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# Assessment of root colonization, identification, and phosphate solubilization activity of root endophytic fungi obtained from hybrid tomato *Lycopersicon esculentum* Mill. var. TNAU CO3

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Land plants, in general, rely heavily on positive interactions with fungal endophytes for growth, disease resistance, and stress tolerance. Root colonization, isolation and tri-calcium phosphate (TCP) solubilizing activity of root endophytic fungi (REF) obtained from hybrid tomato (*Lycopersicon esculentum* Mill. var. TNAU CO3) plants grown in natural fields were examined. All field-collected tomato roots were positively colonized by DSE (dark septate endophyte) fungi. A total of 94 culturable fungal isolates belonging to 8 distinct orders, 9 genera, and 11 species were recovered and identified by morphological examination. In *L. esculentum* roots, the endophytic fungal colonization rate (CR%) was noticeably high (62.67%). *Aspergillus niger* was the dominant fungus with a relative abundance (RA%) of 23.4%, while *A. niger*, *Cladosporium cladosporioides*, and *Fusarium oxysporum* had the maximum isolation frequency (IF%). Furthermore, we evaluated the nature of the identified fungal isolates as endophytes by a pot study. Thus, 9 of 11 fungi colonized the test plant roots with clear intracellular structures, including *Alternaria* spp., *C. cladosporioides*, *Colletotrichum* spp., and *F. oxysporum*, which are DSE fungi. Furthermore, four REF were able to solubilize the TCP, with SI% ranging from 1.3 to 2.4%. This study's findings will help to improve our understanding of REF interactions and their potential ability to solubilize inorganic phosphate, as well as the application of these fungi to increase the development and productivity of diverse agricultural plants in sustainable agriculture.

**Keywords:** Dark septate endophyte fungi, crop plants, colonization, PVK agar, phosphate soubilization

## INTRODUCTION

Plant roots are quickly colonized in natural habitats by a vast range of microorganisms, including archaea, bacteria, and endophytic fungus, known as the plant microbiome (Liu *et al*. 2017). Arbuscular mycorrhizal (AM) fungi, the most widespread endophytic fungi in plant roots, form symbioses with about 90% of plant species in all habitats (Smith and Read, 2008).

However, dark septate endophytes (DSE) or root-endophytic fungi (REF) also colonize plant roots alongside AM fungi in tropical, arctic, and alpine habitats (Addy *et al*. 2005; Newsham, 2011). Darkly colored (melanin), septate hyphae, and compact microsclerotial structures define DSE fungi. Plants grown in extreme environments, like heavy metal-polluted soils, low nutrient and high salinity soils, upland agroecosystems, and cold conditions, etc., have high DSE prevalence in their roots. It has also been hypothesized that DSE are mostly non-mycorrhizal plant root-inhabiting fungus.

The majority of previous studies on endophytic fungi have focused on their isolation from aerial plant parts (leaf, stem, flower) and from various crop species such as chilli (*Capsicum annuum*) (Paul *et al*. 2012), potato (*Solanum tuberosum*) (Yasser *et al*. 2019), rice (*Oryza sativa*, *Oryza granulate*) (Naik *et al*. 2009; Yuan *et al*. 2010),

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tomato (*Lycopersicon esculentum*) (Larran *et al*. 2001), etc. Endophytic fungal community diversity varies with plant species, host genotype, host age, tissue type studied, climatic conditions, and geographic ranges (Paul *et al*. 2012). Only a few studies on the diversity of these fungi in plant root systems have been conducted (Addy *et al*. 2005). In comparison to fungal endophytes of plant aerial portions, the identification of DSE fungi in commercially important crop species is limited. The diversity of root endophytic (DSE) fungus in rare wild rice (*Oryza granulate*) in China, and soybean roots was studied by Yuan *et al*. (2010) and Rothen *et al*. (2017), respectively. The DSE fungi are polyphyletic and mainly belong to the orders such as Capnodiales, Eurotiales, Helotiales, Hypocreales, Microascales, Pleosporales and Xylariales (Newsham, 2011; Knapp *et al*. 2012).

Tomato (*Lycopersicon esculentum* Mill. var. TNAU CO3; family Solanaceae) is one of the most widely produced vegetable crops worldwide. In a crop duration of 140-145 days, yields are around 96.2 t/ha. Global tomato output reached more than 180 million tons in 2018, with about 5 million ha of harvested tomato growing area globally by FAOSTAT (2018). Because of its widespread global distribution and consumption, tomato is one of the most significant horticultural crops grown. Besides, very few investigations had been made as far as their associated endophytic fungi are concerned (Yuan *et al*. 2010; Wu *et al*. 2008; Ottesen *et al*. 2013) from varied tomato plant cultivars and segments from around the world (Bogner *et al*. 2016; Toju *et al*. 2019; Panebianco *et al*. 2022).

