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Diversity and Seasonal Distribution of Endophytic Mycoflora of *Catharanthus roseus* (L.) G. Don from Maharashtra

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ABSTRACT

Altogether twenty one species comprising eighteen genera of endophytic fungi were recovered from surface sterilized leaf lamina, leaf mid -rib, stem, petiole and inner bark samples collected from *Catharanthus roseus* from four different locations of the Maharashtra. A total of 395 endophytic isolates were obtained from 1200 plant segments of *C. roseus.* Mitosporic fungi were found to be dominant (74%), followed by ascomycetes (17%) and agonomycetes (9%). The overall colonization and isolation rates of endophytic fungi were significantly higher (χ 2 test, g= 4, P<0.001) in stem when compared to other aerial parts. The overall colonization of endophytes recovered in monsoon season were significantly (χ 2 test, g = 2, P<0.001) higher (33%) followed by summer (16.25%) and winter (15.25%). Similarly, the isolation rate was also found significantly higher during monsoon (0.59) than the summer (0.2) and winter (0.18).

Key words: *Catharanthus roseus*, Diversity, Endophytes, Seasonal distribution

INTRODUCTION

Plants of ethnobotanical importance serve as an important source of endophytic fungal diversity as one of the four-points set of criteria used for the rationale for selection of promising host plants (Gakuubi *et al.,* 2021). *Catharanthus roseus* (L.) G. Don (Apocynaceae), native to the Indian Ocean island of Madagascar, is an herbaceous plant that has long been used to treat diabetes and high blood pressure. The alkaloids generated from *Catharanthus roseus* are a collection of roughly 130 terpenoid indole alkaloids. It is a source of drugs, vincristine and vinblastine, used to treat cancer (Venieraki *et al.,* 2017; Patil and Dusane, 2022). Decoctions of the healthy leaves have also been used to cure a variety of ailments, including ocular irritation, diabetes, bleeding, insect bites, and cancer (Nammi *et al.,* 2003; Kharwar *et al.,* 2008; Siddiqui *et al.,* 2010). Endophytic diversity of *C. roseus* was investigated by various workers from different countries including India and from different geographical locations (Kharwar *et al.,* 2008; Krishnamurthy *et al.,* 2008; Singh and Gaikwad, 2010; Wahida and Khanom, 2016; Akpotu *et al.,* 2017; Sreekanth *et al.,* 2017; Geethanjali *et al.,* 2019; Jayasudha *et al.,* 2021; Srinivas and Nigam, 2021). However, the range of microbial symbionts varies according to the host plant and environmental elements, such as biogeography (Kivlin *et al.,* 2017). The comprehensive endophytic diversity of *C. roseus* from different localities in Maharashtra, in different seasons has been understudied. As part of this study, extensive investigation was conducted to collect *C. roseus* from the four different locations of Maharashtra. The current research reveals the richness and distribution of endophytic fungi in different tissues of *C. roseus* and their seasonal distribution.

MATERIALS AND METHODS

Study area and samples collection

Healthy and any symptomless samples of *C. roseus* were collected from the four different localities of Maharashtra [viz. Pune, Pimpari, Yawat and Lonavala (**Table 1**)]. The samples were collected for a period of two years during the monsoon, winter, and summer seasons from 2006 to 2008. The samples viz., leaf, petiole and stem were collected in pre-sterilized polythene bags and inner bark samples were collected in sterile screw-capped vials. The samples were processed in the lab for the isolation of endophytic fungus within 24 hours of collection.

Isolation of fungal endophytes

In order to get rid of any surface adhesions, the samples were rinsed under running water. The leaf lamina, leaf midrib, petiole, stem, and inner bark were first cut into segments of approximately 5 mm in length with the help of a sterilized surgical blade. Altogether, 1200 plant bits; 300 bits per site; and 60 bits per organ were excised separately. The chopped pieces were first rinsed with 70% ethanol for 1 minute and then with 4% sodium hypochlorite for 30 seconds.

Afterwards, the segments were again immersed in 70% ethanol for 30 seconds. After every step of sterilization, plant bits were washed thoroughly 4 times for about 3 minutes with sterile water and allowed to dry in the laminar airflow (Surynarayanan and Vijaykrishna, 2001).

The sterilized explants were transferred to the Petri plates containing Potato Dextrose Agar (PDA) supplemented with streptomycin sulphate (500 mgL^{-1}). The plates were incubated at 28±1 ^{o}C and checked for fungal growth/development on a regular basis. A fresh PDA plate without antibiotics was used to transfer each individual hyphal tip that sprouted from the borders of the treated plant parts. After some time, the pure endophytic fungal cultures were transferred to a PDA slant and used as a stock culture for further studies. The non-sporulating forms of endophytes obtained in present study were categorized to Agonomycetes (Kirk *et al.*, 2008). For induction of in vitro sporulation, grass leaf technique suggested by Srinivasan *et al.* (1971) was used. Finally, fungal endophytes with induced sporulation were subjected to the identification.

