 HOSTE D BY

Available online at [www.sciencedirect.com](http://www.sciencedirect.com/science/journal/2314808X)

**ScienceDirect**

journal homepage: [http://ees.elsevier.com/ejbas/default.asp](http://http//ees.elsevier.com/ejbas/default.asp)

**Full Length Article**

**Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka**



***Fazilath Uzma*** [***a***](#_bookmark0)***, Narasimha Murthy Konappa*** [***b***](#_bookmark1)***,***

***Srinivas Chowdappa*** [***a***](#_bookmark0)***,***[***\****](#_bookmark2)

a *Fungal Metabolite Research Laboratory, Department of Microbiology and Biotechnology, Jnanabharathi* *Campus, Bangalore University, Bangalore 560 056, Karnataka, India*

b *Department of Studies in Biotechnology, University of Mysore, Manasagangothri, Mysore 570 006, Karnataka, India*

A R T I C L E I N F O A B S T R A C T

*Article history:*

Received 5 March 2016

Received in revised form 15 June 2016

Accepted 29 August 2016

Available online 30 September 2016

*Keywords:* Endophytic fungi Diversity analysis

Extracellular enzymes Medicinal plants

A total of 112 endophytic fungi belonging to 26 genera were isolated from six wild medici- nal plants belonging to Bisle region, Western Ghats of Karnataka, among which *Hedychium flavescens* Carey ex Roscoe and *Hedychium coronarium* J. Koenig are listed as endangered plants in the Red data book. The endophytic fungal diversity and extracellular enzyme activity from the endangered plants are reported for the first time. The diversity of the fungal isolates was analyzed using Simpson’s diversity indices, Shannon–Weiner index and Evenness. The fungal isolates were screened for the production of extracellular enzymes, of which 29% were positive for amylase, 28% for cellulase, 18% for pectinase and 40% for asparaginase activity. None of the endophytic isolates depicted laccase activity.

© 2016 Mansoura University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by->

nc-nd/4.0/).

# Introduction

Endophytic fungal communities colonize plants without causing any notable signs of disease to the host plants [[1]](#_bookmark8). They have ubiquitous distribution within plant tissues and are a repository of varied novel compounds with industrial and pharmaceu- tical potential [[2]](#_bookmark9). Endophytic fungal bioactive compounds from medicinal plants find enormous applications as agrochemi- cals, antibiotics, antiparasitics, antioxidants, biopesticides and

anticancer agents [[3]](#_bookmark10). Endophytes improve the host plant’s re- sistance to adversity by secretion of bioactive metabolites [[4]](#_bookmark11). The diversity of fungal community exists within the tissues of the host plant and among the geographically separated in- dividuals of the same host species [[5]](#_bookmark12). Variation in the fungal diversity may also be associated with location, climate and plant age [[6,7]](#_bookmark13). Diversity analysis of the endophytic fungal assem- blages is an emerging challenge, which leads to the discovery of new species producing novel compounds and a better un- derstanding of their role in ecosystems [[8]](#_bookmark14). Recently, fungal

\* Corresponding author.

*E-mail address:* [srinivasbub@gmail.com](mailto:srinivasbub@gmail.com) (S. Chowdappa). <http://dx.doi.org/10.1016/j.ejbas.2016.08.007>

2314-808X/© 2016 Mansoura University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license ([http://creativecommons.org/licenses/by-nc-nd/4.0/).](http://creativecommons.org/licenses/by-nc-nd/4.0/))

**336** egyptian journal of basic and applied sciences 3 (2016) 33 5 –342

endophytes have been explored for diverse applications owing to their production of extracellular enzymes [[9]](#_bookmark15). The enzymes function so as to obtain nutrition from their host, hydrolyze food substances and are involved in eliciting defense mecha- nisms against pathogens [[10]](#_bookmark16). There is an imperative need to discover and utilize diverse novel enzymes with high stabil- ity for industrial processes. The present work aims to examine the diversity of endophytic fungal assemblages and their ability to produce extracellular enzymes in selected plants.

## *Frequency of endophytic fungi*

The absolute frequency (f) was calculated as the total number of endophytes isolated [[13]](#_bookmark19). Relative frequencies (fr) of isola- tion used to represent fungal species density were calculated as the number of isolates of each species of the endophytic fungi divided by the total number of isolates and expressed in percentage. Isolation rate (IR) of the endophytic fungi was calculated as number of isolates obtained from tissue seg- ments divided by total number of tissue segments [[14]](#_bookmark20). The

colonization rate (CR) of endophytic fungi was expressed as

# Experiment and methods

## *Source of endophytic fungi*

Endophytic fungi were isolated from fresh and healthy tissues of six wild medicinal plants collected from Bisle region, Western

percentage of total number of isolates obtained from differ- ent tissue segments divided by total number of isolates obtained from overall tissue segments incubated [[15]](#_bookmark21).

IR  number of isolates obtained from tissue segments total number of tissue segments

CR  total number of isolates obtained from different tissue segments total number of isolates obtained from overall tissue segments incubated

Ghats of Karnataka and identified as *Tinospora cordifolia* (Willd.) Hook. f. & Thomson, *Piper nigrum* L., *Piper longum* L., *Zingiber officinale* Roscoe, *Hedychium coronarium* J. Koenig and *Hedychium flavescens* Carey ex Roscoe. Herbaria of plant samples were de- posited to National Ayurveda Dietetics Research Institute (Central Council for Research in Ayurveda and Siddha), De- partment of AYUSH, Ministry of Health and Family Welfare, Govt. of India, (New Delhi) Jayanagar, Bangalore, India. The medici- nal uses of the plants are listed in [Table 1](#_bookmark3).

## *Isolation and morphological identification of* endophytic fungi

Standard protocols have been followed for the isolation of en- dophytic fungi as reported in our previous work [[11]](#_bookmark17). The endophytic fungi were identified based on the cultural char- acteristics, morphology of the fruiting bodies and spores, using standard manuals [[12]](#_bookmark18).

## *Data analyses*

Simpson’s diversity index, Simpson’s dominance index (D), Species richness (S), Shannon–Wiener index (H) and Even- ness (E) were calculated [[16,17]](#_bookmark22).

