Sigma J Eng Nat Sci, Vol. 44, No. 1, pp. 162–173, February, 2026

Sigma Journal of Engineering and Natural Sciences
Web page info: https://sigma.yildiz.edu.tr
DOI: 10.14744/sigma.2026.1975

Research Article

Screening of *Trichoderma* spp. for biofertilizer and biocontrol potential in vitro

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ARTICLE INFO

Article history
Received: 14 March 2025
Revised: 01 June 2025
Accepted: 23 August 2025

Keywords:
Biological Control; Microbial Fertilizer; PGPF; Trichoderma

ABSTRACT

Agricultural yields can be significantly increased by using Plant Growth Promoting Microorganism (PGPMs). *Trichoderma* spp., which support plant growth, have been studied as soil microorganisms for many years. This study investigated the PGPM properties of 62 *Trichoderma* species. The properties examined included antagonistic effects against phytopathogens; lytic enzyme activities (β-1,3-glucanase, chitinase, protease, and cellulase); siderophore and indole-3-acetic acid (IAA) production; and phosphate solubility and potassium mobilization capabilities. Therefore, this study aimed to specify the biofertilizer and biocontrol properties of *Trichoderma* spp. that are candidates for use in agriculture. Out of the 62 *Trichoderma* spp. examined, 62.90% exhibited chitinase activity, 83.87% β-1,3-glucanase activity, and all of the strains showed cellulase and protease production. Furthermore, 40 strains produced siderophores, all tested positive for IAA production, and 30 strains were able to solubilize potassium and 58 strains were similarly effective in phosphate solubilization. *Trichoderma* species exhibited inhibition rates ranging from 0-87.92% against *Botrytis cinerea,* 21.75-86.25% against *Fusarium solani,* and 17.64-91.17% against *Rhizoctonia solani*. Two *Trichoderma koningiopsis* species and one *Trichoderma harzianum* species exhibiting high PGPF activity were identified. The microbial fertilizer and biological control properties of *Trichoderma* species molecularly identified in this study show promise for biotechnological applications to reduce chemical inputs in agricultural production.

Cite this article as: Karaca K, Eltem R. Screening of *Trichoderma* spp. for biofertilizer and biocontrol potential in vitro. Sigma J Eng Nat Sci 2026;44(1):162–173.

INTRODUCTION

Plant Growth Promoting Microorganisms (PGPMs) are defined as microorganisms that promote plant growth by increasing the production of various hormones and nutrient uptake, and that can exhibit antagonistic activity against many plant pathogens. Plant growth promoting fungi (PGPFs) are an important group of microorganisms for their ability to stimulate plant growth and activate plant defense mechanisms [1–4].

Trichoderma are attracting great interest due to their multifaceted roles in promoting plant growth and protect

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This paper was recommended for publication in revised form by Editor-in-Chief Ahmet Selim Dalkilic

Published by Yıldız Technical University Press, İstanbul, Turkey

plant diseases. The use of chemicals in agriculture leads to a decrease in biodiversity. By supporting plant nutrition, *Trichoderma* spp. increases soil fertility and contributes to the long term maintenance of soil health [5–7]. *Trichoderma* spp. reduce plant pathogen populations through competition for nutrients and habitat, secondary metabolite production, and mycoparasitism. They also strengthen plant resistance to diseases by enhancing antioxidant defense systems and systemic resistance. Furthermore, they promote plant growth through the release of growth-promoting molecules. *Trichoderma* species produce hydrolytic enzymes that help convert large substrates into smaller, more accessible forms for plant use. Studies on *Trichoderma* spp. have shown that they are extremely resistant to chemical fungicides, as their ability to metabolize different types of pesticides has been proven [5,7,8]. These characteristics allow *Trichoderma* species to be widely used in agriculture as microbial fertilizers and biocontrol agents. *Trichoderma* species have been attracting increasing attention in recent years due to their various functions in sustainable agriculture. By producing lytic enzymes and secondary metabolites, these fungi not only support plant growth through processes like phytohormone synthesis, nutrient solubilization, and siderophore secretion, but they also effectively biocontrol a variety of phytopathogens. Moreover, many recent research have highlighted their capacity to enhance soil health and plant stress tolerance. These characteristics make *Trichoderma* spp. attractive options for biofertilizer formulations and integrated pest management in a range of agroecological environments [9–11]

In this study, the important PGPM properties of *Trichoderma* spp. isolated isolated from different locations were determined. These properties include antagonistic effects against plant pathogens, siderophore and IAA production, lytic enzyme activities, phosphate solubility, and potassium mobilization. This study aimed to contribute to the literature by identifying *Trichoderma* strains with high potential for use in agricultural production.

Therefore, the aim of this study is to evaluate the PGPM potential of *Trichoderma* spp. and to identify promising strains that can be used as biofertilizers and biological control agents in agriculture. Few research have combined molecular identification with a wide range of biofertilizer and biocontrol activities, despite the fact that numerous studies have examined specific plant growth-promoting features of *Trichoderma* spp. By analyzing several PGPM characteristics and linking them to strains that have been genetically identified, this study fills this knowledge gap.

MATERIALS AND METHODS

Microorganisms Used

Sixty of the *Trichoderma* spp. (n=62) used in this study were strains obtained from soil and mushroom compost samples collected from different regions in previous studies [12–15], while two *Trichoderma harzianum* strains were obtained from the Bioengineering Department culture collection. The details of these strains are presented in Table 1. Microscopic structures of the *Trichoderma* spp. were examined using a light microscope at 40× magnifications after staining with 0.05% lactophenol cotton blue. Morphological features such as hyphal structure, conidiophores, and conidia formation were observed and recorded. All of the *Trichoderma* spp. were stored on agar slant at 4 °C and in soil at room temperature.