Phosphorus (P) is one of the key macronutrients that affects plant growth and development after nitrogen, accounting for around 0.2% of plant dry weight (Alori *et al*. 2017). Total P availability (0.5%) and phosphate ion mobility in soil are both low (Chai *et al*. 2010). In acidic soils, a bigger proportion of soil P is bonded to aluminium (Al3+) or iron (Fe3+) or calcium (Ca2+) in alkaline soils (Wakelin *et al*. 2004; Wang *et al*. 2018). To maximise crop production in such deficient soils, natural rock phosphate (RP) or synthetic P fertilisers must be applied (Chai *et al*. 2010; Alori *et al*. 2017). Thus, P solubilization is limited in non-acidic soils, which has a number of negative consequences on soil alkalinization, fertility loss, microbial density reduction, and crop yield (Kanse *et al*. 2015; Alori *et al*. 2017). Thus, using resident microorganisms (including bacteria, fungi, and actinomycetes) that are capable of solubilizing insoluble nutrients in the soil and are more environmentally friendly and competitive than non-endophytic microbes is one alternative strategy for reducing the use of synthetic fertilizers (Wakelin *et al*. 2004; Spagnoletti *et al*. 2017; Wang *et al*. 2018). Several studies have revealed that filamentous fungus are more efficient in solubilizing insoluble phosphates than bacteria (Saxena *et al*. 2015; Spagnoletti *et al*. 2017; Wang *et al*. 2018). *Aspergillus*, *Cladosporium*, *Curvularia*, *Fusarium*, *Trichoderma*, and *Penicillium* spp. are common P-solubilizing fungi found both in the rhizosphere and as endophytes (Chai *et al*. 2010), and are commonly used for phosphate solubilization and as organic acid producers (Wang *et al*. 2018). As a result, P-solubilizing fungi are critical for delivering P to plants and allowing the long-term use of P fertilizers. Furthermore, Saxena *et al*. (2015) observed that inoculating P-solubilizing fungi boosted the growth and production of chickpea and maize, among other crops, in soil modified with or without RP. As a result, their findings could greatly contribute in the development of creative, efficient, and long-term solutions for boosting crop quality and yield while minimizing the use of toxic chemicals. Hence, we investigated the root colonization of DSE fungi in filed roots, as well as the endophytic fungal communities inhabiting the root segments of *L. esculentum* var. TNAU CO3 plants and to screen the potential for tricalcium phosphate (TCP) solubilization activities.

## MATERIALS AND METHODS

### *Site and Plant material*

The study was carried out in a conventional agricultural fields situated on Thiruvengaivasal village (10° 24' 29" N Latitude; 78º 47' 20" E Longitude; altitude 578 m a.s.l) Pudukkottai, which relatively plain topography of Tamil Nadu, India. Root and soil samples belonging to tomato (*Lycopersicon esculentum* Mill. var. TNAU CO3)

plants were collected separately by digging a soil core (0–20 cm depth) around the root zones of five randomly selected individuals of tomato crops at a distance of 10 m apart during fruiting stage of the plant and February 2023. Fine roots (~ 2 g) of tomato plants were collected by excavating from trunk to feeder roots to ensure that the roots of the intended species were collected. The soil (approximately 500 g per plant) attached or adjacent to the roots of each sampled plant was placed in individual polythene bags, labeled, and brought to the laboratory. After air-drying in shade, the soil samples were pooled and used for the analysis of soil properties. The collected fine roots were gently washed with water and subjected to the isolation of fungal endophytes and anatomical observation of DSE fungal colonization, as described below.

### *Assessment of root endophytic (DSE) fungal colonization*

For anatomical observation of endophytic (DSE) fungal colonization, the roots (with tips) were firstly washed thoroughly under tap water and cut into 1- cm long segments, and processed for clearing in 2.5% KOH at 90 ˚C for 120 mins in a water bath, depending on the degree of lignifications of the roots (Koske and Gemma, 1989). The cleared roots were washed and acidified with 5N HCl solution for minimum 15-20 mins, and stained with 0.05% of trypan blue overnight at room temperature. The stained root fragments were mounted on slides in lactoglycerol, squashed, and examined for the presence of DSE fungal structures like hyphae (DSH), and microsclerotia (MI) under an light microscopy (Olympus BX51, Japan) at 40× magnification. The root colonization intensity of DSE was estimated using the magnified intersection method (McGonigle *et al*. 1990).

### *Isolation, culture and identification of endophytic fungi*

Symptomless and feeder roots of *L. esculentum* var. TNAU CO3, were initially surface sterilized by the modified method (Addy *et al*. 2005), in which the root segments were first treated with 0.5% (w/v) Sodium Hypochlorite solution for 1 min, rinsed with sterile distilled water for 2 min, and again treated with 70% (v/v) Ethanol for 2 min. The roots were then rinsed thrice with sterile distilled water. For control, the 1 mL final sterile water rinse was plated and observed during the post-incubation period. The absence of fungal growth indicated the root surface was sterile. The surface sterilized roots were kept in dry autoclaved blot paper for 10 min to remove the moisture, cut into 0.5 to 1.0 cm fragments using a sterile surgical blade, and then five root bits were transferred on to each Petri dish (90 mm diam.) containing Potato Dextrose Agar (PDA, includes 200 g/L fresh potato extract, 20 g/L dextrose, and 20 g/L agar, pH 5.6) amended with 150 mg/L of Streptomycin Sulphate. The inoculated plates were incubated at room temperature (28 ± 2 ºC) and checked daily for 7 days. The actively growing fungal colony from the root tips was removed and transferred into fresh PDA plates (Devi *et al*. 2022). Each colony was subcultured at least three more times until a visually uniform culture was obtained. After, purification the fungal cultures were maintained on PDA slants at 4 ºC. The pure cultures of each fungal endophyte were identified using the morphological features of fungi (Domsch *et al*. 1980).