## *Preliminary screening of endophytic fungi for* extracellular enzymes

The endophytic fungal isolates from selected plants were screened for enzyme production by plate assay method and were assessed by placing 5 mm mycelial plugs on solid media with substrates specific to the respective enzymes: starch to test amylase, hexadecyl trimethyl ammonium bromide to test pectinase, carboxy methyl cellulose to test cellulase, 1-napthol to test laccase and L-asparagine to test for asparaginase. After incubation at room temperature for 7 days, the enzyme ac- tivity was determined [[18,19]](#_bookmark23).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Table 1 – List of selected medicinal plants and their uses** | | | | |
| Sl. No | Medicinal Plant | Family | Herbarium Accession no. | Medicinal uses of the plants |
| 1 | *Tinospora cordifolia* (Willd.) Hook. f. & Thomson | *Menispermaceae* | RRCBI-8976 | Antipyretic and anti-asthmatic. |
| 2 | *Piper nigrum* L. | *Piperaceae* | RRCBI-MUS135 | Treatment of vertigo, asthma and sinusitis. |
| 3 | *Piper longum* L. | *Piperaceae* | RRCBI-AP -2591 | Arthritis and respiratory tract infections. |
| 4 | *Zingiber officinale* Roscoe | *Zingiberaceae* | RRCBI-AP.4046 | Throat infections, common colds & fever. |
| 5 | *Hedychium coronarium* J. Koenig | *Zingiberaceae* | RRCBI-7331 | Nausea, halitosis, vomiting, and stimulant |
| 6 | *Hedychium flavescens* | *Zingiberaceae* | RRCBI-MUS.145 | Anti-rheumatic, stimulant and antipyretic |
|  | Carey ex Roscoe |  |  |  |

## *Statistical analysis*

The experiments were performed in triplicate and the means were analyzed statistically with the SPSS program version 20. The analyses of variance were according to the rules of the ANOVA. The significant differences between the means were determined through Duncan’s multiple range Test (DMRT).

# Results

## *Endophytic colonization in medicinal plants*

A total of 112 isolates were obtained from 480 tissue frag- ments grouped into twenty six fungal taxa consisting of hyphomycetes (38.4%), ascomycetes (34.6%), coelomycetes (15.38%) and zygomycetes (7.69%) based on their morphol- ogy. The extent of colonization varied among the different plant parts with the leaves harboring more endophytic fungi than the other tissues. The isolation rates (IR) was high for *T. cordifolia* whereas low IR was observed for *P. nigrum* and *Z. officinale*. The fungal colonization rate (CR) differed among the six plant species ([Table 2](#_bookmark4)). The fungal taxa *Aspergillus, Alternaria, Cla- dosporium, Colletotrichum, Fusarium, Mucor, Penicillium, Rhizopus* and Mycelia sterilia had high relative frequency of occur- rence but fungal taxa *Acremonium, Cylindrocephalum, Dreshclera, Lasiodiplodia, Myrothecium, Nigrospora, Paecilomyces* and *Torula* were found in less frequency ([Table 3](#_bookmark5)). All the plant samples were found to be associated with various endophytic fungi with dif- ferent isolation rates (IR) and colonization rates (CR). The

|  |
| --- |
| **Table 2 – Colonization and isolation rates of endophytic fungi in six medicinal plants.** |
| Sl. no Medicinal Tissue Colonization Isolation plant segments rate rate (IR)  CR (%) |
| 1. *Tinospora cordifolia* Leaf 32 0.4   (Willd.) Hook. f. & Petiole 12 0.15  Thomson Stem 36 0.45  Roots 20 0.25   1. *Piper nigrum* L. Leaf 15.38 0.1   Petiole 15.38 0.1  Stem 30.76 0.2  Roots 38.46 0.25   1. *Piper longum* L. Leaf 40.74 0.55   Petiole 22.22 0.3  Stem 29.62 0.4  Roots 7.40 0.1   1. *Zingiber officinale* Leaf 35.71 0.25   Roscoe Petiole 14.28 0.1  Stem 28.57 0.2  Roots 21.42 0.15   1. *Hedychium* Leaf 40 0.3   *coronarium* Petiole 26.66 0.2  J. Koenig Stem 20 0.15  Roots 13.33 0.1   1. *Hedychium flavescens* Leaf 44.44 0.4   Carey ex Roscoe Petiole 27.77 0.25  Stem 16.66 0.15  Roots 11.11 0.1 |

absolute and relative frequencies of occurrence of each endo- phytic fungal species are depicted in [Table 3](#_bookmark5).

### T. cordifolia

The 25 endophytic fungi were isolated from 80 tissue seg- ments of *T. cordifolia*, of which 8 fungal isolates were obtained from leaves, 3 fungal isolates from petiole, 9 fungal isolates from stem and 5 fungal isolates were from roots. They were grouped into 11 genera, of which *Aspergillus* sp. (6.22%), *Cladosporium* sp. (2.67%), Mycelia sterilia (3.56%), and *Fusarium* sp. (2.67%) were the most common and obtained from more than one tissue type ([Table 3](#_bookmark5)). The colonization and isolation rates were higher in stem tissues (36%) and leaves (32%) than in the other tissues ([Table 2](#_bookmark4)). Simpson and Shannon–Wiener’s diversity indices were higher in stem and leaves. The species richness was also greater in the stem and leaves. There was little difference in species evenness among the tissues studied ([Table 4](#_bookmark6)).

### P. nigrum

A total of 13 endophytic fungal isolates were obtained from 80 tissue segments of *P. nigrum*. Among them, 2 fungal iso- lates were from leaves, 2 fungal isolates were from petiole, 4 fungal isolates were from stem and 5 fungal isolates were from roots. They were grouped into 9 genera. The most frequently occurring fungal endophytes were *Aspergillus* sp. (3.56%) and *Phoma* sp. (1.78%) ([Table 3](#_bookmark5)).The richness of endophytic fungi varied in different tissues of *P. nigrum.* The Simpson and Shannon–Wiener’s diversity indices were higher in stem and roots. The species richness was also greater in the roots and stem ([Table 4](#_bookmark6)).