Phytopathogenic fungi such as *Botrytis cinerea*, *Fusarium solani*, and *Rhizoctonia solani*, found in the Industrial Microbiology culture collection of the Department of Bioengineering, Ege University, were used to determine the antagonistic activity of *Trichoderma* species [16].

Assessment of Antagonıstıc Effect of *Trichoderma* Spp. Against Phytopathogenıc Fungi

The antagonistic effect of *Trichoderma* species against *Rhizoctonia solani*, *Botrytis cinerea*, and *Fusarium solani* was determined by dual culture testing. Under aseptic conditions, *Trichoderma* spp. and phytopathogenic discs were placed on sterile Potato Dextrose Agar (PDA). Petri plates containing only phytopathogenic fungi, not *Trichoderma* spp., were used as a control group. After incubating the Petri dishes at 28 °C for 5 days, the inhibition percentages were determined [17–21].

The formula was used to calculate the growth inhibition:

Growth inhibition (%) = $\frac{(C-T)}{C} x100$

C: Colony diameter (cm) of the phytopathogenic fungi on the control plate.

Table 1. Sources of *Trichoderma* spp. from different samples

Sampling codes	Sampling types	Sampling places
3, 4	*Pleurotus ostreatus* compost	Izmır, Türkiye
7, 11	*Agaricus bisporus* compost	Kocaeli, Türkiye
6, 8, 12	*Agaricus bisporus* casing soil	Kocaeli, Türkiye
13, 14, 15, 16, 17, 18, 19, 20	Heavy metal contamination soil samples	Trabzon, Türkiye
EGE-K-38, EGE-K-49	Soil samples	Izmir, Türkiye

T: Colony diameter (cm) of the phytopathogenic fungi on the plate where the antifungal activity was tested.

Evaluation of Lytic Enzyme Activities

Spore suspensions were prepared from activated *Trichoderma* species at a concentration of 10^8 spores/mL using a 0.1% Tween 80 solution. These suspensions were inoculated 2% (v/v) rate into *Trichoderma* Liquid Enzyme (TLE) Medium, which was modified by the addition of 0.5% colloidal chitin, (14.3 g/L corn step solid, 10 g/L soy peptone, 2 g/L monopotassium phosphate, 0.3 g/L magnesium sulfate heptahydrate, 0.3 g/L glucose, 5 g/L colloidal chitin, 1 mL/L stock salt solution; Stock salt solution: 0.002 g/L $CoCl_2.6H_2O$, 0.0016 g/L $MnSO_4.4H_2O$, 0.005 g/L $FeSO_4.7H_2O$, 0.0014 g/L $ZnSO_4.7H_2O$) for the production of β-1,3 glucanase, chitinase, and protease, and into *Trichoderma* Cellulase Production Medium (20 g/L carboxymethtl cellulose (CMC), 15 g/L KH_2PO_4, 5 g/L $(NH_4)_2SO_4$, 0.6 g/L $CaCl_2.2H_2O$, 0.5 g/L $MgSO_4.7H_2O$) for cellulase production. *Trichoderma* spp. were incubated at 180 rpm for 5 days at 30 °C [18,22,23]. Inoculum-free production medium was used as a negative control.

The 3,5-dinitrosalicylic acid (DNS) method described by Miller (1959) was used to determine β-1,3-glucanase, chitinase, and cellulase activities [24,25]. To determine chitinase activity, 0.6 mL of supernatant was mixed with 1 mL of 0.1 M acetate buffer (colloidal chitin, 1% (w/v)), and the mixture was incubated at 45 °C for 1 h. For β-1,3-glucanase activity, 50 μL of supernatant was mixed with 100 μL of 0.05 M acetate buffer (laminarin, 1% (w/v)), and incubated for 30 min at 40 °C. To determine cellulase activity, 200 μL of supernatant was mixed with 0.1 M acetate buffer containing 500 μL of 1% carboxymethyl cellulose (CMC) and incubated for 15 min at 50 °C. Following the incubation periods, 0.75 mL of DNS reagent was added, and the mixtures were boiled at 100 °C for 10 min. Then, all samples were centrifuged at 10,000 rpm for 5 min, and the absorbances of the supernatants were measured at 530 nm for chitinase and at 540 nm for cellulase and β-1,3-glucanase. Standard curves establish using N-acetylglucosamine for chitinase and glucose for β-1,3-glucanase and cellulase were used to calculate enzyme activities [18,22,25,26].

To determine protease activity, 50 μL of supernatant was mixed with 450 μL of 0.1 M phosphate buffer (azokazein, 1% (w/v)) and the mixture was incubated for 30 min at 50 °C. The reaction was stopped by adding 250 μL of 25% (w/v) TCA. The absorbance of the samples was measured at 440 nm after mixing the supernatant and 1 N NaOH in a 1:1 ratio. One unit (U) of protease activity was defined as the amount of enzyme that produces 1 μmol of free amino acids in 1 minute, and results were expressed in U/mL [18].

To determine the amount of protein produced by *Trichoderma* species, 100 μL of supernatant and 1 mL of Bradford reagent were mixed, and the mixture was incubated for 5 min. Absorbance values at a wavelength of 595 nm were determined by spectrophotometer. Protein concentration was calculated from a standard curve prepared using bovine serum albumin (BSA) [23]. The specific activity (U/mg) was determined by dividing the enzyme activity (U/mL) by the protein concentration (mg/mL).

Evaluation of Siderophore Production

Activated *Trichoderma* spp. were inoculated into the center of Chromium Azurol S (CAS) agar plates. After incubating *Trichoderma* spp. for 10 days, strains showing zone/orange zone formation were evaluated as positive for siderophore production [27]. The siderophore production index was determined using the following formula: Siderophore Production Index = (Zone Diameter (cm) / Colony Diameter (cm)).

