








Original Article

Functional characterization of endophytic fungi from *Tamarix ramosissima*

Mostafa Ebadi¹ , Solmaz Najari¹ , Leila Zarandi Miandoab¹ , Hamideh Abdolrahmani¹ , Nader Chaparzadeh¹ , Ali Ebadi² 

¹Department of Biology, Faculty of Basic Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran

²Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Karaj, Iran

 <https://doi.org/10.22092/MI.2025.369742.1314>

ABSTRACT

Endophytic fungi are commonly found in the root endosphere and can enhance plant growth through various mechanisms. This study aimed to isolate cultivable endophytic fungi associated with the roots of *Tamarix ramosissima* and evaluate their plant growth-promoting properties. Thirty-five fungal endophytes belonging to the phylum Ascomycota, representing four different genera, were obtained from the endosphere of *T. ramosissima*: *Alternaria*, *Aspergillus*, *Fusarium*, and *Talaromyces*. These fungal endophytes showed different abilities to solubilize phosphate and produce indole-3-acetic acid (IAA). The isolates of *T. allahabadensis* (T3) and *A. niger* (T4) showed different efficiencies in phosphate solubilization. Almost all fungal isolates produced IAA, with the highest concentration (0.699 µg/ml) found in the isolate of *F. solani* (T11). Among the tested endophytes, only *A. fumigatus* (T15) significantly increased wheat root length (75% increase vs control, $p < 0.05$). The extensive root system of *T. ramosissima* may be due to symbiosis with IAA-producing endophytic fungi, which enhance root development and water uptake in dry conditions. Additionally, these fungi can increase soil phosphorus levels, further promoting plant growth.

KEYWORDS

Ascomycota, *Aspergillus fumigatus*, Endosphere, IAA, Root developing.

INTRODUCTION

Phosphorus is one of the most important limiting factors for plant growth and one of the most immobile and least available nutrients. Its deficiency leads to a drastic decline in plant productivity in most soils. Phosphorus is one of the components of DNA, RNA, and energy-carrying molecules in plants and plays an essential role in biochemical processes (Nesme et al. 2018). Phosphorus is a macronutrient, and farmers use large amounts of chemical phosphorus fertilizers to achieve optimal crop production. Due to the low mobility of phosphorus in soil, the efficiency of chemical fertilizers is very low (only 10–25%). Frequent use to compensate for this deficiency not only reduces economic productivity but also contributes to environmental pollution (Bononi et al. 2020). A

reduction in the efficiency of phosphate fertilizers in agricultural soils is caused by the high reactivity of phosphorus with some soil elements, such as calcium, iron, and the formation of insoluble phosphorus minerals (Delgado et al. 2014). It has been reported that 95–99% of soil phosphorus is in the form of insoluble minerals that are not available to plants (Elias et al. 2016). An alternative approach to reducing consumption and increasing the efficiency of chemical fertilizers is the use of indigenous microflora, particularly those capable of dissolving insoluble mineral elements in the soil. Some soil microorganisms can dissolve insoluble phosphorus minerals and increase their availability in the soil (Tian et al. 2021). In the past three decades, phosphate-dissolving

Received: 07 June. 2025

Revised: 27 June 2025

Accepted: 28 June 2025

Published online: 29 June 2025

✉ Corresponding Author: Mostafa Ebadi; Email: ebadi2023@gmail.com



Mycologia Iranica is licensed under a "Creative Commons Attribution 4.0 International (CC-BY 4.0)"

Published by Iranian Mycological Society (IrMS)—<https://mij.areeo.ac.ir>

microorganisms have been reported and the phosphate-dissolving potential of various bacteria and fungi has been evaluated. Among the phosphate-dissolving microorganisms, *Pseudomonas* and *Bacillus* species are the most common among bacteria and *Aspergillus* and *Penicillium* species are the most common among fungi. Among these microorganisms, the ability of phosphate-dissolving fungi is greater than that of bacteria, as they have a hyphae-like structure and spread deeper in the soil (Kuhad et al. 2011). These microorganisms also have other properties, such as the ability to produce phytohormones that can improve plant growth (Naziya et al. 2019).

Phytohormones are organic compounds that are involved in plant growth, physiological processes, and defense mechanisms. The microbiome associated with plants can alter phytohormone homeostasis and manipulate physiological responses under environmental stress. Indole-3-acetic acid (IAA or auxin) is one of the phytohormones that stimulate plant growth. It plays an important role in increasing root biomass and the uptake of plant nutrients (Kuhad et al. 2011). The importance of stimulatory substances to improve plant growth has been investigated in recent years. Endophytic fungi can improve and increase plant growth by producing growth-stimulating substances such as auxin (Meents et al. 2019, Prasad et al. 2017). The ability of fungi to produce auxin varies, and even different isolates of the same species can differ in this respect (Raja et al. 2017). Many physiological processes, including growth, cell differentiation, germination, vascular tissue formation, photosynthesis, and root elongation, are affected by auxin. IAA, which is produced by endophytic fungi, is responsible for stimulating root growth, nitrogen uptake, increasing photosynthesis and expanding host growth in the presence of many abiotic stress factors. For example, auxin produced by *Phoma* and *Penicillium* increases host growth and the uptake of essential nutrients such as calcium, potassium and magnesium under salt stress. Due to the high pH and abundance of calcium ions in many Iranian soils, the available form of phosphorus is primarily below the threshold and required level for plant growth. The use of native phosphate-solubilizing strains is a biological approach to solving this problem. Studies have shown that the positive interaction of the host plant with fungal endophytes can increase the plant's tolerance to biotic and abiotic stress factors (de Carvalho et al. 2020). Thanks to their functions such as dissolving phosphate, producing the hormone auxin, siderophores, increasing the synthesis of biological compounds, helping to regulate osmosis, regulating stomata, increasing the absorption of minerals and altering nitrogen accumulation, endophyte fungi improve the growth and adaptation of plants to biotic and abiotic stress conditions (Anyasi and Atagana 2019). Therefore, it is necessary to identify and utilize these microorganisms to increase phosphorus absorption in the root zone and achieve sustainable agriculture.

Tamarix belongs to the Tamaricaceae family and comprises approximately 54 species, primarily

distributed in countries such as Pakistan, Afghanistan, Iran, Turkmenistan, South Kazakhstan, Western China, and the Eastern Mediterranean regions (Di Tomaso 1998). *Tamarix* species exhibit high adaptability to climate change, particularly in arid and semi-arid environments. Consequently, these species host a variety of microbial endophytes that enhance plant tolerance to both biotic and abiotic stressors. Despite this, no studies have been conducted to assess the potential plant growth-promoting properties of endophytic fungi isolated from *Tamarix* species in Iran. This study aimed to isolate and identify culturable endophytic fungi from the roots of *T. ramosissima* and to characterize their growth-promoting traits.

