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Assessment of *Trichoderma* Species Isolated From Volcanic Soil of a Durian Field in Sisaket Province, Thailand for Plant Growth Promotion and Biocontrol Potential

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Abstract

Trichoderma species are ubiquitous saprophytic fungi commonly found in soil, with potential as fungal biocontrol agents for plant disease control and growth promotion. This research aimed to assess the attributes of *Trichoderma* sp. as plant growth promoters, focusing on nitrogen fixation, siderophore production, plant nutrient solubilization and indole-3-acetic acid production. Out of 19 isolates tested, 6 (T05, T19, T25, T27, T28 and T33) showed superior plant growth promotion abilities. These isolates were further evaluated for their capacity to enhance the germination of Khao Dawk Mali 105 rice seeds. Seeds were soaked in a suspension of conidia at a concentration of 1×10^{12} conidia/mL, and germination rates were measured. The germination rate ranged between 77.67 - 86.67 %, statistically significantly higher than the control rate of 33.33 %. Among the isolates, T05 exhibited the highest promotion of plant growth, evidenced by root length (7.27 cm), shoot length (8.50 cm), fresh weight (0.54 g) and dry weight (0.27 g), all significantly different from the control. Additionally, the potential of isolates to inhibit the fungal pathogen *Colletotrichum* sp. MN-3 was assessed using a dual culture method, with T35 showing the highest inhibition percentage (60.90 %). Morphological classification and nucleotide sequencing of the ITS1-5.8S-ITS2 region of rDNA gene identified the isolates as *T. harzianum*, *T. reesei*, *T. asperellum* and *T. longibrachiatum*. These findings underscore the effectiveness of *Trichoderma* sp. in promoting plant growth and suppressing plant pathogenic fungi.

Keywords: Anthracnose, Plant growth promotion, Rice, *Trichoderma* species, Seed germination

Introduction

The utilization of microbial agents for enhancing plant growth and controlling plant pathogens has gained considerable attention in agricultural research. Among these agents, species of the genus *Trichoderma* have emerged as promising candidates due to their ubiquitous presence in soil environments and their multifaceted roles as plant growth promoters and biocontrol agents. Compared to other pathogen-controlling microbes, *Trichoderma* species offer several advantages: They have a rapid growth rate, which allows them to quickly colonize the rhizosphere and outcompete pathogenic fungi, they produce a wide range of enzymes and secondary metabolites that can degrade the cell walls of pathogens, they are known to induce systemic resistance in plants, enhancing the plant defense mechanisms and they have a broad spectrum of activity against various plant pathogens. Additionally, *Trichoderma* species are generally recognized as safe for both plants and humans, making them an environmentally friendly and sustainable option for biocontrol [1]. *Trichoderma* species have been widely recognized for their ability to colonize diverse ecological niches, including soil, rhizosphere and phyllosphere, owing to their saprophytic lifestyle and competitive advantages in nutrient utilization [2]. These fungi have evolved intricate mechanisms to interact with plants, ranging from direct stimulation of plant growth *via* the synthesis of phytohormones such as indole-3-acetic acid (IAA) [3] to indirect mechanisms such as solubilization of unavailable nutrients like phosphate and production of siderophores, facilitating iron uptake by plants [4].

Moreover, *Trichoderma* spp. have shown remarkable efficacy in suppressing various plant pathogens through mechanisms such as competition for nutrients and space, mycoparasitism and production of antimicrobial compounds [5]. This biocontrol potential of *Trichoderma* has been extensively exploited in integrated pest management strategies to mitigate the detrimental effects of phytopathogens, thereby reducing reliance on chemical pesticides and fostering sustainable agricultural practices [6]. *Trichoderma* exhibit immense potential in agriculture due to their dual roles as biocontrol agents against phytopathogens and as promoters of plant growth. Their ability to colonize plant roots and interact with both plants and other microorganisms underscores their importance in soil ecosystems. *Trichoderma* species offer promising alternatives to chemical pesticides, particularly as pathogens often develop resistance. Their mechanisms of action, including mycoparasitism, nutrient competition, and induction of plant defenses, make them valuable assets in sustainable agricultural practices. As significant contributors to biofertilization and biostimulation, *Trichoderma* represents a cornerstone in the development of green technologies aimed at enhancing crop productivity while minimizing environmental impact [7]. However, despite the widespread application of *Trichoderma*-based formulations in agriculture, the effectiveness of individual isolates varies considerably depending on their inherent genetic diversity, physiological characteristics and ecological adaptation [2]. Therefore, comprehensive screening and characterization of *Trichoderma* isolates are imperative to identify strains with optimal plant growth promotion and biocontrol attributes for specific agricultural settings.

In this context, the present study aimed to evaluate the plant growth promotion and biocontrol potential of *Trichoderma* species isolated from the volcanic soil of a durian (*Durio zibethinus* L.) field in Sisaket Province, Thailand. Through a combination of *in vitro* assays and molecular characterization techniques, we sought to elucidate the mechanisms underlying the beneficial interactions between *Trichoderma* spp. and host plants, as well as their antagonistic effects against phytopathogens. By delineating the functional diversity and taxonomic identity of *Trichoderma* isolates, this research endeavors to provide valuable insights into harnessing these fungi as sustainable alternatives for enhancing crop productivity and for disease management in agricultural systems.

Materials and methods

Microorganisms

Nineteen isolates of *Trichoderma* sp. were collected from the rhizosphere of volcanic soil in a durian field in Sisaket Province, Thailand. These isolates were obtained by the Microbiology Laboratory, Department of Biology, Faculty of Science, Mahasarakham University. All isolates were preserved in 20 % glycerol at −20 °C in 2020 and tested in 2023.

Efficacy of *Trichoderma* sp. in promoting plant growth

Nitrogen fixation

Testing the nitrogen fixation of *Trichoderma* sp. used the method of Zhang *et al.* [8]. Cultures of *Trichoderma* sp. growing on potato dextrose agar (PDA) for 5 - 7 days were drilled with a cork borer (diameter 0.5 cm) cultured on Nitrogen-lacking agar that lacks a nitrogen source as a component. Incubated at 28 °C for 3 days. The results were checked by observing the growth of colonies on the culture medium.

Siderophore production

Testing the production of siderophore compounds by *Trichoderma* sp. used the method of Dolatabad *et al.* [9]. A cork borer (diameter 0.5 cm) was used to take a core of PDA medium on which *Trichoderma* sp. had been grown for 5 - 7 days. The agar pieces were grown on CAS blue agar and incubated at 28 °C for 7 days. The production of siderophores was examined as revealed by a blue zone around the colony of *Trichoderma* sp.

Phosphate solubilization

The phosphate solubilizing ability of *Trichoderma* sp. was assessed using the method of Dolatabad *et al.* [9]. Cores (0.5 cm) cut from PDA on which *Trichoderma* sp. had been growing for 5 - 7 days were transferred to Sperber medium (adjusted to pH 7.2 before autoclaving) and grown at 28 °C for 7 days to check for phosphate solubility and observe clear surrounding colonies of *Trichoderma* sp.

Potassium solubilization

The efficiency of *Trichoderma* sp. in dissolving potassium was tested using the method of Hewedy *et al.* [10] by growing *Trichoderma* sp. on Aleksandrow agar medium and incubating it at 28 °C for 7 days. The growth of the fungus growing on the medium plate and observe the clear area around the colony.