### *Evaluation of endophytic fungal diversity*

The occurrence and diversity of endophytic fungi communities from tomato roots were analyzed (Devi *et al*. 2022). The colonization rate (CR%) of fungal infection was calculated by dividing number of root segments colonized by fungal species by total number of root segments examined × 100. Moreover, the relative abundance (RA%) was calculated by dividing the total number of isolates representing a single taxon by the total number of taxa obtained from root tissues × 100; the isolation frequency (IF%) was calculated by dividing the total number of isolates representing a each taxon by the total number of root segments incubated × 100 (Devi *et al*. 2022).

### *Analysis of soil properties*

Soil properties were assessed in three subsamples. The collected rhizosphere soil pH and Electrical conductivity (EC) were determined at room temperature in the aqueous solution of soil: water (1:1, v: v) using digital pH and

conductivity meters (ELICO, India). Soil texture, organic carbon (OC), total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) were determined (Jackson, 1971).

### *Determination of endophytic nature of isolated fungi by pot experiment*

Pigeon pea (*Cajanus cajan* L) seeds (var CO7) were obtained from Tamil Nadu Agricultural University, Coimbatore, India. Seeds were surface sterilized by immersing them in 2% (w/v) sodium hypochlorite solution for 1 min, followed by immersion in 70% (v/v) ethanol for 3 min and then rinsing with four changes of sterile distilled water. The effectiveness of surface sterilization was confirmed by imprinting the sterilized pigeon pea seeds on PDA medium and checking for contamination after three days (Priyadharsini and Muthukumar, 2017). The surface sterilized seeds were placed in plastic pots (85×50 mm, HW) containing sterilized tomato field soil : sand mixture (1 : 1, v : v) at the rate of five seeds per pot. Fungal inoculation was effected by placing 1 cm diameter fungal plugs obtained from the each identified fungal culture adjacent to the root system. Fungus-free PDA plugs served as controls. The pots with tomato seedlings were watered on alternate days to maintain the moisture at ~60% of the water holding capacity of the soil. The seedlings were harvested 20 days after inoculation and the fresh feeder roots of each pot per fungus was taken to assess the root colonization by isolated fungi and to identify its endophytic nature (Priyadharsini and Muthukumar, 2017).

### *Screening of tri-calcium phosphate (TCP) solubilization by DSE fungi*

To assess the TCP solubilization efficiency the isolated endophytic fungi were inoculated (5.0 mm mycelial disk) on Pikovskaya's (PVK) agar medium (Pikovskaya, 1948) containing 5 g/L of $Ca_3(PO_4)_2$ at pH 7.2. The inoculated culture plates (in triplicate) were incubated at 28 ± 2 ºC for 7 days. A halo/clear zone around the fungal colony represented phosphate solubilization and was measured (Priyadharsini and Muthukumar, 2017). The fungi with potential phosphate solubilization were selected for further studies. Moreover, for qualitative estimation of phosphate solubilization in solid medium, the solubilizing index was also calculated according to Wakelin *et al*. (2004) as follows,

Solubilization index (SI) = Colony diameter + Clearing zone / Colony diameter

### *Data analysis*

All the experimental results related to soil nutrient analysis, root DSE colonization and phosphate solubilization were expressed as mean ± SD.

## RESULTS AND DISCUSSION

### *Tomato root colonization by DSE fungi*

Dark septate and other root invading fungal endophytes are a varied collection of fungi renowned for their dominance in plant belowground tissues worldwide (Surendirakumar *et al*. 2023). These endophytes are thought to play significant roles in fungal communities in harsh, nutrient-limited environments (Yuan *et al*. 2010). In the present study, roots of tomato (*L. esculentum* var. TNAU CO3) were colonized by DSE fungi, as indicated by the presence of melanized septate hyphae (Dsh), and microsclerotia (Mi) cells (Fig. 1). Muthukumar and Tamilselvi (2010) recorded that crop plants were readily colonized by DSE fungi from agricultural fields in Tamil Nadu, southern India. However, the DSE fungal associations in tomato varieties were earlier reported from different tropical, subtropical, and temperate agroecosystems (Kubota and Hyakumachi, 2004; Muthukumar and Tamilselvi, 2010; Muthukumar and Sathya, 2017). The total percentage of DSE colonization in the examined tomato roots were 15.2%, which is lower than that of other studies by Muthukumar and Tamilselvi (2010), who recorded the same variable of DSE fungi in different crop plant roots that ranged from 1% to 61.3%. In general, DSE fungal colonization was abundant in the roots of plants grown in nutrient-stressed environments with steep altitudinal gradients.

