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# Diversity of endophytic fungal community in *Jatropha heynei* N.P. Balakr

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Endophytic fungi co-exist symbiotically with their host plants in a diverse range of geographic and environmental conditions. Forty seven endophytic fungal species belonged to 26 genera and 16 families were recovered from root, leaf and fruit regions of *Jatropha heynei* by four isolation media such as Potato dextrose agar (PDA), Malt extract agar (MEA), Water agar (WA) and Czapek dox agar (CZA). These fungal communities were grouped into 35 asexual ascomycetes (anamorphs) of 18 genera, and 11 sexual ascomycetes (telomorphs) from 6 genera. Colonization frequency of endophytic fungi was more in WA followed by PDA, MEA and CZA media. Some fungi were media-specific and some of them were found in all of the media tested. Seasonal specificity was observed. *Alternaria tenuissima*, *Ulocladium* sp, *Exserohilum echinochloae* and *Xylaria curta* were isolated only during the winter season, while *Cladosporium herbarum*, *Zygorhynchus* sp., were exclusively isolated only in the rainy season. The genus *Fusarium* was the most abundant in root and in the summer season, while *Alternaria*, *Exserohilum* and *Colletotrichum* were equally abundant in the leaf and during winter season. This study emphasizes the significance of incubation techniques, plant parts and seasons in determining endophytic fungal assemblages in *Jatropha heynei*.

**Keywords:** Ascomycetes, Czapek dox agar, Endophytic fungi, *Jatropha heynei*, Malt extract agar, Potato dextrose agar, Water agar.

## INTRODUCTION

Endophytes are microbes that colonize living, internal plant tissues without harming the host (Bacon & White, 2000). Endophytic fungi are found in all vascular plants (Zhang *et al.* 2006).However, scientists have explored in detail only the endophytic communities of a few plants ( Hyde and Soytong 2008). Environmental conditions, as well as the age of the host species or tissue, determine the distribution of endophytes in a host.

The number of endophytic species that can be recovered from the host decreases with extreme climates. Endophytic fungi have critical physiological and ecological roles in the lives of their host (Malinowski *et al.* 2004). Endophytes are now recognized as a vital part of biodiversity. Endophytic mycoflora distribution varies depending on the host. Their ability to stimulate plant growth, increase disease and pest resistance in host plants, improve plant ability to withstand environmental stress and recycle nutrients in exchange for space and nutrition provided by plants have been attributed to the secondary metabolites produced by them (Strobel and Daisy 2003). Endophytic communities have previously been found in different geographical locations in a variety of plants (Huang *et al.*2008; Shankar *et al.* 2008). It has been observed that some endophytic fungi produce chemical substances that are comparable to those produced by their hosts (Aly *et al.*2010) and have been shown to be a potential source of novel natural products useful in medicine, agriculture, and industry (Lin *et al.* 2010). Endophytes have been reported in medicinal plants (Strobel, 2002), which defend their hosts from pathogenic pathogens and are unexpected producers of metabolites valuable to the pharmacological and agricultural sectors. Recent research has shown that diverse secondary metabolites are produced by the endophytic fungi harboured inside the plants rather than by the plants themselves, emphasizing the significance of the contribution of microbial life to plant thermotolerance, drought resistance, and other crucial survival strategies (Pieterse *et al.* 2018). There is a crucial need to better understand the variety of endophytic fungi

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in dry tropical regions since there is a paucity of data on endophytic diversity in this location, where climatic conditions are extreme to severe and annual rainfall is very less. As a result, investigating the presence of endophytic mycoflora in *Jatropha heynei*, a valued rare medicinal herb, is crucial. Further, the study was taken up to provide a comparative account on the diversity of endophytic fungal assemblages in arial and belowground parts and also the influence of different seasons on the occurrence of endophytic fungi in *J.heynei*.

## MATERIALS AND METHODS

### *Selection of study region and plant species*

Three study sites located in the dry tropical region of Karnataka in Surammanahalli village (14°37¹583 N and 76°372 163 E) of Chitradurga district was selected for the study. The study region consisted of red sandy loam soil and receives an average annual rainfall of 593.5 mm and experiences a temperature of 32–36 °C. *Jatropha heynei* was selected for the study and characterized based on its morphological characteristics (Gamble, 1934) (Fig. 1).The identity of the plant species was confirmed with Dr. K. G. Bhat, an eminent plant taxonomist of Karnataka. The herbarium specimen was prepared and deposited in the Herbarium Centre at the Department of Studies in Applied Botany, Kuvempu University, Shankaraghatta. (Voucher no-KU/AB/05).