MATERIALS AND METHODS

Isolation of endophytes from *Tamarix ramosissima*

Plant specimens of *T. ramosissima* were collected from natural habitats in Tabriz, Iran (N46.63148046, E 38.15551580), in May 2021. A voucher specimen (ASMUH-10331) was deposited at the herbarium of Azarbaijan Shahid Madani University. Root samples were washed with running tap water for 10 minutes to remove soil particles and cut into 1 cm sections. For surface sterilization, the root pieces were submerged in 70% ethanol for 1 minute, in a 0.5% sodium hypochlorite solution for 3 minutes, and in 70% ethanol for 30 seconds, then washed three times with sterile distilled water. They were allowed to dry on a paper towel. After drying, the root pieces were placed on potato dextrose agar (PDA) supplemented with 50 mg/L chloramphenicol to inhibit bacterial growth. All plates were incubated at 25 °C for 7–10 days to isolate endophytic fungi. Pure fungal isolates were obtained by selecting individual colonies from the PDA plates and transferring them to fresh PDA medium, followed by incubation at 25 °C for 10 days.

Molecular identification and phylogenetic analysis

For molecular identification, the pure isolates were inoculated into 100 ml of potato dextrose broth (PDB, Difco) media and incubated at 24 °C for one week on a rotary shaker at 110 rpm. The mycelia were harvested by filtration, dried for 48 hours at 50 °C, and stored at 4 °C for further study. Total genomic DNA of the fungal isolates was extracted following the method proposed by Zhu et al. (1993). The purified genomic DNA was used as a template for rDNA amplification using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Toju et al. 2012).

Amplification was carried out in a thermocycler (SensoQuest, Germany) with the following program: 94 °C for 2 min, 35 cycles at 94 °C for 20 s, 57 °C for 30 s, 72 °C for 2 min, followed by a final extension step of 10 min at 72 °C. The PCR products were then electrophoresed at 85 V on 1.2% agarose gels, and the resulting bands were visualized under a UV transilluminator.

The obtained sequences (Pishgam Biotech Co.) were deposited in the NCBI GenBank and compared with existing sequences using BLAST searches. Phylogenetic

analyses were conducted using MEGA software version 6.0, with sequence alignment performed using MUSCLE (Tamura et al. 2007). The phylogenetic tree was constructed using the neighbor-joining (NJ) method with a p-distance substitution model and bootstrapping of 1000 replications.

Qualitative and Quantitative Assessment of Phosphate Solubilization

The evaluation of fungi's ability to solubilize phosphate was conducted on Sperber media, consisting of 10 g glucose, 0.5 g yeast extract, 0.1 g CaCl_2 , 0.25 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5 g $\text{Ca}_3(\text{PO}_4)_2$, and 15 g agar dissolved in 1000 ml distilled water. Plugs of PDA with each fungal isolate were transferred to the centers of Sperber medium and incubated at 26 °C, with observations made daily for 7 days. The growth of phosphate-solubilizing fungal colonies is indicated by the formation of a clear zone around the colony (Mursyida et al. 2015).

The quantitative assessment of fungi's phosphate solubilization ability was conducted using a colorimetric assay method (Lynn et al. 2013). A plug of the isolate was inoculated into 100 mL of liquid Sperber medium (Sperber 1958) and incubated in a shaking incubator at 25 °C and 120 rpm for 7 days. After centrifugation of the culture at 6000 rpm for 45 minutes to separate fungal mycelia from the supernatant, 1 ml of the supernatant was mixed with 1 ml Vanadate-molybdate reagent (Sigma, Aldrich) and incubated for 10 minutes at room temperature. The yellow color formation indicated phosphate concentration, which was measured using a spectrophotometer (Unico 2100 UV-Vis) at a wavelength of 470 nm.

Quantitative Estimation of Indole-3-Acetic Acid Production

The measurement of IAA was conducted using a colorimetric method described by Gordon and Weber (1951). Czapek Dox (CD) medium supplemented with 5 mg/l L-tryptophan was used for this purpose. In 100 mL Erlenmeyer flasks containing 20 mL CD medium, each flask was inoculated with 5 mm fungal plugs and incubated at room temperature for 6 days on a rotary shaker at 120 rpm. After centrifugation of the flask contents at 6000 rpm for 10 minutes, 1 mL of the supernatant of each isolate was mixed with 2 mL of Salkowski reagent (HClO_4 (70%) + FeCl_3 (0.5 M)) and incubated in the dark for 30 minutes. The development of a pink color indicated IAA production, with absorbance measured at 530 nm using a spectrophotometer. The IAA concentration was determined using an IAA standard curve generated from serial dilutions of IAA solution.

Impact of Endophytic Fungal Inoculation on Root Growth

The endophytic fungal isolates (T3-T17) were used as bio-inoculants to evaluate their effect on wheat plant (*Triticum aestivum* L.) root growth, measured as root length (cm). Wheat seeds were surface-sterilized with 2.5% sodium hypochlorite,

washed with distilled water, soaked in CD broth media (Merck, Darmstadt, Germany) (supplemented with 5 mg/l L-tryptophan) inoculated with fungal isolates, and incubated for 24 hours. The soaked seeds were then transferred to sterilized cups with wet filter paper and incubated for 7 days at room temperature in the dark. CD broth without fungal inoculations served as the control. The average root length of wheat was calculated for each treatment based on ten samples.

Statistical Analysis

Each experiment was replicated three times, and data were analyzed using SPSS ver. 17 with one-way analysis of variance (ANOVA) in a completely randomized design. Mean comparisons were determined using Tukey's test ($p < 0.05$).

RESULTS

Morphological and Phylogenetic analysis of endophytic fungi

In this study, it was found that the roots of *T. ramosissima* were associated with endophytic fungi. A total of 35 fungal endophytes were isolated from the root fragments and classified into 9 groups based on their morphological characteristics, such as colony shape, surface texture, growth rate, and colony color (both obverse and reverse sides) (Table 1). The fungal isolates exhibited circular colony shapes, except for T4, 7, 8, and 12, which had irregular shapes on PDA. The isolates displayed abundant uniform to cottony mycelium with growth rates ranging from 2.1 to 7.3 cm after 5 days of incubation at 25 °C. The colony colors on both the obverse and reverse sides of the isolates varied from white to black and cream to tan on PDA.