In addition, Jumpponen and Trappe (2005) stated that under adverse conditions, DSE fungi can act as mutualists by extracting water and nutrients from the rhizosphere and transporting them to

**Table 1:** Taxonomic distribution and identification of root-endophytic (REF) fungi associated with *L. esculentum* plants

| Phylum | Order | Family | Genus | Species | Strain ID |
|---|---|---|---|---|---|
| Ascomycota | Pleosporales | Pleosporaceae | *Alternaria* | *Alternaria* spp. | STRE-01 |
| | Eurotiales | Trichocomaceae | *Aspergillus* | *A. flavus* | STRE-02 |
| | | | | *A. niger* | STRE-03 |
| | Sordariales | Chaetomiaceae | *Chaetomium* | *C. globosum* | STRE-04 |
| | Capnodiales | Davidiellaceae | *Cladosporium* | *C. cladosporioides* | STRE-05 |
| | Glomerellales | *Glomerellaceae* | *Colletotrichum* | *Colletotrichum* spp. | STRE-06 |
| | Hypocreales | Nectriaceae | *Fusarium* | *F. oxysporum* | STRE-07 |
| | | | *Gibberella* | *G. moniliformis* | STRE-08 |
| | Eurotiales | Trichocomaceae | *Penicillium* | *P. echinatum* | STRE-09 |
| | | | | *P. expansum* | STRE-10 |
| | Hypocreales | Hypocreaceae | *Trichoderma* | *T. longibrachitum* | STRE-11 |

**Table 2:** Absolute (AF) frequency, relative abundance (RA%) and isolation frequency (IF %) of endophytic fungi isolated from the roots of *L. esculentum* plants

| Root fungal endophytes | *L. esculentum* var. TNAU CO 3 | | |
|---|---|---|---|
| | AF | RA (%) | IF (%) |
| *Alternaria* spp. | 3.00 | 3.19 | 33.33 |
| *Aspergillus flavus* Link | 12.00 | 12.77 | 100 |
| *Aspergillus niger* Tiegh | 22.00 | 23.40 | 100 |
| *Chaetomium globosum* Kunze | 5.00 | 5.32 | 66.67 |
| *Cladosporium cladosporioides* (Fresen.) G.A. de Vries | 9.00 | 9.57 | 100 |
| *Colletotrichum* spp. | 5.00 | 5.32 | 66.67 |
| *Fusarium oxysporum* Schltdl., Flora Berolinensis, Pars secunda | 11.00 | 11.70 | 100 |
| *Gibberella moniliformis* Wineland | 6.00 | 6.38 | 66.67 |
| *Penicillium echinatum* E. Dale | 6.00 | 6.38 | 66.67 |
| *Penicillium expansum* Link | 8.00 | 8.51 | 66.67 |
| *Trichoderma longibrachitum* Rifai | 7.00 | 7.45 | 66.67 |
| No. of species= 11 | 94.00 | 100.00 | |

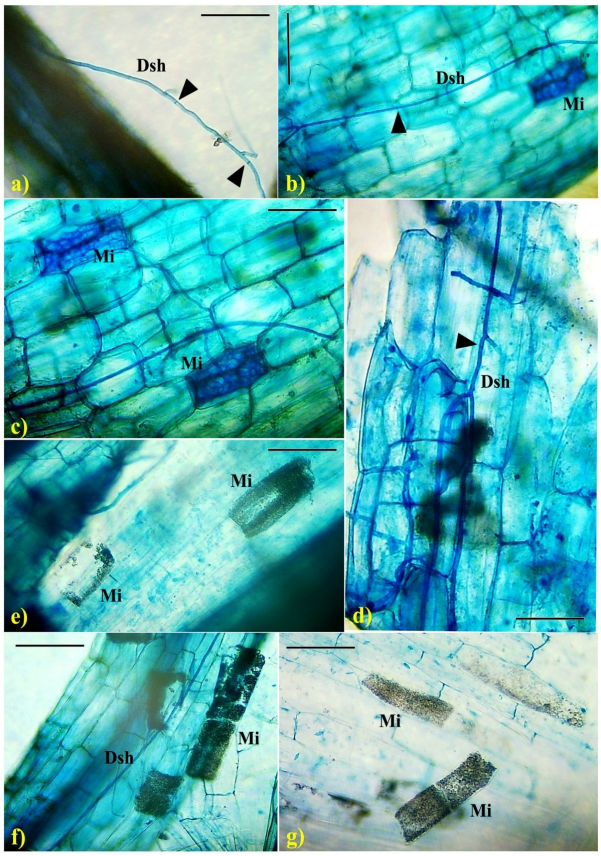


**Fig.1**: Dark septate endophyte (DSE) fungal colonization structures in roots of tomato (*Lycopersicon esculentum* var. TNAU CO3) plants. a, b, d) Different dark septate hyphae (Dsh); c, e, f, g) Microslerotia (Mi) structures in roots of tomato plant. Scale bars = 40 µm