### *Isolation and characterization of endophytic fungi in Jatropha heynei*

Apparently, healthy plant samples like leaf, stem and root were collected in sterile moistened polypropylene covers and processed in the laboratory within 24 hours. The plant samples were water cleaned, surface-disinfected, segmented (1-cm) in aseptic condition and incubated on potato dextrose agar, malt extract agar, czapek dox agar or on water agar media (Shivanna and Vasanthakumari, 2011) amended with chloramphenicol (100 mg $L^{-1}$) and incubated for 5-7 days at 23±2 °C under a 12/12 h light/ dark UV light (350-400 nm) regime. Surface disinfection was successfully tested by briefly pressing plant sample fragments onto agar and incubating imprints (Unterseher and Schnittler, 2009).The fungal species occurring on the incubated segment surfaces were identified based on the morphological characteristics of fruiting bodies and spores ( Rao and Manoharachary, 1990). Some of the endophytic fungi were selected for molecular characterization. The ITS regions of the DNA of the candidate fungal species were selected for characterization by the cetyltrimethylammonium bromide (CTAB) method (Aamir *et al.* 2015). Primers (ITS1: 5¹- TCC GTA GGT GAA CCT CGG -3¹ and ITS4: 5¹- TCC TCC GCT TAT TGA TAT GC -3¹) were applied to amplify the ITS region, or internal transcribed spacer. The mixture for the PCR amplification reaction included 1 µL of diluted genomic DNA and 25 µL of PCR master mix (Taq polymerase, 10X buffer, dNTPs, Primers and sterile water). A preliminary denaturation of 94 °C for five minutes was followedby 30 cycles of amplification, denaturation (94 °C for one minute), annealing (50 °C for 30 seconds), and extension (72 °C for one minute), with the final extension being carried out at 72 °C for seven minutes. After the process, the PCR products were purified, the amplified DNA fragments were sequenced using Sanger's method, and the amplification of the ITS region was confirmed by electrophoresis. The MAFFT algorithm (Katoh *et al*. 2019) was used to generate 100 alternative guide trees using nrITS sequences. Data sets comprising of 8 nrITS sequences, along with those retrieved from the NCBI GenBank, were utilized to test the alignment confidence score on the GUIDANCE web server (http:// guidance.tau.ac.il). In BioEdit v.7.2.5, the columns with less than 93 % confidence scores were removed and aligned using GUIDANCE outputs. The alignment file is then utilized in RAxML GUI v.2.0.0.0 to perform a maximum likelihood analysis with 1,000 bootstrap replications using the GTRGAMMA+I model as proposed by jModelTest v.2.1.10 (Darriba *et al.* 2012).

### *Statistical analyses*

The data from two years 2019-20 and 2020-21 (replicate trials) were determined for homogeneity by ANOVA ($P<0.05$). The determination of the

colonization frequency and relative abundance of endophytic fungi was conducted by Suryanarayanan *et al.* (2000). The occurrence data of endophytic fungi were analyzed using Simpson and Shannon diversity and evenness indices, as well as species richness employing a bootstrap of 9999 at a 95% confidence interval. Further statistical analyses were carried out using rarefaction indices at a 95% confidence interval, principal component analysis (PCA), correlation, and phylogenetic analysis which was performed utilizing RAxML GUI v.2.0.0.0 employing the maximum likelihood method. These analyses were conducted using PAST ver. 2.17 by Darriba *et al.* (2012).

## RESULTS

### *Diversity of endophytic fungal species in Jatropha heynei influenced by different incubation methods*

In total, 2484 mycelial fungal endophytes were isolated from 10919 plant segments by four incubation methods during 2019-20 to 2020-21 in three different seasons, yielding a colonization frequency of 22.74%. These isolates represented 47 species of fungi from 26 genera and16 families, as well as seven isolates of nonsporulating fungi which were isolated from 1006 segments of *Jatropha heynei*. The fungal isolates were classified as 35 asexual ascomycetes from18 genera, 11sexual ascomycetes from6genera, and one mucoromycete species (Table 1). Among 47 fungal isolates,7 endophytic fungal species were isolated in all four incubation methods which belonged to 5 genera and 4 families. Table 2 lists the fungal species and their families isolated using each method. Water agar medium (26.79 %) had the highest endophytic fungal incidence in *J. heynei*, followed by potato dextrose agar (22.71 %) malt extract agar (19.52 %) and czapek dox agar media (19.34 %) (Fig. 2).

*Cladosporium cladosporioides* (4.98 %) and *Penicillium citrinum* (4.10 %) have the highest incidence on PDA, followed by *Nigrospora oryzae*( 1.71 %), *Aspergillus niger* (1.62 %), while *C. cladosporioides* (3.15 %), *N. oryzae* (3.09 %), *P. citrinum* (2.66 %) and *Exserohilum rostratum*(1.30 %) were predominantly exprssed in MEA. On the other hand, *Fusarium* sp.(3.7 %), *Colletotrichum truncatum*(3.08 %), *Alternaria alternata* (2.56 %) and *Penicillium citrinum* (1.77 %) were found in high abundance on WA. Further, *Cladosporium cladosporioides*(6.03 %) *Penicillium citrinum* (3.15 %) *Fusarium* sp. (2.22 %) and *Aspergillus flavus*(2.17 %) were occurred frequently in CZA. The incidence of morphotypes was higher on MEA than PDA followed by CZA and WA (Table 1). Certain fungal isolates only expressed in the PDA, MEA, WA or CZA methods. Certain other isolates, on the other hand, grew in more than one medium (Fig.3). *Cladosporium*, *Penicillium*, *Fusarium* sp, and *Alternaria*, are some of the species expressed in all four incubation methods. However, fungal species such as *Epicoccum nigrum*, *Pithomyces* sp., and *Zygorhynchus* sp., were found exclusively in PDA, where as a *Xylaria curta* was found exclusively on MEA. On the other hand*Alternaria longipes*, *Alternaria tenuissima*, *Exserohilum echinochloae*, *Nigrospora* sp. *Phoma* sp*., Ulocladium* sp., and *Trichoderma viride*, were found only on WA.