Zinc solubilization

The efficiency of *Trichoderma* sp. in zinc solubilization was tested using the method of Hewedy *et al.* [10] by growing the fungus on Tris minimal agar medium with D-glucose (1.728 g/L) with 0.1 % Zn carbonate ($ZnCO_3$) added as a source of zinc (Zn), incubated at 28 °C for 7 days in the dark.

Indole-3-acetic acid (IAA) production

Indole-3-acetic acid production by *Trichoderma* sp. was tested using the method of Chutima and Lumyong [11] by growing *Trichoderma* sp. in potato dextrose broth (PDB) mixed with tryptophan (2 mg/mL). Five cores (extracted with 0.5 cm diameter cork borer) taken from the *Trichoderma* sp culture were added to the prepared medium, shaken at 150 rpm, 28 °C for 13 days and then centrifuged at 1,500 rpm for 30 min to collect the supernatant which was used to measure the amount of IAA in the fungal liquid medium by colorimetry. Colorimetric assay used 2 mL of supernatant to which was added 2 drops of orthophosphoric acid, 4 mL of Salkowski reagent, after which the change in color was observed then observing the change in color. A pink color indicated that IAA was present and the absorbance was measured with a spectrophotometer at a wavelength of 530 nm and concentration determined by comparing with standard IAA solutions.

Efficiency of *Trichoderma* sp. in promoting the germination of Khao Dawk Mali 105 rice seeds

A spore suspension was prepared by adapting the method of Mukherjee and Kumar [12]. *Trichoderma* sp. was cultivated on PDA at 28 °C for 7 - 10 days. The fully grown culture was then scraped off and the harvested spores were suspended in sterile distilled water at 10^{12} conidia/mL (concentration was determined with a hemocytometer).

Khao Dawk Mali 105 rice seeds were sterilized by soaking them in 10 % Clorox for 5 min, then the seeds were washed with sterile distilled water 2 times, 5 min each time, allowed to dry.

Germination was tested using a modification of the method of Fontenelle *et al.* [13] by growing seeds on paper (top of paper) according to the ISTA method by soaking 100 g of Khao Dawk Mali 105 rice seeds in 10 mL *Trichoderma* sp. spore suspension (10^{12} conidia/mL) for 30 min. Then, seeds were placed in plastic boxes, with 10 seeds per box. The experiment used 7 treatments, each replicated in triplicate. In treatments 1 - 6 Khao Dawk Mali 105 rice seeds were soaked with different strains of *Trichoderma* sp. (T05, T19, T25, T27, T28 and T33). These 6 isolates were chosen based on their superior plant growth-promoting potential observed in preliminary screenings. Treatment 7 consisted of Khao Dawk Mali 105 rice seeds without *Trichoderma* sp. Seven days after planting, the following growth data were measured and recorded: Including seedling height, stem height, root length, fresh weight and dry weight. Growth promotion was calculated as a percentage for 3 parameters: Germination percentage, germination index and seedling vigor index, using the following formulae:

$$\text{Germination percentage} = (\text{Number of normal seedlings}/\text{Total number of seeds})\times 100 \tag{1}$$

$$\text{Seedling vigor index (SVI)} = \text{Germination percentage}\times\text{Seedling length} \tag{2}$$

$$\text{Germination index (GI)} = \text{Sum of (Number of normal seedlings/Number of days after germination)} \tag{3}$$

Efficacy of *Trichoderma* sp. in inhibiting *Colletotrichum* sp. isolate MN-3

Nineteen isolates of *Trichoderma* were assessed for their ability to inhibit *Colletotrichum* sp. MN-3 using the dual culture technique. Incubation was at 28 °C for 3 - 7 days, during which the growth radius of the pathogen hyphae was measured. Percentage inhibition of radial growth (PIRG) and percentage overgrowth (POG) were calculated following Sutthisa [14]. %PIRG was calculated using the formula:

$$\%PIRG = [(R1 - R2)/R1]\times 100 \tag{4}$$

where: R1 represents the average colony radius size of the pathogenic fungus on control plate. R2 represents the average colony radius size of the pathogenic fungus on the test plate.

Calculate of %POG, employed the formula:

$$\%POG = ((C1 - C2)/D)\times 100 \tag{5}$$

where: C1 is the average radius of fungal coverage on the day of observation. C2 is the average radius of fungal coverage before the study period. D represents the duration of the study in days.

Identification of *Trichoderma* sp.

Morphological characterization of *Trichoderma* isolates was conducted based on colony characteristics, specifically hyphal morphology, hyphal color and pigmentation on PDA plates. Microscopic examination of conidia and phialides was performed using compound microscopy. For molecular identification, genomic DNA was extracted from pure cultures of *Trichoderma* isolates using the

PureDireX Genomic DNA Isolation Kit (BIO-HELIX), following the manufacturer's instructions. The quantity and quality of extracted DNA were assessed using gel electrophoresis. Subsequently, the DNA samples were sent for analysis of the ITS1-5.8S-ITS2 region rDNA gene nucleotide sequence at Macrogen Company, Korea. The obtained nucleotide sequences were compared with sequences available in the GenBank database using BLAST analysis. Finally, a phylogenetic tree was constructed using the neighbor-joining method to determine the taxonomic relationships among the *Trichoderma* isolates.

Results and discussion

Efficacy of *Trichoderma* sp. in promoting plant growth

Nitrogen fixation

An assessment of nitrogen fixation ability among all 19 *Trichoderma* sp. isolates revealed that 11 isolates grew on nitrogen-lacking agar, indicating that they had capacity to fix nitrogen. The other 8 isolates did not grow in this medium. Notably, isolates T09, T19, T25, T27, T33 and T46 exhibited the highest nitrogen fixation levels, rated at +++ (**Figure 1**, **Table 1**).

Siderophore production

Examination of siderophore production across 19 *Trichoderma* sp. isolates revealed that 10 isolates demonstrated growth on Chrome Azurol S (CAS) agar. The other 9 isolates did not grow in this medium. Notably, isolates T28 and T33 exhibited the highest siderophore production, rated at +++, while isolate T47 displayed growth on CAS agar but did not induce a color change in the culture media (**Figure 2**, **Table 1**).

Phosphate solubilization

The phosphate solubility test conducted on all 19 isolates of *Trichoderma* sp. found that 13 isolates were able to grow on Pikovskaya agar medium, with isolate T11 growing on the medium at a level of +++ and formed a clear zone around the colony with a solubility level + and which was best able to dissolve phosphate. The remaining 6 isolates did not grow on Pikovskaya agar medium. The possible cause for their lack of growth could be due to their inability to utilize the specific nutrients present in the medium or sensitivity to certain components of Pikovskaya agar. These isolates might require different environmental conditions or specific nutrients not provided in this medium for optimal growth. There were 6 isolates that grew well on the medium at the level +++ but did not form a clear zone around the colony (**Figure 3**, **Table 1**).