Table 1: Physiography of sampling sites/localities of *Catharanthus roseus*

Locality	Latitude/ Longitude	Max. Temp. (°C) (summer)	Min. Temp. (°C) (winter)	Max. Annual rainfall (mm)	Altitude (MSL)	Habitat/ Forest type
Lonavala	N 18º45'03.3" E 73º24'08.7"	38	12	4,500	625	Cultivated
Pimpari	N 18º37'06.2" E 73º48'12.3"	39	10-12	722	559	
Pune	N 18º31'18.4" E 73º49'53.6"	39	12	722	559	
Yawat	N 18º 28'015" E 74º 17'000"	40	12	375	597	

Morphological identification of isolated endophytic fungi

Preliminary identification was done by studying the cultural characteristics of the fungi, i.e., colony growth, colour, shape, etc. The morphological characters were examined by preparing semi-permanent slides of different sporulating structures of endophytic fungi such as conidiophores and conidia (Hyphomycetes); pycnidia, condiogenous cells, and conidia (Coelomycetes); and ascocarp, asci, and ascospores (Ascomycetes) from pure colonies of each endophytic culture. Slides were studied under an Olympus CX-21 binocular research microscope.

Statistical analysis

Statistical analysis of the collected data was done. The diversity and richness of fungal endophytes isolated from different tissues of *C. roseus* were quantified using various indices such as Colonisation rate, Colonization frequency, Isolation rate, Simpson's diversity index (1-λ'), Shannon-Wiener index (H') and Pielou's evenness (J'). The number of plant-tissue segments infected by one or more fungus divided by the total number of inoculation segments was used to compute the colonisation rate (Petrini *et al.*, 1982). Isolation rate was determined as the number of isolates obtained from plant-tissue segments divided by the total number of segments inoculated (Wang and Guo, 2007). The density of colonization (rD%) or colonization frequency (CF%) of a single endophyte species was calculated by the method of Fisher and Petrini (1987):

$$\mathbf{rD\% = (N_{col}/N_t) \times 100}$$

Where $\mathbf{N_{col}}$ = Number of segments colonized by each fungus

$\mathbf{N_t}$ = Total number of segments inoculated

Simpson's diversity index (1-λ'), Shannon-Wiener index (H') and Pielou's evenness (J') were calculated using Primer software to determine the endophytic species diversity of *C. roseus* growing in four different localities.

RESULTS

A total of 395 fungal isolates belonging to 21 species in 17 genera were isolated from 1200 tissue segments of *C. roseus* (L.) G. Don (**Table 2**). All the non-sporulating forms were allocated to a group of agonomycetes (**Table 2**). Mitosporic fungi were found to be the most prevalent among the total endophytic fungi observed in this investigation (74%) followed by Ascomycetes (17%) and Agonomycetes (9%) (**Figure 1**).

Table 2: Occurrence of fungal endophytes of *Catharanthus roseus* collected from different localities and in different seasons

Sr.	Endophyte Fungi	NFCCI no.	Yawat	Pune	Lonavala	Pimpari	M*	W*	S*
1	*Alternaria alternata*	645	+	+	+	+	+	+	+
2	*Botryosphaeria parva*	2083	-	+	-	+	+	-	-
3	*Chaetomium globosum*	643	+	+	+	+	+	+	+
4	*Cladosporium oxysporum*	642	+	+	+	+	+	+	+
5	*C. tenuissimum*	VPG-1	+	+	+	+	+	+	+
6	*Colletotrichum dematium*	2288	-	+	-	+	+	-	-
7	*C. gloeosporioides*	641	+	+	+	+	+	+	+
8	*Curvularia lunata*	1399	+	+	+	+	+	-	+
9	*Fusarium oxysporum*	2085	+	+	-	+	+	+	+
10	*F. sporotrichioides*	2081	+	+	+	-	-	+	-
11	*Gliocladium* sp.	2262	+	+	+	+	+	+	+
12	*Myrothecium advena*	648	-	+	+	+	+	+	-
13	*Myrothecium roridum*	2265	+	-	-	-	+	+	-
14	*Nigrospora sphaerica*	640	+	+	+	+	+	+	+
15	*Periconia digitata*	2086	+	-	+	+	+	-	-
16	*Phoma* sp. 1	VPG-2	+	+	+	+	+	+	+
17	*Phomopsis* sp.	2263	+	+	+	-	+	+	+
18	*Phyllosticta conjac*	649	+	+	-	-	+	-	-
19	*Scytalidium lignicola*	2082	+	+	+	-	+	+	+
20	*Sordaria fimicola*	VPG-3	+	+	-	-	+	+	-
21	*Xylaria* sp. 1	VPG-4	+	+	+	+	+	-	+
22	NS Gr. I	VPG-5	+	+	+	+	+	+	+
23	NS Gr. II	VPG-6	+	+	+	+	+	+	+

