

# Diversity and antimicrobial potential of endophytic fungi from aromatic plants of Bhadra Wildlife Sanctuary, Western Ghats, Karnataka

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## ARTICLE INFO

### Article history:

Received on: October 21, 2020  
Accepted on: March 05, 2021  
Available online: September 01, 2021

### Key words:

Endophytic fungi, aromatic plants, Baba Budangiri, Lakkavalli, Mullayanagiri, antimicrobial activity

## ABSTRACT

Endophytic fungi are the main sources of novel bioactive compounds because of their unique inhabitation ability. This work focus on studying the diversity of fungal endophytes from eleven aromatic plants in three locations of Bhadra Wildlife Sanctuary i.e. Baba Budangiri, Lakkavalli, Mullayanagiri and screened for antimicrobial potential. Three hundred and forty-three endophytic fungi were isolated, and the highest colonization frequency was in *Plectranthus amboinicus* (60%) collected from the Baba Budangiri region. *Fusarium* sp., was the most prominent genus isolated from all plant samples. The relative density (RD) of isolates varied within plant parts, with leaf fragments of the Baba Budangiri region having the highest RD 42% and root segments showed the least RD value from all three locations. The Shannon- Wiener and species richness was highest in the Lakkavalli region. The Jaccard's similarity indices of fungal endophytes compared between the three sites ranged from 0.33-0.38. The antimicrobial activity showed that the endophyte *Chaetomium globosum* had maximum inhibition of 22 mm against *Escherichia coli* and *Bacillus subtilis*. Also, a wide range of antifungal activity was exhibited by *C. globosum*, inhibiting the radial growth of *Fusarium oxysporum* by 74.5%. *C. globosum* can be further exploited for the production of bioactive compounds.

## 1. INTRODUCTION

Microorganisms are important forms of life, occupying virtually every possible niche on earth [1]. It has been estimated that, including endophytic fungi, there are 5 million different fungal species on our planet [2]. Endophytic fungi are those organisms that reside within plant tissues throughout their life cycle without causing any symptoms of a disease [3]. De Bary first introduced the term “endophyte” in 1866, where “endon” means inside and “phyton” means plant [4]. There exists a symbiotic relationship between the plant and the endophytic fungi, where the plant provides nutrients to the microbe [5]. In turn, the microbe produces secondary metabolites that guard the host plant against many external factors like an attack by animals, insects, microbes and

drought, nutrient depletion in soil, etc. [6,3]. It is reported that some endophytes have evolved the ability to produce metabolites similar to their host plant, which may be due to co-evolution or genetic transfer between the endophyte and the plant [7]. Such a mutual interrelationship between endophytic fungi and their host plants can induce the latter to produce active metabolites, which can be used in pharmaceuticals and agriculture [8,9]. The origin, sustenance level, and ecological niche of the host plants are considered during isolation of endophytic fungi [10]. The Western Ghats being older than the Himalayan Mountains represents a unique ecosystem [11]. Its diverse topography and environmental conditions are the reasons for the rich floral diversity it possesses, which includes a wide variety of aromatic plant species [12]. Bhadra Wildlife Sanctuary, which is situated amid the Western Ghats region, is enclosed by Mullaianagiri, Baba Budangiri, Gangegiri, and Hebbegiri hills, and the river Bhadra flows through it [13]. The sanctuary is covered with moist deciduous, dry deciduous, evergreen, semi-evergreen, scrub, plantations, and grassland [14].

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Any plant that produces and exudes chemical substances that activate the olfactory nerves are called aromatic plants, and these substances are used in making perfumes, pharmaceutical, food, and liquor industries [15,16]. Essential oils produced by such plants are of high economic value [17]. Botanical surveys conducted on the Western Ghats during 2005–2009 have revealed that there are more than 126 aromatic plant species belonging to 61 genera under 17 families. The Lamiaceae family is the most frequently occurring, followed by the family Rutaceae [12]. Many of the aromatic plant species belong to family Lauraceae, Umbelliferae, and Myrtaceae [18]. Regardless of the numerous reports on wild aromatic plants of this region, the diverse microbiome associated with it remains unexplored. Therefore, in the present investigation, an attempt has been made to evaluate the diversity of fungal endophytes from 11 aromatic plants collected from 3 different locations of Bhadra Wildlife Sanctuary and screened for antimicrobial potential.

## 2. MATERIALS AND METHODOLOGY

### 2.1 Plant Samples and Study Site

The plant samples were collected in the month of September 2016, in three different locations of Bhadra Wildlife Sanctuary, Western Ghats, Karnataka, i.e., Baba Budangiri, Lakkavalli, and Mullayangiri. The geographical location and its climatic conditions are shown in Figure 1 and Table 1.

### 2.2 Isolation of Endophytic Fungi

The collected plant samples were washed thoroughly and surface sterilized by immersing them in 75% ethanol (1 minute), 2.5% sodium hypochlorite solution (v/v) for 1 minute, and 75% ethanol for 30 seconds. They were cut into 5 × 5 mm segments after drying

and placed on sterile potato dextrose agar (PDA) amended with 50 mg/l streptomycin [22]. The plates were incubated at 27°C for 3 weeks, and the fungi emerging from the explants were subcultured on to fresh PDA plates, and pure cultures were obtained [23].

### 2.3 Identification of Endophytic Fungi

The isolated endophytic fungi were stained with lactophenol cotton blue and the structure of fruiting bodies and spores were observed under a microscope at 40× resolution and identified using standard manuals [24].