Potassium solubilization

Experiments assessing potassium solubility across all 19 isolates of *Trichoderma* sp. revealed that 9 isolates exhibited growth on Aleksandrow agar medium containing potassium carbonate. The remaining 10 isolates did not grow on Aleksandrow agar medium. The possible cause for their lack of growth could be due to their inability to metabolize potassium carbonate or other components present in the medium. These isolates might require different environmental conditions or specific nutrients not provided in this medium for optimal growth. Specifically, isolates T19, T20, T25, T27, T35 and T46 demonstrated robust growth, scoring at level +++. However, despite their strong growth, they did not generate clear zones around the colony (**Figure 4**, **Table 1**).

Zinc solubilization

In the assessment of zinc solubility across 19 isolates of *Trichoderma* sp., it was observed that 12 isolates thrived on Tris minimal agar supplemented with Zn carbonate. The remaining 7 isolates did not grow on Tris minimal agar supplemented with Zn carbonate. The possible cause for their lack of growth could be due to their inability to utilize Zn carbonate as a nutrient source or sensitivity to the specific conditions of the Tris minimal agar. These isolates might require different environmental conditions, specific nutrients, or a different pH level not provided in this medium for optimal growth. Notably, isolates T19 and T27 exhibited robust growth, scoring at level +++. Despite their vigorous growth, they did not produce clear rings around the colonies, indicating zinc dissolution albeit at a lower solubilization rate, precluding the formation of distinct circles. Conversely, isolate T05 showed moderate growth at the ++ level and formed clear rings around the colonies at a similar level (**Figure 5**, **Table 1**).

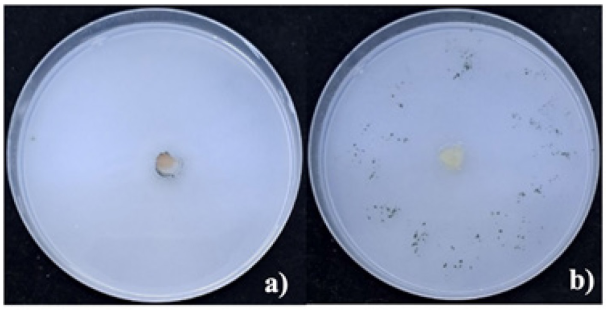

Figure 1 Illustrates the nitrogen fixation test conducted on *Trichoderma* sp. using nitrogen-lacking agar; (a) negative results and (b) positive result.

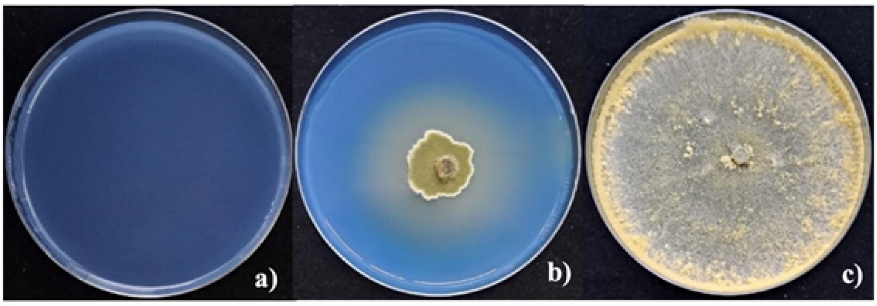

Figure 2 Evaluation for siderophore production by *Trichoderma* sp. on Chrome Azurol S (CAS) aga. (a) negative control, (b) The results were positive, with little growth and color change in the media, (c) illustrates a positive result, characterized by moderate growth and a noticeable alteration in the color of the medium.

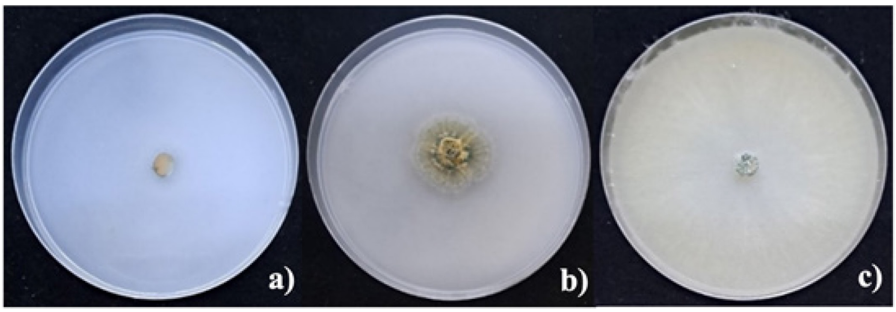

Figure 3 Testing the phosphate solubility of *Trichoderma* sp. on Pikovskaya agar added with tricalcium phosphate. (a) negative result, did not grow, (b) positive result, grows and forms a clear zone, (c) The result is positive, grows but does not form a clear zone.

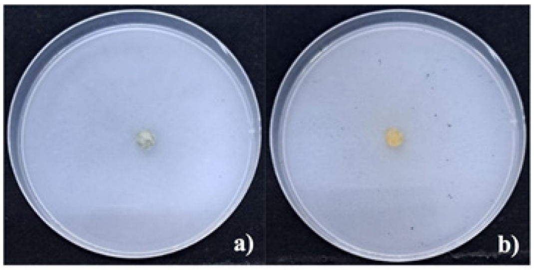

Figure 4 Testing the potassium solubility of *Trichoderma* sp. on Aleksandrow agar; (a) negative result and (b) positive result, grows but does not occur in the clear zone.

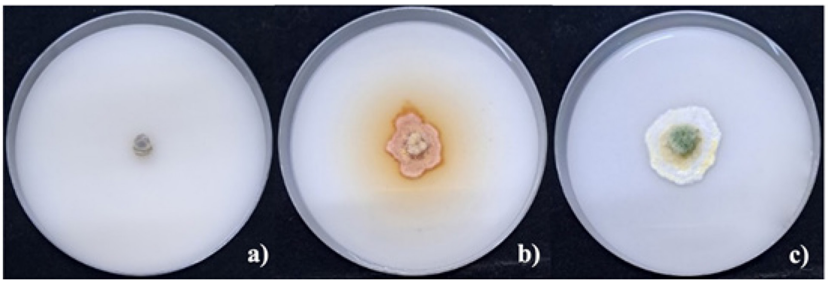

Figure 5 Testing the zinc solubility of *Trichoderma* sp. on Tris minimal agar added with Zn carbonate. (a) negative result, did not grow, (b) positive result, grows but does not occur in clear zone, (c) positive result, grow and form clear zone around the colony.

Indole-3-acetic acid (IAA) production

In the evaluation of indole-3-acetic acid (IAA) production among 19 isolates of *Trichoderma* sp., it was determined that 12 isolates exhibited a positive response, evidenced by a color change in the culture medium from yellow to pink. Notably, isolates T25 and T27 demonstrated the highest IAA production, both achieving a level of +++. They yielded 34.82 and 30.32 ug/mL of IAA, respectively (**Table 1**).

Table 1 Plant growth promoting properties of *Trichoderma* sp.