### *Seasonal diversity of endophytic fungi*

The assemblage of fungal endophytes in *Jatropha heynei* was influenced by the seasons. The rainy season (27.54 %) was the most favourable to the expression of endophytic fungal isolates, followed by the winter (26.66 %) and summer (20.27 %). According to the present study, *Penicillium citrinum*, *Aspergillus niger*, *Cladosporium cladosporioides* and *Aspergillus flavus* predominantly occurredin the rainy season, followed by *C. cladosporioides*, *A. niger*, *P. citrinum*, *Alternaria alternata* and *Nigrospora oryzae* were dominant in winter season. While, *Fusarium* sp., *A. niger*, *Chaetomium* sp., and *C. cladosporioides* were highly expressed during the summer season.

Principle components analysis (Beta diversity) demonstrated highly significant seasonality in the culturable endophytic fungal communities by clearly separating samples from the three seasons. The various samples collected during the rainy, winter and summer seasons resembled endophyte communities linked to seasonality and

**Table 1:** The colonization frequency of fungal endophytes in *Jatropha heynei* by potato dextrose agar (PDA), malt extract agar (MEA) czapek dox agar (CZA) and water agar (WA) methods

| | Incubation methods / Colonization frequency (%) | | | |
|---|---|---|---|---|
| Fungal species | PDA | MEA | WA | CZA |
| Anamorphic ascomycetes | | | | |
| *Acremonium* sp. | 0.66 | 0 | 0.65 | 0 |
| *Alternaria* spp. | 1.04 | 2.12 | 3.34 | 0 |
| *Albifimbria verrucaria* | 0.11 | 0 | 0.19 | 0 |
| *Aspergillus* spp. | 2.66 | 1.14 | 1.51 | 5.81 |
| *Cladosporium* spp. | 4.98 | 3.15 | 2.46 | 6.19 |
| *Colletotrichum* spp. | 0.59 | 0 | 3.67 | 0 |
| *Epicoccum nigrum* | 0.04 | 0 | 0 | 0 |
| *Exserohilum* spp. | 0.40 | 1.68 | 1.93 | 0 |
| *Fusarium* spp. | 0.02 | 0 | 3.74 | 3.04 |
| *Penicillium* spp. | 4.31 | 2.88 | 1.77 | 3.53 |
| *Pestalotiopsis* spp. | 1.02 | 0.76 | 1.14 | 0 |
| *Pithomyces* sp. | 0.11 | 0 | 0 | 0 |
| *Talaromyces pinophilus* | 0.09 | 0 | 0.09 | 0 |
| *Trichoderma* spp. | 0.14 | 0 | 0.16 | 0.38 |
| *Torula sp.* | 0.09 | 0 | 0 | 0 |
| *Ulocladium* sp. | 0 | 0.32 | 1.77 | 0 |
| *unknown 1* | 0.14 | 0.54 | 0.09 | 0 |
| *Verticillium* sp. | 0.07 | 0 | 0.13 | 0 |
| *Xylaria curta* | 0 | 0.48 | 0 | 0 |
| Total frequency (%) | 18.56 | 13.10 | 22.79 | 18.96 |
| Morphotypes | 10.48 | 13.64 | 4.59 | 9.51 |
| Mucoromycetes | | | | |
| *Zygorhynchus* sp. | 0.02 | 0 | 0 | 0 |
| Teleomorphic ascomycetes | | | | |
| *Chaetomium* spp. | 1.45 | 1.52 | 1.05 | 0.10 |
| *Cochliobolus* spp. | 0.83 | 1.52 | 1.37 | 0.27 |
| *Macrophomina phaseolina* | 0.11 | 0.16 | 0 | 0 |
| *Nigrospora* spp. | 1.71 | 3.99 | 1.31 | 0 |
| *Phoma* sp. | 0 | 0 | 0.16 | 0 |
| *Thielavia* sp. | 0.23 | 0.27 | 0.16 | 0 |
| Total frequency (%) | 4.24 | 6.41 | 4.07 | 0.38 |

Colonization frequency of fungal endophyte occurence was calculated using formula; Data is an average of three replicates

presence/absence data more. The higher-ranked distances clearly showed that differences in the occurrence of fungal endophytes in three seasons were significantly greater than differences within each season. Beta diversity of principle component analysis in *J. heynei* clearly represents the high fungal diversity in the winter season when compared to the rainy and summer (Fig.4).

The rarefaction curve (Alpha diversity) clearly demonstrated that, depending on the season, an increase in endophytic fungal diversity is correlated with an increase in the number of isolations. The species richness of fungal endophytes was high in winter and rainy followed by summer season (Fig.5).

