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RESEARCH ARTICLE

Application of *Trichoderma asperellum* as a biofertilizer to optimize plant growth in wick hydroponic systems

Kenanga Murni Abdul Razak[1], Nur Ain Izzati Mohd Zainudin[1, 2*], Nor Azwady Abd Aziz[1], and Mohd Hafiz Ibrahim[1]

[1]Universiti Putra Malaysia, Faculty of Science, Department of Biology, 43400, Serdang, Selangor, Malaysia.
[2]Universiti Putra Malaysia, Institute of Plantation Studies, Laboratory of Sustainable Agronomy and Crop Protection, 43400, Serdang, Selangor, Malaysia.
*Corresponding author (ainizzati@upm.edu.my)
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ABSTRACT

Hydroponic approaches are increasingly being applied in sustainable agriculture due to their efficient resource utilization and controlled environmental conditions. This study evaluated the potential of *Trichoderma asperellum* as a biofertilizer in a wick hydroponic system, which emphasis on its effects on plant growth, root development, and water and nutrient uptake in pak choy (*Brassica rapa* L. subsp. *chinensis* (L.) Hanelt). The treatment involving *T. asperellum* application prior to sowing, combined with full-strength nutrient solutions A and B, resulted in the highest colony-forming unit (cfu) counts, plant height (16.07 ± 2.62 cm), and number of leaves (14.33 ± 1.33) compared with the control and other treatments. In addition, these plants exhibited greater water content (0.51 ± 0.18) and chlorophyll content (32.00 ± 1.82 SPAD units; 16.05 ± 0.06 mg g^{-1} *via* spectrophotometer), indicating enhanced plant health and vigor. In conclusion, *T. asperellum* can significantly enhance the growth and development of pak choy in wick hydroponic systems, highlighting its potential as a sustainable biofertilizer for hydroponic agriculture.

Key words: Biostimulant, chlorophyll content, microbial fertilizer, soil-free cultivation, *Trichoderma* species.

INTRODUCTION

Biofertilizers are biological formulations containing beneficial microorganisms that enhance nutrient uptake, stimulate plant growth, and protect crops from diseases (Odoh et al., 2020). Various microbes have been identified as plant growth-promoting organisms (Zhao et al., 2023), and they are widely applied as sustainable alternatives to chemical fertilizers, which can degrade soil quality over time. According to Pal et al. (2015), integrating biofertilizers with chemical fertilizers can increase crop yields by 10% to 40% and reduce chemical fertilizer use by approximately 25% to 30%.

Trichoderma species are widely recognized as biocontrol agents and biofertilizers due to their ability to produce phytohormones and stimulate plant growth (Hasan et al., 2021; Reghmit et al., 2022; Gonçalves et al., 2023). They enhance nutrient solubilization, improve root architecture, and secrete growth-promoting compounds such as cytokinins, gibberellins, and indoleacetic acid, thereby supporting plant health and productivity (He et al., 2020). While their role in soil-based systems is well studied, their potential in hydroponics remains less explored.

Hydroponics, a soil-less cultivation technique using nutrient-rich water solutions, is especially useful in areas with limited arable land (Al-Shrouf, 2017). This method offers precise control of nutrients, water, light, and temperature, leading to consistent yields and reduced risks from climate variability (Kailashkumar et al., 2023). A commonly used hydroponic nutrient solution is the AB formulation, which provides essential nutrients and improves crop yield (Pangaribuan et al., 2022).

This study aims to investigate the potential of *T. asperellum* to enhance plant growth and improve nutrient and water uptake in a hydroponic wick system. Exploring this could provide insights into the application of *Trichoderma* spp. for sustainable plant production in controlled environments.

MATERIALS AND METHODS

Plant and fungal source

Pak choy (*Brassica rapa* L. subsp. *chinensis* (L.) Hanelt, Dwarf type GE380) seeds were obtained from D'Syira Enterprise, Serdang, Selangor, Malaysia. The seeds were cultivated using the wick hydroponic system in a plant house located at the Department of Biology, Faculty of Science, Universiti Putra Malaysia (UPM), Selangor, Malaysia. Environmental conditions were maintained at 67.5% humidity, 2000 lux light intensity, with daytime and nighttime temperatures of 28-30 °C and 24-26 °C, respectively. *Trichoderma asperellum* strain B1902 (Figure 1) was obtained from the Laboratory of Mycology, Department of Biology, Faculty of Science, UPM. The fungal culture was originally isolated from banana plantation soil in Dengkil, Selangor, Malaysia, and its identification was confirmed through morphological and molecular characterization (Hasan et al., 2020).

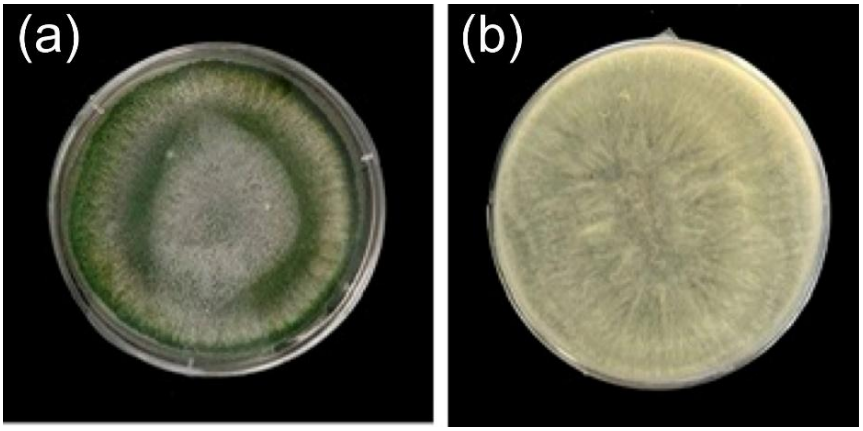

Figure 1. *Trichoderma asperellum* B1902 culture on PDA after 7 d of incubation. Top view (a) and bottom view (b) of the fungal culture.

