the application of FCF showed promising potential as a means to promote growth in plants. Endophyte-produced plant hormones can significantly affect the metabolic pathways, leading to changes in the net photosynthesis and stomatal conductance of plants (Spiering et al., 2006).

Figures 7 and 8 display the growth, biomass, and gas exchange data of jambu inoculated with *T. asperellum*, *B. bassiana*, and *M. anisopliae* in both protected and unprotected growing systems during the dry season. There was a significant interaction between the growing systems (p ≤ 0.05). Figure 7 displays the results of the Jambu growth variables, indicating a significant difference between the growing systems. Specifically,



Lowercase letters compare means within the same growing system and uppercase letters compare means among growing systems by Tukey's test (p ≤ 0.05)

Figure 6. Net photosynthesis rate (A; A), stomatal conductance (g_s; B), intercellular CO_2 concentration (C_i; C), and instantaneous carboxylation efficiency (iCE; D) of *Acmella oleracea* treated with an entomopathogenic fungus (*Beauveria bassiana* or *Metarhizium anisopliae*) or a growth promoter (*Trichoderma asperellum*) in protected and unprotected systems during the rainy season



Lowercase letters compare means within the same growing system and uppercase letters compare means among growing systems by Tukey's test (p ≤ 0.05)

Figure 7. Plant height (A), collar diameter (B), chlorophyll index (C), robustness index (D), shoot fresh mass (E), shoot dry mass (F) of *Acmella oleracea* treated with an entomopathogenic fungus (*Beauveria bassiana* or *Metarhizium anisopliae*) or a growth promoter (*Trichoderma asperellum*) in protected and unprotected systems during the dry season

protected cultivation yielded better outcomes for plant height, collar diameter, and the robustness index (Figures 7A, B and D). As stated previously, there appears to be an indirect correlation between plant height growth and the fungus' capacity to produce growth hormones. However, only *B. bassiana* treatment significantly altered the chlorophyll content (SPAD) (Figure 7C).

The temperature during the experiment in the dry season ranged from 27 to 32 °C, higher than the temperature during the rainy season, and the humidity was low. As a result, there was only 34 mm of rainfall during the month, making it the driest month. The average temperature was 27.5 °C. Overall, these conditions are less conducive to fungal growth (INMET, 2021).

In the two assessed planting periods and systems, the *M. anisopliae* and *T. asperellum* treatments resulted in superior growth, biomass, and physiological variables compared with the *B. bassiana* treatment. Similarly, Siqueira et al. (2020) demonstrated that endophytic colonization of tomato plants by *Metarhizium robertsii* and *Metarhizium humberi* increased plant growth, as evidenced by an increase in plant height, root length, and dry weight of the shoots and roots compared with non-inoculated plants.

In the protected system, jambu inoculated with *T. asperellum* and *M. anisopliae* showed the greatest accumulation of fresh mass in plant height, with an increase of 172 and 150%, respectively, compared with the control (Figure 7E). The increase from *B. bassiana* treatment was much lower (a 28% increase compared with the control). In the unprotected system, *M. anisopliae* treatment produced the highest aerial fresh mass accumulation (a 96% increase compared with control), but the *T. asperellum* and *B. bassiana* treatments also showed positive results (an increase of 59 and 52%, respectively; Figure 7E).

The gas-exchange-based physiological responses of jambu treated with fungal isolates in two growing systems during the dry season are presented in Figure 8. The protected system showed significantly higher A, g_s, and iCE (Figures 8A, B, and D) compared with the unprotected system. There were no significant differences regarding C_i between the growing systems (Figure 8C).