Evaluation of Indole-3-Acetic Acid (IAA) Production

Agar discs taken from each activated *Trichoderma* spp. were inoculated into half-concentration Tryptic Soy Broth (TSB) enriched with 0.5% (w/w) L-tryptophan and incubated for 7 days at 30 °C and 180 rpm. After incubation, the biomass and culture medium were separated by centrifugation. 2 mL of Salkowski reagent was mixed with 1 mL of supernatant and incubated for 20 min. The absorbances of the samples were then measured at 530 nm. The standard curve was prepared with different concentrations of IAA. TSB medium without inoculum was used as a negative control. [18,28].

Evaluation of Phosphate Solubility

1 mL of 10^8 spores/mL *Trichoderma* spore suspension was inoculated into National Botanical Research Institute's Phosphate (NBRIP) medium (10 g/L glucose, 5 g/L tricalcium phosphate, 5 g/L $MgCl_2·6H_2O$, 0.25 g/L $MgSO_4·7H_2O$, 0.2 g/L KCl, 0.1 g/L ammonium sulfate). After incubation at 30 °C for 7 days at 180 rpm, the samples were centrifuged at 9000 rpm for 10 min. 0.8 mL of ascorbic acid and 4 mL of supernatant were mixed and incubated for 10 min. The absorbance of the samples was measured at 700 nm. A standard curve was constructed using different dissolved phosphate concentrations [18,29].

Evaluation of Potassium Mobility

Activated *Trichoderma* spp. were inoculated spot method onto Modified Alexandrov Agar (10 g/L glucose, 0.5 g/L $MgSO_4·7H_2O$, 0.005 g/L $FeCl_3$, 0.1 g/L calcium carbonate, 2 g/L calcium orthophosphates, 5 g/L potassium silicate and, 0.025% bromotymmol blue). The potassium mobilization of *Trichoderma* species after 10 days of incubation at 27°C was determined by the diameter of the zone formation and the appearance of an orange color [30].

Identification of Microorganisms

ITS species identification of *Trichoderma* spp. (n=3) was performed through external analysis obtained from BM Software Consulting and Lab. Systems Ltd. MEGA software (v11) was used to generate the phylogenetic tree.

Statistical Analysis

All experiments were conducted in triplicate, and standard deviations were calculated. In this study, normality tests were not performed; therefore, the data were analyzed using one-way ANOVA followed by Tukey's post hoc test at a significance level of $p<0.05$, under the assumption of normal distribution. PASW Statistics software (v18) was used for statistical analyses. Means followed by the same letter in parentheses belong to the same statistical group. Letter combinations in parentheses (e.g., (ab)) represent a distinct group and should not be interpreted as equivalent to either 'a' or 'b' groups alone.

RESULTS AND DISCUSSION

A total of 62 *Trichoderma* spp. were used in this study. *T. pleuroticola* 3-2, *T. pleuroticola* 4-2, *T. harzianum* 6-2, *T. pleuroticola* 6-5, *T. virens* 7-2, *T. afroharzianum* 7-4, *T. pleuroticola* 8-3, *T. pleuroticola* 8-7, *T. pleuroticola* 11-2, and *T. harzianum* EGE-K-38 are *Trichoderma* strains that have been identified in previous studies [13,31]. The strains were examined under a microscope. Figure 1 presents the growth of the *Trichoderma harzianum* EGE-K-16-3 strain on PDA plates and its distinct morphological structures observed under the light microscope.

Controlling plant diseases through chemical methods carries many undesirable risks in terms of health, safety, and the environment. Therefore, biological control is considered a powerful and environmentally friendly approach to reducing damage caused by plant pathogens. Evaluation the antagonistic effects of *Trichoderma* species against various phytopathogens is considered important in studies [32]. The inhibition rate results against phytopathogenic fungi are shown in Table 2 and Figure 2.

Based on a review of various studies, it is believed that this research will make significant contributions to the existing literature. In particular, the evaluation of the *Trichoderma* strains identified in this study in different research studies is of great importance. In our study, the *T. harzianum* EGE-K-49 showed the highest antagonistic activity against *F. solani* with an inhibition rate of 86.25%. Zhan et al. (2023), Savaş et al. (2021), and Stracquadanio et al. (2020) reported the effects of *Trichoderma* strains against various pathogens such as *Lasiodiplodia theobromae, B. cinerea,* and *Fusarium* in their studies [33-35].

Table 2. Percentage inhibition values of *Trichoderma* species against phytopathogenic fungi (*B. cineria*, BC; *F. solani*, FS and *R. solani*, RS)

Inhibition percent range	BC	FS	RS
	Number of *Trichoderma* spp.		
0-40	1	1	1
41-60	9	7	0
61-80	41	40	34
81-99	11	14	27