Isolate	Nitrogen fixing[1/]	Siderophore production[1/]		Nutrient Solubilization[1/]						IAA production	
				Phosphate		Potassium		Zinc		Color change	Volume (µg/mL)[2/]
		Growth	Color change	Growth	Dissolution	Growth	Dissolution	Growth	Dissolution		
T04	-	-	-	+	+	-	-	+	-	-	0.00 ± 0.00^{f}
T05	-	+	+	+	-	++	-	++	++	+	21.75 ± 0.00^{bcde}
T06	-	-	-	+	-	+	-	++	+	+	10.50 ± 0.00^{ef}
T07	-	-	-	++	+	-	-	-	-	+	18.91 ± 0.00^{cde}
T09	+++	-	-	-	-	-	-	+	+	-	0.00 ± 0.00^{f}
T10	-	-	-	+	+	-	-	-	-	+	33.02 ± 0.00^{ab}
T11	-	+	+	+++	+	-	-	-	-	+	23.86 ± 0.00^{abcd}
T19	+++	+++	++	+++	-	+++	-	+++	+	+	29.24 ± 0.10^{abc}
T20	+	-	-	-	-	+++	-	-	-	++	33.25 ± 0.10^{ab}
T22	-	-	-	-	-	-	-	+	-	-	0.00 ± 0.00^{f}
T25	+++	+++	+	+++	-	+++	-	++	-	+++	34.82 ± 0.00^{a}
T27	+++	+	+	+++	-	+++	-	+++	+	+++	30.32 ± 0.00^{abc}
T28	++	+++	+++	+++	-	-	-	+	+	-	0.00 ± 0.00^{f}

Isolate	Nitrogen fixing[1/]	Siderophore production[1/]		Nutrient Solubilization[1/]						IAA production	
				Phosphate		Potassium		Zinc		Color change	Volume (µg/mL)[2/]
		Growth	Color change	Growth	Dissolution	Growth	Dissolution	Growth	Dissolution		
T33	+++	+++	+++	+++	-	-	-	+	-	+	22.66 ± 0.00[abcde]
T35	-	+	+	-	-	+++	-	+	-	++	29.42 ± 0.00[abc]
T42	+	-	-	-	-	-	-	-	-	-	0.00 ± 0.00[f]
T46	+++	++	+	+++	-	+++	-	-	-	-	0.00 ± 0.00[f]
T47	+	+	-	-	-	+	+	-	-	-	21.41 ± 0.00[bcde]
T49	+	-	-	+	+	-	-	+	+	+	16.25 ± 0.00[de]

[1/] - = not detect, + = slight, ++ = moderate, +++ = high; [2/] The means followed by the same letter were not significantly different according to Least significant differences (LSD) $p = 0.05$.

In this study, we observed the growth-promoting effects of 4 *Trichoderma* species (*T. harzianum*, *T. reesei*, *T. asperellum* and *T. longibrachiatum*) on plant growth. Our findings align with previous research indicating the beneficial role of *Trichoderma* species in enhancing plant growth and improving nutrient uptake [1,6]. Specifically, *T. harzianum* has been reported to stimulate plant growth through various mechanisms, including the production of phytohormones and the induction of systemic resistance in plants [15]. Similarly, *T. asperellum* has been shown to promote plant growth by solubilizing phosphates and enhancing nutrient uptake [16]. Additionally, *T. reesei* has demonstrated the ability to improve plant growth by producing cellulolytic enzymes, which contribute to the breakdown of organic matter and release of nutrients [15]. Furthermore, *T. longibrachiatum* has been implicated in enhancing plant growth by suppressing plant pathogens through the production of antifungal compounds [1].

In previous research [17], *Trichoderma* species have been recognized for their biofertilizer role, attributed to their ability to produce phytohormones and promote plant growth. Fifteen strains of *Trichoderma* spp. (T1 - T15), isolated from olive rhizosphere soil in northern Algeria, underwent screening for their phytohormone production and growth-promoting metabolites. Various *in vitro* assays, including phosphatase, siderophore, hydrogen cyanide and ammonia production were used to assess the plant growth-promoting potential of these *Trichoderma* spp. Furthermore, quantitative evaluations of gibberellic acid and indole-3-acetic acid (IAA) were conducted *via* colorimetric assays. The results underscored the significant capacity of *Trichoderma* spp. to generate plant growth-promoting biomolecules. Noteworthy findings included isolate T10 which exhibited the highest phosphate solubilization index on Pikovskaya medium and T8 which demonstrated high nitrogen-fixing abilities. Quantitative analysis revealed varying levels of indole-3-acetic acid and gibberellic acid production across strains, with isolate T11 consistently yielding the highest amounts of both phytohormones. In another study by Bader *et al.* [18] found that 12 strains of *Trichoderma* sp. inhibited (by over 50 %) growth of 4 strains of a pathogenic fungus growth (FCCT 16, FCCT 58, FCCT 199-2 and FCCT 363-2) and also exhibited notable IAA production (13.38 to 21.14 mg/mL) and phosphate solubilization (215.80 to 288.18 mg/mL of calcium phosphate). Inoculation of tomato plants with these strains resulted in increased chlorophyll content, shoot length and fresh and dry weights of both shoot and roots, alongside a reduction in Fusarium wilt disease by 10 to 30 %. Additionally, research has demonstrated that *Trichoderma aggressivum* f. *europaeum* exhibits significant biostimulant capabilities in promoting the growth of pepper and tomato seedlings under commercial plant nursery and greenhouse conditions. Direct seed application *in vitro* did not enhance germination rates or seedling vigor. Irrigation with *T. aggressivum* f. europaeum spores in the substrate notably improved seedling quality, which was particularly evident in the 66.66 % increase in tomato root growth at a dosage of 10^6 spores/plant [19].

Efficiency of *Trichoderma* sp. in promoting the germination of Khao Dawk Mali 105 rice seeds

The efficiency of 6 isolates of *Trichoderma* sp. (T05, T19, T25, T27, T28 and T33) in promoting the germination of Khao Dawk Mali 105 rice seeds was evaluated. After 7 days, all *Trichoderma* isolates significantly increased seed germination compared to the control, with percentage increases ranging from 76.67 to 86.67 %. The vigor index was highest for isolates T05, T19, T25 and T27, ranging from 733.33 to 674.67. Additionally, all isolates improved the germination index, ranging from 12.88 to 11.37, compared to the control (4.99).

Furthermore, testing on Khao Dawk Mali 105 rice variety revealed that isolates T05, T19, T25 and T27 significantly increased stem length (8.50 - 8.00 cm) and fresh weight (0.54 - 0.45 g), with isolate T05 showing the highest dry weight (0.27 g). Isolate T33 exhibited the greatest root length of 10.63 cm. These results indicate the potential of *Trichoderma* sp. in enhancing the growth and development of rice seedlings (**Table 2**).

Similar to Swain *et al.* [20] who reported a novel application of *Trichoderma* strains collected from tree barks for enhancing rice plant growth, health management and paddy straw degradation; 7 different species of *Trichoderma* were characterized using both morphological and molecular tools. Among them, *Trichoderma hebeiensis* and *Trichoderma erinaceum* emerged as particularly effective strains. These strains exhibited robust control over 4 significant rice pathogens: *Rhizoctonia solani* (100 %), *Sclerotium oryzae* (84.17 %), *Sclerotium rolfsii* (66.67 %) and *Sclerotium delphinii* (76.25 %). Seed bio-priming with these *Trichoderma* strains led to reduced mean germination time, enhanced seedling vigor and increased total chlorophyll content, ultimately resulting in higher yields for 2 rice varieties, Annapurna and Satabdi. Additionally, all 7 strains accelerated rice straw decomposition by producing elevated levels of straw-degrading enzymes measured as total cellulase, endoglucanase, xylanase and laccase. Moreover, these *Trichoderma* strains demonstrated the production of plant growth-promoting substances like indole acetic acid, soluble phosphate and prussic acid, which not only promoted plant growth but also inhibited rice pathogen populations. Furthermore, they induced the expression of defense enzymes such as catalase, peroxidase, superoxide dismutase, polyphenol oxidase and total phenolics, indicating the rice crop's enhanced stress tolerance.