PCR amplification of the ITS rDNA region produced a single band ranging from 500 to 600 bp in size. The phylogenetic tree analysis revealed that the isolates could be categorized into four main clades representing different genera: *Alternaria* (T8), *Aspergillus* (T4, T7, T12, and T15), *Fusarium* (T5, T11, and T17), and *Talaromyces* (T3). BLAST analysis of the fungal isolates showed a 99–100% identity with the ITS sequences of related species (Table 2). Species names were assigned based on the highest similarity of the ITS sequences to known species in the NCBI database. Although the ITS region is considered the primary DNA barcode for fungi, it may not always be sufficient for precise species-level identification. Therefore, the names assigned to the obtained isolates represent the closest known species based on sequence similarity and do not necessarily indicate definitive species identification. Therefore, based on the ITS sequence analysis, the fungal isolates were identified as follows: *Alternaria alternata* (T8), *Aspergillus niger* (T4), *Aspergillus bicephalus* (T7), *Aspergillus terreus* (T12), *Aspergillus fumigatus* (T15), *Fusarium oxysporum* (T5), *Fusarium solani* (T11), *Fusarium redolens* (T17), and *Talaromyces allahabadensis* (T3) (Fig. 1).

Table 1. Morphological characters of endophytic fungi isolated from *Tamarix ramosissima* on PDA.

Morphotype	Colony character				
	Shape	Surface texture	Growth rate (cm)	Obverse color	Reverse color
T3	Circular	Glabrous	2.2 ± 0.1	White green	Yellow orange
T4	Circular	Cottony	3.1 ± 0.2	Black	Sunny green
T5	Irregular	Floccose	2.3 ± 0.2	White pink	Pink
T7	Irregular	Glabrous	2.1 ± 0.1	White	Orange
T8	Irregular	Granular	6.1 ± 0.4	Silver grey	Black
T11	Circular	Cottony	7.3 ± 0.2	White	Canary
T12	Irregular	Floccose	4.8 ± 0.2	Plum	Yellow orange
T15	Circular	Granular	3.7 ± 0.1	Bright green	Cream
T17	Circular	Cottony	5.2 ± 0.2	White	Tan

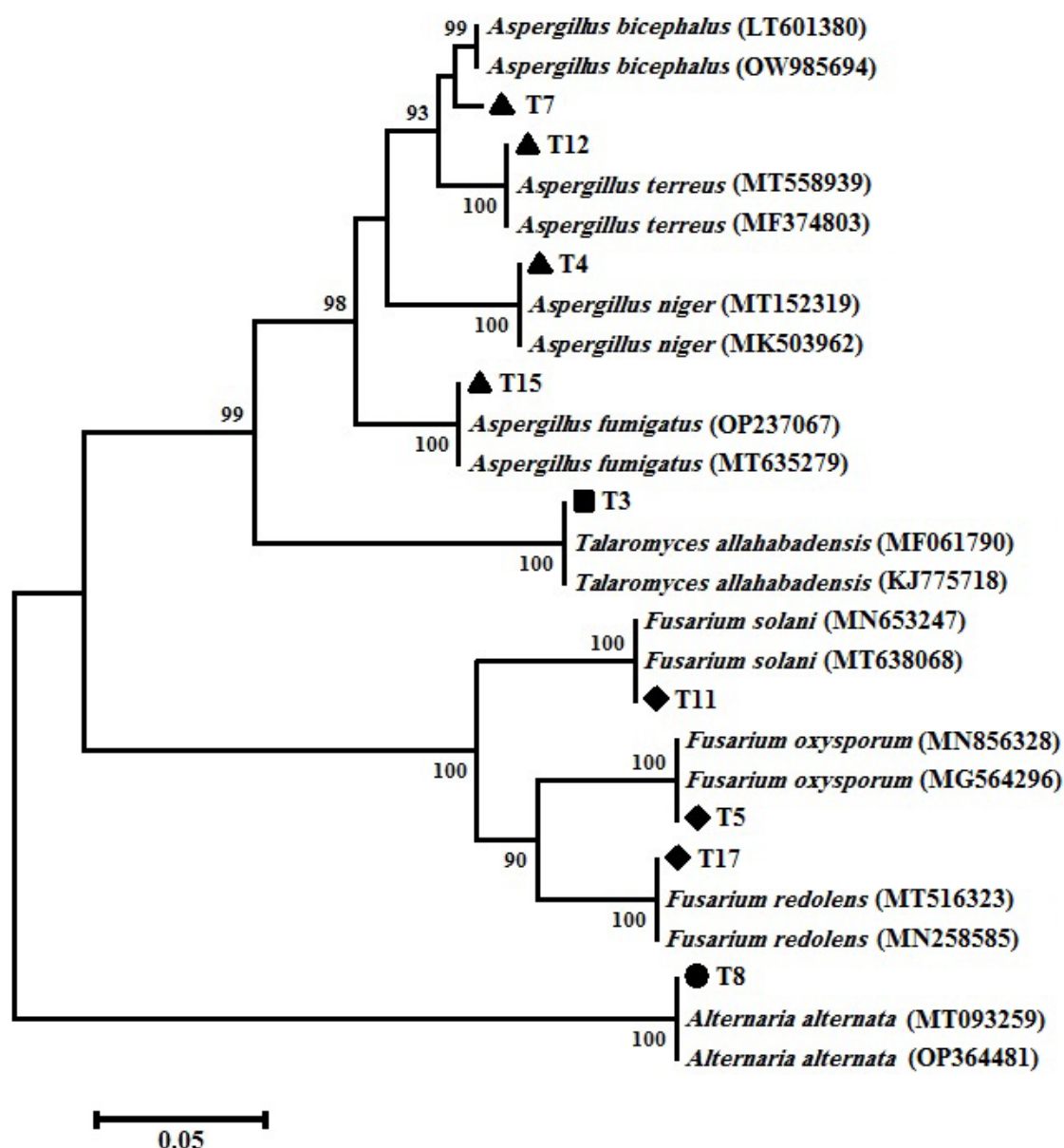
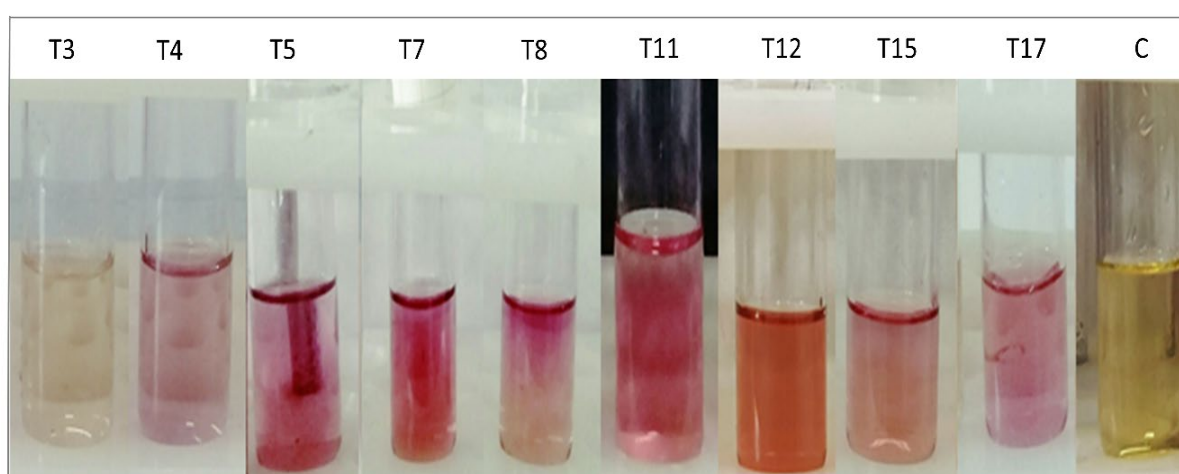
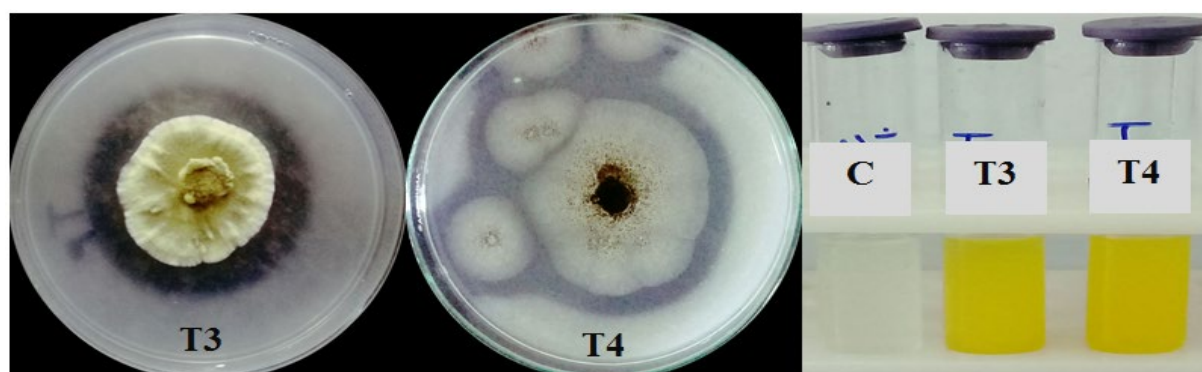