### P. longum

Twenty seven isolates from 80 tissue segments of *P. longum* were obtained, from which 11 fungal isolates were from leaves, 6 fungal isolates were from petiole, 8 fungal isolates were from stem, and 2 fungal isolates were from roots; these were grouped into 13 genera, in which *Aspergillus* sp., *Penicillium* sp., and Mycelia sterilia were the most commonly occurring and were obtained from more than one tissue type. The frequencies of occurrence of these endophytes were 7.13%, 3.56% and 2.67% respectively ([Table 3](#_bookmark5)).The colonization rate was highest in leaves (40.74%), followed by stem (29.62%) ([Table 2](#_bookmark4)). The Simpson and Shannon–Wiener’s diversity indices were higher in leaves and petiole. The species richness was greater in the leaves ([Table 4](#_bookmark6)).

### Z. officinale

Fourteen isolates, from which 5 fungal isolates were from leaves, 2 fungal isolates were from petiole, 4 fungal isolates were from rhizome and 3 fungal isolates were from adventitious roots, were obtained from *Z. officinale* and grouped into 11 genera. *Cla- dosporium* sp. (1.78%) was the most frequently isolated endophyte from only one tissue type (Leaf). Except *Cladospo- rium* sp., the remaining endophytic fungi had a low frequency of occurrence that is obtained only once from each tissue type (petiole, rhizome and adventitious roots) ([Table 3](#_bookmark5)).The coloni- zation rates were higher in rhizomes (36%), leaves (35.71%) and then in the other tissues of *Z. officinale* ([Table 2](#_bookmark4)). The richness of endophytic fungi varied among the different tissues of *Z. officinale.* The Simpson’s dominance of endophytic fungi was

**338**

egyptian journal of basic and applied sciences 3 (2016) 33 5 –342

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 3 – Absolute (f) and relative frequency (fr) of endophytic fungal isolates from selected medicinal plants.** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Sl. no | Endophytic fungi | L | *Tinospora cordifolia*  P S | | R | L | *Piper nigrum*  P S | | R | L | *Piper longum*  P S | | R | L | *Zingiber officinale*  P S | | R | L | *Hedychium flavescens*  P S | | R | L | *Hedychium coronarium*  P S | | R | Absolute frequency (f) | Relative frequency fr (%) |
| 1 | *Acremonium* sp. |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  | 1 | 0.892 |
| 2 | *Alternaria* sp. |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  | 1 |  | 1 | 1 | 1 |  |  |  | 1 | 6 | 5.357 |
| 3 | *Aspergillus* sp. | 3 | 1 | 3 |  |  |  | 2 | 2 | 3 | 1 | 3 | 1 |  |  | 1 |  |  |  |  |  |  | 2 |  |  | 22 | 19.642 |
| 4 | *Bipolaris* sp. |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  | 1 |  |  |  |  |  | 1 |  | 3 | 2.678 |
| 5 | *Chaetomium* sp. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  | 1 | 0.892 |
| 6 | *Cladosporium* sp. |  | 1 | 2 |  |  |  |  |  |  |  |  |  | 2 |  |  |  |  |  | 1 |  |  |  |  |  | 6 | 5.357 |
| 7 | *Colletotrichum* sp. |  |  |  | 1 |  |  | 1 |  | 1 |  |  |  |  |  | 1 |  | 1 | 1 |  |  |  |  |  |  | 6 | 5.357 |
| 8 | *Curvularia* sp. |  |  | 1 |  |  |  |  |  | 1 |  | 1 |  |  | 1 |  |  |  |  |  |  |  |  |  |  | 4 | 3.571 |
| 9 | *Cylindrocephalum* sp. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  | 1 | 0.892 |
| 10 | *Dreshclera* sp. |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | 0.892 |
| 11 | *Fusarium* sp. | 1 |  | 1 | 1 |  |  |  |  |  |  | 1 |  | 1 |  |  |  | 1 |  | 1 |  |  | 1 |  |  | 8 | 7.142 |
| 12 | *Lasiodiplodia* sp*.* |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | 0.892 |
| 13 | *Mucor* sp. | 1 |  |  | 1 |  |  |  |  | 1 |  | 1 |  |  |  |  | 1 |  | 1 |  |  |  |  |  |  | 6 | 5.357 |
| 14 | Mycelia sterilia | 1 | 1 | 1 | 1 |  | 1 |  |  |  | 1 | 1 | 1 |  |  |  | 1 |  | 1 |  |  | 1 |  |  |  | 11 | 9.821 |
| 15 | *Myrothecium* sp*.* |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  | 1 | 0.892 |
| 16 | *Nigrospora* sp. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | 1 |  | 2 | 1.785 |
| 17 | *Paecilomyces* sp. |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 | 0.892 |
| 18 | *Penicillium* sp. | 1 |  |  |  |  |  |  | 1 | 2 | 1 | 1 |  |  |  |  |  |  |  |  |  | 1 |  |  | 1 | 8 | 7.142 |
| 19 | *Pestalotiopsis* sp. |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  | 1 |  | 1 |  |  |  |  |  |  |  | 3 | 2.678 |
| 20 | *Phoma* sp. |  |  |  |  | 1 | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 2 | 1.785 |
| 21 | *Phomopsis* sp. |  |  |  | 1 |  |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  | 3 | 2.678 |
| 22 | *Pithomyces* sp. |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  | 2 | 1.785 |
| 23 | *Rhizopus* sp. | 1 |  |  |  |  |  |  |  |  |  |  |  | 1 | 1 |  |  | 1 | 1 |  | 1 |  |  |  |  | 6 | 5.357 |
| 24 | *Sordaria* sp. |  |  |  |  |  |  |  | 1 |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 2 | 1.785 |
| 25 | *Torula* sp. |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | 1 |  |  |  |  |  |  |  | 1 | 0.892 |
| 26 | *Trichoderma* sp. |  |  | 1 |  |  |  |  | 1 |  |  |  |  |  |  |  |  |  |  |  |  | 2 |  |  |  | 4 | 3.571 |
|  | TOTAL | 8 | 3 | 9 | 5 | 2 | 2 | 4 | 5 | 11 | 6 | 8 | 2 | 5 | 2 | 4 | 3 | 8 | 5 | 3 | 2 | 6 | 4 | 3 | 2 | 112 | 99.984 |
|  | Species richness | 6 | 3 | 6 | 5 | 2 | 2 | 3 | 4 | 8 | 6 | 6 | 2 | 4 | 2 | 4 | 3 | 8 | 5 | 3 | 2 | 5 | 3 | 3 | 2 |  |  |
| L, Leaf; P, Petiole; S, Stem; R, Root. | | | | | | | | | | | | | | | | | | | | | | | | | | | |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 4 – Diversity indices of endophytic fungi isolated from *T. cordifolia, P. nigrum* and *P. longum.*** | | | | | | | | | | | | | |
| Sl. No | Indices | L | *Tinospora*  P | *cordifolia*  S | R | L | *Piper*  P | *nigrum*  S | R | L | *Piper*  P | *longum*  S | R |
| 1 | Simpson’s dominance | 0.218 | 0.333 | 0.209 | 0.200 | 0.500 | 0.500 | 0.375 | 0.280 | 0.157 | 0.166 | 0.218 | 0.500 |
| 2 | Simpson’s diversity | 0.781 | 0.666 | 0.790 | 0.800 | 0.500 | 0.500 | 0.625 | 0.720 | 0.843 | 0.833 | 0.781 | 0.500 |
| 3 | Species Richness | 6 | 3 | 6 | 5 | 2 | 2 | 3 | 4 | 8 | 6 | 6 | 2 |
| 4 | Shannon–Wiener | 1.667 | 1.097 | 1.676 | 1.609 | 0.693 | 0.693 | 1.039 | 1.331 | 1.971 | 1.791 | 1.667 | 0.693 |
| 5 | Evenness | 0.930 | 0.998 | 0.935 | 0.999 | 0.999 | 0.999 | 0.946 | 0.960 | 0.948 | 0.999 | 0.930 | 0.999 |
| L, Leaf; P, Petiole; S, stem; R, root. | | | | | | | | | | | | | |