### 2.4 Data Analyses

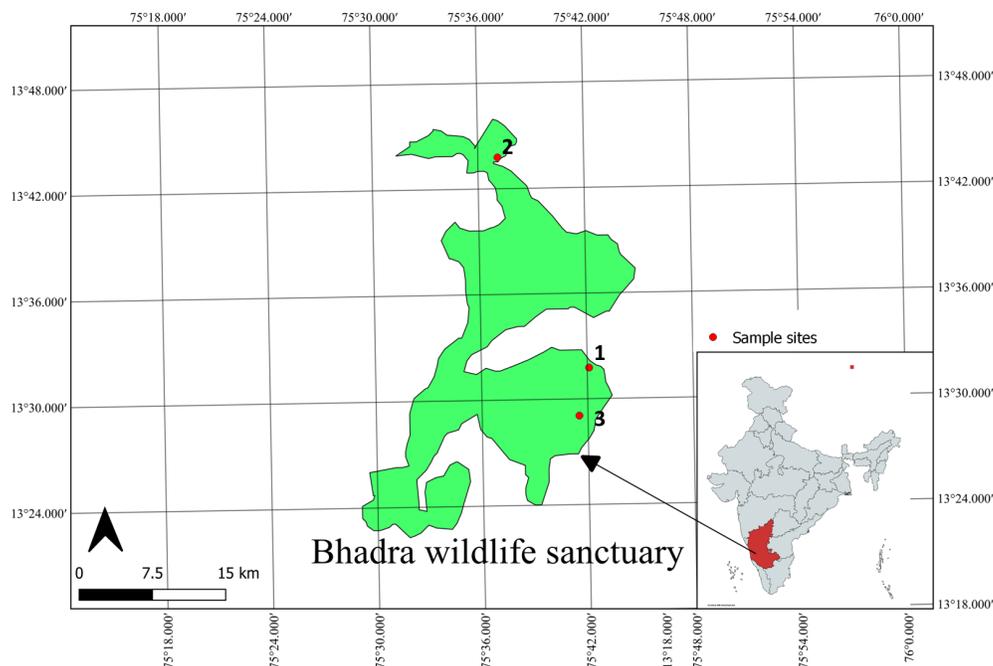
The colonization frequency (CF) and the percentage of the dominant endophytic fungi were calculated using the following formulas [25]:

$$CF\% = \frac{\text{Number of segments colonized by an endophyte}}{\text{Total number of explants placed}} \times 100$$

$$\text{Percentage of dominant fungi\%} = \frac{\text{Number of isolates of a particular species collected}}{\text{Total number of explants analyzed}} \times 100$$

The isolation rate (IR) determines the extent of various fungal endophytes colonizing in the sample in different explants and determined by the following formula [26]:

$$IR = \frac{\text{Total number of isolates yielded by a sample}}{\text{Total number of explants in that sample}} \times 100$$



**Figure 1.** Location of sample collection – Bhadra Wildlife Sanctuary: 1, Baba Budangiri; 2, Lakkavalli; and 3, Mullayangiri.

**Table 1.** Location and climatic conditions of the sample site in Bhadra Wildlife Sanctuary.

Description	Collection site		
	Baba Budangiri	Lakkavalli	Mullayanagiri
Coordinates	13.284134°N 75.997893°E	13°22' to 13°47'N 75°29' to 75°45'E	13°23'27.5"N 75°43'17"E
Elevation	843 m (2,766 ft)	300 m (1,000 ft)	1,925 m (6,316 ft)
Annual rainfall	100–150 cm	120–260cm	150–200cm
Temperature	20°C–25°C	19°C–35°C	20°C–25°C

Source: [19–21].

The relative abundance (RA) of each genus was also calculated by using the following formula [27]:

$$RA = \frac{\text{Number of isolates belonging to a particular species}}{\text{Total number of endophytic fungi isolated}} \times 100$$

The relative percentage of occurrence (RPO%) of different fungi groups was calculated using the following formula [28]:

$$RPO (\%) = \frac{\text{Number of isolates from particular fungal genera}}{\text{Total number of isolates of all the fungal genera}} \times 100$$

Camargo's index was used to determine dominant endophytic fungi among the isolates. A species is defined as dominant when it satisfies  $RA > 1/S$ , where "S" is species richness.  $RA = f/n$ , where, "f" is the number of times the isolate occurred and "n" is the total isolates [29].

Shannon–Weiner diversity and Simpson's diversity indices, species richness, and evenness were determined using the following formula and the data were analyzed as follows [30]:

$$\text{Shannon–Weiner diversity index } (H) = \sum (Pi) (\ln Pi)$$

where Pi is the RA of species ( $Pi = n/N$ ) and  $\ln Pi$  is the natural log of Pi value to the base 2.

$$\text{Simpson's Diversity index } (D) = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

$$\text{Gini Simpson's Diversity} = 1 - \sum_i (n_i/N)^2 \text{ [31].}$$

where N is the total number of isolates and n is number of individuals belonging to a particular species.

Species evenness (E) and was calculated using the following formula:  $E = H/\ln S$  [32].

Species richness =  $S/HN$ , where S = the total number of species [32].

## 2.5 Jaccard's Similarity Index (JI)

To measure the range of similarity of fungal endophytes shared between the three sample sites, JI was analyzed using the following formula:

$$JI = a(b + c) - a$$

where a represents the number of species isolated in any two sample sites, b is the number of species present only in location 1,

and c is the number of species present only in location 2. JI ranges from 0 to 1, where 0 signifies no overlap or no taxa shared and 1 signifies complete overlap or all taxa shared between the two sample sites analyzed [33].

## 2.6 Antimicrobial Activity

### 2.6.1 Test Organisms

The endophytic fungal isolates were screened for antibacterial activity against both Gram-positive and Gram-negative human pathogenic bacteria like *Staphylococcus aureus* (NCIM-2076), *Bacillus subtilis* (NCIM-5698), *Escherichia coli* (NCIM-2065), *Pseudomonas aeruginosa* (NCIM-2036), and *Klebsiella pneumoniae* (NCIM-2706) [34]. Plant pathogenic fungi like *Fusarium oxysporum* (NCIM-1072), *Alternaria alternata* (NCIM-718), and *Aspergillus niger* (NCIM-1004) were tested for antifungal activity. The test cultures were procured from National Collection of Industrial Microorganisms (NCIM) Pune, Maharashtra, India.

### 2.6.2 Antibacterial Activity of Endophytic Fungi

Antibacterial activity of endophytic fungi was carried out by agar plug assay. Each isolate was cultivated on PDA media at 30°C for 7 days. Then, disks were cut from the PDA plate (6 mm diameter) and transferred to the Mueller-Hinton agar media previously seeded with  $1.5 \times 10^5$  CFU/ml of 24-hours-old test bacteria. Streptomycin (30 µg/ml) and sterile distilled water were loaded as positive control and negative control, respectively. The petri plates were refrigerated at 4°C for 8 hours for complete diffusion of antibacterial compounds present in the fungal disks and later incubated at 37°C for 12 hours. After incubation, the inhibition zone around the fungal disks was measured in millimeters using a scale [35].

### 2.6.3 Antifungal Activity of Endophytic Fungi

Antifungal activity of endophytic fungi was carried out by dual culture assay against three plant pathogens: *A. alternata*, *F. oxysporum*, and *A. niger*. Small plugs (3 mm in diameter) of test cultures were placed at one corner of the sterile PDA plate. On the same petri plate, a 7-day-old endophytic fungal culture was inoculated at the opposite end. The inoculated plates were kept at 27°C for 24–48 hours. Test pathogens without the endophytes served as control plates. Filter paper disks inoculated with 10 µl of fungicide Derosil plus® (with  $10^{-1}$  dilution) and sterile distilled water were used as the positive and negative controls, respectively, and growth was regularly observed in both the petri plates. The

inhibition percentage was calculated by using the following formula [36]:

$$\text{Inhibition \%} = \frac{R-r}{R} \times 100$$

where  $R$  is the radius of the pathogen in control plate and  $r$  is the radius of the pathogen with endophytic fungi.