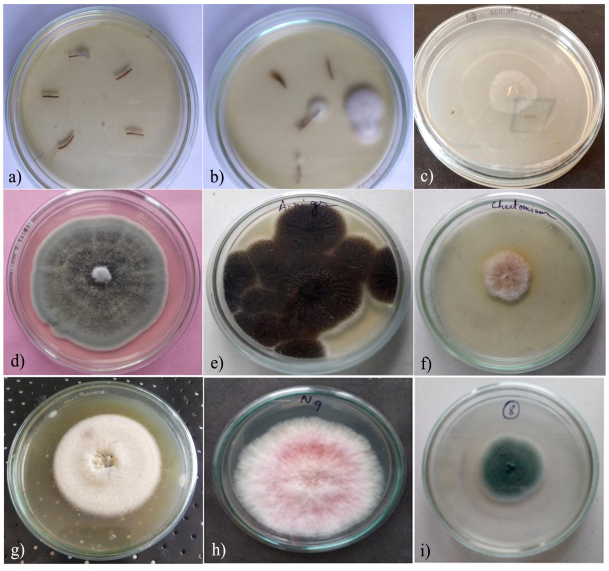


**Fig. 2**: Isolation, pure culture and colony characteristics of the root endophytic fungi (REF) from tomato plants. a) Inoculation of surface sterilized tomato roots; b, c) Colonization of endophytic fungus from root tips; d) *Alternaria* spp.; e) *Aspergillus niger*; f) *Chaetomium globosum*; g) *Fusarium oxysporum*; h) *Gibberella moniliformis*; and I) *Penicillium echinatum*.

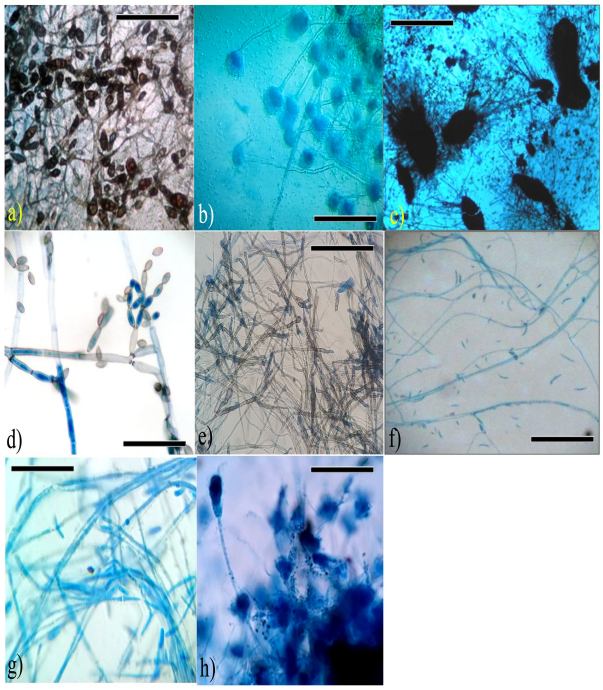


**Fig. 3**: Morphological characters of identified root endophytic fungal species from tomato plants. a) *Alternaria* spp.; b) *Aspergillus niger*; c) *Chaetomium globosum*; d) *Cladosporium cladosporioides*; e) *Colletotrichum* spp.; f, g) *Fusarium oxysporum*; and h) *Penicillium echinatum*. Scale bars = 40 µm

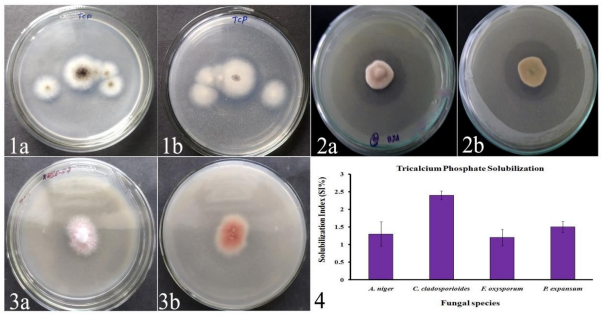


**Fig. 4:** Tri-calcium phosphate (TCP) solubilization activity of endophytic fungi associated with Tomato plant roots. 1a,1b) *Aspergillus niger*, 2a,2b) *Cladosporium cladosporioides*, 3a,3b) *Fusarium oxysporum*, 4) TCP solubilization index

plant roots. However, studies have also shown that association of DSE fungi in plant is mutualistic rather than parasitic (Priyadharsini and Muthukumar, 2017). Soil elements such as N, P, and K influenced the colonization by DSE fungal structures. DSE response to soil nutrients appears to vary with soil conditions and the availability microbial density (Priyadharsini and Muthukumar, 2017). Furthermore, an understanding of the factors influencing the DSE fungal associations and the functions would enable the researchers to explore their possible exploitation in sustainable agriculture.