higher in the petiole. The Shannon–Wiener’s diversity indices were higher in leaves and rhizomes ([Table 5](#_bookmark6)).

### H. flavescens

A total of 18 endophytic fungal isolates were attained from 80 tissue segments of *H. flavescens* (8 fungal isolates from leaves, 5 fungal isolates from petiole, 3 fungal isolates from stem and 2 fungal isolates from roots) and were grouped into 12 genera. The species of *Alternaria* and *Rhizopus* occurred in low fre- quency of 2.67% ([Table 3](#_bookmark5))*.* The colonization rate was highest in leaves (44.44%) and petiole (27.77%) than in the other tissues of *H. flavescens* ([Table 2](#_bookmark4)). The species richness was high in the leaves followed by petiole ([Table 5](#_bookmark6)).The Simpson’s domi- nance of endophytic fungi was higher in the roots. Both Simpson and Shannon–Wiener’s diversity indices were higher in leaves and petiole.

### H. coronarium

Fifteen endophytic isolates were obtained – 6 fungal isolates from leaves, 4 fungal isolates from petiole, 3 fungal isolates from stem and 2 fungal isolates from roots which were grouped into 12 genera. The colonization rates were highest in leaves (40%) and petiole (26.6%) ([Table 2](#_bookmark4)). The Simpson and Shannon– Wiener’s diversity indices were higher in leaves. The species richness was also greater in the leaves ([Table 5](#_bookmark6)). The genera *Aspergillus* and *Cladosporium* were the most frequently iso- lated in the petiole and leaf tissues ([Table 3](#_bookmark5)).

* + 1. *Screening of the endophytic fungi for extracellular enzyme production*

The fungal isolates were subjected for extracellular enzyme production. Eighty isolates were able to produce the extracel- lular enzymes ([Table 6](#_bookmark7)) with the exception of laccase which none of the fungal isolates produced. The incubation period influenced enzyme production and varied from 3 to 7 days. In

our study, 29% of the isolates hydrolyzed starch and were posi- tive for amylase activity. The maximum production of amylase was from Pn-7 (*Lasiodiplodia* sp*.*), Pl-13 (*Aspergillus* sp.) and Zo-3 (*Cladosporium* sp.). The isolates of *H. coronarium* and *H. flavescens* were weak producers of amylase ([Table 6](#_bookmark7)). Cellulolytic activ- ity was observed in 28.18% of the total isolates. Prominent cellulolytic activity was observed in Tc-8 (Mycelia sterilia), Pl- 13 (*Aspergillus* sp.), Pl-10 (*Penicillium* sp.), Pl-21 (*Pestalotiopsis* sp*.)* and Hc-3 (Mycelia sterilia) ([Table 6](#_bookmark7)). The isolates demonstrat- ing pectinase enzyme were lower compared to other enzymes. Maximum pectinase activity was observed in *T. cordifolia* iso- lates (24%). The isolates from *P. nigrum, H*. *coronarium* and *H. flavescens* were weak producers of pectinase. Significant pec- tinase activity was detected in Tc-8 (Mycelia sterilia), Zo-3 (*Cladosporium* sp.), Pl-24 (*Phomopsis* sp.) and Hf-8 (*Mucor* sp) ([Table 6](#_bookmark7)). None of the isolates screened demonstrated laccase activity, indicating the disparity in enzyme production by the fungal species which is mostly dependent on the host habitat. Asparaginase activity was depicted by 39% of the endophytic isolates. The highest L-asparagine producing isolates were ob- served from *P. longum* where 13 of the 27 isolates produced asparaginase. The isolates Tc-25 (*Fusarium* sp*.*), Zo-14 (*Rhizo- pus* sp.) and Zo-7 (*Aspergillus* sp.) demonstrated high asparaginase activity ([Table 6](#_bookmark7)).

# Discussion

The plant tissues, namely the leaf, petiole, stem and roots of the medicinal plants, were used. The members of *Hedychium* (*Zingiberaceae*) are listed as critically endangered species of India in the Red data book [[20]](#_bookmark25). However, not much work has been done on endophytic fungal isolations from the plants belong- ing to *Hedychium* sp. to the best of our knowledge. Also, the studies on endophytic fungi from these plants have revealed