The experiments for antimicrobial activity were carried out in triplicates and results are recorded in mean  $\pm$  standard deviation.

### 3. RESULTS AND DISCUSSION

#### 3.1 Isolation and Identification of Endophytic Fungi

A total of 343 endophytic fungi were isolated from 11 aromatic plants including *Cymbopogon citretus* L., *Mentha arvensis* L., *Ocimum basilicum* L., *Ocimum tenuiflorum* L., *Origanum majorana* L., *Oxalis corniculata* L., *Plectranthus amboinicus* L., *Rosmarinus officinalis* L., *Ruta graveolens* L., *Thymus vulgaris* L., and *Zingiber officinale* L., which were collected from Baba Budangiri, Lakkavalli, and Mullayanagiri regions of Bhadra Wildlife Sanctuary (Table 2) (Fig. 2).

##### 3.1.1 Baba Budangiri

Among the 343 isolates, 114 fungi representing 19 genera were found to be associated with plants collected from Baba Budangiri region. The highest CF was recorded in *P. amboinicus* with 60%, followed by *O. tenuiflorum* (46%). *Amorphotheca* sp., *Bipolaris* sp., *Cladosporium* sp., and *F. oxysporum* were some of the

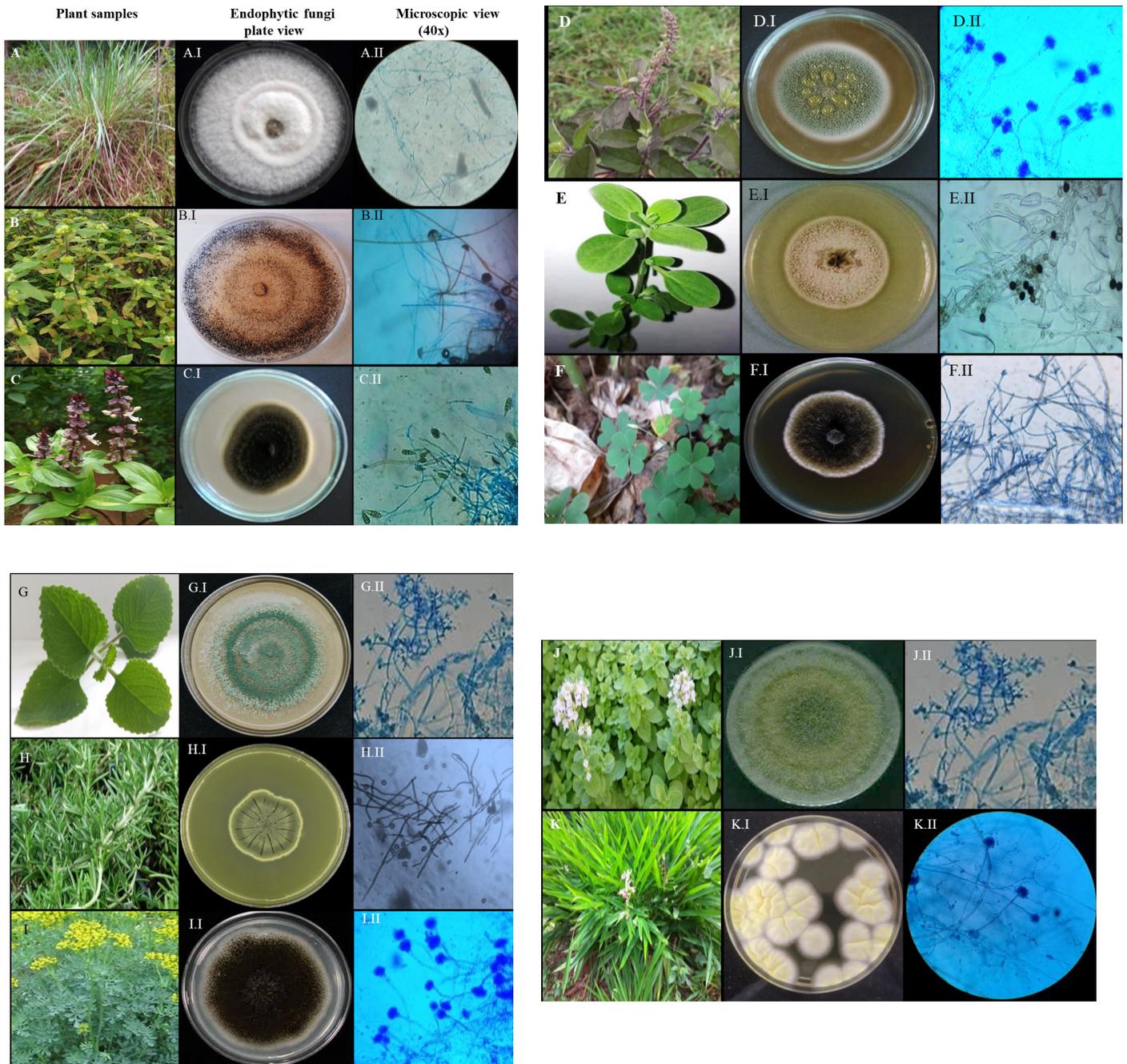
commonly occurring fungi in this region. *O. majorana* and *P. amboinicus* had the highest IR of 0.36 and *Z. officinale* showed the least IR of 0.15. *O. tenuiflorum* exhibited maximum species richness of 13.52, followed by *M. arvensis* (12.52), and the minimum species richness was recorded in *T. vulgaris* (5.52). *P. amboinicus* harbored the maximum endophytic fungi among the 11 plants assessed with RA of 12.84. Camargo's index indicated that endophytic fungal species differed majorly between different plant samples, except for *M. arvensis* and *O. tenuiflorum*, which showed a similar Camargo's index of 0.07 (Table 3). *F. solani* and *Cladosporium* sp. were found to be the most dominant fungi to occur in this region (Fig. 3a).