Preparation of fungal inoculum

The fungus was cultured on 15 cm diameter potato dextrose agar (PDA) plates and incubated at room temperature (28 ± 2 °C) for 7 d. Spores were harvested from the 7-d-old PDA cultures of *T. asperellum* B1902 using sterile distilled water and scraped with a sterile glass hockey stick. The resulting conidial suspension was filtered through a double layer of cheesecloth to remove mycelial debris (Saharan et al., 2015). The filtered suspension was transferred into 50 mL centrifuge tubes and pre-frozen at -80 °C for 20 h to prepare for freeze-drying. The samples were then freeze-dried using a freeze dryer (FreeZone 6L, Labconco Corporation, Kansas City, Missouri, USA) for 20 h (Liu et al., 2014).

Plant preparation and hydroponic setup

The wick system kit was purchased from AeroFarmCity Company (Shah Alam, Selangor, Malaysia), while nutrient solutions A and B were obtained from TZ Agro Riyadh Company, Serdang, Selangor. Solution A consists of 18 kg calcium nitrate ($Ca(NO_3)_2$), 3 kg potassium nitrate (KNO_3), 180 g Fe, and water to make up a total volume of 100 L. Solution B consists of 9 kg potassium nitrate (KNO_3), 11 kg magnesium sulphate ($MgSO_4$), 4 kg monopotassium phosphate (MKP), 50 g Mn, 34 g Zn, 140 g boric acid (H_3BO_3), 7 g Cu, 4 g sodium molybdate (Na_2MoO_4), and water to make up a total volume of 100 L. The nutrient solution was prepared by mixing 20 mL solution A and 20 mL solution B into 10 L water. Coco peat was used as the planting medium, with 300 g placed

in each pot. Coco peat is ideal for hydroponics due to its high-water retention, good drainage, and ability to support nutrient absorption and root aeration.

Three pak choy seedlings were planted in each pot, with four different treatments and four replicates per treatment. The treatments involved the application of *T. asperellum* B1902 freeze-dried powder at a concentration of 4×10^7 cfu g^{-1} mixed with 300 g coco peat (Table 1). Plant growth was monitored daily, and nutrient solution levels in the hydroponic bottles were replenished weekly. The experiment was repeated twice. Four treatments were established: (i) Control with nutrient solutions A and B only, (ii) T1 with solutions A and B plus *T. asperellum* B1902 applied 14 d before sowing and at 14 and 28 d after sowing, (iii) T2 with solutions A and B plus B1902 applied at 14 and 28 d after sowing, and (iv) T3 with half-strength solutions A and B plus B1902 applied at 14 and 28 d after sowing.

Table 1. *Trichoderma* treatment design. Solution A consists of 18 kg calcium nitrate ($Ca(NO_3)_2$), 3 kg potassium nitrate (KNO_3), 180 g Fe, and water to make up a total volume of 100 L. Solution B consists of 9 kg potassium nitrate (KNO_3), 11 kg magnesium sulphate ($MgSO_4$), 4 kg mono potassium phosphate, 50 g Mn, 34 g Zn, 140 g boric acid (H_3BO_3), 7 g Cu, 4 g sodium molybdate (Na_2MoO_4), and water to make up a total volume of 100 L. The nutrient solution was prepared by mixing 20 mL solution A and 20 mL solution B into 10 L water.

Treatments	Control	T1	T2	T3
Solutions A and B	Yes	Yes	Yes	Yes (half-strength)
Freeze-dried *T. asperellum* B1902 (4×10^7 cfu 300 g^{-1} cocopeat) treatment	None	Yes 14 d before, 14 and 28 d after sowing	Yes 14 and 28 d after sowing	Yes 14 and 28 d after sowing

Determination of survival of *Trichoderma* based on colony-forming units (cfu)

The survival of *Trichoderma* in coco peat was assessed using the soil dilution technique. Samples were collected on days 10, 20, 30, and 40 after inoculation. For each sampling, 10 g inoculated medium was mixed with 100 mL sterile distilled water and agitated at 100 rpm for 10 min using an orbital shaker. Serial dilutions up to 10^3 were prepared, and 1 mL from each dilution was plated on *Trichoderma* selective medium (TSM) (Elad et al., 1981). The plates were incubated at 28 ± 2 °C for 4 d. Colonies were counted daily and recorded as cfu. Data were analyzed using one-way ANOVA via the Statistical Package for the Social Sciences (SPSS) version 29.0 (IBM Corp., Armonk, New York, USA).

Measurement of growth parameters

Growth parameters such as plant height, number of leaves, fresh and dry weight, root and shoot lengths, and chlorophyll content were recorded. Plant height and number of leaves were measured on days 10, 20, 30, and 40. After 40 d, the plants were harvested for biomass analysis. Shoots were cut at the base, and roots were gently washed with tap water to minimize loss. Excess moisture was removed with paper towels. Fresh weights of shoots and roots were recorded using a digital scale. For dry weight, plant samples were oven-dried at 70 °C for 24 h (Hasan et al., 2020) and then reweighed. The root-to-shoot ratio and water content were calculated. Water content was determined using the formula by Xiong and Choong (2014): Water content (w/w) = (Total fresh weight – Total dry weight)/Total fresh weight.