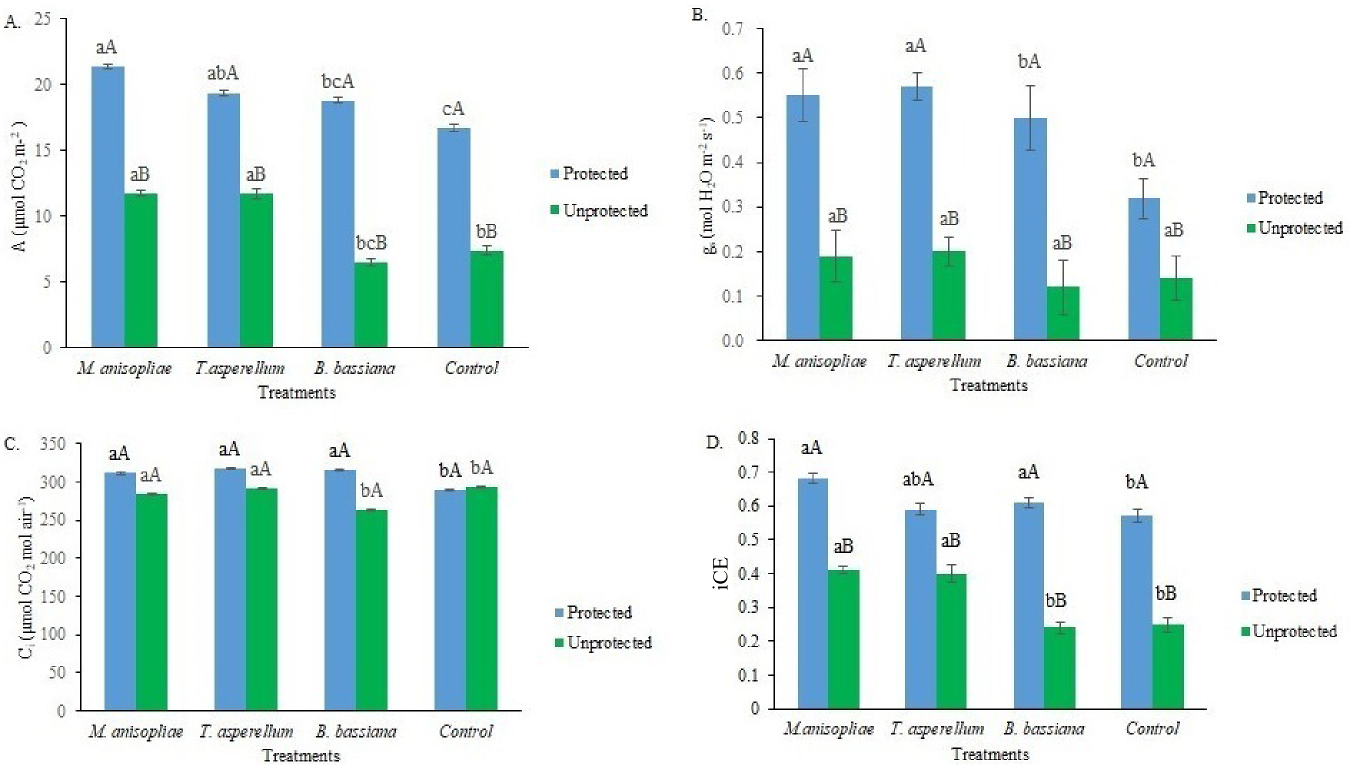
The *B. bassiana*, *M. anisopliae*, and *T. asperellum* treatments increased A, g_s, C_i, and iCE in both seasons, particularly during the rainy season. The most pronounced increases occurred in the protected cultivation system during both seasons. These changes can benefit the plant by supporting the absorption of mineral nutrients (Cho et al., 2007). Additionally, there is an increase in the production of photoassimilates, which are used to form tissues in the plant, resulting in a positive correlation between photosynthesis and biomass production (Lemos Neto et al., 2017).

An increase in C_i typically corresponds to an increase in g_s. Consequently, the primary cause of lower photosynthetic performance is the limitation of stomatal aperture, which is due to an increase in CO_2 diffusion into the substomatal chamber with a larger stomatal aperture (Augé et al., 2015). Microorganisms are likely to play a role in increasing stomatal aperture. Most of the treatments in both evaluated growing systems resulted in reduced carboxylation efficiency, implying ineffective utilization of CO_2 in photosynthesis (Konrad et al., 2005).

The results from the present study confirm the hypothesis that the entomopathogenic fungi *B. bassiana* and *M. anisopliae* and the growth promoter *T. asperellum* promote jambu growth in the suspended cultivation system and enhance photosynthetic performance in both the protected and unprotected systems. The protected system demonstrated the most favorable results in terms of growth, development, physiological responses, and microorganism reactions, probably due to the more favorable conditions for microorganisms and plants.

The findings indicate that jambu inoculated with the entomopathogenic fungi *M. anisopliae* and *B. bassiana* in a protected environment exhibit improved growth and biomass variables as well as increased photosynthetic activity that is similar to the growth-promoting activities attributed to *T. asperellum* in previous research (Kumar et al., 2021). These findings are significant because they demonstrate that entomopathogenic fungi can also act as biostimulants to stimulate jambu growth, an action that is in addition to their global recognition and use as controllers of insects. These microorganisms employ both direct and indirect mechanisms to promote growth, including nutrient acquisition and phytohormone production. In this case, their actions are vital for both the development and preventative protection of the plants. Despite a history of pests previously observed in the commercial area used for different crops, no pests were observed during the trials.

The use of beneficial fungi, such as those evaluated in this study, can improve jambu's gas exchange variables and thus be very advantageous for its development. Even in climatic conditions and planting systems that were not favorable to the satisfactory development of jambu, the microorganisms could increase the transpiration rate of jambu, denoted by changes in some of the evaluated variables, as well as other photosynthetic



Lowercase letters compare means within the same system and upper case letters compare means among systems by Tukey's test ($p \leq 0.05$)

Figure 8. Net photosynthesis rate (A; A), stomatal conductance (g_s; B), intercellular CO_2 concentration (C_i; C), and instantaneous carboxylation efficiency (iCE; D) of *Acmella oleracea* treated with an entomopathogenic fungus (*Beauveria bassiana* or *Metarhizium anisopliae*) or a growth promoter (*Trichoderma asperellum*) in protected and unprotected systems during the dry season

variables. The present study is the first to demonstrate that the fungi *B. bassiana*, *M. anisopliae*, and *T. asperellum* are capable of significantly promoting jambu growth, especially during periods of climatic conditions in which the plant can respond better, as well as the type of growing system most suited to the crop.

Conclusions

1. The application of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* promotes growth, increasing the biometric and physiological variables of *A. oleracea*, similarly to the growth promoter *T. asperellum*. However, *M. anisopliae* outperforms *T. asperellum*, particularly during the rainy seasons and in a protected cultivation system.

2. The optimum growth of *A. oleracea* occurs during the rainy season in a protected and suspended system.

3. The entomopathogenic microorganisms *M. anisopliae* and *B. bassiana* and the growth promoter *T. asperellum* are promising growth promoters that can improve biometric and physiological variables. They represent a practical and ecologically sound alternative for growing *A. oleracea* in peri-urban commercial agricultural areas.

4. The consistent use of these microorganisms could lead to sustainable agricultural practices, providing a viable solution to current challenges.

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