Fig. 1. Phylogenetic analysis of endophytic fungi isolated from *Tamarix ramosissima* based on the internal transcribed spacer. The phylogenetic tree was constructed using the neighbor-joining method (1000 bootstrap replications).

Table 2. The ITS sequence identification of the endophytic fungal isolates from *Tamarix ramosissima*.

Fungal Isolate Code	GenBank Accession Number	Homolog Sequences	Sequence Identity %	Closest Accession Number
T8	OR345327	<i>Alternaria alternata</i>	100	OR018293
T7	OR345326	<i>Aspergillus bicephalus</i>	99.79	LT601380
T15	OR345330	<i>Aspergillus fumigatus</i>	99.82	MT635279
T4	OR345324	<i>Aspergillus niger</i>	100	MT152319
T12	OR345329	<i>Aspergillus terreus</i>	100	MT558939
T5	OR345325	<i>Fusarium oxysporum</i>	100	MG564296
T17	OR345331	<i>Fusarium redolens</i>	100	MT516323
T11	OR345328	<i>Fusarium solani</i>	100	MT638068
T3	OR345323	<i>Talaromyces allahabadensis</i>	100	KP281439

**Fig. 2.** Qualitative production of IAA by fungal endophytes isolated from *Tamarix ramosissima*. T3: *Talaromyces allahabadensis*, T4: *Aspergillus niger*, T5: *Fusarium oxysporum*, T7: *Aspergillus bicephalus*, T8: *Alternaria alternata*, T11: *Fusarium solani*, T12: *Aspergillus terreus*, T15: *Aspergillus fumigatus*, T17: *Fusarium redolens*.**Fig. 3.** Phosphate solubilizing activity of endophytic fungi obtained from *Tamarix ramosissima* on Sperber medium. T3: *Talaromyces allahabadensis*, T4: *Aspergillus niger*.**Indole-3-acetic acid production ability of isolates**

The potency of endophytic fungal isolates for IAA production was quantitatively evaluated using the colorimetric method. When Salkowski reagent was added to the Czapek Dox medium, almost all fungal isolates (except *T. allahabadensis*) developed a pink color to varying degrees (Fig. 2). IAA production by the isolates in the Czapek Dox medium containing 5 mg/ml of L-tryptophan ranged from 0.04 to 0.699 µg/ml. The highest amount of IAA was produced by *F.*

solani (T11) and *A. terreus* (T12), while the lowest value was obtained by *T. allahabadensis* (T3).

Phosphate solubilization activity of isolates

In this study, the phosphate solubilizing activity of all fungal isolates was qualitatively assessed on Sperber medium supplemented with $\text{Ca}_3(\text{PO}_4)_2$ as an inorganic phosphate source. Among the endophytic fungal isolates, only two isolates, *T. allahabadensis* (T3) and *A. niger* (T4), exhibited clear zones, indicating their phosphate solubilizing ability.

Talaromyces allahabadensis (T3) showed the highest solubilization zone with a diameter of 8 mm (Fig. 3).

A total of 10 isolates were tested for the quantitative estimation of phosphate-solubilizing activity in the Sperber medium. Upon addition of the Vanadate-molybdate reagent, only the culture medium of *T. allahabadensis* (T3) and *A. niger* (T4) changed color to bright yellow. The maximum phosphate dissolution was observed in *T. allahabadensis* (T3) and *A. niger* (T4) isolates, with concentrations of 0.260 and 0.298 µg/ml, respectively (Fig. 4).

Effect of Endophytic Fungi Inoculation on Wheat Root Growth

The data analysis revealed that only *A. fumigatus* (T15) significantly enhanced wheat root length compared to the non-inoculated control ($p < 0.05$) (Fig. 5). Other inoculated strains showed no statistically significant effects on root growth. Notably, while *A. alternata* (T8) exhibited reduced root length compared to *A. fumigatus*, this difference was not statistically significant when compared to the control group.

DISCUSSION

Isolation and identification of endophytes are crucial steps in studying phylogeny, diversity, plant interactions, and the potential use of biological

inoculants to enhance plant growth and adaptation. Additionally, it is important to explore the sources of biologically active molecules with industrial and medicinal significance (Strobel and Daisy 2003). Fungal identification is challenging due to their vast diversity and morphological similarities among species. Therefore, molecular phylogeny methods are essential for accurate species identification. DNA barcoding systems utilize a short standard region (typically between 400 and 800 bp) for species identification, with the ITS region being a significant molecular marker known for its high accuracy in fungal identification. The ITS region has been widely used for a diverse range of fungi (Lacap et al. 2003, Begerow et al. 2010). In this study, phylogenetic analysis revealed that the isolates were grouped into distinct clades, and their precise taxonomic placement was determined based on the ITS region analysis. The isolates obtained in this study belong to the *Ascomycota* branch, which is consistent with the dominance of *Ascomycota* species as endophytic fungi as shown in previous studies (Khalil et al. 2021). Among the studied samples, *Aspergillus* species (*A. bicephalus*, *A. fumigatus*, *A. niger*, *A. terreus*) and *Fusarium* species (*F. oxysporum*, *F. redolens*, and *F. solani*) exhibited the highest species richness. These species have been previously reported as endophytes in various hosts (Perrone and Gallo 2017).