Table 2 Efficiency of *Trichoderma* sp. in promoting seed germination of Khao Dawk Mali 105 rice variety.

Treatment	Germination (%)	Vigor index	Germination index	Shoot length (mm)	Root length (mm)	Fresh weight (g)	Dry weight (g)
T05	86.67 + 5.77^{a}	733.33 + 23.09^{a}	12.88 + 2.79^{a}	8.50 + 0.87^{a}	7.27 + 1.00^{ab}	0.54 + 0.04^{a}	0.27 + 0.10^{a}
T19	80.00 + 0.00^{a}	674.67 + 36.07^{a}	12.75 + 2.16^{a}	8.43 + 0.45^{a}	5.80 + 0.75^{b}	0.52 + 0.02^{a}	0.18 + 0.00^{ab}
T25	83.33 + 11.55^{a}	699.00 + 145.49^{a}	11.98 + 1.69^{a}	8.37 + 1.03^{a}	7.10 + 2.23^{ab}	0.46 + 0.11^{a}	0.18 + 0.02^{ab}
T27	76.67 + 5.77^{a}	690.00 + 65.57^{a}	11.72 + 0.58^{a}	8.00 + 0.50^{a}	7.00 + 0.87^{ab}	0.45 + 0.07^{a}	0.19 + 0.03^{ab}
T28	76.67 + 5.77^{a}	611.67 + 140.74^{ab}	11.37 + 1.92^{a}	8.00 + 1.80^{a}	6.30 + 0.60^{ab}	0.45 + 0.12^{ab}	0.15 + 0.02^{ab}
T33	80.00 + 10.00^{a}	521.67 + 160.03^{ab}	12.75 + 1.48^{a}	6.67 + 2.36^{ab}	10.63 + 1.47^{a}	0.54 + 0.03^{ab}	0.18 + 0.02^{ab}
Control	33.33 + 15.28^{b}	162.67 + 70.60^{b}	4.99 + 1.43^{b}	4.90 + 0.87^{b}	4.20 + 0.61^{b}	0.12 + 0.08^{b}	0.07 + 0.04^{b}

[1/] The means followed by the same letter were not significantly different according to Least significant differences (LSD) $p = 0.05$.

Efficacy of *Trichoderma* sp. in inhibiting *Colletotrichum* sp. isolate MN-3

The efficiency of all 19 isolates of *Trichoderma* sp. in inhibiting the growth of *Colletotrichum* sp. MN3 mycelium was investigated using the dual culture method. Results indicated that all *Trichoderma* spp. isolates demonstrated inhibition of *Colletotrichum* sp. MN3 mycelium growth, with inhibition percentages ranging from 60.61 to 84.85 %. Additionally, all isolates were able to grow and cover the *Colletotrichum* sp. MN3 mycelium, with coverage percentages varying from 23.75 to 76.25 % (**Figure 6**, **Table 3**). Our study explored the biocontrol potential of 4 *Trichoder*ma species (*T. harzianum*, *T. reesei*, *T. asperellum* and *T. longibrachiatum*) against *Colletotrichum* sp. Previous research has extensively documented the biocontrol efficacy of *Trichoderma* species against a wide range of plant pathogens [1,6]. Specifically, *T. harzianum* and *T. asperellum* have been identified as promising biocontrol agents against *Colletotrichum truncatum* in chilli plants, inducing systemic resistance and reducing lesion development [21]. Similarly, *T. reesei* has shown efficacy in reducing *Fusarium* dieback in tea crops and enhancing defensive enzyme activities [22]. Moreover, Kim *et al.* [23] demonstrated the effectiveness of *T. atroviride* and *T. longibrachiatum* in controlling red pepper anthracnose caused by *C. acutatum*, both *in vitro* and in the field, highlighting their potential as eco-friendly alternatives to chemical fungicides.

As in the report of Cruz-Quiroz *et al.* [24], an integrated assessment was conducted to determine the potential of various *Trichoderma* strains against *Colletotrichum gloeosporioides* (the phytopathogen used in the assay to evaluate the inhibition potential by *Trichoderma)*. *Trichoderma harzianum*, *Trichoderma longibranchiatum*, *Trichoderma yunnanense*, *Trichoderma asperellum* (T2 - T10 and T2 - T31) and *Trichoderma* sp. were evaluated. The *Trichoderma* strains showed a percentage growth inhibition of *C. gloeosporioides* of up to 22.5 %. All assessed *Trichoderma* strains demonstrated promising potential as pest control agents against *C. gloeosporioides.* Sutthisa *et al.* [25] investigated the efficacy of *T. asperellum* MSU007 in controlling *Colletotrichum* sp. via dual culture technique. *T. asperellum* MSU007 was able to inhibit the mycelium growth of Colletotrichum sp. with 50.76 - 74.85 % inhibition.

Table 3 Efficiency of *Trichoderma* sp. on inhibition to mycelium growth of *Colletotrichum* sp. MN-3 by dual culture technique, 4 days after inoculation.

Isolate	Mycelium growth inhibition (%)[1/]	Overgrowth (%)[1/]
T04	57.58 ± 4.29^{g}	76.25 ± 5.30^{a}
T05	62.12 ± 2.14^{fg}	66.25 ± 1.77^{ab}
T06	84.85 ± 0.01^{a}	53.75 ± 8.84^{cde}
T07	69.70 ± 8.58^{cde}	52.50 ± 0.00^{cdef}
T09	65.15 ± 15.00^{ef}	30.00 ± 3.54^{gh}
T10	72.73 ± 4.29^{bcd}	50.00 ± 0.00^{def}
T11	69.70 ± 0.0^{cde}	56.25 ± 5.30^{bcde}
T19	65.15 ± 6.42^{ef}	55.00 ± 3.54^{bcde}
T20	60.61 ± 4.29^{fg}	52.50 ± 3.54^{cdef}
T22	66.67 ± 12.86^{def}	55.00 ± 3.54^{bcdef}
T25	69.70 ± 0.00^{cde}	51.25 ± 1.77^{cdef}
T27	65.16 ± 15.00^{ef}	23.75 ± 5.30^{h}
T28	65.155 ± 15.00^{ef}	60.00 ± 3.54^{bcd}
T33	77.28 ± 10.71^{b}	56.25 ± 5.30^{bcde}
T35	74.24 ± 2.14^{bc}	46.25 ± 1.77^{ef}
T42	66.67 ± 12.86^{def}	52.50 ± 7.07^{cdef}
T46	66.67 ± 4.29^{def}	62.50 ± 14.14^{bc}
T47	65.67 ± 1.56^{ef}	51.25 ± 1.77^{cdef}
T49	60.61 ± 8.57^{fe}	41.25 ± 5.30^{fg}

[1/] The means followed by the same letter were not significantly different according to Least significant differences (LSD) $p = 0.05$.