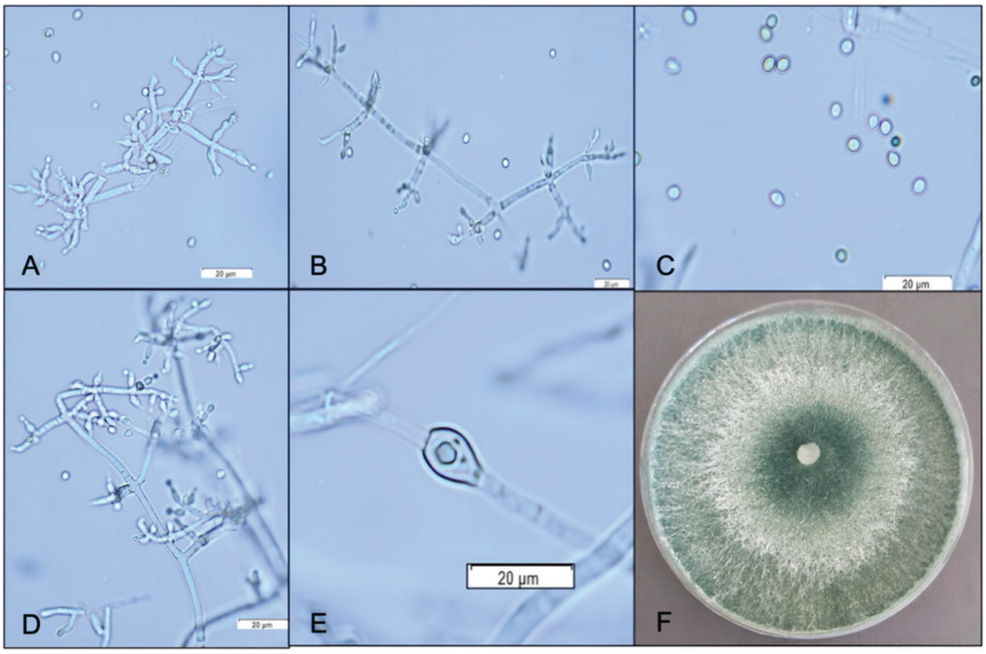

Figure 1. Light microscopic observation at 40x magnifications and PDA plates cultures of *Trichoderma harzianum* EGE-K-16-3. **A, B, D)** Conidiophores, **C)** Conidia, **E)** Chlamydospores, **F)** Cultures on PDA plates.

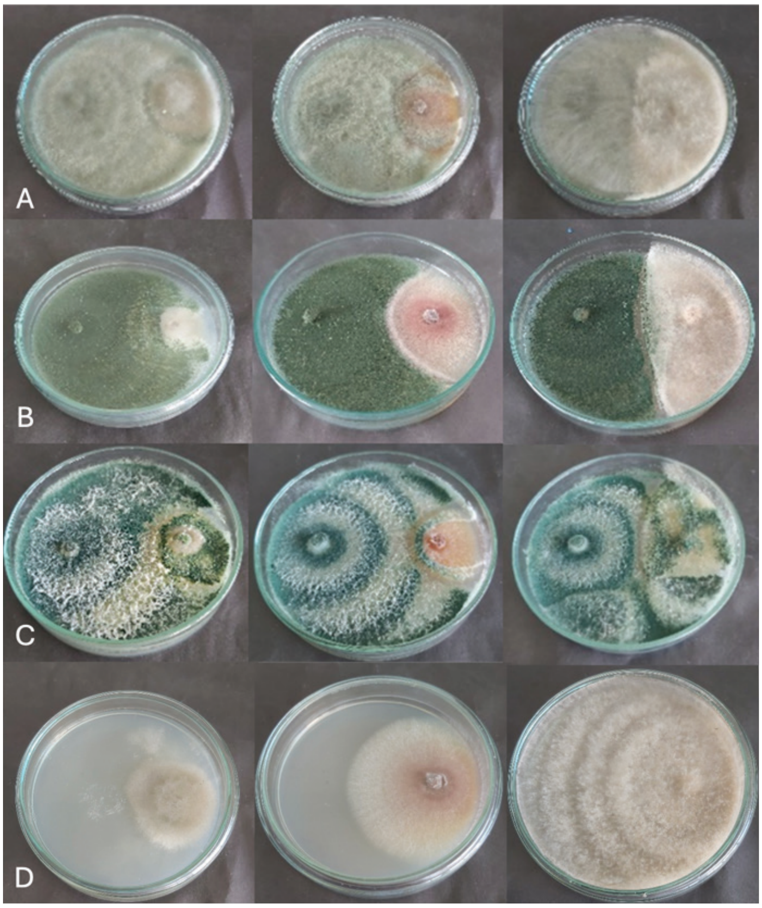

Figure 2. In the dual culture test, *Trichoderma* spp. showed antagonistic activity against phytopathogenic fungi. **A)** *Trichoderma pleuroticola* 4-2, **B)** *Trichoderma harzianum* EGE-K-49, **C)** *Trichoderma harzianum* EGE-K-38, **D)** Control group of phytopathogenic fungi (The phytopathogenic fungi are *B. cinerea*, *F. solani*, and *R. solani*, from left to right).

In this study, the highest inhibition rate against *B. cinerea* was obtained with the *Trichoderma* sp. 19-4 strain, at 87.92%. *Trichoderma* strains are also included in studies aimed at reducing pesticide use within the scope of Integrated Pest Management (IPM). The high antagonistic activities obtained against phytopathogenic fungi in this study contribute to the literature. If field studies are carried out with the *Trichoderma* spp. to be determined, the results to be obtained are very important in this respect. [36,37]. In a study conducted in the literature, the antifungal effects of *Trichoderma* strains on *Fusarium* spp. were investigated in vitro and in vivo. They found an inhibition rate of 71 to 80% in the dual test. In vivo, they reached an inhibition rate of 47 to 51% in chickpea plants. In the literature, differences between in vivo and in vitro are seen as an expected situation [38].

Among the 62 *Trichoderma* spp. examined, strains 52, 39, 62, and 62, respectively, were found to be positive for β-1,3-glucanase, chitinase, protease, and cellulase enzyme production. Chitinase activity ranged from 0.01–0.40 U/mL (0.15–30.66 U/mg), with the highest production observed in *Trichoderma* sp. 13-15. Cellulase activity varied between 1.40–31.43 U/mL (45.99–1231.89 U/mg), with the highest specific activity observed in *Trichoderma* sp. 13-4. β-1,3-glucanase activity was found to be between 0.58–3.38 U/mL (7.69–103.64 U/mg), with the highest production belonging to the *T. afroharzianum* 7-4. Protease activity ranged from 8.00–261.33 U/mL (123.30–7610.18 U/mg), with the highest specific activity determined in the *Trichoderma* sp. 19-3. As seen in Figure 3, the numerical values for β-1,3-glucanase and chitinase activities are relatively low, showing a narrow range of variation. In contrast, cellulase and protease activities have higher numerical values and exhibit a wide range of variation.