**Table 2 :** Family-wise species diversity of endophytic fungi in *Jatropha heynei*

| Isolation methods | Species | Genera | Family | Morphotypes |
|---|---|---|---|---|
| PDA | 38 | 22 | 16 | 4 |
| MEA | 23 | 14 | 10 | 5 |
| WA | 35 | 21 | 14 | 2 |
| CZA | 16 | 7 | 6 | 3 |
| DNA sequence* | 14 | 12 | 8 | 0 |

The taxonomic classification of endophytic fungal species and their corresponding families in *Jatropha heynei*. Isolation methods (PDA- potato dextrose agar, MEA- malt extract agar and MB- moist blotter); * DNA sequencing was done to confirm the identification of certain fungal strains that were indistinguishable based on their morphological characteristics..

**Table 3:** The colonization frequency of endophytic fungi in root, leaf and fruit regions of *Jatropha heynei* by potato dextrose agar (PDA), malt extract agar (MEA) czapek dox agar (CZA) and water agar (WA) methods

| | Plant parts / Colonization frequency (%) | | |
|---|---|---|---|
| Fungal species | Leaf | Root | Fruit |
| *Acremonium* sp. | 0 | 2.71 | 0 |
| *Alternaria alternata* | 9.34 | 0.35 | 0 |
| *Alternaria tenuissima* | 1.24 | 0 | 0 |
| *Alternaria longipes* | 0.62 | 0 | 0 |
| *Albifimbria verrucaria* | 0.80 | 0 | 0 |
| *Aspergillus candidus* | 0.58 | 2.65 | 0 |
| *Aspergillus flavus* | 2.7 | 5.76 | 8.88 |
| *Aspergillus niger* | 2.51 | 16.67 | 2.44 |
| *Aspergillus ochraceus* | 0.73 | 5.27 | 0 |
| *Chaetomium globosum* | 0.78 | 0.09 | 0 |
| *Chaetomium indicum* | 0.24 | 0 | 0 |
| *Chaetomium* sp. | 4.46 | 00.16 | 1.21 |
| *Cladosporium cladosporioides* | 13.02 | 11.8 | 3.77 |
| *Cladosporium herbarum* | 0.24 | 0.2 | 4.66 |
| *Colletotrichum* sp. | 2.68 | 0 | 0 |
| *Colletotrichum truncatum* | 5.10 | 0 | 3.32 |
| *Curvularia lunata* | 3.55 | 0.2 | 5.43 |
| *Curvularia pallescence* | 0.80 | 0 | 0 |
| *Curvularia eragrostidis* | 0 | 0.4 | 0 |
| *Epicoccum nigrum* | 0.04 | | 0 |
| *Exserohilum rostratum* | 4.93 | 0 | 2.32 |
| *Exserohilum holmii* | 1.7 | 0.5 | 0 |
| *Exserohilum echinochloae* | 0.28 | 0 | 0 |
| *Fusarium falciforme* | 0.24 | 0.27 | 0 |
| *Fusarium solani* | 0.19 | 0.21 | 0 |
| *Fusarium* sp. | 0.34 | 14.5 | 0 |

(Contd. part table 3)

| | | | |
|---|---|---|---|
| *Nigrospora oryzae* | 8.01 | 0.43 | 0 |
| *Nigrospora* sp. | 0.18 | 0 | 0 |
| *Penicillium citrinum* | 8.52 | 12 | 17.99 |
| *Penicillium commune* | 0.4 | 0.1 | 1.33 |
| *Penicillium chrysogenum* | 0.19 | 0.2 | 0 |
| *Pestalotiopsis clavispora* | 0.21 | 0 | 0 |
| *Pestalotiopsis guepinii* | 0 | 0.6 | 0 |
| *Pestalotia microspora* | 0.28 | 0 | 0 |
| *Neopestalotiopsis* sp. | 0.43 | 0 | 0 |
| *Phoma* sp. | 0.37 | 0 | 0 |
| *Pithomyces* sp. | 0.24 | 0 | 0 |
| *Talaromyces pinophilus* | 0.12 | 0.27 | 0 |
| *Trichoderma harzianum* | 0 | 0.21 | 0 |
| *Trichoderma viride* | 0.05 | 0.16 | 0 |
| *Torula* sp. | 0.67 | 0.5 | 0 |
| *Ulocladium* sp. | 3.82 | 0 | 0 |
| *unknown 1* | 1.7 | 0.62 | 0 |
| *Thielavia* sp. | 0.24 | 0.5 | 0 |
| *Verticillium* sp. | 0.24 | 0.5 | 0 |
| *Xylaria curta* | 0.21 | 0 | 0 |
| *Zygorhynchus* sp. | 0.04 | 0 | 0 |
| Total frequency | 13.57 | 13.46 | 1.21 |