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 5 – Diversity indices for endophytic fungi isolated from *Z. officinale, H. flavescens* and *H. coronarium.*** | | | | | | | | | | | | | |
| Sl. No | Indices | L | *Zingiber*  P | *officinale*  Rh | Ad. R | *Hedychium*  L P | | *flavescens*  S R | | *Hed*  L | *ychium*  P | *coronarium*  S R | |
| 1 | Simpson’s dominance | 0.280 | 0.500 | 0.250 | 0.333 | 0.125 | 0.200 | 0.333 | 0.500 | 0.222 | 0.370 | 0.333 | 0.500 |
| 2 | Simpson’s diversity | 0.720 | 0.500 | 0.750 | 0.666 | 0.875 | 0.800 | 0.666 | 0.500 | 0.777 | 0.625 | 0.666 | 0.500 |
| 3 | Species Richness | 4 | 2 | 4 | 3 | 8 | 5 | 3 | 2 | 5 | 3 | 3 | 2 |
| 4 | Shannon–Wiener | 1.331 | 0.693 | 1.386 | 1.098 | 2.079 | 1.604 | 1.096 | 0.693 | 1.560 | 1.039 | 1.098 | 0.693 |
| 5 | Evenness | 0.960 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 | 0.999 | 0.969 | 0.946 | 0.999 | 0.999 |
| L, Leaf; P, Petiole; S, stem; R, root; Rh, Rhizome; Ad. R, Adventitious roots. | | | | | | | | | | | | | |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 6 – Endophytic fungal isolates screened for extracellular enzymes on solid media.** | | | | | | | | |
| Sl. No | Medicinal plant | Code | Endophytic fungal isolate | Amylase | Cellulase | Pectinase | Laccase | Asparaginase |
| 1 | *Tinospora cordifolia* | Tc2 | *Fusarium* sp*.* | 29.00d ± 0.51  0a  36.33f ± 0.59  34.33e ± 0.29  0a  18.33b ± 0.78  0a  0a  38.33g ± 0.78  0a  0a 0a  24.66c ± 0.29  29.00d ± 0.51  0a  0a  19.33b ± 0.29  69.00d ± 0.51  0a  49.00c ± 0.51  0a  0a 0a  44.00i ± 0.51  0a  0a 0a 0a  31.66f ± 0.29  0a  0a 0a 0a 0a  52.66j ± 0.29  24.00bc ± 0.51  0a  0a  29.33e ± 0.59  25.66d ± 0.29  36.00g ± 0.51  0a  0a 39.00h ± 0.51  29.66e ± 0.29  0a  23.33b ± 0.59  24.66c ± 0.29  0a  41.00d ± 0.51  53.33f ± 0.78  29.00c ± 0.51  0a  14.66b ± 0.29  0a  0a 0a  42.66e ± 0.29  0a  0a 0a  29.00c ± 0.51  0a  11.66b ± 0.29  0a  0a 0a  12.00b ± 0.51  0a  44.66d ± 0.29  0a  39.66c ± 0.29  0a  52.66d ± 0.29  0a  0a 0a  19.66b ± 0.29  0a  0a | 0a 0a 0a  55.33f ± 1.07  15.33c ± 0.78  21.66d ± 0.29  0a  0a  13.66b ± 0.78  0a  21.00d ± 0.51  24.00e ± 0.23  24.00e ± 0.51  0a  0a  21.66b ± 0.29  0a  0a 0a 0a  43.33d ± 0.29  26.00c ± 0.51  0a  46.00f ± 0.51  49.00g ± 0.51  32.33c ± 0.29  37.00e ± 0.51  34.00d ± 0.51  0a  49.00g ± 0.57  0a  54.66i ± 0.33  0a  52.33h ± 0.29  71.00k ± 0.51  0a  54.66i ± 0.29  0a  0a 0a 0a  54.00i ± 0.51  56.33j ± 0.29  0a  21.66d ± 0.29  0a  0a 0a 0a  40.33c ± 0.29  0a  0a 0a 0a  35.33b ± 0.29  0a  0a 0a 0a  26.66c ± 0.29  65.00d ± 0.51  0a  0a 0a 0a  20.66b ± 0.29  0a  0a 0a 0a  29.33c ± 0.29  0a  0a 0a 0a  17.66b ± 0.29  0a  0a 0a  42.66d ± 0.29 | 0a 0a 0a  72.33f ± 0.78  69.33e ± 0.59  41.66c ± 0.78  0a  0a 41.00c ± 0.51  0a  0a 53.00d ± 0.51  39.33b ± 0.59  0a  9.00b ± 0.51  0a  0a 0a 0a 0a 0a  23.66c ± 0.29  0a  0a 0a 0a  10.66ab ± 0.29  0a  0a 0a 0a 0a 0a 0a 0a 0a 0a  18.33b ± 0.29  0a  0a 0a 0a 0a 0a  61.66c ± 0.29  0a  0a 81.00d ± 0  0a  39.66d ± 0.29  63.33e ± 0.78  0a  0a 0a 0a  26.66c ± 0.29  0a  10.33b ± 0.59  0a  0a 0a 0a 0a 0a  14.00b ± 0.51  0a  19.00c ± 0.51  0a  0a 0a  27.33c ± 0.29  0a  0a 0a  62.66d ± 0.29  0a  0a 0a  15.66b ± 0.29  0a | 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a 0a | 25.00b ± 0.59  47.66cd ± 1.30  43.66cd ± 0.78  34.66bc ± 0.29  54.00d ± 0.51  0a  59.00d ± 0.51  75.00e ± 0.29  0a  23.66ab ± 0.78  24.00b ± 0.78  36.00bc ± 0.51  79.66e ± 0.29  0a  0a  69.66e ± 0.29  65.66d ± 0.29  34.00b ± 0.51  0a  0a 69.00e ± 0.51  64.00c ± 0.51  39.00c ± 0.51  0a  0a 0a  47.66gh ± 0.29  0a  47.66gh ± 0.33  39.00c ± 0.57  40.33de ± 0.33  39.66cd ± 0.29  39.33cd ± 0.59  41.00e ± 0.51  0a  45.00f ± 0.48  24.33b ± 0.63  0a  0a 0a 0a  48.00h ± 1.03  0a  0a  39.66cd ± 0.29  46.66g ± 0.29  0a  32.00b ± 0.51  0a  0a 0a  63.33d ± 0.29  0a  72.33e ± 0.29  0a  41.66c ± 0.29  0a  72.33e ± 1.30  0a  23.66c ± 0.78  0a  31.00e ± 0.51  0a  51.00g ± 0.51  0a  15.66b ± 0.29  0a  24.66d ± 0.29  32.66f ± 0.29  0a  41.33d ± 0.29  65.00e ± 1.03  0a  0a 0a  11.66b ± 0.29  0a  23.66c ± 0.29  0a |
| 2  3 | (Willd.) Hook. f. & Thomson | Tc6  Tc7 | *Aspergillus* sp*.*  *Penicillium* sp. |
| 4 |  | Tc8 | Mycelia sterilia |
| 5 |  | Tc9 | *Cladosporium* sp. |
| 6 |  | Tc10 | *Aspergillus* sp. |
| 7 |  | Tc12 | *Curvularia* sp*.* |
| 8 |  | Tc15 | *Trichoderma* sp. |
| 9 |  | Tc16 | *Cladosporium* sp. |
| 10 |  | Tc17 | *Fusarium* sp. |
| 11 |  | Tc18 | *Cladosporium* sp. |
| 12 |  | Tc19 | *Aspergillus* sp. |
| 13 |  | Tc20 | *Aspergillus* sp. |
| 14 |  | Tc25 | *Fusarium* sp*.* |
| 15 | *Piper nigrum* | Pn1 | *Phoma* sp*.* |
| 16  17 | L. | Pn2  Pn6 | *Paecilomyces* sp*.*  *Aspergillus* sp. |
| 18 |  | Pn7 | *Lasiodiplodia* sp. |
| 19 |  | Pn8 | *Aspergillus* sp. |
| 20 |  | Pn9 | *Aspergillus* sp. |
| 21 |  | Pn11 | *Sordaria sp.* |
| 22 |  | Pn12 | *Aspergillus* sp. |
| 23 |  | Pn13 | *Trichoderma* sp. |
| 24 | *Piper longum* | Pl1 | *Mucor* sp. |
| 25  26 | L. | Pl2  Pl3 | *Penicillium* sp.  *Aspergillus* sp. |
| 27 |  | Pl4 | *Colletotrichum* sp. |
| 28 |  | Pl5 | *Penicillium* sp. |
| 29 |  | Pl7 | *Dreshclera* sp. |
| 30 |  | Pl8 | *Aspergillus* sp. |
| 31 |  | Pl9 | *Pithomyces* sp. |
| 32 |  | Pl10 | *Bipolaris* sp*.* |
| 33 |  | Pl11 | *Curvularia* sp. |
| 34 |  | Pl12 | *Fusarium* sp. |
| 35 |  | Pl13 | *Aspergillus* sp. |
| 36 |  | Pl14 | *Curvularia s*p. |
| 37 |  | Pl15 | *Aspergillus* sp. |
| 38 |  | Pl16 | *Aspergillus* sp. |
| 39 |  | Pl17 | *Mycelia sterilia* |
| 40 |  | Pl18 | *Mucor* sp. |
| 41 |  | Pl19 | *Penicillium* sp. |
| 42 |  | Pl20 | *Aspergillus* sp. |
| 43 |  | Pl21 | *Pestalotiopsis* sp*.* |
| 44 |  | Pl22 | *Mycelia sterilia* |
| 45 |  | Pl24 | *Phomopsis* sp. |
| 46 |  | Pl25 | *Penicillium* sp. |
| 47 |  | Pl26 | *Aspergillus* sp. |
| 48 |  | Pl27 | *Mycelia sterilia* |
| 49 | *Zingiber officinale* | Zo1 | *Aspergillus* sp. |
| 50  51 | Roscoe | Zo2  Zo3 | *Rhizopus* sp.  *Cladosporium* sp. |
| 52 |  | Zo4 | *Cladosporium* sp. |
| 53 |  | Zo5 | *Alternaria* sp. |
| 54 |  | Zo6 | *Curvularia* sp. |
| 55 |  | Zo7 | *Aspergillus* sp. |
| 56 |  | Zo8 | Mycelia sterilia |
| 57 |  | Zo11 | Mycelia sterilia |
| 58 |  | Zo13 | *Mucor* sp. |
| 59 |  | Zo14 | *Rhizopus* sp. |
| 60 | *Hedychium coronarium* | Hc2 | *Trichoderma* sp. |
| 61  62 | J. Koenig | Hc3  Hc4 | Mycelia sterilia  *Penicillium* sp. |
| 63 |  | Hc5 | Mycelia sterilia |
| 64 |  | Hc7 | *Alternaria* sp. |
| 65 |  | Hc8 | *Penicillium* sp. |
| 66 |  | Hc9 | *Fusarium* sp. |
| 67 |  | Hc11 | *Aspergillus* sp. |
| 68 |  | Hc12 | *Bipolaris* sp. |
| 69 |  | Hc13 | *Nigrospora* sp. |
| 70 |  | Hc15 | *Aspergillus* sp. |
| 71 | *Hedychium flavescens* | Hf3 | *Colletotrichum* sp. |
| 72  73 | Carey ex Roscoe | Hf4  Hf7 | *Fusarium* sp.  *Bipolaris* sp. |
| 74 |  | Hf8 | *Pithomyces* sp. |
| 75 |  | Hf9 | *Mucor* sp. |
| 76 |  | Hf10 | *Alternaria* sp. |
| 77 |  | Hf11 | Mycelia sterilia |
| 78 |  | Hf12 | *Rhizopus* sp. |
| 79 |  | Hf12 | *Fusarium* sp. |
| 80 |  | Hf14 | *Cladosporium* sp. |
| Values followed by lower case alphabets in the column are statistically equivalent (P < 0.05) according to the Duncan multiple range test. | | | | | | | | |