When the results in the literature are examined, the enzyme activities determined as a result of this study are similar to previous studies [18,22,39-41]. Chitinase activity results in *Trichoderma* isolates ranged from 0.1-4.5 U/mL. These studies indicated differences in activity among different *Trichoderma* species and incubation periods. [18,39,40]. In this study, enzyme activity was determined only for screening purposes. Similar results to the optimization

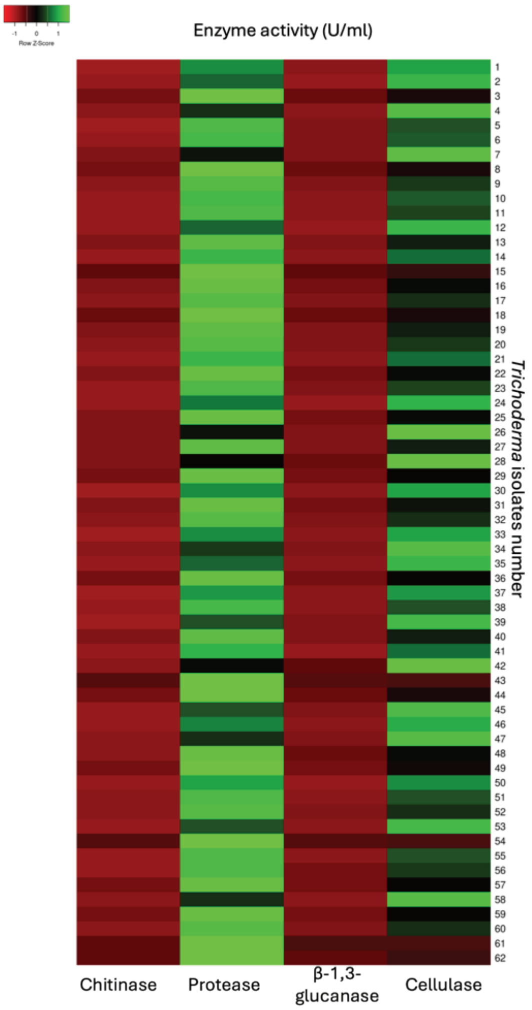

Figure 3. Heatmap including chitinase, protease, β-1,3-glucanase, and cellulase enzyme activity results of *Trichoderma* spp.

studies in the literature indicate that the *Trichoderma* spp. in this study are good enzyme producer candidates. In this regard, it is important to conduct enzyme production optimization studies with the selected *Trichoderma* strains. The fact that different *Trichoderma* spp. showed different results indicates that microorganisms have different characteristics depending on the strain. Even different strains of the same species showed different results. This shows us the importance of screening in a specific study. For example, in our study, *T. pleuroticola* 3-2 exhibited 16.32 U/mg β-1,3-glucanase activity, whereas *T. pleuroticola* 4-2 did not exhibit β-1,3-glucanase activity. Despite belonging to the same species, different results were obtained. Parameters such as the type of sample from which *Trichoderma* spp. was isolated and the location also affect these results.

Although all strains were able to grow on CAS agar medium, zone formation was observed in 40 *Trichoderma* spp. The top row of images in Figure 4 shows the zones formed by *Trichoderma* spp. due to siderophore production. Siderophores produced by microorganisms facilitate iron uptake and can be detected on CAS agar medium thanks to the zones formed as a result of the chelation of Fe from the dye [42,43]. Although studies have shown that hexadecyltrimethylammonium bromide (HDTMA) found in CAS agar causes toxicity in fungi and Gram-positive bacteria, it is thought that *Trichoderma* species may also show high tolerance to the toxic effects caused by HDTMA [42,44,45]. In addition, since siderophores are known to cause biosorption and biological degradation of azo dyes, the transparent zones observed in our study are thought to be degradation [46,47].

In our study, where all strains gave positive results in terms of IAA production, *Trichoderma* sp. 18-13 was determined as the highest IAA producer (111.92 mg/mL). There are limited

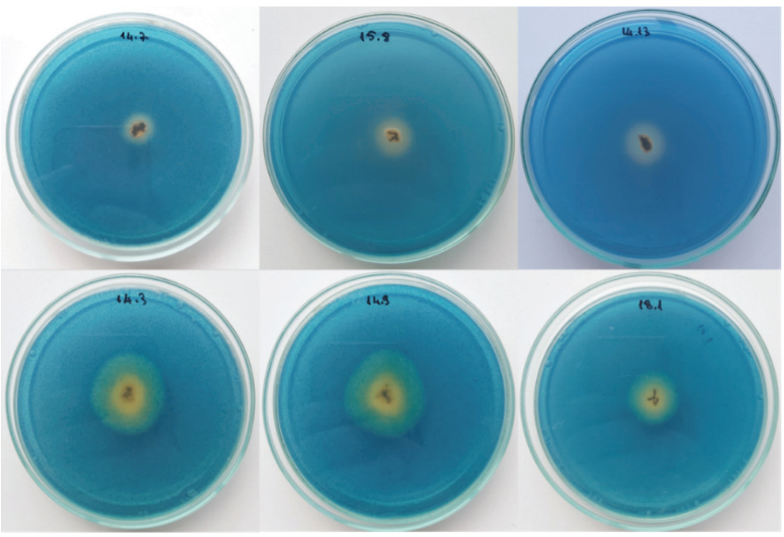

Figure 4. Siderophore results on CAS agar plates. The top row shows clear zone formation indicating siderophore production; the bottom row shows without zone formation.

studies on IAA production by fungi, so it is thought that the results in our study will make a great contribution to the literature [18, 42]. The study results reveal that *Trichoderma* strains are effective microorganisms in terms of IAA production. These characteristics make them promising candidates that can be used in microbial biofertilizer formulations.