Chlorophyll content determination

Chlorophyll content was assessed using both a SPAD meter and spectrophotometric analysis. Leaf greenness was measured using a SPAD-502 chlorophyll meter (Konica Minolta, Osaka, Japan) with readings taken at three points per leaf from the second or third youngest fully expanded leaf. For spectrophotometric analysis, 250 mg leaf tissue were ground in 10 mL 80% acetone, centrifuged at 3000 rpm for 10 min, and the supernatant was brought to a final volume of 25 mL. The absorbance of the extract was measured at 649 and 665 nm using a

SP-UV300 spectrophotometer (Spectrum Instrument, Shanghai, China). The chlorophyll *a* + *b* concentration (mg g^{-1}) was calculated using the equation from Fritschi and Ray (2007):

$$\text{Chl } a + b = [(5.1 \times A\ 665) + (20.04 \times A\ 649)]\ V/m$$

where A is absorbance value at corresponding wavelength, V is volume of chlorophyll solution (L), m is weight of leaf samples (mg). Statistical analysis for growth and chlorophyll data was performed using one-way ANOVA in SPSS v29.0.

RESULTS AND DISCUSSION

Determination of *Trichoderma* colony-forming units (cfu)

The data suggest that *Trichoderma* thrives under this treatment, with steady reproduction and survival. The cfu value in T2 (solution A and B supplemented with *Trichoderma asperellum* B1902 applied on 14 and 28 d after sowing) continuously increased, but T3 (half-strength solution A and B supplemented with B1902 applied 14 and 28 d after sowing) showed an increase in fungal growth, peaking at 175.50 ± 5.9×10^3 cfu g^{-1} on day 30 and decreasing to 156.33 ± 8.6×10^3 cfu g^{-1} by day 40 (Table 2).

Table 2. Colony forming unit (cfu) of *Trichoderma asperellum* B1902 on days 10, 20, 30, and 40. *Colony forming unit (cfu) g^{-1} of *Trichoderma* on Trichoderma selective medium (TSM). Values represent mean ± SE. Different letters in different columns indicate significant differences between media. The nutrient solution was prepared by mixing 20 mL solution A and 20 mL solution B into 10 L water. Solution A: 18 kg $Ca(NO_3)_2$, 3 kg KNO_3, 180 g Fe, and 100 L water; Solution B: 9 kg KNO_3, 11 kg $MgSO_4$, 4 kg MKP, 50 g Mn, 34 g Zn, 140 g H_3BO_3, 7 g Cu, 4 g Na_2MoO_4, and 100 L water; Control: Solution A and B; T1: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 d and 28 d after sowing; T2: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d and 28 d after sowing; T3: Half-strength solution A and B supplemented with B1902 applied 14 d and 28 d after sowing.

Days of inoculation	*T. asperellum* B1902 (mean ×10^3 cfu g^{-1})			
	Control	T1	T2	T3
10	3.67 ± 0.84^a	113.00 ± 9.06^b	2.67 ± 0.71^a	4.33 ± 0.71^a
20	15.83 ± 0.87^a	101.33 ± 4.10^c	66.67 ± 5.65^b	67.50 ± 5.60^b
30	10.67 ± 0.84^a	222.17 ± 20.43^b	173.83 ± 6.77^b	175.50 ± 5.98^b
40	7.00 ± 1.06^a	228.00 ± 37.22^b	178.67 ± 12.21^b	156.33 ± 8.69^b

Meanwhile, control showed the lowest fungal growth from day 10 to day 40, compared to the other three treatments. The cfu values increased across the 4 d but were slightly higher than T2 on day 10. It increased to 15.83 ± 0.87×10^3 cfu g^{-1} on day 20 and decreased to 10.67 ± 0.84×10^3 and 7.00 ± 1.06×10^3 cfu g^{-1} on day 30 and day 40, respectively (Table 2, Figure 2).

The consistently high cfu counts in T1 (solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 and 28 d after sowing) demonstrated how the initial colonization of *T. asperellum* lays the groundwork for long-term persistence. This rapid colonization indicated that the fungal population is strong enough to survive in the hydroponic medium under various environmental conditions, such as nutrient fluctuations and microbial competition. Moreover, *T. asperellum* can create protective layer showing that its population is flourishing in the medium and engaging in active contact with plant roots. This protective layer not only enhances nutrient uptake but also serves as a barrier against root-infecting pathogens, which is critical for maintaining plant health. The interaction ensures a consistent flow of nutrients, promoting fungal survival and growth. These findings align with the research by Yao et al. (2023), which proved that *Trichoderma* can swiftly adsorb to crop roots for propagation after 24 h in the soil, and its hyphae swiftly envelop the roots to form a protective layer, shielding them from pathogens and environmental stresses.