**Table 4 :** Diversity and evenness indices and colonization frequency of fungal endophytes in *Jatropha heynei*

| Sample units | Diversity index | | | Evenness index | | Colonization |
|---|---|---|---|---|---|---|
| | Taxa (S) | Shannon | Simpson (D) | Shannon (J) | Simpson (E) | frequency (%) |
| PDA | 38 | 2.80 | 0.89 | 0.77 | 0.43 | 22.71 |
| MEA | 23 | 2.62 | 0.90 | 0.83 | 0.50 | 19.52 |
| WA | 36 | 3.00 | 0.93 | 0.83 | 0.56 | 26.79 |
| CZA | 16 | 2.13 | 0.83 | 0.77 | 0.53 | 19.34 |
| Root | 29 | 2.36 | 0.87 | 0.70 | 0.36 | 26.85 |
| Leaf | 43 | 2.93 | 0.92 | 0.78 | 0.43 | 27.34 |
| Fruit | 10 | 1.96 | 0.81 | 0.85 | 0.71 | 18.21 |
| Rainy | 26 | 2.66 | 0.90 | 0.81 | 0.54 | 27.54 |
| Winter | 43 | 3.01 | 0.93 | 0.80 | 0.47 | 26.66 |
| Summer | 25 | 2.59 | 0.90 | 0.80 | 0.53 | 20.27 |

Note: The data is derived from three distinct locations and data is subjected to a mean calculation over three replicates.

**Table 5:** NCBI blast analysis of nucleotide sequences of endophytic fungi from *J.heynei*

| Submission No. | Endophytic fungi | Gene markers | Query length/covers | Identity | GenBank accession No. | Closest match |
|---|---|---|---|---|---|---|
| SUB9433304 | *Albifimbria verrucaria* | ITS 1&4 | 819/100% | 100% | MW866572 | MT276081 |
| SUB9433765 | *Xylaria curta* | ITS1&4 | 966/100% | 100% | MW871545 | MN534950 |
| SUB9433791 | *Nigrospora oryzae* | ITS1&4 | 968/100% | 100% | MW871546 | MG571398 |
| SUB9499324 | *Colletotrichum truncatum* | ITS1&4 | 654/100% | 100% | MW947149 | MK611675 |
| SUB12301171 | *Aspergillus terreus* | ITS1&4 | 902/100% | 100% | OP862634 | ONO63269 |
| SUB12300440 | *Fusarium solani* | ITS1&4 | 902/100% | 100% | OP861528 | OP848133 |
| SUB12300433 | *Chaetomium globosum* | ITS1&4 | 773/100% | 100% | OP861526 | MN960568 |
| SUB12300425 | *Fusarium incarnatum* | ITS1&4 | 902/100% | 100% | OP861525 | OP764488 |
| SUB12300356 | *Pestalotiopsis microspora* | ITS1&4 | 970/100% | 100% | OP861509 | MK801280 |
| SUB12300014 | *Talaromyces pinophilus* | ITS1&4 | 750/100% | 100% | OP861505 | MT594355 |
| SUB12299756 | *Exserohilum rostratum* | ITS1&4 | 1066/100% | 100% | OP861483 | MT322139 |
| SUB11967162 | *Alternaria alternata* | ITS1&4 | 787/100% | 100% | OP288199 | MK121458 |
| SUB11476551 | *Macrophomina pahseolina* | ITS1&4 | 699/100% | 100% | ON514451 | MN097200 |
| SUB12300647 | *Pestalotiopsis* sp. | ITS1&4 | 719/100% | 100% | OP861638 | ON180773 |

### *Endophytic fungal assemblages in different parts of J. heynei*

In *Jatropha heynei*, a large number of endophytic fungal isolates were found in the leaf (1482) as well as the root (1470) followed by fruit (133). Table 3 provides more information on the species diversity in the various plant parts. *Cladosporium cladosporioides* (13.02 %), *Alternaria alternata* (9.34 %), *Penicillium citrinum* (8.52 %), *Nigrospora oryzae* (8.01 %), and *Exserohilum rostratum* (4.93 %) are the endophytes with high incidence in leaf, whereas in root fungal endophytes with high incidence found were *Aspergillus niger* (16.67 %), *C. cladosporioides* (11.8 %), *Fusarium* sp.(14.5 %), *P.citrinum* (12 %), and *Aspergillus ochraceus* (5.27 %). Further, *P.citrinum* (17.9 %), *Aspergillus flavus* (8.88 %), *Cladosporium herbarum* (4.66 %) and *Cochliobolus lunatus*(5.43 %) were found to be more prevalent in the fruit region.

### *Diversity indices of endophytic fungi*

The quantitative data on endophyte diversity has been analysed using diversity and evenness indices. Shannon's and Simpson's diversity indices were higher for PDA (38), WA (36), MEA (23) followed by CZA (16). The number of fungal taxa were found abundantly in the leaf (43) and root (29) followed by fruit (10). Additionally, the number of fungal species occurring in the winter season was 43, whereas it was 26 in the rainy season and 25 in the summer season (Table 4).

### *Molecular characterization of endophytic fungi*

The internal transcribed spacer (ITS) is a non-functional RNA region found in structural ribosomal RNAs (rRNA). The ITS region's sequence comparison is frequently employed in taxonomy and molecular phylogeny because of its large copy number of rRNA genes, which allows for easy amplification even fromlittle amounts of DNA, and it has a high degree of variation even among closely related species (Won *et al*. 2005). Certain endophytic fungi, which are frequently isolated and those, found difficult to identify through morphological characters were identified by molecular characterization.