In this study, 58 out of 62 *Trichoderma* spp. strains showed positive results in terms of phosphate solubility, with soluble phosphate levels ranging from 0.31 to 4.72 mg/L. *Trichoderma* sp. 13-16 was identified as the strain with the highest phosphate solubility, at 4.72 mg/L. In studies in the literature, *Trichoderma* strains helped dissolve an average of 0.1-20 mg/L of phosphate [18, 48]. When the data obtained from this study are compared with the literature, it is seen that similar phosphate solubility ranges were obtained, but strains showing higher phosphate solubility have also been reported in the literature.

Potassium mobility of *Trichoderma* spp. was evaluated based on zone formation and orange color development in solid medium (Fig. 5). Positive results for potassium mobility between 0.8–5.45 cm were observed in thirty of

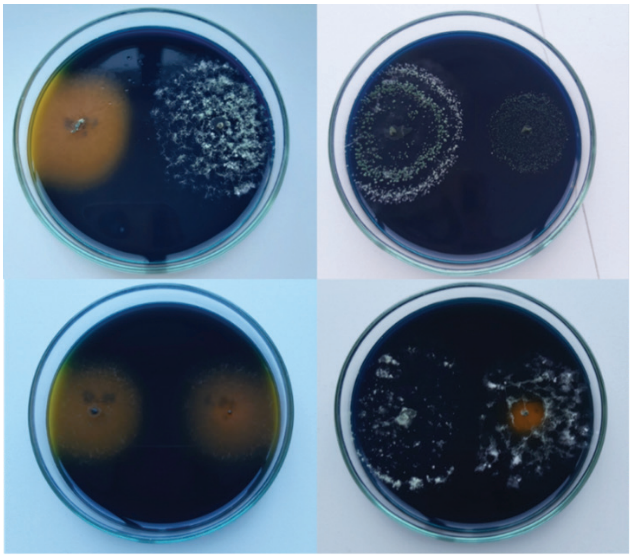

Figure 5. Orange zones formed by *Trichoderma* strains dissolving potassium.

Table 3. *Trichoderma* spp. exhibiting strong antagonistic activity and lytic enzyme production as part of their PGPM potential

***Trichoderma* spp.**	**Antagonictic Activity (% Inhibition rate)**			**Lytic Enzyme Spesific Activity (U/mg)**			
	BC	**FS**	**RS**	**Chitinase**	**β-1,3-glucanase**	**Cellulase**	**Protease**
7-2	87.87±0.01 ab	76.62±1.87 h-k	75.92±1.67 ijk	4.71±3.06 d-i	98.5±15.99 ab	903.37±121.58 bc	1204.30±560.47 d-h
7-4	88.33±0.15 a	79.22±2.35 fgh	75.0±1.0 jkl	3.9±0.13 e-i	103.64±12.99 a	923.73±61.57 b	1751.14±82.77 d-h
8-3	75.75±0.14 i-n	75.32±4.56 i-l	80.55±0.55 fgh	1.24±0.18 hi	12.69±0.42 ijk	548.99±23.71 d-j	232.78±12.45 fgh
13-2	ND	50.0±1.0 v	66.66±1.87 o	11.26±4.0 b-f	60.89±35.59 a-h	372.73±5.42 h-n	3157.68±207.01 c-g
13-4	74.44±1.46 k-q	80.48±2.35 d-g	87.05±11.34 b	7.23±3.53 c-i	45.18±3.82 d-j	1231.89±81.21 a	1822.60±475.60 d-h
13-5	75.94±2.67 i-n	69.49±11.09 op	85.88±2.88 bc	3.05±0.86 f-i	24.02±1.29 g-k	404.66±16.41 g-m	6157.63±113.40 ab
13-7	72.22±8.76 os	75.60±8.97 ijk	86.47±8.75 b	15.58±8.46 bc	48.11±6.61 d-j	369.81±80.23 h-n	2983.79±591.17 c-h
13-13	58.0±1.50 z-(aa)	80.58±5.60 d-g	75.89±1.45 ijk	0.70±0.34 i	19.25±1.17 g-k	776.35±9.39 b-e	1454.22±86.66 d-h
13-15	74.0±0.10 l-q	81.55±2.0 c-f	75.89±0.01 ijk	30.66±4.47 a	89.72±17.84 a-d	355.35±51.18 i-n	5229.08±115.86 abc
13-16	70.0±1.0 r-u	80.58±1.45 d-g	75.89±0.45 ijk	5.96±0.46 d-i	40.0±14.89 e-k	441.82±0.11 g-l	1097.24±189.81 d-h
14-1	72.0±2.50 p-s	86.46±6.78 a	77.67±1.37 hij	4.16±2.43 d-i	24.49±12.28 g-k	431.12±49.93 g-m	1010.19±79.86 d-h
14-7	80.0±1.0 fgh	82.52±5.65 c-f	74.6±1.84 j-m	2.69±0.35 f-i	10.83±0.76 ijk	407.37±23.67 g-m	466.83±33.69 fgh
14-10	52.0±1.50 (ab)	84.46±4.58 abc	75.89±0.01 ijk	3.91±2.53 e-i	41.82±2.37 e-k	601.64±81.87 c-j	892.30±71.05 e-h
14-13	68.0±0.50 tuv	82.52±0.20 c-f	77.60±0.60 hij	0.15±0.02 i	13.23±0.05 ijk	510.45±105.31 e-k	256.42±53.49 fgh
15-1	84.21±1.26 cde	69.23±0.45 op	71.92±2.35 lm	3.94±0.81 e-i	14.35±2.09 ijk	403.53±37.74 g-m	852.71± 146.76e-h
15-8	78.0±20.54 ghi	71.18±2.34 m-p	87.05±5.40 b	1.96±0.46 ghi	13.39±5.68 ijk	444.10±66.74 g-l	547.12±30.21 fgh
16-3	74.44±12.0 k-q	76.82±0.78 h-k	85.88±4.55 bc	3.59±0.45 f-i	20.85±1.23 g-k	664.04±5.64 b-h	2371.32±92.38 c-h
16-4	81.01±0.01 efg	74.57±1.97 j-m	91.17±1.17 a	1.81±0.59 ghi	51.18±18.32 c-j	484.99±29.81 e-k	515.50±36.98 fgh
17-2	74.44±4.87 k-q	75.6±4.56 ijk	86.47±2.56 b	ND	8.34±1.19 jk	393.37±38.94 g-m	248.18±22.56 fgh
18-1	74.44±1.23 k-q	68.29±0.01 pq	87.67±7.88 b	2.8±0.47 f-i	34.98±3.14 f-k	465.39±12.14 f-l	2734.75±280.88 c-h
18-13	86.36±6.79 abc	77.92±4.67 g-j	75.92±3.45 ijk	ND	102.08±5.53 a	418.65±24.04 g-m	1564.02±134.56 d-h
K-38	55.60±1.60 (aa)	84.90±3.27 abc	72.6±15.40 klm	2.01±1.51 ghi	28.20±3.64 g-k	421.78±18.11 g-m	3081.97±170.89 c-h