##### 3.1.2 Lakkavalli

A total of 118 isolates representing 25 different genera were found to be associated with plants from the Lakkavalli region. The highest CF was recorded in *C. citretus* (50%), followed by *O. basilicum* (43.3%). *A. fumigatus*, *A. niger*, *C. clavata*, *F. circinatum*, *F. oxysporum*, and *Phomopsis* were some of the commonly occurring fungi in this region. *P. amboinicus* had the highest IR of 0.52 and *Z. officinale* showed the least IR of 0.2. *C. citretus* exhibited maximum species richness of 14.51, followed by *O. basilicum* (12.51) and minimum species richness was recorded in *Z. officinale* (3.5). *C. citretus* harbored the maximum endophytic fungi among the 11 plants assessed with an RA of 14.41. Camargo's index ranged from 0.07 to 0.28, with *C. citretus* having the minimum value of 0.07 (Table 3). Similar to Baba Budangiri region, *F. solani*, *Cladosporium* sp., and *Cyindrocladium* sp. were found to be the most dominant fungi to occur in this region (Fig. 3b).

**Table 2.** List of selected aromatic plants collected from Bhadra Wildlife Sanctuary and its medicinal uses.

Plant samples	Uses	References
<i>C. citretus</i> L.	It is used in food, drinks, perfumery, body care products, and soap manufacture and also in treating digestive disorders, inflammation, diabetes, nervous disorders, and fever.	[37-40]
<i>M. arvensis</i> L.	It is used as an antimicrobial, antiviral, and insecticidal agent and also in cosmetic, food, and flavour industries.	[41,42]
<i>O. basilicum</i> L.	It is used to treat headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions. It can be used as an ointment for insect bites, and its oil is applied directly to the skin to treat acne.	[43]
<i>O. tenuiflorum</i> L.	It is used for relieving from fever, headache, sore throat, itching skin disorders, and issues like ringworms and shows antiviral, antibacterial, and anticarcinogenic effects. It is commonly used for cold, cough, flu, and chest congestion.	[44]
<i>O. majorana</i> L.	It is traditionally used as a folk medicine rheumatic pain, nervous disorders, cardiovascular diseases, epilepsy, insomnia, skincare, and flatulence to cure against asthma, indigestion, headache, and rheumatism, chest infection, cough, sore throat, and stomach disorders.	[45,46]
<i>O. corniculata</i> L.	It is one of the folk medicine to treat headaches, cure skin diseases, and to treat jaundice, anti-inflammatory, digestive, diuretic, antibacterial, antiseptic, cardiopathy, hepatopathy, dysentery, diarrhea, piles, and skin diseases.	[47]
<i>P. amboinicus</i> L.	It has been traditionally used in treating conditions like cold, asthma, constipation, headache, cough, fever, and skin diseases. The leaves are used as flavoring agent	[48]
<i>R. officinalis</i> L.	It is used to treat dyspepsia, high blood pressure, and rheumatism.	[49]
<i>R. graveolens</i> L.	Useful in treating fever, flatulence, colic, amenorrhoea, epilepsy, and hysteria. The oil acts as a stimulant for uterine and nervous systems. The fresh leaves are used for rheumatism.	[50]
<i>T. vulgaris</i> L.	It is one of the folk medicines used to treat cough, diabetes, and cold and chest infections; and in a syrup form for digestive upset. It is also soothing for sore throat, antiseptic, antibiotic, and antifungal properties.	[51]
<i>Z. officinale</i> L.	It is used to treat hypertension, dementia, muscular aches, pains, sore throats fever arthritis, rheumatism, sprains, cramps, constipation, indigestion, vomiting, infectious diseases, and helminthiasis.	[52]



**Figure 2.** Aromatic plant samples collected from different locations of Bhadra Wildlife Sanctuary and endophytic fungi isolated (plate and microscopic view). A *C. citretus* L. and A.I *F. oxysporum*; B *M. arvensis* L. and B.I *Rhizopus* sp.; C *O. basilicum* L. and C.I *A. alternate*; D *O. tenuiflorum* L. and D.I *P. Crysogenum*; E *O. majorana* L. and E.I *Circinella* sp.; F *O. corniculata* L. and F.I *Cladosporium* sp.; G *P. amboinicus* L. and G.I *Tricoderma* sp.; H *R. officinalis* L. and H.I *Cladosporium* sp.; I *R. graveolens* L. and I.I *A. niger*; J *T. vulgaris* L. and J.I *T. viride*; and K *Z. officinale* L. and K.I *A. fumigatus*.