differences in the colonization rates as well as diversity pattern which have not been documented so far. Previous report sug- gests that fungal endophytes were more frequent in leaf and stem tissues [[14]](#_bookmark20). In contrast, in the species composition of en- dophytic fungi from *Lippia sidoides*, the colonization of leaves (50.41%) was higher than that of stems (35.40%) [[21]](#_bookmark26). In Brazil, 95 endophytic fungi from *Bauhinia forficata* were isolated and reported highest frequency of colonization in the stems [[22]](#_bookmark27). Species of *Aspergillus, Fusarium, Penicillium, Colletotrichum, Cla- dosporium, Curvularia, Mucor and Rhizopus* were dominant in our work and it may be due to high spore production of these fungi and their cosmopolitan nature, which increases their chance to get established as endophytes [[23]](#_bookmark28). Mycelia sterilia, the fungal taxa which failed to sporulate, are also reported in our present work. The species of *Acremonium, Colletotrichum, Chaetomium, Myrothecium, Phomopsis, Fusarium* and *Pestalotiopsis* were the com- monly isolated endophytes from medicinal plants of Western Ghats, Karnataka [[24]](#_bookmark29). There is a dearth of reports on the di- versity of endophytic fungi obtained from *Z. officinale* as many authors have worked on the antagonistic activity of the en- dophytic actinomycetes of *Z. officinale* against phytopathogenic fungi [[25]](#_bookmark30).