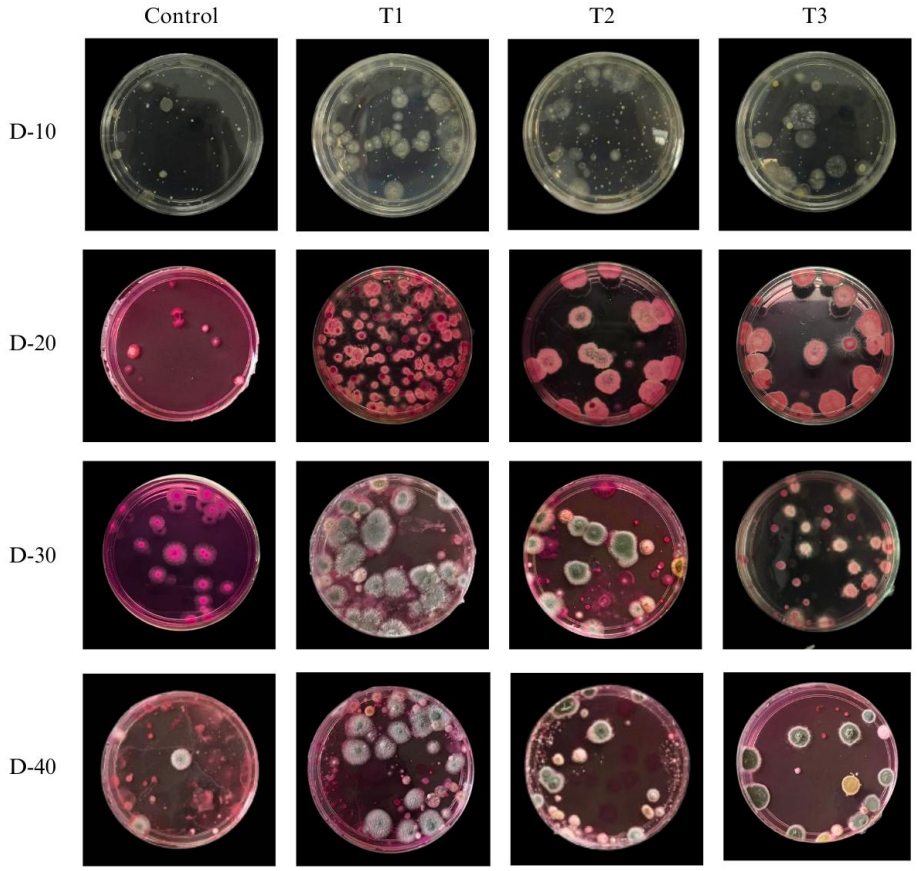

Figure 2. Growth of *Trichoderma asperellum* B1902 on *Trichoderma* selective medium (TSM) on days 10, 20, 30 and 40. Control: Solution A and B; T1: solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 and 28 d after sowing; T2: solution A and B supplemented with *T. asperellum* B1902 applied on 14 and 28 d after sowing; T3: half-strength solution A and B supplemented with B1902 applied 14 and 28 d after sowing.

According to a study by Mahmood et al. (2024), *Trichoderma* species can adapt to low-nutrient environments and still achieve significant growth and biocontrol effectiveness. *Trichoderma* species can effectively use the organic matter that is present in the soil or hydroponic solutions, which could make up for T3's lower nutrient concentration. Furthermore, the presence of plant roots in both T2 and T3 may be advantageous to the fungus because the roots release a variety of compounds that can promote *Trichoderma* growth even in the absence of nutrients. *Trichoderma* in the rhizosphere can also promote plant growth, which may in turn give the fungus additional resources and sustain its cfu count.

Hence, the findings of this study highlight the importance of early inoculation in maximizing *Trichoderma* colonization and efficacy in both soil-based and hydroponic systems. Early establishment of *T. asperellum* can help maintain root zone dominance, enhance nutrient dynamics, and ultimately support healthier plant growth and increased resilience to environmental stressors. These findings have practical implications for sustainable farming systems that utilize biological control agents like *Trichoderma* to reduce reliance on chemical inputs.

Trichoderma enhances the growth of pak choy plants

Table 3 shows the effects of *T. asperellum* B1902 on the growth of pak choy plants in terms of plant height and number of leaves. Across all 4 wk, both the height and number of leaves of pak choy plants showed increasing values in both the control and treated media. The height of T1 and T2 pak choy plants was higher than that of the control set, while T3 was the lowest. Plant height in T1, the medium which differs in extra application of

Trichoderma before sowing, remained the highest from 5.25 ± 0.13 cm on day 10 to 16.07 ± 2.62 cm on day 40 (Table 3). Plant height of T3, on the other hand, stayed the lowest despite the presence of *Trichoderma* due to the half-strength application of solutions A and B. The height was 3.57 ± 0.12 cm on day 10 and increased slightly until day 40 by 6.10 ± 1.07 cm. In comparison to the control, T2 was higher. The growth trend of the pak choy heights in each medium is shown in Figure 3.

Table 3. Plant height and number of leaves of pak choy plants on days 10, 20, 30, and 40. Values represent mean ± SE. Different letters in different rows indicate significant differences between media. The nutrient solution was prepared by mixing 20 mL solution A and 20 mL solution B into 10 L water. Solution A: 18 kg $Ca(NO_3)_2$, 3 kg KNO_3, 180 g Fe, and 100 L water; Solution B: 9 kg KNO_3, 11 kg $MgSO_4$, 4 kg MKP, 50 g Mn, 34 g Zn, 140 g H_3BO_3, 7 g Cu, 4 g Na_2MoO_4, and 100 L water; Control: Solution A and B; T1: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 d and 28 d after sowing; T2: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d and 28 d after sowing; T3: Half-strength solution A and B supplemented with B1902 applied 14 d and 28 d after sowing.

Plant height (cm)				
Day of inoculation	D10	D20	D30	D40
Control	3.90 ± 0.19[a]	5.68 ± 0.75[a]	6.02 ± 1.09[a]	6.38 ± 1.39[a]
T1	5.25 ± 0.13[b]	8.12 ± 0.34[a]	12.3 ± 1.12[c]	16.07 ± 2.62[b]
T2	3.95 ± 0.11[a]	6.57 ± 0.55[a]	9.17 ± 1.10[b]	11.28 ± 2.27[a]
T3	3.57 ± 0.12[a]	4.63 ± 0.16[a]	5.40 ± 0.44[a]	6.10 ± 1.07[a]
Number of leaves				
Day of inoculation	D10	D20	D30	D40
Control	4.50 ± 0.22[a]	7.17 ± 0.60[a]	8.50 ± 0.99[a]	9.00 ± 1.16[a]
T1	4.83 ± 0.31[a]	9.33 ± 0.33[b]	11.50 ± 0.43[b]	14.33 ± 1.33[b]
T2	4.50 ± 0.22[a]	7.50 ± 0.56[a]	8.50 ± 0.92[a]	9.33 ± 1.15[a]
T3	4.17 ± 0.31[a]	6.50 ± 0.43[a]	7.50 ± 0.76[a]	7.83 ± 1.01[a]