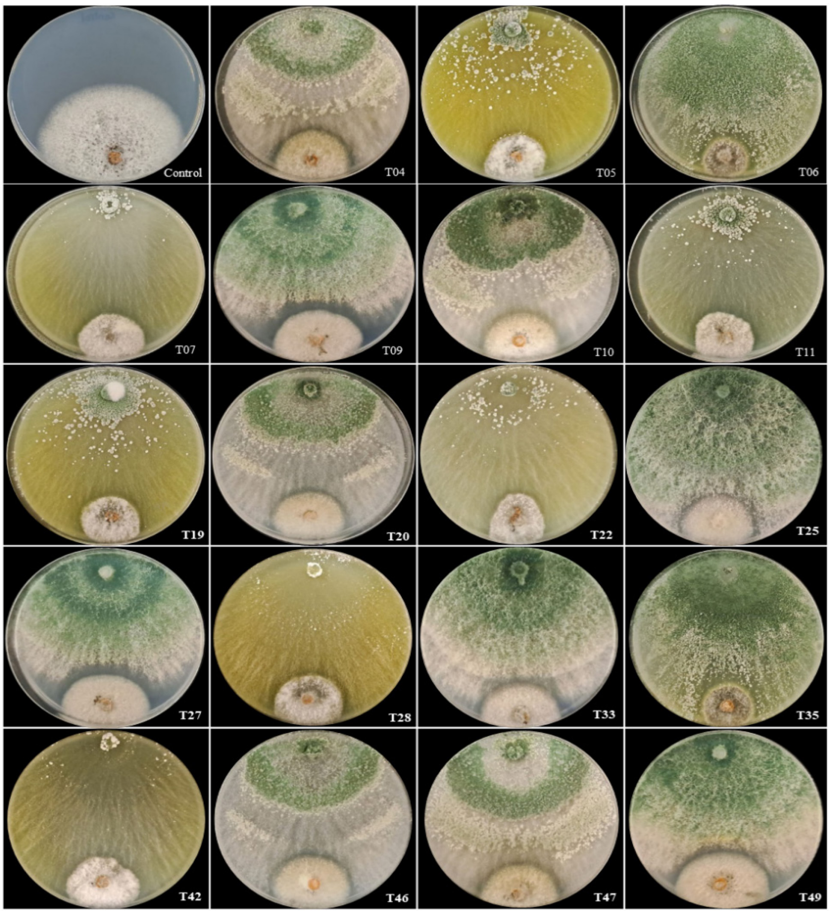

Figure 6 Efficiency of each isolate of *Trichoderma* sp. in inhibiting the mycelium growth of *Colletotrichum* sp. MN-3 by dual culture method after inoculation for 4 days.

Identification of *Trichoderma* sp.

Morphological characteristics

The preliminary morphological characteristics of all 19 isolates of *Trichoderma* sp. were studied, encompassing colony appearance on PDA media, conidiophore branching, conidia shape and the presence of chlamydospores. Following the classification methods outlined by Jaklitsch and Voglmayr [26] *Trichoderma* sp. can be categorized into 4 distinct groups as follows:

Group 1 comprised *Trichoderma asperellum*, exhibiting consistent morphological traits across 5 isolates: T09, T25, T27, T33 and T49. Colonies grown on PDA medium had a dark green hue with a subtle white tint. The mycelia protrude above the medium surface, presenting a fluffy texture without pigment production. The primary branching pattern extended to form either fertile or sterile aerial hyphae, characterized by primary fertile branches and shorter secondary fertile branches; the pyramidal-type, conidia are green, typically sub-globose to ovoidal in shape, with chlamydospores interspersed among the hyphae (**Figure 7**).

Group 2 encompassed *Trichoderma harzianum*, displaying consistent morphological features observed across 5 isolates: T04, T10, T20, T46 and T47. Colonies cultivated on PDA medium exhibited a greenish-yellow circular pattern at the center, surrounded by a white outer layer of fluffy mycelial fibers. Dense aggregations of fibers were notable. Abundant pustule-like conidia were produced without pigment or odor. Conidiophore branching followed a distinct pattern identified as Pachybasium-type, giving rise to either sterile or fertile hairs. Ampulliform filaments bearing green, ellipsoidal conidia were found at the tips of conidiophore stems, with chlamydospores observed intermediate between globose and subglobose hyphae (**Figure 7**).

Group 3 comprised *Trichoderma longibrachiatum*, exhibiting morphological similarities observed in 7 isolates: T05, T07, T11, T19, T22, T28 and T47. Colonies grown on PDA medium displayed fibers attached to the surface, which aggregated to form clusters resembling green pustules and produced yellow pigment. The conidiophores exhibited a branching pattern characteristic of longibrachiatum-type, featuring a long core with solitary phialides. These phialides produced ampulliform or lageniform filaments, yielding green, globose to subglobose or subglobose to ovoid conidia, alongside chlamydospores in globose to sub-globose forms interspersed among the mycelium (**Figure 7**).

Group 4 consisted of *Trichoderma reesei*, sharing morphological characteristics observed in 2 isolates: T06 and T35. Colonies on PDA medium exhibited a distinctive appearance with a central region ranging from light green to dark green encircled by dense mycelial fibers. Abundant pustule-like conidia were produced without pigment formation on the medium. Conidiophores exhibited sparse branching and directly bear phialides from the main core at the same level, classified as verticillium-type. Ampulliform phialides gave rise to green, globose conidia, with chlamydospores observed at the tip of globose to sub-globose hyphae (**Figure 7**).

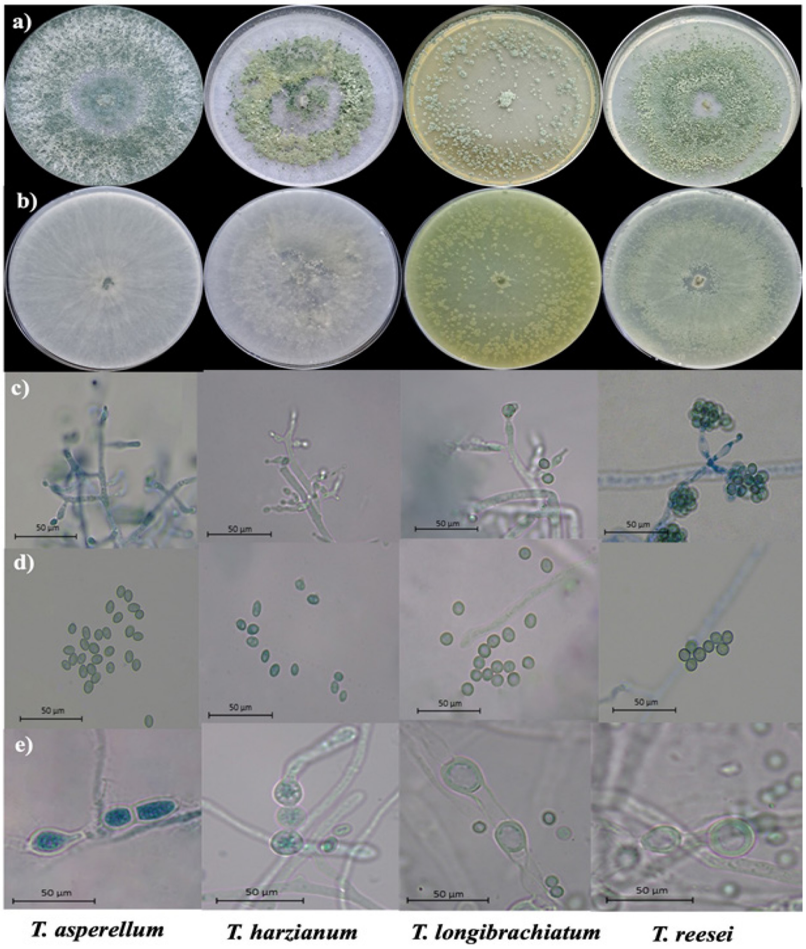

Figure 7 Illustrates the morphological features of a *Trichoderma* sp. colony on a PDA, 7 days after inoculation; (a) front plate, (b) back plate, (c) conidiophores, (d) conidia and (e) chlamydospores.