Diversity indices for fungal endophytes analyzed by Shannon–Weiner and Simpson indices indicated differences in endophytic fungal isolates and species richness. High Simp- son’s diversity indices were noted for *P. longum* and *H. flavescens* whereas lower diversity indices are reported in *P. nigrum.* The colonization rate was higher in the leaves and stems of the medicinal plants as compared to the roots and petiole. Species richness was predominant in leaves. Species richness was higher in leaf segments of the five medicinal plants of Kudremukh region, Western Ghats of Karnataka which is similar to our work [[23]](#_bookmark28).

The Shannon index increases as both the richness and the evenness of the community increase. The Simpson’s domi- nance and diversity were analyzed. As the dominance index increases, the diversity decreases. Species richness relates to count of species, whereas species evenness quantifies the equal abundances of the species in a particular environment. Lesser variation in communities between the species reflects higher species evenness and is independent of species richness.

This study reports *Lasiodiplodia* sp. (Pn-7) as producing maximum amylolytic activity out of all the isolates. From our laboratory, thirty isolates from *Alpinia calcarata* Roscoe were screened, of which the isolate *Cylindrocephalum* sp. gave maximum amylase activity [[26]](#_bookmark31). Among 112 isolates, thirty one isolates have shown cellulolytic activity (27.67%) and the highest cellulolytic activity was demonstrated in *Aspergillus* sp from

*P. longum* (Table-6). Previously, cellulolytic activity was promi- nent in *Talaromyces emersonii* [[19]](#_bookmark24)*.* In another study, 43.33% isolates exhibited cellulase activity with *Cephalosporium* (36.5%) being the prominent cellulase producing form [[27]](#_bookmark32). Moreover, 66% of the isolates from *Brucea javanica* were producers of cel- lulase enzyme [[28]](#_bookmark33). Pectinase activity was observed in 19% of our endophytic isolates Endophytic fungi from *Opuntia ficus*- *indica* Mill were isolated, wherein *Cladosporium cladosporioides* (20.43%) and *Cladosporium sphaerospermum* (15.99%) presented high pectinase activity [[22]](#_bookmark27). Maximum pectinase activity was reported in *T. emersonii* and *Fusarium oxysporum* from *Calophyllum inophyllum* [[19]](#_bookmark24)*.* In fungi, laccase is a ligninolytic enzyme and

is involved in fruiting body formation, fungal plant-pathogen/ host interaction and stress defense. Laccase activity reflects the ability of the fungus to decompose lignocellulosic mate- rials. An interesting observation of our study is that none of the endophytic fungal isolates were able to produce laccase enzyme. Also, none of the endophytic fungi from mangrove an- giosperms were able to produce laccase [[29]](#_bookmark34). The endophytic nature of these fungi might be the reason for the lack of laccase activity, since an active enzyme might damage the host plant. The endophytic *Phomopsis longicolla* of *Bixa orellana* was a sig- nificant producer of laccase enzyme. In addition, *Discosia* sp*.* from *C. inophyllum* and *Chaetomium* sp. from *Alpinia calcarata* produced laccase [[14]](#_bookmark20). Asparaginase activity was depicted by 40% of the isolates. The isolate *Fusarium* sp. (Tc-25) gave the highest asparaginase activity followed by *Aspergillus* sp. (Zo- 7) and *Rhizopus* sp. (Zo-14). Sixteen endophytic fungi were isolated from *Capsicum frutescence* var US 341 and evaluated for L-asparaginase production. Among them, *Aspergillus* sp. was identified as a potential isolate for L-asparaginase [[30]](#_bookmark35). The en- dophytic fungal isolates from seven wild Thai medicinal plants were screened for asparaginase, from which *Colletotrichum* sp. and Mycelia sterilia exhibited good asparaginase activity [[18]](#_bookmark23).

# Conclusion

The present study provides information on colonization of en- dophytic fungi in six important medicinal plants and its diversity analysis. This is a first report on endophytic assem- blages from *Hedychium flavescens* and *H. coronarium*. The extracellular enzymes of endophytic fungi varied from isolate to isolate, hypothesizing that the enzyme production depends on the type of host and its habitats. Further research is re- quired for the synthesis of stable enzymes and bioactive compounds.

# Acknowledgment

The Financial assistance to Fazilath Uzma (F1-17.1/2012-13/ MANF-2012-13-MUS-KAR-11899) granted by Maulana Azad National Fellowship (MANF), University Grants Commission (UGC), New Delhi is gratefully acknowledged.

# Appendix: Supplementary material

Supplementary data to this article can be found online at [doi:10.1016/j.ejbas.2016.08.007](http://dx.doi.org/10.1016/j.ejbas.2016.08.007).