The isolated fungal endophytes amplified ITS sequences were subjected to a BLAST search,


**Fig. 1:** Morphology of host plant *Jatropha heynei* showing **(A) & (B)** Habit **(C)** Flower **(D)** Fruit **(E)** Habitat.

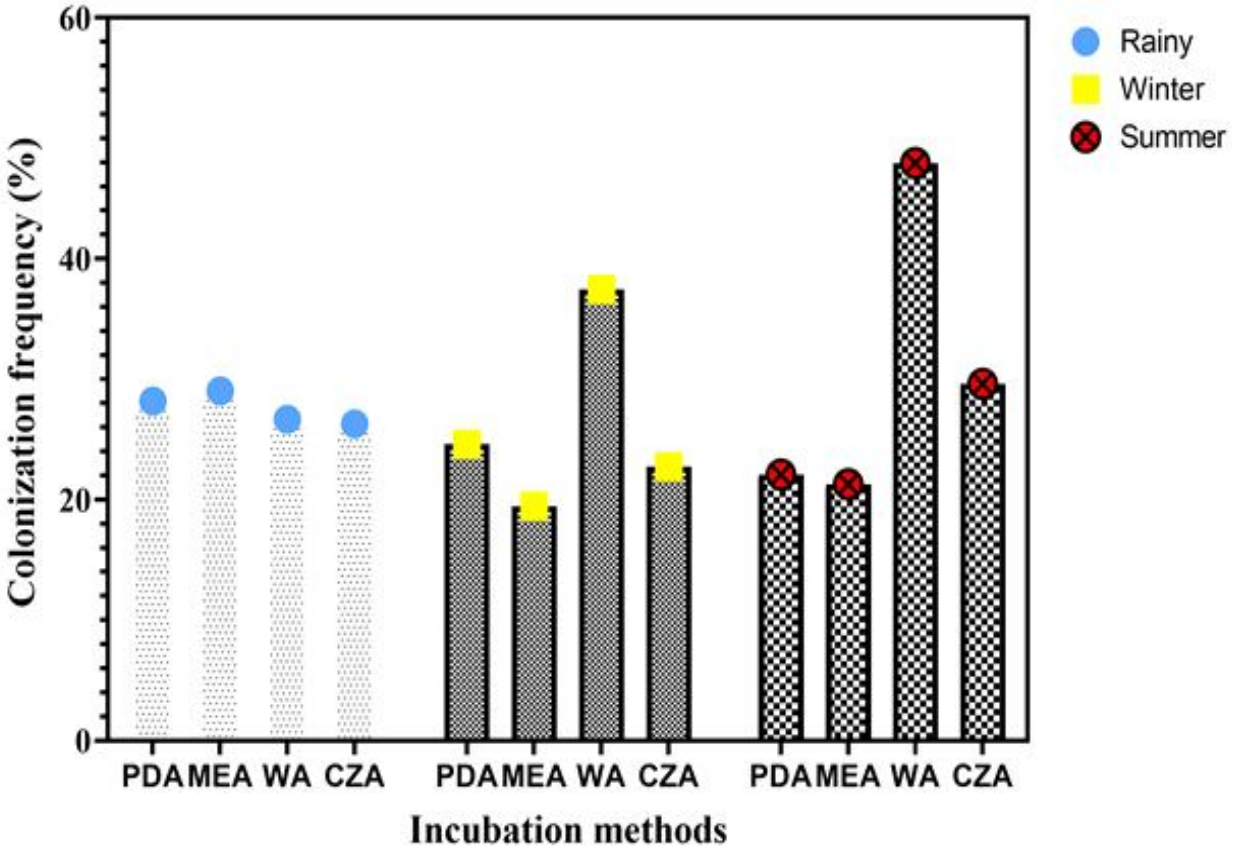


**Fig. 2:**The colonization frequency of endophytic fungal species in *Jatropha heynei* by four incubations methods and three seasons

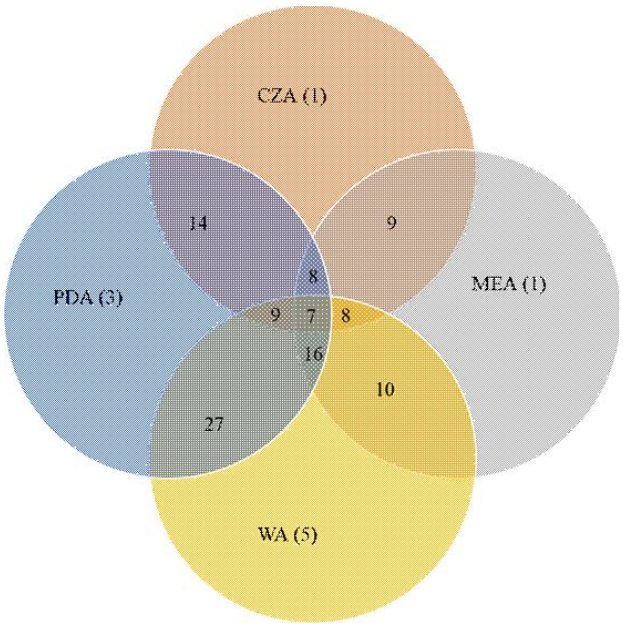


**Fig. 3:** Effect of isolation methods (PDA - Potato dextrose agar; MEA - Malt extract agar; WA –Water agar; CZA – Czapek dox agar method) on the occurrence of endophytic fungi in *J. heynei*