Molecular characterization

After selecting representatives of *Trichoderma* sp. within each group for gene nucleotide sequence analysis of the ITS1-5.8S-ITS2 region rDNA, a comparison with sequences in the GenBank database revealed that all isolates belong to the genus *Trichoderma*. Phylogenetic analysis, conducted using the MEGA11 program and referencing known fungi, demonstrated that the ITS1-5.8S-ITS2 region rDNA gene sequence of *Trichoderma* sp. isolate T04 closely resembled that of *T. harzianum*. Isolates T05 and T07 also exhibited a similar sequence to *T. logibrachiatum*, isolate T06 which shared a nucleotide sequence similarity with *T. reesei*, while isolates T09 and T49 exhibit sequence similarity to *T. asperellum* (**Figure 8**).

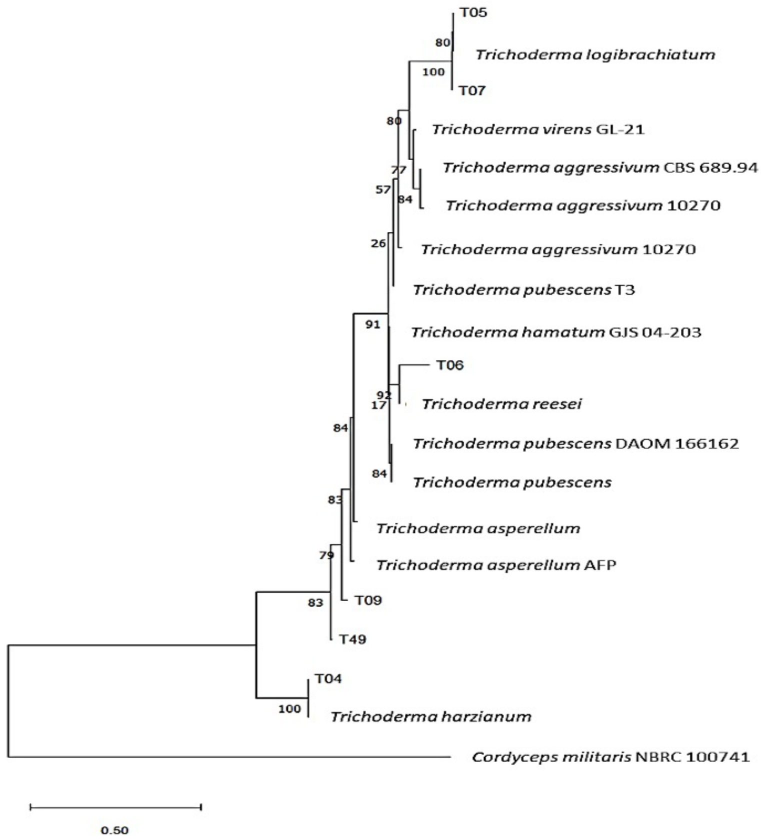

Figure 8 The phylogenetic relationships among *Trichoderma* sp. based on nucleotide sequence data from the ITS1-5.8S-ITS2 region rDNA gene, using the maximum likelihood method.

As reported in previous studies, *Trichoderma* (a soil-borne fungus), holds considerable economic significance in agriculture. However, accurate identification of *Trichoderma* species is essential for commercial use. Traditional methods relying solely on micro-morphological descriptions can be cumbersome and prone to errors. Hence, there is a call for a comprehensive identification approach, integrating morphological characteristics and multilocus gene sequences (including ITS1, ITS regions, TEF-1α and CAL). A study conducted in a wet paddy field in Tuaran, Sabah, Malaysia, isolated and identified 53 *Trichoderma* strains. These were classified into 3 species: *T. asperellum* (43 strains), *T. harzianum* (9 strains) and *T. reesei* (1 strain), based on both morphological traits and gene sequences. Phylogenetic analysis delineated distinct clusters corresponding to *Trichoderma* sections. Accurate species identification is crucial, as *Trichoderma* strains hold potential as biocontrol agents for disease management and crop yield enhancement in agriculture. Adopting a multifaceted identification approach ensures robust and reliable characterization of *Trichoderma* strains for agricultural applications [27]. Saravanakumar and Wang [28] isolated 18 *Trichoderma* strains representing 9 species from soil samples collected in a wetland ecosystem of South Korea. These strains underwent screening for antagonistic activity against 3 pathogens that were *Macrophomina phaseolina*, *Fusarium graminearum* and *Botrytis cinerea* using *in vitro* assays. Among the strains, *T. aureoviride* (strain SKCGW013) exhibited superior antagonistic activity against the tested pathogens.

Conclusions

Based on our comprehensive assessment of 19 *Trichoderma* sp. isolates, encompassing various growth-promoting and biocontrol attributes, several key findings emerge. Firstly, our investigation revealed substantial nitrogen-fixing abilities among the *Trichoderma* strains, with 11 isolates demonstrating growth on nitrogen-lacking agar, indicating their potential to fix nitrogen. Notably, isolates T09, T19, T25, T27, T33 and T46 exhibited the highest levels of nitrogen fixation. Additionally, our study highlighted significant siderophore production and phosphate solubilization capabilities across multiple isolates, indicative of their role in nutrient acquisition and utilization in soil environments. Furthermore, *Trichoderma* strains demonstrated proficient potassium and zinc solubilization abilities, although clear zone formation varied among isolates. This variability underscores the importance of considering multiple factors in assessing nutrient solubilization efficiency. Moreover, our investigation into indole-3-acetic acid (IAA) production highlighted the capacity of several *Trichoderma* isolates to synthesize this phytohormone, which plays a crucial role in plant growth and development.

In terms of promoting seed germination and seedling growth, our findings indicate that *Trichoderma* sp. isolates significantly enhanced both germination rates and seedling vigor in Khao Dawk Mali 105 rice varieties. Additionally, all tested *Trichoderma* sp. isolates exhibited inhibition of *Colletotrichum* sp. MN3 mycelium growth, further highlighting their potential as biocontrol agents against plant pathogens. Our morphological and molecular characterization of *Trichoderma* sp. isolates facilitated accurate species identification, providing valuable insights into their taxonomic classification and genetic diversity.