***Isolation and distribution of root endophytic fungi***

A total of 94 fungi were isolated from 150 root segments of *L. esculentum*. Colonization rate (CR%) of fungi were 62.67%. The isolated root endophytic fungi (REF) were identified on the basis of morphological characteristics (colony colour, reproductive structures and other phenotypic features based on identification manuals) revealed the 11 morphotypes belonging to 9 different genera i.e. *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Gibberella*, *Penicillium* and *Trichoderma* (Fig. 2). Majority of identified fungal species belonged to the Ascomycetous group (Table 1). Out of all, the genera *Aspergillus* and *Penicillium* found to highest with 2 species each. Whereas, the single species, were recovered from each genera like *Alternaria*, *Chaetomium*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Gibberella* and *Trichoderma*, respectively. The identified fungal endophytes from *L. esculentum* as follows, *Alternaria* spp., *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Colletotrichum* spp., *Fusarium oxysporum*, *Gibberella moniliformis*, *Penicillium echinatum*, *Penicillium expansum* and *Trichoderma longibrachitum* (Table 2 and Fig. 3). The relative abundance (%RA) of REF in *L. esculentum* plant roots varied from 3.19% to 23.40% (Table 2). *A. niger* was the most dominant species with high RA%, while *A. niger*, *Cladosporium cladosporioides*, and *Fusarium oxysporum* had the maximum isolation frequency (IF%), and commonly present in all the examined *L. esculentum* roots from natural fields (Table 2).

Previous investigations have reported the diversity and function of dark septate endophytes in the roots of some economically important plant species (Rodriguez *et al*. 2009). It has also been suggested that they may enhance plant health by improving the tolerance to heavy metal pollution and drought stress, thereby reducing the phytopathogenic incidence in differing environmental variables (Xu *et al*. 2015). However, the diversity of root-associated DSE fungi in crops has rarely been investigated (Yuan *et al*. 2010; Surendirakumar *et al*. 2023). Generally, the occurrence and abundance of DSE fungi is comparatively higher in plant roots growing at stressful environments such as high altitudes, temperature, heavy metal contaminated soils and low nutrient sites (Knapp *et al*. 2012).

In our study, a total of 11 root endophytic fungal (REF) species were isolated from Tomato var. TNAU CO3 plants, of these, 5 isolates were identified as DSE fungi, including species of the clade Pleosporales (*Alternaria*), Capnodiales (*Cladosporium*), Glomerellales (*Colletotrichum*), Hypocreales (Nectriaceae- *Fusarium*) etc. Similar distributional patterns were also obtained in studies of fungal endophytes (Newsham, 2011; Knapp *et al*. 2012). Previously, Larran *et al*. (2001) recorded 13 endophytic fungal species from the leaves of tomato var. Tommy grown Argentina. Wu *et al*. (2008) identified 8 fungal taxa by a culture-dependent method using root tissues of tomato plant grown at two different land-use systems of Florida, which is similar to the ranges of fungal endophytes isolated in this study. In contrast, Ottesen *et al*. (2013), Bogner *et al*. (2016), Toju *et al*. (2019) and Panebianco *et al*. (2022) were documented higher number of fungal OTUs using metagenomic approaches (i.e. culture-independent method) in different plant parts like leaves, stem, root, fruit and seeds of tomato plants.

The colonization rates and number of fungal species recovered in Tomato roots, in this study was comparably high with others study. Among the isolated endophytic species, *Aspergillus niger* and *Fusarium oxysporum* were most dominant and commonly distributed which reflects in high relative abundance (%RA) and isolation frequency (%IF). Several studies have indicated that these fungal species can act as endophytes in variety of plant species grown in different ecosystems (Naik *et al*. 2009; Yuan *et al*. 2010; Wu *et al*. 2008). Presence of *Alternaria* spp., *Cladosporium* spp. and *Colletotrichum* spp. is common to majority of plants roots grown in stressed natural environments (Knapp *et al*. 2012). Present findings revealed that the diversity and species occurrence of particular endophytes are not evenly distributed and are mainly influenced by the plant species and available micro-climatic conditions during host plant growth (Naik *et al*. 2009; Knapp *et al*. 2012). Similarly, the

association of other root fungal endophytes isolated in this study, like *Chaetomium globosum*, *Gibberella moniliformis*, *Penicillium echinatum* and *Trichoderma longibrachitum* were also found by different workers from time to time (Yuan *et al*. 2010; Wu *et al*. 2008; Ottesen *et al*. 2013).

***Study of endophytic nature of isolated fungi***

Except for *A. flavus* and *P. expansum*, the majority of *L. esculentum* roots isolated fungal species colonized the test plants (Pigeon pea) in a pot assay. DSE structures (i.e. septate mycelium with or without melanin pigment and compact microsclerotia cells) were not produced in the roots of test plants by *Aspergillus niger*, *Chaetomium globosum*, *Gibberella moniliformis*, *Penicillium echinatum* and *Trichoderma longibrachitum*. However, it generates intracellular flowing hyphae in root cortex cells. This demonstrated the fungal morphotypes were endophytic nature. Furthermore, all of the infected fungi were symptom-free after 20 days. *Alternaria* spp., *Cladosporium cladosporioides*, *Colletotrichum* spp., and *Fusarium oxysporum* invaded the Pigeon pea roots with typical DSE structures. Similar observations were also recorded by Knapp *et al*. (2012).

***Rhizosphere soil properties***

Rhizosphere soils of *L. esculentum* TNAU CO3 had a pH of 6.2, an EC of 0.32 $dSm^{-1}$ and an organic C of 1.95%. Total N, available P and exchangeable K of the soils were 115.4 kg/ha, 8.2 kg/ha and 224.2 kg/ha, respectively. The field soils tested was sandy loam in character.