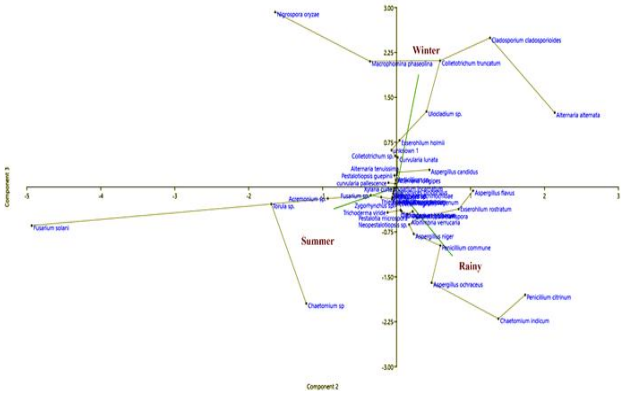


**Fig. 4 :**Beta diversity of dominating endophytic fungal speciesfrom*J. heynei* by principle component analysis in three seasons

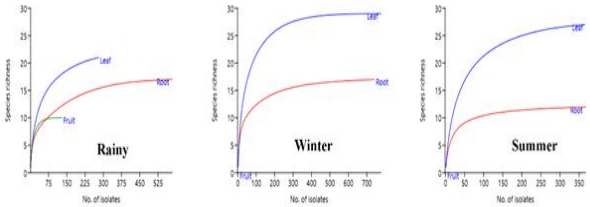


**Fig.5:**The depiction of endophytic fungal speciesfromdifferent parts of *Jatropha heynei*in three seasons by rarefaction curve

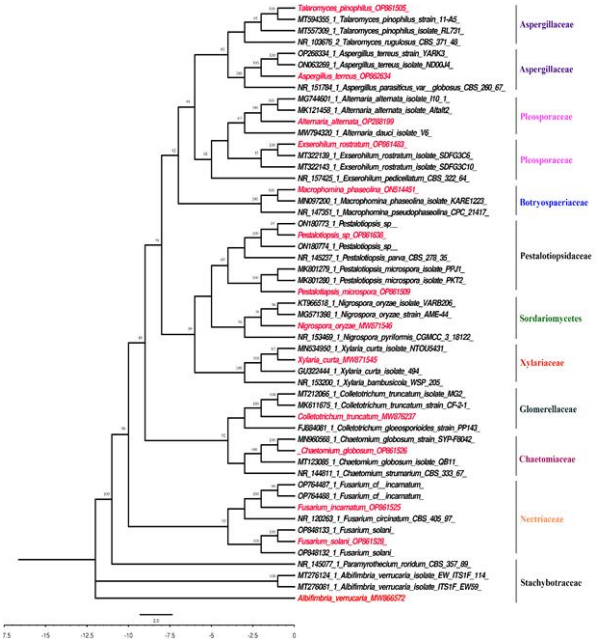


**Fig.6:** Phylogenetic treebased on maximum likelihood analysis of endophytic fungal species (ITS 1-5.8s-ITS4) occurring in *Jatropha heynei*.

as indicated in (Table 5), and yielded strong similarity with the relevant species in the NCBI GenBank database. Molecular identification based on sequence analysis revealed that the isolated endophytic fungi belonged to 12 genera and 8 families include *Albifimbria*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Colletotrichum*, *Exserohilum*, *Fusarium*, *Macrophomina*, *Nigrospora*, *Pestalotiopsis*, *Talaromyces*, and *Xylaria*.

To create the phylogenetic tree, the sequences of sister taxa of the endophytic fungal isolates were obtained from GenBank (NCBI). The phylogenetic tree was constructed using RAxML GUI ver. 2.0.0.0 with maximum likelihood method revealed that the isolates could be classified into different clades with their best-supported bootstrap value According to the phylogenetic tree topology, the query taxa nesting with their closely related fungal community and formed monophyletic clades (Fig.6).

## DISCUSSION

Recent studies has shown that diverse secondary metabolites are produced by the endophytic fungi harboured inside the plants rather than by the plants themselves, highlighting the significance of the contribution of microbial life to plant thermotolerance, drought resistance, and other important survival strategies. The majority of research on endophytes has focused on tree plants rather than herbs and also limited research is focussing on plants inhabiting in dry environments. *Jatropha heynei* is a rare medicinal herb growing in dry tropical regions of Southern India documented for inhabiting numerous fungal endophytes. In the current study, most of the fungal endophytes were dominated by ascomycetous fungi, among them, anamorphic ascomycetes were more frequent than teleomorphic forms. Although the Ascomycota were the most prevalent endophytes, members of the Basidiomycota and Zygomycota were occasionallyisolated as endophytes in prior investigations.