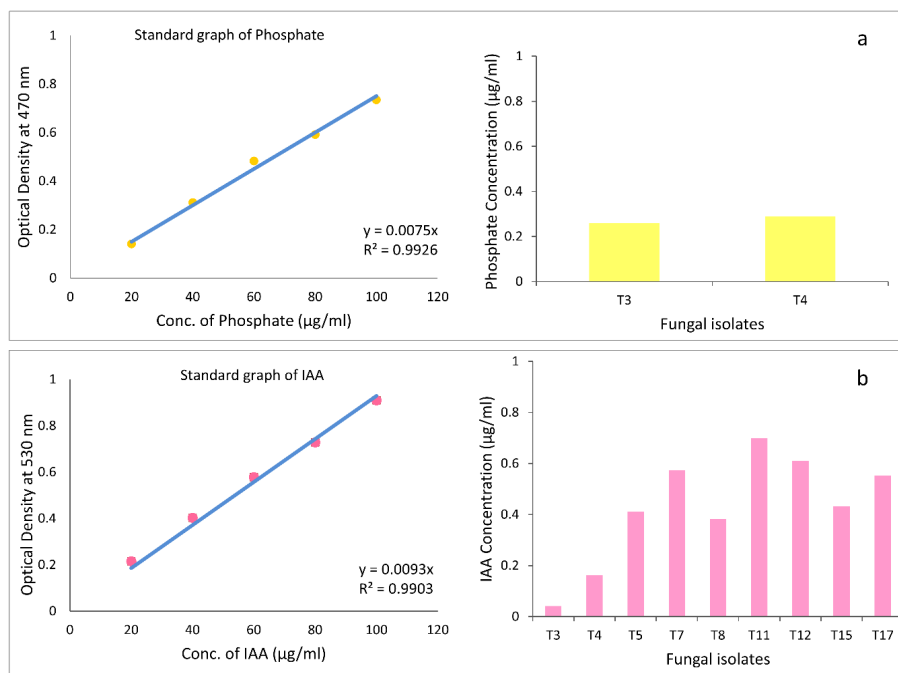


Fig. 4. Phosphate solubilization (a) and IAA production (b) by endophytic fungi isolates. T3: *Talaromyces allahabadensis*, T4: *Aspergillus niger*, T5: *Fusarium oxysporum*, T7: *Aspergillus bicephalus*, T8: *Alternaria alternata*, T11: *Fusarium solani*, T12: *Aspergillus terreus*, T15: *Aspergillus fumigatus*, and T17: *Fusarium redolens*.

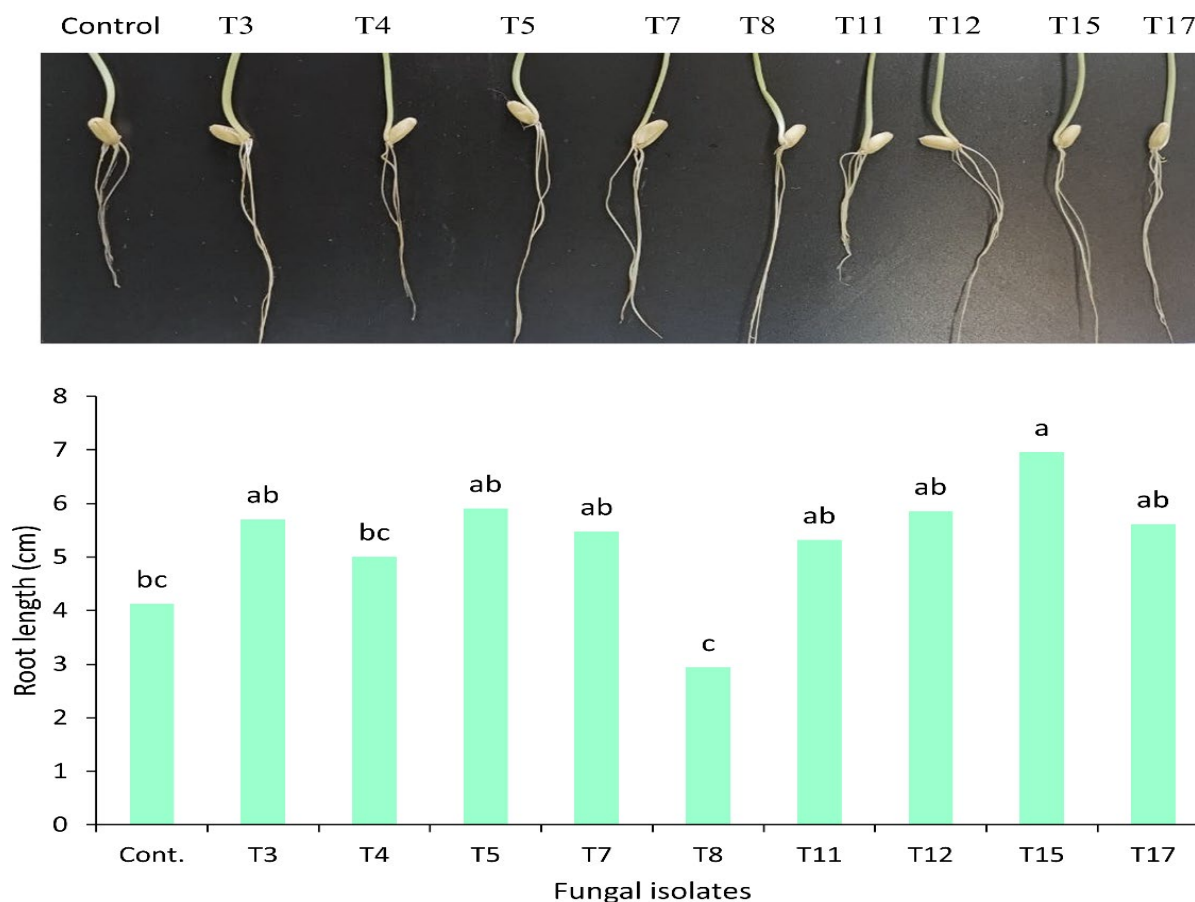


Fig. 5. Effect of endophytic fungi as bioinoculants on root length of wheat. T3: *Talaromyces allahabadensis*, T4: *Aspergillus niger*, T5: *Fusarium oxysporum*, T7: *Aspergillus bicephalus*, T8: *Alternaria alternata*, T11: *Fusarium solani*, T12: *Aspergillus terreus*, T15: *Aspergillus fumigatus*, and T17: *Fusarium redolens*. Different letters (a, b, c, and d) on bars denote that the mean values are significantly different ($p < 0.05$) by the Tukey LSD test.