R E F E R E N C E S

1. [Hyde KD, Soytong K. The fungal endophyte dilemma. Fungal](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0010) [Divers 2008;33:163–73.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0010)
2. [Wang Y, Dai CC. Endophytes: a potential resource for](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0015) [biosynthesis, biotransformation, and biodegradation. Ann](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0015) [Microbiol 2011;61:207–15.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0015)
3. [Pinheiro EA, Carvalho JM, dos Santos DC, Ade OF, Marinho](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0020) [PS, Guilhon GM, et al. Antibacterial activity of alkaloids](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0020) [produced by endophytic fungus Aspergillus sp. EJC08](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0020) [isolated from medical plant *Bauhinia guianensis*. Nat Prod Res](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0020) [2013;27:1633–8.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0020)
4. [Tan RX, Zou WX. Endophytes: a rich source of functional](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0025) [metabolites. Nat Prod Res 2001;18:448–59.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0025)
5. [Saikkonen K. Forest structure and fungal endophytes.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0030) [Fungal Biol Rev 2007;21:67–74.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0030)
6. [Petrini O. Fungal endophytes of tree leaves. In: Andrew IA,](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0035) [Hirano SS, editors. Microbial ecology of leaves. New York:](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0035) [Springer-Verlag; 1991. p. 179–97.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0035)
7. [Asai E, Hata K, Futai K. Effect of simulated acid rain on the](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0040) [occurrence of *Lophodermium* on Japanese black pine needles.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0040) [Mycol Res 1998;102:1316–18.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0040)
8. [Rodriguez RJ, White JFJR, Arnold AE, Redman RS. Fungal](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0045) [endophytes: diversity and functional roles-Tansley review.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0045) [New Phytologist 2009;182:314–30.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0045)
9. [Pavithra NL, Sathish K, Ananda K. Antimicrobial and](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0050) [enzyme activity of endophytic fungi isolated from Tulsi. J](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0050) [Pharm Biomed Sci 2012;16(12).](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0050)
10. [Desire MH, Bernard F, Forsah MR, Assang CT, Denis ON.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0055) [Enzymes and qualitative phytochemical screening of](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0055) [endophytic fungi isolated from *Lantana camara* Linn. Leaves.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0055) [J Appl Biol Biotech 2014;2:1–6.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0055)
11. [Alurappa R, Reddy M, Bojegowda M, Kumar V, Mallesh NK,](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0060) [Chowdappa C. Characterization and bioactivity of oosporein](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0060) [produced by endophytic fungus *Cochliobolus kusanoi* isolated](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0060) [from *Nerium oleander* L. Nat Prod Res 2014;28:2217–20.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0060)
12. [Barnett HL, Hunter BB. Illustrated genera of imperfect fungi.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0065) [St. Paul, Minnesota, USA: APS Press; 1998.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0065)
13. [Larran S, Perelló A, Simón MR, Moreno V. Isolation and](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0070) [analysis of endophytic microorganisms in wheat (*Triticum*](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0070)[*aestivum* L.) leaves. World J Microbiol Biotechnol 2002;18:683–](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0070) [6.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0070)
14. [Huang WY, Cai WZ, Hyde KD, Corke H, Sun M. Biodiversity](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0075) [of endophytic fungi associated with 29 traditional Chinese](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0075) [medicinal plants. Fungal Divers 2008;33:61–75.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0075)
15. [Mahapatra S, Banerjee D. Diversity and screening for](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0080) [antimicrobial activity of endophytic fungi from *Alstonia*](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0080)[*scholaris*. Acta Microbiol Immunol Hung 2010;57(3):215–23.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0080)
16. [Jena SK, Tayung K. Endophytic fungal communities](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0085) [associated with two ethno-medicinal plants of Similipal](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0085) [Biosphere Reserve, India and their antimicrobial](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0085) [prospective. J Appl Pharm Sci 2013;3:S7–12.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0085)
17. [Bezerra Jadson DP, Nascimento Carlos CF, Barbosa Renan do](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0090) [N, da Silva DC, Svedese VM, Silva-Nogueira EB, et al.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0090) [Endophytic fungi from medicinal plant *Bauhinia forficata*:](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0090)

[diversity and biotechnological potential. Braz J Microbiol](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0090) [2015;46:49–57.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0090)

1. [Theantana T, Hyde KD, Lumyong S. Aspaginase production](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0095) [by endophytic fungi from Thai medicinal plants: cytotoxic](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0095) [properties. Int J Integr Biol 2009;7(1):1–8.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0095)
2. [Sunitha VH, Devi ND, Srinivas C. Extracellular enzymatic](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0100) [activity of endophytic fungal strains isolated from medicinal](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0100) [plants. World J Agric Sci 2013;9:1–9.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0100)
3. [Manish M. Current status of endangered Medicinal plant](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0105) [*Hedychium coronarium* and causes of Population decline in](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0105) [the natural forests of Anuppur and Dindori districts of](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0105) [Madhya Pradesh, India. Int Res J Biological Sci 2013;2(3):1–6.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0105)
4. [Siqueira VM, Conti R, Araújo JM, Souza-Motta CM.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0110) [Endophytic fungi from the medicinal plant *Lippia sidoides*](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0110)[Cham and their antimicrobial activity. Symbiosis 2011;53:89–](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0110) [95.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0110)
5. [Bezerra JDP, Santos MGS, Svedese VM, Lima DMM,](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0115) [Fernandes MJS, Paiva LM, et al. Richness of endophytic fungi](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0115) [isolated from *Opuntiaficus-indica* Mill. (Cactaceae) and](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0115) [preliminary screening for enzyme production. World J](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0115) [Microbiol Biotechnol 2012;28:1989–95.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0115)
6. [Raviraja NS. Fungal endophytes in five medicinal plant](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0120) [species from Kudremukh Range, Western Ghats of India. J](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0120) [Basic Microbiol 2005;45:230–5.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0120)
7. [Nalini MS, Sunayana N, Prakash HS. Endophytic fungal](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0125) [diversity in medicinal plants of Western Ghats, India. Int J](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0125) [Biodivers 2014;doi:10.1155/2014/494213.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0125)
8. [Taechowisan T, Chanaphat S, Ruensamran W, Phutdhawong](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0130) [WS. Antibacterial activity of Decursin from Streptomyces sp.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0130) [GMT-8; an endophyte in *Zingiber officinale* Rosc. J Appl Pharm](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0130) [Sci 2013;3:74–8.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0130)
9. [Sunitha VH, Ramesha A, Savitha J, Srinivas C. Amylase](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0135) [production by endophytic fungi *Cylindrocephalum* sp. isolated](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0135) [from medicinal plant *Alpinia Calcarata* (Haw.) Roscoe. Braz J](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0135) [Microbiol 2012;1213–21.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0135)
10. [Smitha SL, Correya NS, Philip R. Marine fungi as a potential](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0140) [source of enzymes and antibiotics. Int J Res Mar Sci](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0140) [2014;3:5–10.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0140)
11. [Choi YW, Hodgkiss IJ, Hyde KD. Enzyme production by](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0145) [endophytes of *Bruceajavanica*. J Agric Sci Technol 2005;1:55–](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0145) [66.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0145)
12. [Maria GL, Sridhar KR, Raviraja NS. Antimicrobial and](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0150) [enzyme activity of mangrove endophytic fungi of southwest](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0150) [coast of India. J Agri Tech 2005;1:67–80.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0150)
13. [Amrutha V, Audipudi G, Supriya NG, Pallavi R, Ganga MP.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0155) [Characterization of L-asparaginase producing endophytic](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0155) [fungi isolated from ripened fruit of capsicum frutescence.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0155) [Int J Pharm Dev Tech 2014;4:52–7.](http://refhub.elsevier.com/S2314-808X(16)30059-8/sr0155)