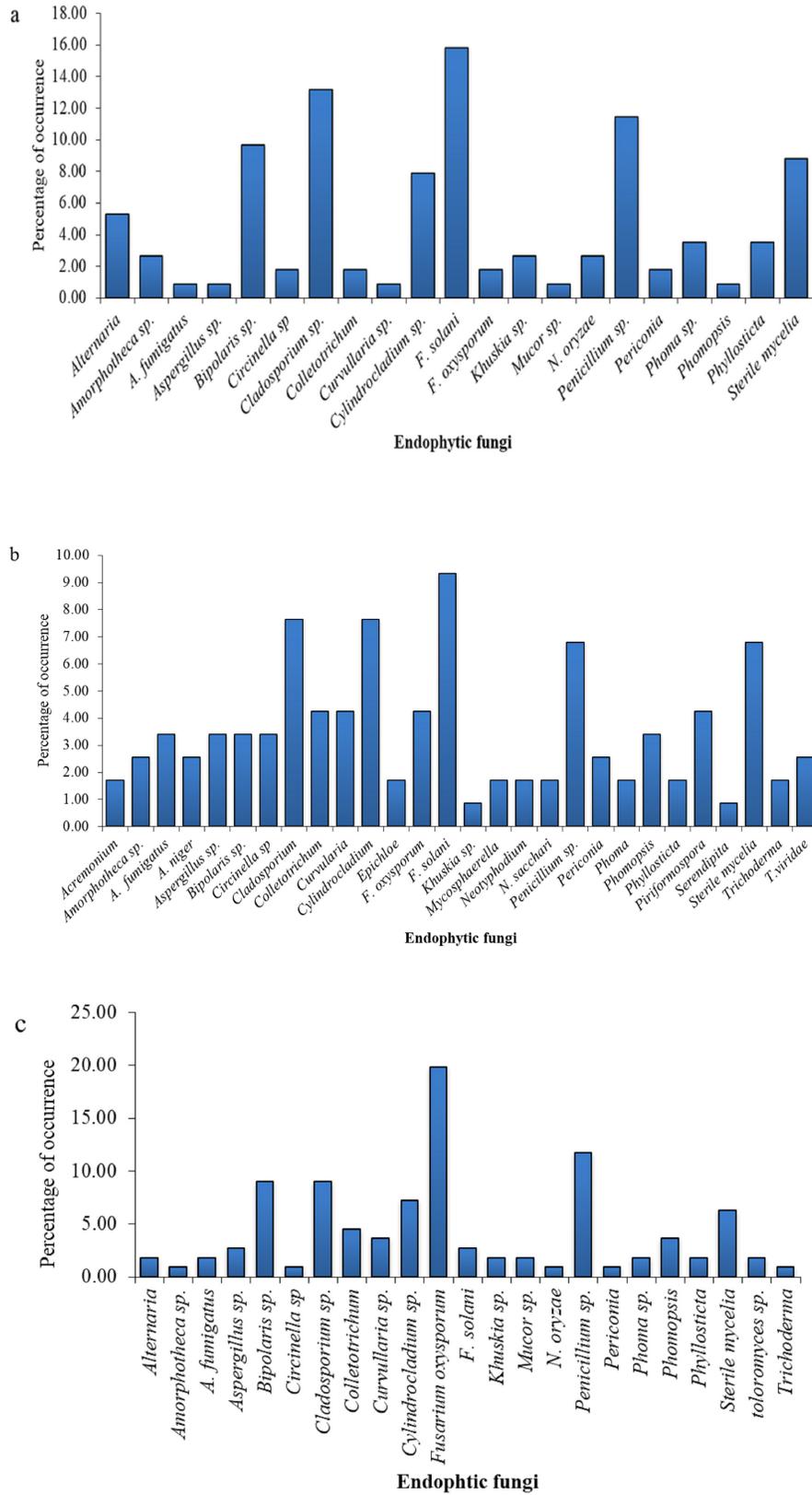
### 3.1.3 Mullayanagiri

A total of 111 fungi representing 21 genera were found to be associated with plants from the Mullayanagiri region. The highest CF was recorded in *O. tenuiflorum* and *P. amboinicus* with 43.3%, followed by *T. vulgaris* with 36.6% colonization. *Amorphotheca* sp., *Cylindrocladium*, *F. solani*, *F. oxysporum*, *Penicillium* sp., *Pestalotiopsis*, and *Phoma* sp. were identified as the commonly occurring fungi. *R. officinalis* had the highest IR of 0.48 and *Z.*

*officinale* showed the least IR of 0.16. *P. amboinicus* exhibited maximum species richness of 12.2, followed by *O. tenuiflorum* (11.42) and minimum species richness was recorded in *O. majorana* (6.51). *P. amboinicus* harbored the maximum endophytic fungi among the 11 plants assessed with RA of 12.51. Carmargo's index ranged from 0.08 to 0.15, with *O. tenuiflorum* and *P. amboinicus* having the minimum value of 0.08 (Table 3). *F. oxysporum* and *Penicillium* sp. were found to occur dominantly in this region

**Table 3** Colonization frequency, isolation rate, relative abundance, species richness, and Camargo's index of the endophytic fungi isolated from different regions of Bhadra Wildlife Sanctuary.

Location	Botanical name	Leaves	Flowers	Petiole	Stem	Root	Total	CF %	IR	RA	Species richness	Camargo's index (I/S)
Baba Budangiri	<i>C. citretus</i> L.	2	3	1	1	1	8	26.67	0.28	9.21	7.52	0.13
	<i>M. arvensis</i> L.	2	4	4	2	1	13	43.33	0.32	10.53	12.52	0.07
	<i>O. basilicum</i> L.	4	2	3	1	1	11	36.67	0.24	7.89	10.52	0.10
	<i>O. tenuiflorum</i> L.	3	3	3	4	1	14	46.67	0.32	10.53	13.52	0.07
	<i>O. majorana</i> L.	4	2	0	3	1	10	33.33	0.36	11.84	9.52	0.11
	<i>O. corniculata</i> L.	2	3	3	1	0	9	30.00	0.28	9.21	8.52	0.12
	<i>P. amboinicus</i> L.	3	3	3	4	5	18	60.00	0.36	12.84	17.52	0.06
	<i>R. officinalis</i> L.	3	3	3	3	0	12	40.00	0.32	10.53	11.52	0.09
	<i>R. graveolens</i> L.	2	4	2	1	1	10	33.33	0.24	7.89	9.52	0.11
	<i>T. vulgaris</i> L.	2	1	1	1	1	6	20.00	0.16	5.26	5.52	0.18
	<i>Z. officinale</i> L.	1	2	1	2	1	7	23.33	0.15	5.26	6.52	0.15
	Total	23	24	21	19	10	118					
Lakkavalli	<i>C. citretus</i> L.	3	3	4	2	3	15	50.00	0.28	8.97	14.51	0.07
	<i>M. arvensis</i> L.	4	3	3	2	0	12	40.00	0.24	7.69	11.51	0.09
	<i>O. basilicum</i> L.	2	4	3	2	2	13	43.33	0.28	8.97	12.51	0.08
	<i>O. tenuiflorum</i> L.	4	3	2	1	0	10	33.33	0.36	11.54	9.51	0.11
	<i>O. majorana</i> L.	4	3	3	2	0	12	40.00	0.32	10.26	11.51	0.09
	<i>O. corniculata</i> L.	4	3	3	0	0	10	33.33	0.24	7.69	9.51	0.11
	<i>P. amboinicus</i> L.	4	4	2	3	0	13	43.33	0.52	16.67	12.5	0.08
	<i>R. officinalis</i> L.	3	3	2	3	0	11	36.67	0.28	8.97	10.51	0.10
	<i>R. graveolens</i> L.	1	2	1	0	0	4	13.33	0.24	7.69	3.51	0.28
	<i>T. vulgaris</i> L.	2	2	2	1	0	7	23.33	0.24	7.69	6.51	0.15
	<i>Z. officinale</i> L.	1	1	2	0	0	4	13.33	0.2	6.41	3.5	0.28
	Total	32	31	27	15	5	111					
Mullayanagiri	<i>C. citretus</i> L.	4	3	2	1	1	11	36.67	0.28	8.54	10.51	0.10
	<i>M. arvensis</i> L.	2	0	2	4	3	11	36.67	0.24	7.32	10.51	0.10
	<i>O. basilicum</i> L.	3	2	3	1	1	10	33.33	0.4	12.20	9.51	0.11
	<i>O. tenuiflorum</i> L.	2	3	3	3	2	13	43.33	0.32	9.76	11.42	0.08
	<i>O. majorana</i> L.	2	2	1	1	1	7	23.33	0.24	6.10	6.51	0.15
	<i>O. corniculata</i> L.	2	2	3	1	1	9	30.00	0.26	6.10	8.51	0.12
	<i>P. amboinicus</i> L.	4	3	3	2	1	13	43.33	0.41	12.20	12.51	0.08
	<i>R. officinalis</i> L.	4	2	3	1	1	11	36.0	0.48	14.63	10	0.10
	<i>R. graveolens</i> L.	4	2	1	1	1	9	30.00	0.28	4.88	8.51	0.12
	<i>T. vulgaris</i> L.	3	2	3	2	1	11	36.67	0.32	9.76	10.52	0.10
	<i>Z. officinale</i> L.	4	3	1	0	1	9	30.00	0.16	8.54	8.51	0.12
	Total	34	24	25	15	8	114					



**Figure 3.** The percentage of dominant (*D*) isolated endophytic fungi from different locations: a, Baba Budangiri; b, Lakkavalli; and c, Mullayanagiri.