In this study, only *A. niger* and *T. allahabadensis* isolates formed a zone on a solid Sperber medium and were able to dissolve phosphate in a liquid Sperber medium. The phosphorus-dissolving ability of *A. niger* and *T. allahabadensis* has been previously documented (Show et al. 2015, Vardasbi et al. 2020). Phosphate-dissolving fungi play a crucial role in breaking down insoluble phosphorus minerals in the soil by producing organic acids, thereby enhancing phosphorus availability to plants (Jain et al. 2017, Doilom et al. 2020). Wang et al. (2018) demonstrated that *A. niger* primarily dissolves phosphorus through the production of oxalic acid, tartaric acid, and citric acid. The variations in the isolates' phosphorus-dissolving capabilities, as indicated by the ratio of halo diameter to colony size, may be attributed to differences in the type, quantity, and release rate of organic acids produced by each isolate in the solid medium (Selvi et al. 2016). Additionally, some of these fungi can convert organic phosphorus in the soil into a mineral form that plants can absorb by producing phosphatase enzymes.

Phosphate-dissolving fungi enhance plant yield by increasing soil-soluble phosphorus. Despite constituting only 0.1 to 0.5% of the total fungal population in soil, phosphate-solubilizing fungi offer numerous advantages for plant nutrition. Fungi, with their hyphae-like structure, have a higher capacity to dissolve phosphate compared to bacteria, penetrating deeper into the soil (Kuhad et al. 2011). Research indicates that Phosphate-solubilizing fungi release significantly higher concentrations of organic acids than bacteria, resulting in greater phosphorus-solubilizing activity. Additionally, endophytic fungi can dissolve all three common forms of phosphate (Ca-, Al-, and Fe-phosphate), making them valuable in both alkaline and acidic soils (Varga et al. 2020). Consequently, they may serve as more effective phosphate solubilizers in soil than the rhizobacteria population.

The results of the present study revealed that all the studied fungal isolates produced indole acetic acid when grown in an L-tryptophan medium, as indicated by the pink color formation (Adnan et al. 2018).

Specifically, endophytes capable of producing auxin exhibit a red/pink color change upon addition of Salkowski's reagent, attributed to the interaction between auxin and iron resulting in complex compound formation. Previous studies have documented the auxin-producing abilities of *Aspergillus* (Imade et al. 2020), *Talaromyces* (Yilmaz et al. 2014), *Fusarium* (Fan et al. 2021), and *Alternaria* (Nira et al. 2022) species. This study represents the first documentation of *A. bicephalus*'s capacity to produce auxin. Studies have shown that different fungal species have distinct pathways for indole-3-acetic acid (IAA) synthesis, with some species possessing multiple IAA synthesis pathways (Suebrasri et al. 2020). Previous research has demonstrated that IAA produced by endophytic fungi can stimulate the formation of lateral roots and promote the growth of hairy roots. Additionally, fungal-derived IAA can indirectly influence plants by boosting plant immune responses and suppressing pathogenic strains (Fu et al. 2015). It has been reported that IAA produced by endophytic fungi can modulate gene expression and antioxidant homeostasis to mitigate disease. For instance, in a study on sesame plants, Jagadish and Cymbopogon found that the endophytic fungus *Penicillium* sp. effectively alleviated oxidative stress induced by *Fusarium* sp. through IAA production (Jagadish and Chowdappa 2021). In another investigation, pretreatment of tomatoes with IAA-producing *Trichoderma* significantly reduced wilt disease caused by *Ralstonia solanacearum* (Rahnama et al. 2016).

Wheat seed inoculation with *A. fumigatus* (T15) significantly enhanced early root growth ($p < 0.05$), potentially through fungal IAA production. Previous studies have also reported the enhancement of crop growth by auxin-producing endophytic fungi (Suebrasri et al. 2020). However, in this study, the increase in wheat root growth did not show a strong correlation with the amount of auxin production by the strains. It is well-documented that microbial auxin secretion facilitates root colonization and the expression of other growth-promoting traits (Mehmood et al. 2018). Furthermore, *A. fumigatus* endophytes have been reported to produce other growth regulators such as gibberellic acid and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme (Bilal et al. 2018), which could explain the observed effect of inoculated endophytic fungi on wheat root growth.

CONCLUSION

The high reactivity of phosphorus with soil and the formation of its insoluble forms significantly reduces the efficiency of using phosphorus chemical fertilizers in agricultural lands. Additionally, in drought conditions, reduced phosphorus mobility and suppressed root development severely limit plant access to phosphorus. In such circumstances, the isolates obtained in this study can effectively enhance

phosphorus availability and promote plant root system development through auxin hormone production. Further research on the fungal isolates for additional growth-promoting characteristics and their potential for colonizing crop roots is necessary to leverage this approach for sustainable production in the face of climate change.

ACKNOWLEDGMENT

The authors would like to thank Azarbaijan Shahid Madani University for conducting and for its financial support of this research.

AUTHOR CONTRIBUTION

M.E.: Supervision, conceptualization, writing original draft, S.N.: Investigation, L.Z.M.: Methodology, Formal analysis, H.A.: Investigation, N.C.: Writing - review & editing, and A.E.: Writing - review & editing, Visualization.

DATA AVAILABILITY

The data used and analyzed for the current study can be obtained from the corresponding author.

DECLARATION

The authors have no conflict of interest to declare.

FUNDING

This study was funded by the Azarbaijan Shahid Madani University (grant number 14041013).

ETHICS APPROVAL

Not applicable.

REFERENCES

- Adnan M, Alshammari E, Ashraf SA, Patel K, Lad K, Patel M. 2018. Physiological and Molecular Characterization of Biosurfactant Producing Endophytic Fungi *Xylaria regalis* from the Cones of *Thuja plicata* as a Potent Plant Growth Promoter with Its Potential Application. *BioMed Research International*. 2018(1): 7362148. <https://doi.org/10.1155/2018/7362148>.
- Anyasi R, Atagana HI. 2019. Endophyte: Understanding the microbes and its applications. *Pakistan Journal of Biological Sciences*. 22: 154-167. <https://doi.org/10.3923/pjbs.2019.154.167>.
- Begerow D, Nilsson H, Unterseher M, Maier W. 2010. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology*. 87: 99-108. <https://doi.org/10.1007/S00253-010-2585-4>/METRICS.
- Bilal L, Asaf S, Hamayun M, Gul H, Iqbal A, Ullah I, Lee IJ, Hussain A. 2018. Plant growth promoting endophytic fungi *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. *Symbiosis*. 76: 117-127.