Endophytic fungi expression appears to be dependent on the method of isolation. The WA method consistently supported greater fungal species expression than the other methods tested. The fungal species are also only expressed in specific isolation methods (Tibpromma *et al.* 2018). This was attributed to either nutrient supplementation or the induction of abiotic or biotic stress conditions on fungal colony growth and expression (Shivanna & Vasanthakumari 2011, Tibpromma *et al*. 2018, Nischitha and Shivanna, 2020). According to Marquez et al. (2012), most endophytic fungal surveys rely on incubation methods/techniques and often prioritize fast-growing species over slow-growing ones, which are commonly excluded (Hyde and Soytong, 2008). The employment of direct isolation methods led to the retrieval of most cultivable fungi, including the morphotype that exhibited a failure to sporulate despite exposure to UV light, nutrient stress, and extended culture.

The host plant as well as the occurrence and colonization frequency of endophytic fungi have been found to be influenced by environmental factors such as temperature, rainfall, and atmospheric humidity that are present during different seasons (Gupta and Chaturvedi, 2017; Gagana *et al.* 2020). *Penicillium citrinum*, *Aspergillus niger*, and *Cladosporium cladosporioides* were predominantly occurred in the rainy season. *C. cladosporioides*, *A. niger, P. citrinum*, and *Alternaria alternata* were dominant

in the winter season. While, *Fusarium* sp., *A. niger*, *Chaetomium* sp., and *C. cladosporioides* were highly expressed during the summer season.

The expression of fungal communities is directly influenced by the seasons. The rainy and winter seasons both favoured high expression of fungal endophytes followed by the summer season. Similar observations were previously reported by research workers (Sudhakar *et al*. 2014; Gagana *et al*. 2020). According to Singh *et al*. (2016), the rainy season in this study likely contributed to the moderate temperature that is favourable for fungal expression. Conversely, nutrient stress brought on by a dry spell prevented some fungi from expressing themselves. The abiotic and biotic factors also have an impact on the association of endophytic fungi with their host plants (Rodriguez *et al*. 2009). Further, the preceding precipitation prior to winter may have solubilized mineral nutrients within the soil, thereby facilitating the growth of plants and endophytic fungi. This phenomenon may account for the elevated frequency of endophytic fungal occurrences observed during winter. Conversely, in certain plants, a scarcity of water may result in the accumulation of non-structural carbohydrates and, consequently, the development of carbon-based defenses, such as tannins. The distribution of endophytes within the plant is regulated by the genes of boththe plant and endophyte and is influenced by environmental factors. The role of seasonal influences on endophytic expression in host plants can be attributed to these environmental factors (Moricca *et al*. 2008). It has also been demonstrated that seasonal variation and isolation techniques influence the expression of microbial communities in plant parts (Singh *et al*. 2016). The rarefication curve showed that the quantification of fungal species was dependent on seasonal variation, and that rainy and winter seasons in addition to their isolation frequencies contributed to the species richness. (Nalini et al. 2014; Li *et al*. 2016).

Diversity indices were high during the winter and low during the summer, with the highest diversity indices found in the leaf. This observation suggests that PDA and the winter season have an effect on the diversity of endophytic fungi, especially in the leaf. Rarefaction indices suggested that the diversity and frequency of endophytic fungal species increased with the number of isolations in all seasons, and was particularly high during the winter season. Seasonality in fungal endophyte communities may be caused by the associations these fungi have with plants during periods of elevated plant productivity (Giauque and Hawkes, 2016). Some endophytes can establish themselves in the plant under conditions of active plant growth, whereas the growth of other endophytes declines as plant metabolic activity rises (Martins *et al.* 2016).

A high frequency of endophytic fungi has been documented in the leaves than in root and fruit in *Jatropha heynei*. Contrarily, some endophytic fungi like *Fusarium sp*., have been found to be more prevalent in the root than the leaf region and some species like *Ulocladium sp*., *Alternaria spp*., and *Colletotrichum* spp., were only occurred in leaf tissues. Earlier studies showed that specific fungal isolates in the root were also documented in the leaf regions of plants (Parmar *et al*. 2018; Nischitha and Shivanna 2020). According to the observations mentioned above, some endophytic fungal isolates may be localized to either the root, leaf or fruit regions, while others may be associated with all parts of the host plant. This provided additional evidence that some endophytic fungi have evolved life history strategies to integrate with the entire plant system and provide the host plant with a number of survival strategies.

## CONCLUSION

In conclusion, we found that the assemblages of the endophytic fungi in *Jatropha heynei* were influenced by the isolation method, localization in plant regions, and seasons. The findings emphasize the importance of considering these factors when investigating the diversity, abundance, and functional characteristics of endophytic fungi within different parts of plant species. The asexual ascomycetes occurred in high frequency rather than the teleomorphic forms. Certain fungal isolates were distinctive to the isolation method and plant region, while others were common. Certain fungal endophytes with aerial regions were also found in the root of

the plant and also few species of fungal endophytes exhibited tissue specificity. Furthermore, the molecular characterization of biologically important endophytic fungi and those that found difficulty in identification by morphological methods were analyzed by the Internal Transcribed Spacer (ITS) region which is a common approach in studying their diversity and taxonomy. The ITS region is a highly conserved region of the fungal genome that is flanked by the 18S and 28S rRNA genes and it provides valuable information for phylogenetic and taxonomic analysis of endophytic fungi.

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