Each value is the mean ± SD of three replicates. Values with the same letter in a column are not significantly different (Tukey's test, $P > 0.05$).
BC, *Botrytis cinerea*; FS, *Fusarium solani*, and RS, *Rhizoctonia solani*.
ND, not detected

Table 4. *Trichoderma* spp. exhibiting phosphate solubilization, IAA production, siderophore production, and potassium mobilization.

Trichoderma spp.	Phosphate Solubilization (mg/L)	IAA Production (mg/L)	Siderophore Production Indeks	Potassium Mobilization (Zone diameter, cm)
7-2	2.63±0.07 h-t	80.18±1.25 a-e	1.06±0.01 op	ND
7-4	4.61±0.16 ab	43.10±3.62 e-m	1.13±0.01 nop	ND
8-3	4.15±0.04 a-f	67.24±44.68 a-g	1.33±0.23 i-p	ND
13-2	3.00±0.52 f-r	36.34±6.25 e-m	1.55±0.05 f-o	4.15±0.05 e-h
13-4	2.15±0.51 p-v	23.10±0.32 g-m	1.88±0.38 c-h	4.60±0.01 bcd
13-5	2.95±0.19 g-s	17.07±1.89 i-m	2.0±0.18 c-g	3.85±0.05 hi
13-7	3.05±0.09 e-q	55.29±11.61 c-m	2.30±0.38 bc	4.15±0.15 e-h
13-13	2.60±0.01 i-t	95.97±14.94 abc	1.50±0.15 f-p	3.90±0.40 hi
13-15	2.28±0.36 n-t	109.98±0.16 a	1.75±0.22 d-i	4.55±0.15 cde
13-16	4.72±0.20 a	36.04±4.16 e-m	1.43±0.14 h-p	4.0±0.01 ghi
14-1	3.48±0.01 b-m	60.95±5.65 b-k	ND	1.10±0.30 jkl
14-7	2.49±0.01 l-t	33.63±8.48 f-m	1.77±0.08 d-i	3.65±0.15 i
14-10	2.57±0.04 j-t	51.28±0.60 c-m	2.0±0.01 c-g	4.65±0.25 bcd
14-13	1.86±0.04 r-w	34.92±10.97 e-m	2.36±0.07 bc	4.10±0.10 fgh
15-1	2.27±0.19 n-t	47.78±11.23 d-m	1.80±0.20 d-i	4.40±0.10 d-g
15-8	2.19±0.65 o-u	92.69±0.06 a-d	2.36±0.22 bc	4.45±0.05 def
16-3	3.69±0.09 a-k	37.16±2.75 e-m	4.69±0.13 a	ND
16-4	0.74±0.06 vwx	68.12±5.97 a-g	2.0±0.37 c-f	4.45±0.05 def
17-2	3.07±0.20 d-q	60.65±9.96 b-l	1.60±1.50 f-n	5.45±0.05 a
18-1	0.52±0.48 x	14.64±0.43 klm	ND	1.25±0.35 jk
18-13	3.52±0.15 b-m	111.92±0.35 a	1.11±0.04 nop	ND
K-38	3.87±0.27 a-g	63.01±0.19 b-i	1.25±0.05 j-p	ND

ND, not detected

Each value is the mean ± SD of three replicates. Values with the same letter in a column are not significantly different (Tukey's test, P > 0.05).

the 62 *Trichoderma* spp. Mycelial growth was detected in some petri dishes even without zone formation. It is known that *Trichoderma* strains provide potassium solubility by producing various organic acids (citric, lactic, acetic etc.) [49]. The potassium solubilities of *Trichoderma* spp., which are not widely available in the literature, were also determined within the scope of our study. By determining the properties such as IAA production, phosphate solubility and potassium mobility, it was observed that *Trichoderma* strains were also strong in microbial fertilizer properties. From now on, it is thought that microbial fertilizer studies should be used frequently in addition to biocontrol studies.

Analyses identified 22 *Trichoderma* spp. with the highest performance from a total of 62 strains. Table 3 and Table 4 present the quantitative results of the PGPF characteristics exhibited by these strains and their statistical comparisons. Among these, strains 13-15, 14-13, and 16-3, which exhibited superior performance across multiple PGPM assays, were chosen for molecular identification.