- <https://doi.org/10.1007/S13199-018-0545-4/METRICS>.
- Bononi L, Chiaramonte JB, Pansa CC, Moitinho MA, Melo IS. 2020. Phosphorus-solubilizing *Trichoderma* spp. from Amazon soils improve soybean plant growth. *Scientific Reports*. 10: 1-13. <https://doi.org/10.1038/s41598-020-59793-8>.
- de Carvalho JO, Broll V, Martinelli AHS, Lopes FC. 2020. Endophytic fungi: Positive association with plants. In *Molecular Aspects of Plant Beneficial Microbes in Agriculture*. (Sharma V, Salwan R, Al-Ani LKT, Eds): 321-332. Academic Press, Cambridge, MA, USA. <https://doi.org/10.1016/B978-0-12-818469-1.00026-2>.
- Delgado M, Mendez J, Rodríguez-Herrera R, Aguilar CN, Cruz-Hernández M, Balagurusamy N. 2014. Characterization of phosphate-solubilizing bacteria isolated from the arid soils of a semi-desert region of north-east Mexico. *Biological Agriculture and Horticulture*. 30: 211-217. <https://doi.org/10.1080/01448765.2014.909742>.
- Di Tomaso JM. 1998. Impact, Biology, and Ecology of Saltcedar (*Tamarix* spp.) in the Southwestern United States. *Weed Technology*. 12: 326-336. <https://doi.org/10.1017/S0890037X00043906>.
- Doilom M, Guo JW, Phookamsak R, Mortimer PE, Karunarathna SC, ... et al. 2020. Screening of Phosphate-Solubilizing Fungi From Air and Soil in Yunnan, China: Four Novel Species in *Aspergillus*, *Gongronella*, *Penicillium*, and *Talaromyces*. *Frontiers in Microbiology*. 11: 1-24. <https://doi.org/10.3389/fmicb.2020.585215>.
- Elias F, Woyessa D, Muleta D. 2016. Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia. *International Journal of Microbiology*. 2016(1): 5472601. <https://doi.org/10.1155/2016/5472601>.
- Fan S, Wang Q, Dai J, Jiang J, Hu X, Subbarao KV. 2021. The Whole Genome Sequence of *Fusarium redolens* Strain YP04, a Pathogen that Causes Root Rot of American Ginseng. *Phytopathology*. 111: 2130-2134. <https://doi.org/10.1094/PHYTO-03-21-0084-A/ASSET/IMAGES/LARGE/PHYTO-03-21-0084-AT2.JPEG>.
- Fu SF, Wei JY, Chen HW, Liu YY, Lu HY, Chou JY. 2015. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. *Plant Signaling & Behavior*. 10(8): 1048052. <https://doi.org/10.1080/15592324.2015.1048052>.
- Gordon SA, Weber RP. 1951. Colorimetric estimation of indoleacetic acid. *Plant Physiology*. 26(1): 192. <https://doi.org/10.1104/PP.26.1.192>.
- Imade YN, Rukayat OK, Olubunmi TA, Busayo TA. 2020. Isolation of an emerging thermotolerant medically important Fungus, *Lichtheimia ramosa* from soil. *African Journal of Microbiology Research*. 14: 237-241. <https://doi.org/10.5897/ajmr2020.9358>.
- Jagadish R, Chowdappa S. 2021. Phytochemical Analysis, Extracellular Enzymes and Antioxidant Activity of Endophytic Fungi from *Cymbopogon citratus* L. *International Journal of Botany Studies*. 6: 509-516.
- Jain R, Saxena J, Sharma V. 2017. The ability of two fungi to dissolve hardly soluble phosphates in solution. *Mycology*. 8: 104-110. <https://doi.org/10.1080/21501203.2017.1314389>.
- Khalil AMA, Hassan SED, Alsharif SM, Eid AM, Eweis EED, ... et al. 2021. Isolation and Characterization of Fungal Endophytes Isolated from Medicinal Plant *Ephedra pachyclada* as Plant Growth-Promoting. *Biomolecules*. 11(2): 140. <https://doi.org/10.3390/BIOM11020140>.
- Kuhad RC, Singh S, Lata, Singh A. 2011. Phosphate-Solubilizing Microorganisms. In *Bioaugmentation, Biostimulation and Biocontrol*. (Singh A, Parmar, N, Kuhad R, Eds): 65-84. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-19769-7_4.
- Lacap D, Hyde K, Liew E. 2003. An evaluation of the fungal 'morphotype' concept based on ribosomal DNA sequences. *Fungal Diversity*. 12: 66.
- Lynn TM, Win HS, Kyaw EP, Latt ZK, San San Yu. 2013. Characterization of Phosphate Solubilizing and Potassium Decomposing Strains and Study on their Effects on Tomato Cultivation. *International Journal of Innovation and Applied Studies*. 3: 959-966.
- Meents AK, Furch ACU, Almeida-Trapp M, Özyürek S, Scholz SS, ... et al. 2019. Beneficial and pathogenic Arabidopsis root-interacting fungi differently affect auxin levels and responsive genes during early infection. *Frontiers in Microbiology*. 10: 1-14. <https://doi.org/10.3389/fmicb.2019.00380>.
- Mehmood A, Hussain A, Irshad M, Khan N, Hamayun M, ... et al. 2018. IAA and flavonoids modulates the association between maize roots and phytostimulant endophytic *Aspergillus fumigatus* greenish. *Journal of Plant Interaction*. 13: 532-542. <https://doi.org/10.1080/17429145.2018.1542041>.
- Mursyida E, Mubarik N, Tjahjoleksono A. 2015. Selection and identification of phosphate-potassium solubilizing bacteria from the area around the limestone mining in Cirebon quarry. *Research Journal of Microbiology*. 10: 270-279.
- Naziya B, Murali M, Amruthesh KN. 2019. Plant Growth-Promoting Fungi (PGPF) Instigate Plant Growth and Induce Disease Resistance in *Capsicum annum* L. upon Infection with *Colletotrichum capsici* (Syd.) Butler & Bisby. *Biomolecules*. 10(1): 41. <https://doi.org/10.3390/BIOM10010041>.
- Nesme T, Metson GS, Bennett EM. 2018. Global phosphorus flows through agricultural trade. *Global Environmental Change*. 50: 133-141. <https://doi.org/10.1016/J.GLOENVCHA.2018.04.004>.