***Phosphate solubilization by REF in PVK Agar***

All fungal isolates were evaluated for their ability to solubilize TCP, an insoluble inorganic phosphate source in PVK agar. Only four isolates *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, and *Penicillium expansum* produced phosphate solubilization halo zones with SI%s of 1.3 to 2.4%. (Fig. 4). This is similar with the findings of Wakelin *et al*. (2004) and Kanse *et al*. (2015) who reported that soil isolates of *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* solubilized calcium phosphate. These results could be attributed to organic acid generation (Saxena *et al*. 2015; Spagnoletti *et al*. 2017; Wang *et al*. 2018) and the accompanying pH shifts (Priyadharsini and Muthukumar, 2017). Numerous investigations demonstrated that the difference in solubilization index among different P sources revealed the influence of P sources on the fungus' ability to synthesise organic acids. The incubation period, carbon and nitrogen sources, type of inoculation fungus, and presence and types of P sources in the medium were all linked to changes in endophytic fungi's P solubilization abilities (Priyadharsini and Muthukumar, 2017). Furthermore, because of their efficacy in supporting plant growth, phosphate-solubilizing fungi found naturally as endophytes have been identified as a source of P fertilizer. Based on our findings, we believe that these promising strains could be employed as biofertilizers in this location to boost agricultural production.

## CONCLUSION

The isolation of endophytic fungi from hybrid tomato (*L. esculentum* mill. var. TNAU CO3) roots revealed the presence of multiple endophytic species, according to the current study's findings. Furthermore, we determined that DSE fungus colonised all of the tested roots, suggesting the frequency of endophytic association in this farmed regions. P is the soil's least accessible nutrient, although it is essential for plant growth. Only four fungal strains shown potential phosphate solubilization activity and a high solubilization index in PVK medium with *Cladosporium cladosporioides*. As a result, more study on phosphate solubilization from various P sources, the formation of organic acids and plant growth hormone, and the evaluation of plant growth promotion in crop species is required in the future.

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## DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

## REFERENCES


Addy, H.D., Piercey, M.M., Currah, R.S. 2005. Microfungal endophytes in roots. *Can. J. Bot*. **83**: 1-13.

Alori, E.T., Glick. B.R., Babalola, O.O. 2017. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front. Microbiol*. **8**: 971.

Bogner, C.W., Kariuki, G.M., Elashry, A., Sichtermann, G., Buch, A.K., Mishra, B., Thines, M., Grundler, F.M.W., Schouten, A. 2016. Fungal root endophytes of tomato from Kenya and their nematode biocontrol potential. *Mycol. Prog*. **15**: 1-17.

Chai, B., Wu, Y., Liu, P., Liu, B., Gao, M. 2010. Isolation and phosphate-solubilizing ability of a fungus, *Penicillium* sp. from soil of an alum mine. *J. Basic Microbiol.* **51**: 5–14.

Devi, W.S., Surendirakumar, K., Singh, M.S. 2022. Distribution of endophytic fungi associated with *Meriandra bengalensis* Benth. and assessment of their bioactive potential in vitro. *Vegetos* **35**: 995-1006.

Domsch, K.H., Gams, W., Anderson, T.H. 1980. Compendium of soil fungi. Academic Press, London.

FAOSTAT 2018. Statistical Databases- 2018, Accessed February 2023. http://faostat.fao.org/site/342/default.aspx

Jackson, M.L. 1971. Soil chemical analysis. Prentice Hall, New Delhi.

Kanse, O.S., Whitelaw-Weckert, M., Kadam, T.A., Bhosale, H.J. 2015. Phosphate solubilization by stress-tolerant soil fungus *Talaromyces funiculosus* SLS8 isolated from the Neem rhizosphere. *Ann. Microbiol*. **65**: 85–93.

Knapp, D.G., Pintye, A., Kovács, G.M. 2012. The dark side is not fastidious–dark septate endophytic fungi of native and invasive plants of semiarid sandy areas. *PLOS ONE* ***7***: e32570.

Koske, R.E., Gemma, J.N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. *Mycol. Res*. **92**: 486-488.

Kubota, M., Hyakumachi, M. 2004. Morphology and colonization preference of arbuscular mycorrhizal fungi in *Clethra barbinervis*, *Cucumis sativus*, and *Lycopersicon esculentum*. *Mycoscience* **45**: 206-213.

Larran, S., Monaco, C., Alippi, H.E. 2001. Endophytic fungi in leaves of *Lycopersicon esculentum* Mill. *World J. Microbiol. Biotechnol.* **17**: 181-184.

Liu, H., Li, T., Ding, Y., Yang, Y., Zhao, Z. 2017. Dark septate endophytes colonizing the roots of 'non-mycorrhizal'plants in a mine tailing pond and in a relatively undisturbed environment, Southwest China. *J. Plant Interact*. **12**: 264-271.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New Phytol.* **115**: 495-501.

Muthukumar, T., Sathya, R. 2017. Endorrhizal fungal association and colonization patterns in Solanaceae. *Polish Bot. J.* **62**: 287-299.