The selection of isolates for molecular identification was based on their PGPM traits, considering spectrophotometric quantitative results (lytic enzymes, IAA, phosphate solubilization), percent inhibition in dual culture tests, and semi-quantitative traits such as siderophore and potassium solubilization. *Trichoderma* sp. 13-15 was selected because it showed positive results for all PGPM characteristics, exhibited the highest IAA production, and demonstrated strong antagonistic activity (>70%) against phytopathogens. *Trichoderma* sp. 14-13 was selected for its overall positive PGPM profile and notably high antagonistic activity against *F. solani* (82.52%). Finally, *Trichoderma* sp. 16-3 was chosen due to its high antagonistic effect (>70%), statistically significant siderophore production, and high activity in all four lytic enzymes.

Molecular identification of three *Trichoderma* spp. exhibiting high PGPM activity revealed that two strains were *T. koningiopsis* and one strain was *T. harzianum* (Table 5). Molecular identification has revealed two different strains of *T. koningiopsis*. The fact that the strains showing high PGPM activity gave similar results confirms the relationship between them. One strain was also identified as *Trichoderma harzianum*. This species is one of the most known and studied species among *Trichoderma*. This result supports the literature that it is an important species in terms of PGPM properties. Figure 6 shows the phylogenetic tree.

Table 5. ITS species identification of three *Trichoderma* spp.

Trichoderma spp. No	Accession number	*Trichoderma* strain No	Similarity ratio
13-15	PP127882	*T. koningiopsis* EGE-K-13-15	99.77%
14-13	PP127885	*T. koningiopsis* EGE-K-14-13	99.83%
16-3	PP127886	*T. harzianum* EGE-K-16-3	100%

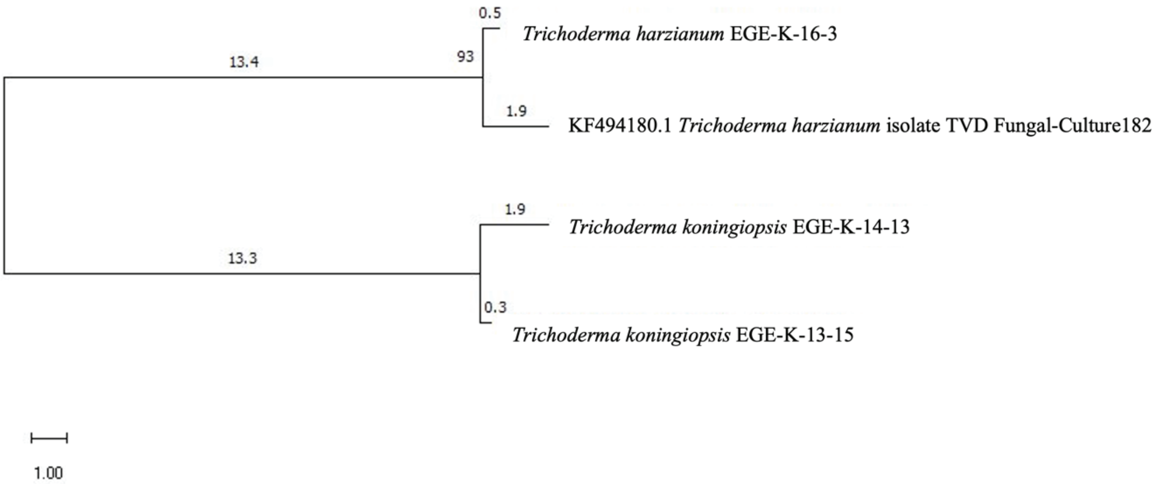

Figure 6. Phylogenetic tree of selected *Trichoderma* strains.

Our study has shown that *Trichoderma* spp. have significant potential as both biocontrol agents and plant growth-promoting microorganisms. In particular, strains *T. koningiopsis* EGE-K-13-15, *T. koningiopsis* EGE-K-14-13, and *T. harzianum* EGE-K-16-3 exhibited high levels of biological activity, demonstrating the high ecological adaptability of *Trichoderma* spp. and highlighting their promising potential as multifunctional bioinoculants for sustainable and integrated agricultural practices.

In addition to the characterization and molecular identification of *Trichoderma* strains for commercialization, effective bioprocessing should also be developed. Developing the production process of *Trichoderma* strains, determining appropriate production conditions and post-production formulation studies are important for commercialization. These parameters, which affect the product's shelf life, must be considered in terms of both application and the continuity of PGPM effectiveness [50,51].

CONCLUSION

Trichoderma species are important in agriculture due to their plant growth-promoting and biological control properties. This study highlights the importance of *Trichoderma* species as plant growth promoters in agricultural applications. Our in vitro results show that *Trichoderma* spp. have remarkable potential in promoting plant growth.

Future studies are recommended to conduct field trials to validate the efficacy of promising *Trichoderma* strains under realistic agricultural conditions. The objectives of this study should be to evaluate their effects on disease control, plant growth promotion, and crop yield under different soil types and climatic conditions. Furthermore, examining the formulation structure and resistance of bio-inoculants developed from these strains is of great importance for their commercial use. In vitro screening studies, however, remain critical as a rapid and cost-effective preliminary evaluation stage that allows for the identification of promising strains based on their functional properties under controlled conditions. Co-inoculation studies with other PGPMs and evaluation of compatibility with chemical pesticides and fertilizers will also make significant contributions to integrated management strategies in sustainable agriculture.

In industrial production, optimizing the production environment contents and physical production parameters should be the next step. It will be a great contribution to the literature to carry out the mentioned studies with three *Trichoderma* strains whose biofertilizer and biological control properties have been determined and molecular identification has been made.

ACKNOWLEDGEMENTS

We would like to thank TUBITAK (The Scientific And Technological Research Council Of Türkiye) for their financial support to project number 122Z711 under the 1002 - Short-Term Support Module.

AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

STATEMENT ON THE USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was not used in the preparation of the article.

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