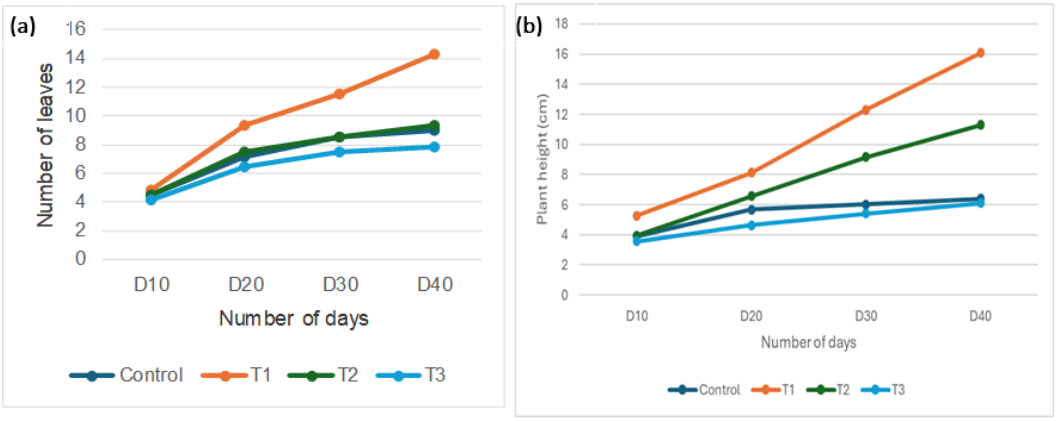

Figure 3. Effect of *Trichoderma asperellum* B1902 on height (a) and number of leaves (b) of pak choy plants on days 10, 20, 30 and 40. The nutrient solution was prepared by mixing 20 mL solution A and 20 mL solution B into 10 L water. Solution A: 18 kg $Ca(NO_3)_2$, 3 kg KNO_3, 180 g Fe, and 100 L water; Solution B: 9 kg KNO_3, 11 kg $MgSO_4$, 4 kg MKP, 50 g Mn, 34 g Zn, 140 g H_3BO_3, 7 g Cu, 4 g Na_2MoO_4, and 100 L water; control: solution A and B; T1: solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 and 28 d after sowing; T2: solution A and B supplemented with *T. asperellum* B1902 applied on 14 and 28 d after sowing; T3: half-strength solution A and B supplemented with B1902 applied 14 and 28 d after sowing.

The early application allows *Trichoderma* to improve soil microbial health and create a protective and nutrient-rich environment for seeds. Faster germination, robust early growth, and improved overall biomass occur due to pre-conditioning of the growth medium. *Trichoderma* encourages root development, plant growth, and seed germination, as well as an increase in the plant's height and weight. In this study, plants in T1 were higher than those in other treatments across 4 wk. Significant variation was also observed in the number of leaves in different treatments. It was observed that T1 has the maximum number of leaves and T3 has the minimum. The positive effects of *Trichoderma* spp. on plant growth, including root development, shoot height, leaf production, and overall plant vigor, are well-documented across a variety of plant species. *Trichoderma* spp. produce auxins and other phytohormones that increase root surface area, improving nutrient and water uptake, which is particularly advantageous under nutrient-limited conditions.

Trichoderma has also been shown to increase root development and seedling height, which enhances plant vigor overall. A study by Hasan et al. (2020) on the ability of *T. asperellum* in inducing tomato growth on two different media, determined that the shoot and root length of cherry tomato plants grown on coconut fiber inoculated with *T. asperellum* are significantly higher than those grown on oil palm empty fruit bunch (EFB) with the application of the fungus. Sugianti and Zulhaedar (2017) also claimed that the organic fertilizer enriched with *Trichoderma* considerably increased plant height and leaf production in mustard and green mustard varieties.

The slower growth and fewer number of leaves of plants in T3 may occur because of the plants' inability to sustain the metabolic activity under reduced nutrient availability. Despite that, the presence of *Trichoderma* in the medium treated with half-strength solutions A and B improved nutrient uptake efficiency, resulting in minimal or no adverse effects on plant growth. This finding aligns with the study by Shabani et al. (2024), which determined that lettuce treated with *T. harzianum* and 50% concentration of nutrient solution showed a similar growth trend to plants in the *Trichoderma*-inoculated treatment with 100% application of nutrient solution. Nonetheless, it is crucial to note that *Trichoderma* is not a replacement for essential nutrients, as severe nutrient deficiencies may still impair plant growth and yield.

Therefore, the growth and development of hydroponic plants largely depend on solutions A and B, as solution A provides essential macronutrients, including calcium and potassium nitrate, while solution B supplies micronutrients and additional compounds such as magnesium sulfate. A reduction in the concentration of these solutions may result in nutrient shortages, which may hinder plant growth, reduce yields, and affect overall plant health. However, *Trichoderma* can mitigate the effects of moderate nutrient shortages by producing organic acids and extracellular enzymes that solubilize nutrients like P and essential micronutrients, making them more accessible to plants. Additionally, *Trichoderma* enhances plant resistance to abiotic stresses, such as nutrient limitations, by generating growth-promoting compounds and activating plant defense mechanisms, thereby supporting plant growth even under nutrient-deficient conditions.

Trichoderma enhances plant root development and water content

Table 4 demonstrates the differences in shoot and root lengths, root/shoot ratio, and water content of pak choy plants in four different treatments on day 42. Pak choy plants treated with *T. asperellum* in T1 had the highest values of shoot and root lengths at 16.07 ± 2.62 and 12.00 ± 2.00 cm, respectively. The lengths were the lowest in T3, in which the shoot length was 6.10 ± 1.07 cm and the root length was 4.68 ± 0.45 cm. However, a significant difference between T3 and the control set can only be observed in shoot length and root/shoot ratio. Meanwhile, plants in the control set had significantly lower values of shoot and root lengths compared to those in T2.