(Fig. 3c). Our findings are in accordance with previous report in 2017, where fungal isolates like *F. oxysporum*, *Pestalotiopsis* spp., *Macrophomina* spp., *Cladosporium* spp., and *Phomopsis* were isolated from *O. majorana* L. and *F. oxysporum* being the frequently isolated fungi [53]. Eighteen endophytic fungal species were found to be associated with five medicinal plants of Western Ghats, with *Curvularia clavata* being the dominant fungi [33]. *Chaetomium globosum*, *A. niger*, *Fusarium* spp., and *Penicillium* spp. were found to be some of the frequently isolated fungi from seven medicinal herbs in the Western Ghats [54].

### 3.2 Relative Density (RD) of Endophytic Fungi in Three Sample Sites of Bhadra Wildlife Sanctuary

The RD of fungal isolates varied within different parts of the host plant. Leaf fragments collected from the Baba Budangiri region had the highest RD of 42%, followed by the Mullayanagiri region (36%). Root segments showed the least fungal communities from all three areas suggesting that the endophytes have a preference for leaf colonization (Fig. 4). Similar results of higher colonization rates in the leaves than in root, stem, and petiole were reported in 2016 [24]. *Cladosporium cladosporioides*, *Drechslera* sp., and *Colletotrichum* were some of the frequently isolated fungi from *C. citrtus*, and leaf samples contained more endophyte [37].

### 3.3 Relative Percentage of Occurrence (RPO%) for Different Genera of Endophytic Fungi

The endophytic fungi of different genera isolated from three sample sites followed a similar pattern of occurrence. Hyphomycetes were the commonly occurring genera with 81.08%, 72.88%, and 61.4% from Lakkavalli, Baba Budangiri, and Mullayanagiri regions, respectively (Fig. 5). These findings support earlier reports on endophytic fungi isolated from shrubby medicinal plants in the Malnad region of Bhadra Wildlife Sanctuary with the highest colonization rates of Hyphomycetes (28.0%), Coelomycetes (26%), and Ascomycota (8.6%) [55]. Nalini et al. [53], reported 1,529 isolates obtained from 7 medicinal plants of Western Ghats, and colonization was seen more in stem (80.37%) than in other plant parts analyzed. Maximum isolates belonged to genera

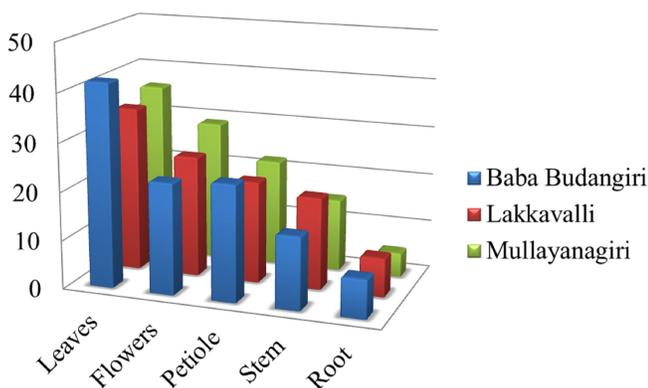
*Coelomycetes* (65%) [54]. Another work in 2016 reported RPO of Hyphomycetes (50%) to be maximum, followed by Coelomycetes (33.3%) and ascomycetes (8.3%) for endophytic fungi isolated from *Adenium obesum* [56].

### 3.4 Host Specificity of Endophytic Fungi

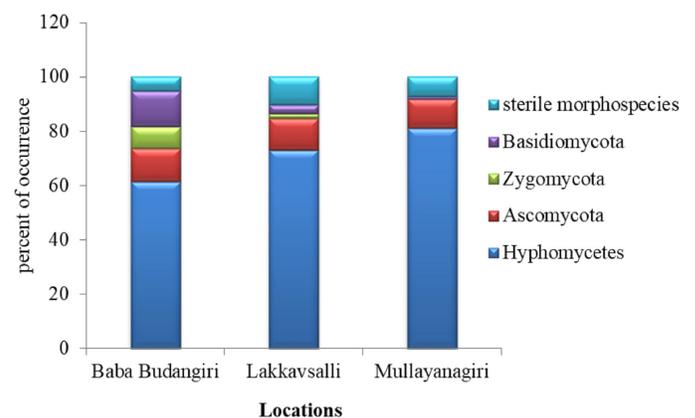
In Lakkavalli region, endophytic fungus like *Cylindrocladium* was found to be associated with seven plant samples mostly belonging to family Lamiaceae, thereby exhibiting a wide host range, whereas species like *Epichloe* sp., *Khuskia* sp., *Nigropora sacchari*, *Periconia*, *Phomopsis* sp., and *Phyllosticta* sp. were restricted to single host plants. In Mullayanagiri, *F. oxysporum* was isolated from nine different host plants, whereas *Trichoderma* sp. and *Nigrospora oryzae* were associated only with *O. basilicum*. Similarly, in the Baba Budangiri region, *P. chrysogenum* was associated with six host plants belonging to the Lamiaceae family. *Mucor* sp. and *Periconia* sp. were seen only in *T. vulgaris* and *R. officinalis*, respectively. Thus, it can be concluded that endophytic fungi may occur more commonly in the host plants belonging to the same family. Likewise, host specificity was exhibited by *A. fumigatus*, which was found to be associated with *C. citretus* collected from all the three sample sites. Similarly, *Cylindrocladium* sp., *F. solani*, and *Curvularia* sp. were isolated from *O. basilicum*, *O. majorana*, and *R. graveolens*, respectively, collected from all the three regions of Bhadra Wildlife Sanctuary.