Muthukumar, T., Tamilselvi, V. 2010. Occurrence and morphology of endorhizal fungi in crop species. *Trop. Subtrop. Agroecosyst.* **12**: 593-604.

Naik, B.S., Shashikala, J., Krishnamurthy, Y.L. 2009. Study on the diversity of endophytic communities from rice (*Oryza sativa* L.) and their antagonistic activities in vitro. *Microbiol. Res*. **164**: 290-296.

Newsham, K.K. 2011. A meta analysis of plant responses to dark septate root endophytes. *New Phytol.* **190**: 783-793.

Ottesen, A.R., González Peña, A., White, J.R., Pettengill, J.B., Li, C., Allard, S., Brown, E. 2013. Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). *BMC Microbiol.* **13**: 1-12.

Panebianco, S., Lombardo, M.F., Anzalone, A., Musumarra, A., Pellegriti, M.G., Catara, V., Cirvilleri, G. 2022. Epiphytic and endophytic microorganisms associated to different cultivar of tomato fruits in greenhouse environment and characterization of beneficial bacterial strains for the control of post-harvest tomato pathogens. *Int. J. Food Microbiol.* **379**: 109861. https://doi.org/10.1016/j.ijfoodmicro.2022.109861.

Paul, N.C., Deng, J.X., Sang, H.K., Choi, Y.P., Yu, S.H. 2012. Distribution and antifungal activity of endophytic fungi in different growth stages of chili pepper (*Capsicum annuum* L.) in Korea. *Plant Pathol. J.* **28**: 10-19.

Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiology* **17**: 362–370.

Priyadharsini, P., Muthukumar, T. 2017. The root endophytic fungus *Curvularia geniculata* from *Parthenium hysterophorus* roots improves plant growth through phosphate solubilization and phytohormone production. *Fungal Ecol.* **27**: 69-77.

Rodriguez, R.J., White, J.F., Arnold, A.E., Redman, A.R.A. 2009. Fungal endophytes: diversity and functional roles. *New Phytol.* **182**: 314-330.

Rothen, C., Miranda, V., Aranda-Rickert, A., Fracchia, S., Rodríguez, M.A. 2017. Characterization of dark septate endophyte fungi associated with cultivated soybean at two growth stages. *Appl. Soil Ecol.* **120**: 62-69.

Saxena, J., Saini, A., Ravi, I., Chandra, S., Garg, V. 2015. Consortium of phosphate-solubilizing bacteria and fungi for promotion of growth and yield of Chickpea (*Cicer arietinum*). *J Crop Improv.* **29**: 353–369.

Smith, S.E., Read, D.J. 2008. Mycorrhizal symbiosis. Academic Press, London.

Spagnoletti, F.N., Tobar, N.E., Fernández Di Pardo, A., Chiocchio, V.M., Lavado, R.S. (2017) Dark septate endophytes present different potential to solubilize calcium, iron and aluminum phosphates. *Appl. Soil Ecol.* **111**: 25–32.

Surendirakumar, K., Pandey, R.R., Muthukumar, T. 2023. Distribution, molecular characterization and phosphate solubilization activity of culturable endophytic fungi from crop plant roots in North East (NE) India. *Vegetos* https://doi.org/10.1007/s42535-023-00743-w.

Toju, H., Okayasu, K., Notaguchi, M. 2019. Leaf-associated microbiomes of grafted tomato plants. *Sci. Rep.* **9**: 1787.

Wakelin, S.A., Warren, R.A., Harvey, P.R., Ryder, M.H. 2004. Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *Biol. Fertil. Soils* **40**: 36–43.

Wang, X., Wang, C., Sui, J., Liu, Z., Li, Q., Ji, C., Song, X., Hu, Y., Wang, C., Sa, R., Zhang, J., Du, J., Liu, X. 2018. Isolation and characterization of phosphofungi, and screening of their plant growth-promoting activities. *AMB Expr.* **8**: 1–12.

Wu, T., Chellemi, D.O., Graham, J.H., Rosskopf, E.N. 2008. Assessment of fungal communities in soil and tomato roots subjected to diverse land and crop management systems. *Soil Biol. Biochem.* **40**: 1967–1970.

Xu, R., Li, T., Cui, H., Wang, J., Yu, X., Ding, Y., Wang, C., Yang, Z., Zhao, Z. 2015. Diversity and characterization of Cd-tolerant dark septate endophytes (DSEs) associated with the roots of Nepal alder (*Alnus nepalensis*) in a metal mine tailing of southwest China. *Appl Soil Ecol.* **93**: 11–18.

Yasser, M.M., Mousa, A.S.M., Marzouk Marym, A., Tagyan, A.I. 2019. Molecular identification, extracellular enzyme production and antimicrobial activity of endophytic fungi isolated from *Solanum tuberosum* L. *Biosci. Biotechnol. Res. Asia* **16**: 135–142.

Yuan, Z.L., Zhang, C.L., Lin, F.C., Kubicek, C.P. 2010. Identity, diversity, and molecular phylogeny of the endophytic mycobiota in the roots of rare wild rice (*Oryza granulate*) from a nature reserve in Yunnan, China. *Appl. Environ. Microbiol.* **76**: 1642–1652.