Figure 4 shows the effect of *T. asperellum* B1902 on shoot and root lengths, respectively. Regarding root/shoot ratio, pak choy plants with the highest value were in the control set at 0.82 ± 0.29, followed by T3 at 0.81 ± 0.05 and T1 at 0.75 ± 0.33. Meanwhile, the root/shoot ratio of those in T2 was the lowest at 0.66 ± 1.37. A higher root/shoot ratio indicates that roots are longer, searching for the source of nutrients. When *T. asperellum* was applied, there was an increase in the water content of the pak choy plants.

The highest water content can be seen in the plants grown in T1 with the addition of B1902 applied 14 d before sowing by 0.51 ± 0.18 w/w, while the lowest water content was recorded in those in T3 by 0.26 ± 0.37 w/w. Pak choy plants in T1 gave the highest value in shoot and root lengths and water content among the four treatments. However, it shows nonsignificant difference from the other treatments in root length and water

content. On the other hand, it is significant with T2 in shoot length and shoot/root ratio. The values in the shoot/root ratio were slightly different in trend, as control was the highest, while T2 was the lowest.

Table 4. Root/shoot ratio and water content of pak choy plants on day 42. Values represent mean ± SE. Different letters in different rows indicate a significant difference between media. The nutrient solution was prepared by mixing 20 mL of solution A and 20 mL solution B into 10 L water. Solution A: 18 kg $Ca(NO_3)_2$, 3 kg KNO_3, 180 g Fe, and 100 L water; Solution B: 9 kg KNO_3, 11 kg $MgSO_4$, 4 kg MKP, 50 g Mn, 34 g Zn, 140 g H_3BO_3, 7 g Cu, 4 g Na_2MoO_4, and 100 L water; Control: Solution A and B; T1: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 d and 28 d after sowing; T2: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d and 28 d after sowing; T3: Half-strength solution A and B supplemented with B1902 applied 14 d and 28 d after sowing.

Treatment	Shoot length	Root length	Root/shoot ratio	Water content
	cm	cm		w/w
Control	6.38 ± 1.39[a]	5.10 ± 0.94[a]	0.82 ± 0.29[b]	0.41 ± 0.19[a]
T1	16.07 ± 2.62[b]	12.00 ± 2.00[b]	0.75 ± 0.33[a]	0.51 ± 0.18[a]
T2	11.28 ± 2.27[b]	7.23 ± 1.54[a]	0.66 ± 1.37[a]	0.41 ± 0.18[a]
T3	6.10 ± 1.07[a]	4.68 ± 0.45[a]	0.81 ± 0.05[b]	0.26 ± 0.37[a]

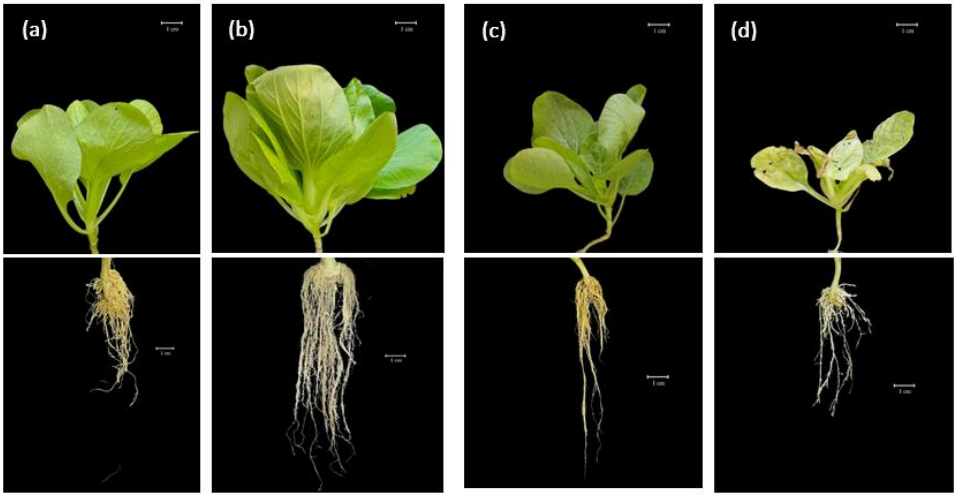

Figure 4. Effect of *Trichoderma asperellum* B1902 on shoot growth and root growth of pak choy plants on day 42. The nutrient solution was prepared by mixing 20 mL solution A and 20 mL solution B into 10 L water. Solution A: 18 kg $Ca(NO_3)_2$, 3 kg KNO_3, 180 g Fe, and 100 L water; Solution B (9 kg KNO_3, 11 kg $MgSO_4$, 4 kg MKP, 50 g Mn, 34 g Zn, 140 g H_3BO_3, 7 g Cu, 4 g Na_2MoO_4, and 100 L water. Control: solution A and B (a); solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 and 28 d after sowing (b); solution A and B supplemented with *T. asperellum* B1902 applied on 14 and 28 d after sowing (c); T3: half-strength solution A and B supplemented with B1902 applied 14 and 28 d after sowing (d).

Trichoderma inoculation has been linked in some studies to a higher root/shoot ratio, which suggests improved root growth in comparison to shoot growth. For example, a study by Yao et al. (2023) found that inoculating some wheat varieties with *Trichoderma* increased the ratio of root/shoot biomass, indicating a shift towards greater root development. *Trichoderma harzianum* or *T. asperellum* can promote root elongation and branching in adverse growing environments, such as nutrient-poor soils or drought stress. It is well known that these strains generate phytohormones, like auxins, that encourage root development and raise the root/shoot ratio.