### 3.5 Diversity Indices

The Shannon–Wiener index indicated that Baba Budangiri region had a higher index value of 3.19. Simpson's *D* depicts that Lakkavalli region had higher species diversity with an index value of 0.95. Baba Budangiri and Mullayanagiri regions have similar Simpson's *D* of 0.91. These results are also supported by Gini Simpson's diversity index indicating that plants collected from the Lakkavalli region showed greater fungal diversity when compared to the other two regions. The *E* and species richness were also compared between different regions and it was found that Lakkavalli region had comparatively higher *E* (0.72) and species richness (5.87) (Table 4).



**Figure 4.** Relative isolations of fungal endophytes from different plant parts of aromatic plants collected from Baba Budangiri, Mullayanagiri, and Lakkavalli.



**Figure 5.** RPO% of different groups of endophytic fungi from different locations of Bhadra Wildlife Sanctuary.

**Table 4.** Diversity indices of endophytic fungi isolated from different regions of Bhadra Wildlife Sanctuary.

Indices	Location		
	Baba Budangiri	Lakkavalli	Mullayanagiri
Simpson's diversity index	0.91	0.95	0.91
Evenness	0.49	0.72	0.47
Richness	4.43	5.87	4.88
Shannon–Wiener Index (H)	3.19	2.76	2.70
Gini Simpson index	0.09	0.05	0.09

**Table 5.** Antibacterial activity of endophytic fungi isolated from selected aromatic plants of Bhadra Wildlife Sanctuary.

Locations	Fungal isolates	Zone of inhibition in mm				
		<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>E. coli</i>
Baba Budangiri	<i>Curvularia</i> sp.	16 ± 0.1	13.3 ± 1.3	11.9 ± 1.2	11.5 ± 1.22	1.21 ± 0.12
	<i>Penicillium</i> sp.	17 ± 0.2	14.4 ± 0.3	12.8 ± 0.98	1.80 ± 1.27	1.1 ± 0.24
	<i>Aspergillus</i> sp.	18 ± 1	13.6 ± 0.8	12 ± 1.40	1.74 ± 1.38	1.3 ± 90.12
	<i>Curvularia lunata</i>	11.6 ± 1.5	12.9 ± 0.6	2.08 ± 0.6	1.25 ± 0.88	0.74 ± 0.18
	<i>Colletotrichum</i> sp.	9.6 ± 0.2	12.9 ± 0.1	2.37 ± 0.2	1.06 ± 0.81	0.49 ± 0.23
	<i>Chaetomium</i> sp.	15.3 ± 1.5	11.8 ± 1.5	1.56 ± 0.1	10.5 ± 0.4	0.24 ± 0.15
	<i>C. lunata</i>	13.3 ± 0.2	11.74 ± 0.9	1.27 ± 0.33	0.58 ± 0.28	0.3 ± 0.09
Lakkavalli	<i>Bipolaris</i> sp.	12.3 ± 1.5	11.25 ± 0.6	0.90 ± 0.5	0.42 ± 0.1	0.22 ± 0.07
	<i>Rhizopus oryzae</i>	2.33 ± 1.3	11.25 ± 0.6	0.9 ± 0.2	0.42 ± 0.1	0.22 ± 0.07
	<i>Talaromyces</i> sp.	20 ± 0.3	14 ± 0.93	13.3 ± 0.3	19.4 ± 1.1	12.6 ± 0.32
	<i>Curvularia</i> sp.	12 ± 1.5	14.1 ± 0.9	2.58 ± 1.1	11.89 ± 1.4	12 ± 0.1
	<i>F. oxysporum</i>	18.6 ± 1.3	14.1 ± 0.9	2.58 ± 1.2	1.89 ± 1.4	1.34 ± 0.18
	<i>Chaetomium globosum</i>	22 ± 0.65	14.3 ± 0.2	3.15 ± 0.5	1.53 ± 1.12	22 ± 0.3
	<i>Piriformospora</i> sp.	20 ± 0.2	14.9 ± 0.2	3 ± 0.46	2.25 ± 1.64	11.5 ± 0.2
	<i>Alternaria</i> sp.	17.33 ± 1	2.85 ± 5.34	3.72 ± 0.7	1.89 ± 1.36	0.97 ± 0.3
	<i>Curvularia</i> sp.	17 ± 1.36	15.5 ± 0.1	4.66 ± 0.3	1.8 ± 1.5	10.9 ± 0.9
	<i>Penicillium</i> sp.	12.3 ± 0.5	13.6 ± 0.76	2.9 ± 0.4	1.35 ± 1.1 0.6 ± 0.2	1.35 ± 1.1 0.6 ± 0
Mullayanagiri	<i>Alternaria</i> sp.	19 ± 0.2	14.47 ± 0.3	2.99 ± 1.1	1.98 ± 1.42	1.28 ± 0.24
	<i>Penicillium</i> sp.	21 ± 2.6	15.2 ± 0.6	3.66 ± 1.1	2.24 ± 1.57	1.34 ± 0.31
	<i>Phyllosticta</i> sp.	13.6 ± 0.2	3.62 ± 0.2	2.67 ± 0.6	1.48 ± 1.04	0.81 ± 0.24
	<i>Curvularia</i> sp.	12 ± 0.2	4.64 ± 0.3	3.7 ± 1.2	2.07 ± 1.4	1.37 ± 0.24
	<i>Alternaria</i> sp.	18.3 ± 2.1	4.1 ± 0.80	3.0 ± 1.08	1.93 ± 1.3	11.21 ± 0.2

\*Results expressed in mean ± standard deviation from triplicate samples.

### 3.6 Jaccard's Similarity Index (JI)

The range of similarity between endophytes associated with the three regions of Bhadra Wildlife Sanctuary was computed using a JI. The highest overlap (JI = 0.38) was observed for the fungal communities from Lakkavalli and Mullayangiri, followed by Baba Budangiri and Mullayangiri (JI = 0.34), and Baba Budangiri and Lakkavalli showed the least of similarities (0.33). The overlap between all the three regions was found to be 0.24. These results indicate that the distribution of endophytic fungi in the three sample sites of Bhadra Wildlife Sanctuary is similar and range toward even distribution.

### 3.7 Antibacterial Activity of Endophytic Fungi

A total of 343 endophytic fungi were screened for antibacterial activity against five human pathogenic bacteria. Among the 343, about 312 (91%) isolates exhibited antimicrobial activity against

one or more bacteria in which 45% of the isolates were from the Lakkavalli region, and 23% from Mullayanagiri and Baba Budangiri region each. Twenty-four isolates (7%) showed potential activity against all the pathogens tested (Table 5). *Chaetomium globosum* (PAL3) isolated from the leaf of *P. amboinicus* collected from the Lakkavalli region exhibited maximum activity with the inhibition zone of 22 mm against *E. coli* and *B. subtilis*. Similar work was reported in 2019 in which *A. flavus*, an endophytic fungi isolated from *P. amboinicus*, inhibited *E. coli* and *S. aureus* with zone of inhibition of 24.91 mm [57]. Similarly, the endophytic fungi *A. terreus* isolated from leaves of *T. vulgaris* showed the highest antibacterial activity against *E. coli* and *S. aureus* [57].