- Nira ST, Hossain MF, Hassan NUM, Islam T, Akanda AM. 2022. *Alternaria* leaf spot of broccoli caused by *Alternaria alternata* in Bangladesh. *Plant Protection Science*. 58: 49-56. <https://doi.org/10.17221/44/2020-PPS>.
- Perrone G, Gallo A. 2017. *Aspergillus* species and their associated mycotoxins. *Methods in Molecular Biology*. 1542: 33-49. https://doi.org/10.1007/978-1-4939-6707-0_3/COVER.
- Prasad MR, Sagar BV, Devi GU, Triveni S, Rao SRK, ... et al. 2017. Isolation and Screening of Bacterial and Fungal Isolates for Plant Growth Promoting Properties from Tomato (*Lycopersicon esculentum* Mill.). *International Journal of Current Microbiology and Applied Sciences*. 6: 753-761. <https://doi.org/10.20546/ijemas.2017.608.096>.
- Rahnama K, Jahanshahi M, Nasrollanejad S, Fatemi MH, Shahiri Tabarestani M. 2016. Identification of Volatile Organic Compounds from *Trichoderma virens* (6011) by GC-MS and Separation of a Bioactive Compound via Nanotechnology. *International Journal of Engineering*. 29: 1347-1353. <https://doi.org/10.5829/idosi.ije.2016.29.10a.04>.
- Raja HA, Miller AN, Pearce CJ, Oberlies NH. 2017. Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. *Journal of Natural Products* 80: 756-770. https://doi.org/10.1021/ACS.JNATPROD.6B01085/ASSET/IMAGES/LARGE/NP-2016-01085V_0007.JPEG.
- Selvi K, Paul J, Vijaya V, Saraswathi K. 2016. Analyzing the Efficacy of Phosphate Solubilizing Microorganisms by Enrichment Culture Techniques. *Biochemistry & Molecular Biology Journal*. 3(1): 1-7. <https://doi.org/10.21767/2471-8084.100027>.
- Show PL, Oladele KO, Siew QY, Aziz Zakry FA, Lan JCW, ... et al. 2015. Overview of citric acid production from *Aspergillus niger*. *Front Life Science*. 8: 271-283. <https://doi.org/10.1080/21553769.2015.1033653>.
- Sperber JJ. 1958. The incidence of apatite-solubilizing organisms in the rhizosphere and soil. *Australian Journal of Agricultural Research*. 9(6): 778-781. <https://doi.org/10.1071/AR9580778>.
- Strobel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*. 67(4): 491-502. <https://doi.org/10.1128/MMBR.67.4.491-502.2003>.
- Suebrasri T, Harada H, Jogloy S, Ekprasert J, Boonlue S. 2020. Auxin-producing fungal endophytes promote growth of sunchoke. *Rhizosphere*. 16: 100271. <https://doi.org/10.1016/J.RHISPH.2020.100271>.
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*. 30(12): 2725-2729. <https://doi.org/10.1093/molbev/mst197>.
- Tian J, Ge F, Zhang D, Deng S, Liu X. 2021. Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle. *Biology*. 10(2) 158. <https://doi.org/10.3390/BIOLOGY10020158>.
- Toju H, Tanabe AS, Yamamoto S, Sato H. 2012. High-Coverage ITS Primers for the DNA-Based Identification of Ascomycetes and Basidiomycetes in Environmental Samples. *PLoS One*. 7: e40863. <https://doi.org/10.1371/JOURNAL.PONE.0040863>.
- Vardasbi H, Saremi H, Fotouhifar KB, Kaveh H, Javan-Nikkhah M. 2020. Novel endophytic species of *Talaromyces* sect. *Talaromyces* associated with saffron plant to the mycobiota of Iran. *Mycologia Iranica*. 7: 219-229. <https://doi.org/10.22043/MI.2021.124383>.
- Varga T, Hixson KK, Ahkami AH, Sher AW, Barnes ME, ... et al. 2020. Endophyte-Promoted Phosphorus Solubilization in *Populus*. *Frontiers in Plant Science*. 11: 567918. <https://doi.org/10.3389/fpls.2020.567918>.
- Wang X, Wang C, Sui J, Liu Z, Li Q, ... et al. 2018. Isolation and characterization of phosphofungi, and screening of their plant growth-promoting activities. *AMB Express*. 8: 1-12. <https://doi.org/10.1186/S13568-018-0593-4/FIGURES/6>.
- Yilmaz N, Visagie CM, Houbraken J, Frisvad JC, Samson RA. 2014. Polyphasic taxonomy of the genus *Talaromyces*. *Studies in Mycology* 78: 175-341. <https://doi.org/10.1016/J.SIMYCO.2014.08.001>.
- Zhu H, Qu F, Zhu LH. 1993. Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. *Nucleic Acids Research*. 21: 5279. <https://doi.org/10.1093/NAR/21.22.5279>.

توصیف عملکردی قارچ‌های اندوفیت جدا شده از گونه *Tamarix ramosissima*

مصطفی عبادی[✉]، سولماز نجاری^۱، لیلا زرنندی میان‌دوآبی^۱، حمیده عبدالرحمانی^۱، نادر چپرزاده^۱، علی عبادی^۲

^۱گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه شهید مدنی آذربایجان، تبریز

^۲پژوهشکده کشاورزی هسته‌ای، پژوهشگاه علوم و فنون هسته‌ای، کرج، ایران

چکیده

قارچ‌های اندوفیت معمولاً در اندوسفر ریشه یافت می‌شوند و می‌توانند رشد گیاه را از طریق مکانیسم‌های مختلف افزایش دهند. این مطالعه با هدف جداسازی قارچ‌های اندوفیت قابل کشت مرتبط با ریشه‌های *Tamarix ramosissima* و ارزیابی خواص آنها در افزایش رشد گیاه انجام شد. سی و پنج اندوفیت قارچی متعلق به شاخه *Ascomycota*، و از چهار جنس مختلف، از اندوسفر *T. ramosissima* به دست آمد: *Aspergillus*، *Fusarium*، *Talaromyces* و *Alternaria*. این اندوفیت‌های قارچی توانایی‌های متفاوتی در حل کردن فسفات و تولید ایندول-۳-استیک اسید (IAA) نشان دادند. جدایه‌های (T3) *Talaromyces allahabadensis* و *Aspergillus niger* (T4) کارایی متفاوتی در حل کردن فسفات نشان دادند. تقریباً همه جدایه‌های قارچی IAA تولید کردند، که بالاترین غلظت (699 میکروگرم در میلی‌لیتر) در جدایه (T11) *Fusarium solani* یافت شد. در میان اندوفیت‌های آزمایش‌شده، تنها *Aspergillus fumigatus* (T15) طول ریشه گندم را به طور قابل توجهی افزایش داد (۷۵٪ افزایش در مقایسه با شاهد، $p < 0.05$). سیستم ریشه‌ای گسترده *T. ramosissima* ممکن است به دلیل همزیستی با قارچ‌های اندوفیت تولیدکننده IAA باشد که توسعه ریشه و جذب آب را در شرایط خشک افزایش می‌دهند. علاوه بر این، این قارچ‌ها می‌توانند سطح فسفر خاک را افزایش دهند و رشد گیاه را بیشتر تقویت کنند.

کلمات کلیدی: آسکومیکوتا، ایندول استیک اسید، اندوسفر، توسعه ریشه، *Aspergillus fumigatus*.