In this study, pak choy plants in T1 and T2 have a lower root/shoot ratio compared to T3 and the control group, indicating that the conditions of T1 and T2 treatments are favorable due to the presence of *T. asperellum*. Although T3 was also *Trichoderma*-inoculated, it showed a significant difference ($p > 0.05$) from T1 and T2, as nutrient deficiency restricts the root development and shoot elongation. This is because the plants prioritize survival overgrowth, thereby diminishing the overall impact of the fungus. This study's findings align with the study by Sehim et al. (2023), which identified an increase in shoot fresh weight during the *Trichoderma-Fusarium* interactions, suggesting that *T. asperellum* promotes shoot development, which leads to a decreased root/shoot ratio. The data revealed an increase in length of shoots and number of leaves, suggesting that the plant devoted more resources to growth above ground. This suggested *T. asperellum* produced plant growth regulators like gibberellins and cytokinins, which encourage shoot elongation and leaf expansion. *Trichoderma* colonization may also improve nutrient availability and uptake, which could lessen the need for the plant to make significant investments in root expansion and lower the root/shoot ratio.

Awad-Allah et al. (2022) stated that regardless of the presence or absence of the *Fusarium solani* pathogen, cherry tomato plants treated with *T. harzianum* alone or in combination with *T. viride*, showed notable effects on plant fresh and dry weights as well as a stronger root/shoot ratio when compared to non-infected controls. Plant fresh and dry weights increased most when a mixture of the two *Trichoderma* isolates was treated under infection stress, improving the root/shoot ratio. This indicated that *T. viride* and *T. harzianum* appear to have synergistic effects as biocontrol agents.

Under optimal conditions, *T. asperellum* decreases the ratio by promoting shoot growth due to the production of plant growth regulators. Additionally, by promoting nutrient solubilization and increasing root efficiency, *Trichoderma* improves nutrient availability and uptake. Plants consequently devote more resources to the growth of their shoots rather than making significant investments in the expansion of their roots, which results in a lower root/shoot ratio. As nutrients are easily accessible in this situation, the plant can afford to give above-ground growth priority to optimize photosynthesis and biomass production.

The water content of plants in T1 was also higher compared to control, T2 and T3, proving that *T. asperellum* aids in improving water uptake and increasing water content. The findings are supported by the study by Oljira et al. (2020) that identified that plants grown from seeds coated with *Trichoderma* isolates showed significantly elevated water use efficiency, suggesting that the fungal isolates dramatically improved efficiency compared to the bacterial isolate and control. This is because *Trichoderma* can colonize plant roots and aid in the plants' ability to withstand abiotic stressors, including drought (Oljira et al., 2020). Similar results can be observed in a study by Hasan et al. (2021), which obtained increasing water content in the shoots and roots of tomato plants that were treated with *T. asperellum*.

Furthermore, Yu et al. (2023) examined the effect of *T. longibrachiatum* improving drought resistance and growth of *Pinus massoniana* (Masson pine), revealing increased plant growth under drought stress and increased uptake of N, P, and K. This suggests that *Trichoderma* improved the absorption of water and nutrients. In addition, a study by Cañada-Coyote et al. (2021) involving mutants of *T. harzianum* revealed that plants under drought stress that were inoculated with a particular mutant had a higher relative water content (82.9%) than plants inoculated with the wild-type strain. This suggests that some strains of *Trichoderma* may be more successful in enhancing plant water retention in stressful situations.

Trichoderma stimulates chlorophyll content

Chlorophyll content of plants' leaves in T1 is significantly highest at 32.00 ± 1.82 on SPAD and 16.05 ± 0.06 on spectrophotometer (Table 5). In contrast, the values are significantly lower for those in T3, where the SPAD meter showed a reading of 5.27 ± 1.18, and it was lower in the spectrophotometer at 0.90 ± 0.36. In this study, pak choy plants in T1 with healthier and darker green colour of leaves resulted in higher chlorophyll content, compared to the control set and the other treatments, T2 and T3, using both methods. The T1 had the highest chlorophyll content, according to both methods, followed in decreasing order by T2, control, and T3.

The findings depict that *Trichoderma* positively affects the chlorophyll synthesis or chloroplast activity in the plants. This is because of the induction of plant growth regulators, as stated in a study by Yao et al. (2023), where indole-3-acetic acid (IAA), gibberellins (GA), abscisic acid (ABA), and cytokinins are among the plant growth regulators produced by *Trichoderma* species. These substances are essential for increasing the synthesis of chlorophyll, which improves plant health. In comparison to the control group, the study discovered that

Trichoderma inoculation increased the amount of chlorophyll *a*, chlorophyll *b*, and total chlorophyll in the leaves of treated plants. These growth regulators work together to support plant growth, improve photosynthetic efficiency, and boost overall vitality, which leads to an increase in chlorophyll levels.

Table 5. Chlorophyll content of pak choy plants on day 42 using SPAD chlorophyll meter and spectrophotometer. Values represent mean ± SE. Different letters in different rows indicate a significant difference between media. The nutrient solution was prepared by mixing 20 mL solution A and 20 mL solution B into 10 L water. Solution A: 18 kg $Ca(NO_3)_2$, 3 kg KNO_3, 180 g Fe, and 100 L water; Solution B: 9 kg KNO_3, 11 kg $MgSO_4$, 4 kg mono potassium phosphate, 50 g Mn, 34 g Zn, 140 g H_3BO_3, 7 g Cu, 4 g Na_2MoO_4, and 100 L water; Control: Solution A and B; T1: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d before, 14 d and 28 d after sowing; T2: Solution A and B supplemented with *T. asperellum* B1902 applied on 14 d and 28 d after sowing; T3: Half-strength solution A and B supplemented with B1902 applied 14 d and 28 d after sowing.