### 3.8 Antifungal Activity of Endophytic Fungi

The antifungal activity of the fungal isolates was evaluated for the antagonistic property. A total of 160 endophytic fungi (46.6%)

**Table 6.** Antifungal activity of endophytic fungi against phytopathogens.

Locations	Fungal isolates	Growth inhibition in percentage		
		<i>A. alternaria</i>	<i>F. oxysporum</i>	<i>A. niger</i>
Baba Budangiri	<i>Alternaria</i> sp.	36.5 ± 1.7	45.2 ± 0.5	43.4 ± 1.3
	<i>Penicillium</i> sp.	17.3 ± 1.3	5.2 ± 0.8	32.9 ± 0.3
	<i>Curvularia</i> sp.	22.5 ± 1.1	55.8 ± 0.8	22.8 ± 0.8
	<i>Piriformospora</i> sp.	21.9 ± 1.6	41.9 ± 0.6	41.7 ± 0.8
	<i>F. oxysporum</i>	20.6 ± 0.7	32.7 ± 0.2	19.0 ± 0.6
	<i>Phyllosticta</i> sp.	46.5 ± 1	41.8 ± 0.6	34.3 ± 0.3
	<i>Cylindrocladium</i> sp.	16.6 ± 1.8	52.5 ± 0.5	18.5 ± 0.5
	<i>A. fumigatus</i>	36.0 ± 1.6	74.5 ± 0.2	17.4 ± 1.2
Lakkavalli	<i>Penicillium</i> sp.	35.2 ± 2.3	67.0 ± 1	16.1 ± 1.7
	<i>Penicillium</i> sp.	25.1 ± 2.5	15.8 ± 0.4	16.5 ± 0.9
	<i>Phyllosticta</i>	60.5 ± 0.9	68.4 ± 0.8	24.9 ± 0.6
	<i>Toloromyces</i> sp.	41.8 ± 1.8	26.0 ± 0.9	20.0 ± 1.1
	<i>C. globosum</i>	73.7 ± 1.7	74.5 ± 0.9	19.0 ± 0.3
	<i>Phomopsis</i> sp.	35.4 ± 1.2	55.5 ± 1.2	35.3 ± 0.5
	<i>Curvularia</i> sp.	67.2 ± 0.8	46.4 ± 0.3	18.3 ± 1
	<i>A. niger</i>	43.0 ± 0.9	23.8 ± 0.3	19.4 ± 0.2
Mullayanagiri	<i>Chaetomium</i> sp.	37.4 ± 0.6	22.7 ± 0.6	15.4 ± 0.7
	<i>F. oxysporum</i>	78.0 ± 0.8	74.9 ± 0.2	43.8 ± 0.4
	<i>Phomopsis</i> sp.	35.8 ± 1.1	21.7 ± 1.1	34.6 ± 0.3
	<i>Colletotrichum</i> sp.	42.2 ± 0.8	35.5 ± 0.4	19.5 ± 1
	<i>Alternaria</i> sp.	54.0 ± 1.3	27.4 ± 0.8	16.9 ± 1.2
	<i>N. oryzae</i>	67.3 ± 0.6	55.6 ± 0.9	53.7 ± 1.8
	<i>Rhizopus oryzae</i>	25.1 ± 1.7	45.5 ± 0.2	15.8 ± 0.4
	<i>C. lunata</i>	45.8 ± 0.2	63.9 ± 0.6	24.3 ± 1.3

\*Results expressed in mean ± standard deviation from triplicate samples.

were found active against one or more test pathogens. More than 15% inhibition in radial growth against all the tested pathogens was recorded in 27 (7.8%) isolates (Table 6). The most notable inhibition was demonstrated by *C. globosum* (PAL3) from Lakkavalli against *F. oxysporum*, giving 74.5% inhibition in radial growth. Similar work was reported in which, 24 isolates among the 72 endophytic fungi from *Cedrus deodara*, *Pinus roxburgii*, and *Abies pindrow* inhibited 3 or more phytopathogens by at least 50% in dual culture [22].

#### 4. CONCLUSION

India has many unique ecological niches harboring varieties of plants. One such region is situated in the Western Ghats, India, which contains diversified plants, animals, and microorganisms. This present study reveals the composition and variability of fungal endophytes associated with 11 aromatic plants from 3 different regions of Bhadra Wildlife Sanctuary, Western Ghats, Karnataka. A total of 343 endophytic fungi were isolated from Baba Budangiri, Lakkavalli, and Mullayanagiri regions. *P. amboinicus* showed better association with endophytic fungi from all the regions with the highest CF and IR, whereas *Z. officinale* demonstrated the least fungal association among the

selected plant samples. Host specificity was observed for specific endophytes like *C. globosum*, which was associated with *C. citretus* from all the three sample sites. Diversity indices were measured by Shannon–Weiner, Simpson’s diversity and Gini Simpson’s diversity index, and high diversity indices were noted for the Lakkavalli region, followed by Mullayanagiri and Baba Budangiri regions. JI was used to assess the similarity of fungal species from the three sample sites. Lakkavalli and Mullayanagiri (JI = 0.38) showed the highest fungal overlap. All the isolates were subjected to antimicrobial activity against pathogenic bacteria and fungi. 91% and 46.6% of the isolates showed antibacterial and antifungal activity, respectively, against one or more tested pathogens. *Chaetomium globosum* from the Lakkavalli region was the most potential isolate that can be further exploited for the production of bioactive compounds.

#### 5. ACKNOWLEDGMENTS

This work has been funded by the department of backward classes’ government of Karnataka for providing Ph.D–OBC fellowship. The authors thank the Department of Microbiology and Biotechnology, Bangalore University, Bangalore, for providing the facility needed in carrying out this research work.

## 6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

## 7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 8. ETHICAL APPROVALS

Not applicable.

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#### How to cite this article:

Jagadish R, Chowdappa S. Diversity and antimicrobial potential of endophytic fungi from aromatic plants of Bhadra Wildlife Sanctuary, Western Ghats, Karnataka. *J Appl Biol Biotechnol* 2021; 9(05):1–12.