In conclusion, our study underscores the multifaceted beneficial attributes of *Trichoderma* sp. in promoting plant growth, enhancing nutrient availability and suppressing plant pathogens. These findings contribute to a deeper understanding of the agricultural potential of *Trichoderma* sp. isolates and emphasize the importance of employing integrated approaches for their effective utilization in sustainable agriculture. Our research has limitations: Conducting the study under controlled laboratory conditions may not fully replicate field conditions, the mechanisms underlying observed beneficial effects were not explored at the molecular level, and only a subset of *Trichoderma* isolates was tested for each nutrient solubility. Further research is recommended to address these limitations, including conducting extensive field trials to validate laboratory findings and assess long-term effects on crop yield and soil health, investigating molecular and genetic mechanisms behind plant growth promotion and pathogen suppression, screening a larger number of Trichoderma isolates to identify superior or unique beneficial traits, expanding research to include multiple crop species for broader applicability and studying interactions between Trichoderma isolates and other soil microbes to determine their effectiveness. Addressing these areas can enhance the practical application of Trichoderma in sustainable agriculture for maximum benefit.

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References

[1] GE Harman. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* 2006; **96**, 190-4.

[2] GE Harman, CR Howell, A Viterbo, I Chet and M Lorito. *Trichoderma* species - opportunistic, avirulent plant symbionts. *Nat. Rev. Microbiol*. 2004; **2**, 43-56.

[3] A Viterbo, U Landau, S Kim, L Chernin and I Chet. Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. *FEMS Microbiol. Lett*. 2010; **305**, 42-8.

[4] GE Harman and N Uphoff. Symbiotic root-endophytic soil microbes improve crop productivity and provide environmental benefits. *Scientifica* 2019; **2019**, 9106395.

[5] M Lorito, SL Woo, GE Harman and E Monte. Translational research on *Trichoderma*: From 'omics to the field. *Annu. Rev. Phytopathol.* 2010; **48**, 395-417.

[6] M Shoresh, GE Harman and F Mastouri. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol*. 2010; **48**, 21-43.

[7] R Tyśkiewicz, A Nowak, E Ozimek and J Jaroszuk-Ściseł. The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci.* 2022; **23**, 2329.

[8] F Zhang, C Chen, F Zhang, L Gao, J Liu, L Chen, X Fan, C Liu, KeZhang, Y He, C Chen and X Ji. *Trichoderma harzianum* containing 1-aminocyclopropane-1-carboxylate deaminase and chitinase improved growth and diminished adverse effect caused by *Fusarium oxysporum* in soybean. *J. Plant Physiol.* 2017; **210**, 84-94.

[9] HK Dolatabad, MJ Nikkhah and WT Shier. Evaluation of antifungal, phosphate solubilisation, and siderophore and chitinase release activities of endophytic fungi from *Pistacia vera*. *Mycol. Prog.* 2017; **16**, 777-90.

[10] OA Hewedy, A Mansour, MG Ali, KSA Lateif, MH Ismaiel and RM El-Meihy. Comprehensive characterization and screening of different *Trichoderma* Isolates as plant growth promoters: Insight to metal solubilization, enzymatic activity, and antagonistic effect. *Res. Square* 2022; **40**, 1-26.

[11] R Chutima and S Lumyong. Production of indole-3-acetic acid by Thai native orchid-associated fungi. *Symbiosis* 2012; **56**, 35-44.

[12] A Mukherjee and S Kumar. An eco-friendly approach of biocontrol of aphid (*Aphis gossypii* Glover) by *Trichoderma harzianum*. *Environ. Monit. Assess*. 2022; **195**, 102-17.

[13] ADB Fontenelle, SD Guzzo, CMM Lucon and R Harakava. Growth promotion and induction of resistance in tomato plant against *Xanthomonas euvesicatoria* and *Alternaria solani* by *Trichoderma sp. Crop Prot.* 2011; **30**, 1492-500.

[14] W Sutthisa. Biological control properties of *Cyathus* spp. to control plant disease pathogens. *J. Pure Appl. Microbiol*. 2018; **12**, 1755-60.

[15] HA Contreras-Cornejo, L Macías-Rodríguez, C Cortés-Penagos and J López-Bucio. *Trichoderma* spp. improve growth of *Arabidopsis* seedlings under salt stress through enhanced root development, osmoregulation, and Na^+ exclusion. *Plant Physiol*. 2009; **151**, 1-10.

[16] F Mastouri, T Björkman and GE Harman. *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Mol. Plant-Microbe Interact*. 2012; **25**, 1264-71.

[17] R Abdenaceur, B Farida, D Mourad, H Rima, O Zahia and SH Fatma. Effective biofertilizer *Trichoderma* spp. isolates with enzymatic activity and metabolites enhancing plant growth. *Int. Microbiol.* 2022; **25**, 817-29.

[18] AN Bader, GL Salerno, F Covacevich and VF Consolo. Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). *J. King Saud Univ. Sci*. 2020; **32**, 867-73.

[19] B Sánchez-Montesinos, F Diánez, A Moreno-Gavíra, FJ Gea and M Santos. Role of *Trichoderma aggressivum* f. *europaeum* as plant-growth promoter in Horticulture. *Agronomy* 2020; **10**, 1004.

[20] H Swain, T Adak, AK Mukherjee, S Sarangi, P Samal, A Khandual, R Jena, P Bhattacharyya, SK Naik, ST Mehetre, MS Baite, S Kumar and NW Zaidi. Seed biopriming with *Trichoderma* strains isolated from tree bark improves plant growth, antioxidative defense system in rice and enhance straw degradation capacity. *Front. Microbiol.* 2021; **12**, 633881.

[21] M Yadav, MK Dubey and RS Upadhyay. Systemic resistance in chilli pepper against anthracnose (caused by *Colletotrichum truncatum*) induced by *Trichoderma harzianum*, *Trichoderma asperellum* and *Paenibacillus dendritiformis*. *J. Fungi* 2021; **7**, 307.

[22] AK Pandey, MK Samota, AJ Tanti and A Babu. *Trichoderma reesei* induces defense-related biochemical markers associated with resistance to Fusarium dieback in tea crop. *Biol. Contr*. 2023; **180**, 105200.

[23] SH Kim, Y Lee, K Balaraju and Y Jeon. Evaluation of *Trichoderma atroviride* and *Trichoderma longibrachiatum* as biocontrol agents in controlling red pepper anthracnose in Korea. *Front. Plant Sci.* 2023; **14**, 1201875.

[24] RD la Cruz-Quiroz, S Roussos, R Rodríguez-Herrera, D Hernandez-Castillo and CN Aguilar. Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains. *Karbala Intern. J. Mod. Sci*. 2018; **4**, 237e243.

[25] W Sutthisa, A Popranom, A Taddeetrakool and S Khankhum. Development of *Trichoderma* formulation and application to control durian anthracnose disease. *Trends Sci.* 2024; **21**, 7276.

[26] WM Jaklitsch and H Voglmayr. Biodiversity of *Trichoderma* (Hypocreaceae) in southern Europe and macaronesia. *Stud. Mycol*. 2015; **80**, 1-87.

[27] A Asis, SA Shahriar, L Naher, S Saallah, HNN Fatihah, V Kumar and S Siddiquee. Identification patterns of *Trichoderma* strains using morphological characteristics, phylogenetic analyses and lignocellulolytic activities. *Mol. Biol. Rep*. 2021; **48**, 3285-301.

[28] K Saravanakumar and MH Wang. Isolation and molecular identification of *Trichoderma* species from wetland soil and their antagonistic activity against phytopathogens. *Physiol. Mol. Plant Pathol.* 2020; **109**, 101458.