	Chlorophyll content	
Treatment	SPAD chlorophyll meter	Spectrophotometer (Chl *a* + *b*)
	SPAD value	mg g^{-1}
Control	22.50 ± 1.84^{b}	10.03 ± 0.07^{b}
T1	32.00 ± 1.82^{c}	16.05 ± 0.06^{c}
T2	26.37 ± 1.13^{b}	10.10 ± 0.07^{b}
T3	5.27 ± 1.18^{a}	0.90 ± 0.36^{a}

Santos et al. (2021) claimed *Trichoderma* species can produce volatile organic compounds (VOCs) that dramatically increase the amount of chlorophyll in plants, which improves photosynthetic efficiency and growth. In the study, *Arabidopsis thaliana* showed increases in chlorophyll content of 82.5% and 89.3%, respectively, when exposed to VOCs from *T. aggressivum* and *T. pseudokoningii*. Additionally, the chlorophyll content of tomato plants treated with VOCs from *T. viride* increased by 70.7% after 14 d and by 100.1% after 21 d. These indicate that due to the VOCs released by *Trichoderma*, the chlorophyll levels increase, and plant health and productivity are improved.

Besides that, high chlorophyll levels also occur due to better nutrient uptake. *Trichoderma* ensures that plants get nutrients like N and Mg, which are necessary to produce chlorophyll, by improving nutrient availability and uptake. Better chlorophyll synthesis is supported by this enhanced nutritional status. This finding is in agreement with a study by Andrzejak et al. (2021) that investigated the positive influence of *Trichoderma* inoculation on chlorophyll levels in leaves upon better uptake of essential nutrients, including N, P, K, Ca, Zn, and Fe. This enhanced nutrient absorption contributed to improved plant growth and health. Hasan et al. (2020) also emphasized the efficiency of *Trichoderma* species in absorbing nutrients, where it was found that total chlorophyll contents of plants treated with *T. asperellum* B1902 were higher. The *Trichoderma*-treated plants also had much darker green leaves, as opposed to the control group with lighter green colour and yellowish leaves of Fusarium-treated plants.

Chlorophyll content in T3 was at its lowest among other treatments, indicating that the pak choy plants were in poor health or suboptimal conditions due to the insufficient nutrients applied. Significant difference ($p > 0.05$) was obtained in comparison to the control because the production of chlorophyll is reliant on the availability of certain nutrients, including N, Fe, and P, for the photosynthesis process. A study by Song et al. (2024) supports these findings, where it demonstrated that *T. viride* inoculation failed to raise chlorophyll levels in low-P environments, reducing both chlorophyll content and efficiency of photosystem II. It showed that *Trichoderma* was unable to make up for the deficiency of available P.

Aside from facing nutrient deficiencies, plants in T3 had yellowish leaves, compared to control, T1 and T2, probably due to lower resistance level to diseases or pests. Zhou et al. (2015) also claimed that disruption in nutrient absorption can result in nutrient deficiencies that exacerbate leaf yellowing. In addition to bacterial infections, insect pests such as aphids, whiteflies, and leafhoppers feed on plant sap, often causing damage to plant cells and chloroplasts. It can induce stress responses in plants that ultimately lead to chlorosis, a condition

in which the leaves turn yellow due to insufficient chlorophyll production. Furthermore, pests like root-feeding nematodes can damage the plant roots, which impairs nutrient uptake, especially essential nutrients such as N, Mg, and Fe. This disruption in nutrient absorption can result in nutrient deficiencies that exacerbate chlorosis and yellowing of leaves (Zhou et al., 2015).

Also, Singh et al. (2019) found that a lack of N causes the amount of chlorophyll to decrease, hindering plant growth even with the presence of *Trichoderma*. It concluded that *Trichoderma* could enhance N uptake under ideal circumstances, but its presence does not completely counteract the effects of N insufficiency. Hence, plants with low chlorophyll content have difficulties in converting light energy into chemical energy, causing reduced growth and development. Chlorosis will also occur, causing the leaves to appear yellowish, which indicates insufficient chlorophyll. According to these results, *Trichoderma* can improve nutrient absorption by promoting root development and increasing the bioavailability of essential nutrients in the rhizosphere. It also improves plant stress tolerance by producing bioactive compounds and stimulating plant defense mechanisms. However, under conditions of severe nutrient deficiency, its ability to support plant growth is limited, as insufficient nutrient availability directly impairs critical physiological processes such as chlorophyll synthesis. This results in reduced chlorophyll content and overall plant vitality, indicating that while *Trichoderma* can mitigate moderate nutrient stress, it cannot fully compensate for extreme deficiencies.

CONCLUSIONS

Application of *Trichoderma asperellum* to hydroponic pak choy plants significantly enhances the growth. The highest levels of fungal growth and plant performance were consistently shown by application of *Trichoderma* before sowing. Significant improvements in plant height, leaf count, and chlorophyll content were also recorded. This study indicates that although *Trichoderma* plays a crucial role in enhancing nutrient availability, it is unable to make up for the absence of essential nutrients. Therefore, nutrient sufficiency remains important for optimal plant growth, even in the presence of beneficial microbial inoculants, because *Trichoderma* only enhances nutrient utilization, not producing nutrients.

Author contribution

Conceptualization: N.A.I.M.Z. Methodology: N.A.I.M.Z., K.M.N.R. Software: K.M.N.R., N.A.A. Validation: N.A.I.M.Z., N.A.A., M.H.I. Formal analysis: N.A.I.M.Z., K.M.N.R., N.A.A., M.H.I. Investigation: N.A.I.M.Z., K.M.N.R. Resources: N.A.I.M.Z. Data curation: K.M.N.R. Writing-original draft: N.A.I.M.Z. Writing-review & editing: N.A.I.M.Z. Visualization: N.A.I.M.Z. Supervision: N.A.I.M.Z., N.A.A. Project administration: N.A.I.M.Z. Funding acquisition: N.A.I.M.Z. All co-authors reviewed the final version and approved the manuscript before submission.

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