M*, Monsoon; W*, Winter; S*, Summer

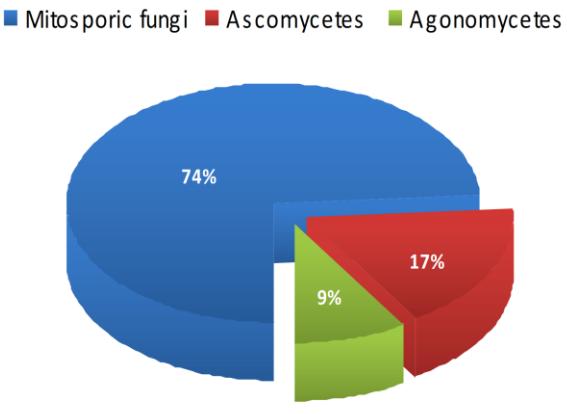

Figure 1: Group wise distribution of fungal endophytes of *Catharanthus roseus*

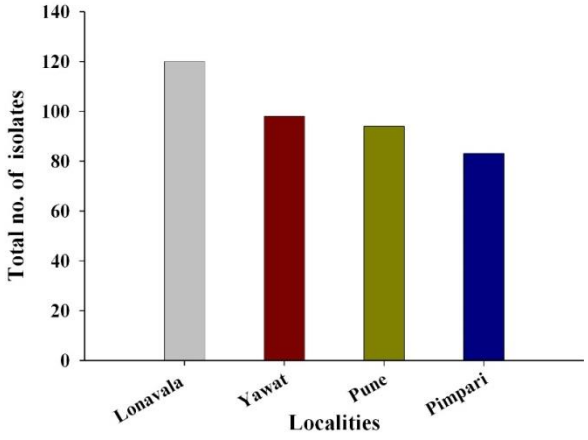

Figure 2: Endophytic fungal isolates obtained from *Catharanthus roseus* collected from different localities

Table 3: Diversity indices and evenness of fungal endophytes of *Catharanthus roseus* collected from different localities

Study site	Simpson's diversity index (1-λ')	Shannon-Wiener index (H')	Evenness index (Pielou's evenness index) J'
Yawat	0.936	2.387	0.918
Pune	0.948	2.942	0.938
Lonavala	0.917	2.62	0.906
Pimpari	0.936	2.747	0.933

Endophytic fungi from Pune exhibited the highest species diversity with highest evenness when collection localities are compared in terms of Shannon-Wiener index (2.942), Simpson diversity index (0.948), and Evenness (0.938). Yawat had the lowest Shannon-Wiener index, while Pimpari (2.747) and Lonavala (2.62) had better Shannon-Wiener indices (2.387) than Yawat. Although close (0.936/0.936), the Simpson diversity index for endophytes from Yawat and Pimpari was considerably lower than that from Pune. The Simpson diversity index for endophytes from Lonavala has the lowest value (0.917). Endophytes from Pimpari showed better evenness (0.933) as compared to Yawat and Lonavala (0.918 and 0.906, respectively) (**Table 3**).

The endophytic fungus *Alternaria alternata* was recovered in all the seasons and from all the selected localities. Besides, *Chaetomium globosum*, *Cladosporium oxysporum*, *C. tenuissimum*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Gliocladium* sp., *Nigrospora sphaerica*, *Phoma* sp. 1, *Phomopsis* sp., *Scytalidium lignicola*, NS Gr. I and II were also isolated in all the seasons. The other endophytic isolates selectively obtained in a particular seasons were *Fusarium sporotrichioides* in winter and *Botryosphaeria parva*, *C. dematium*, *Periconia digitata* and *Phyllosticta conjac* in monsoon season (**Table 2**).

Endophytic fungi *A. alternata*, *C. globosum*, *C. oxysporum*, *C. tenuissimum*, *C. gloeosporioides*, *Curvularia lunata*, *Gliocladium* sp., *N. sphaerica*, *Phoma* sp. 1, *Xylaria* sp. 1, NS Gr. I and II were isolated from all collection sites. The isolate *Myrothecium roridum* has only been found in Yawat area (**Table 2**).

The percent colonization density (rD%) of isolated endophytes was recorded maximum for *A. alternata* (4.3%), followed by *C. gloeosporioides* (4.25%) and NS Gr. I (3%) (**Figure 3**). However, rest of the endophytic fungi had colonization frequency between 0.5-2%. The colonization density of a few endophytes recorded to be ≤ 0.5% were *C. dematium* (0.5%), *P. digitata* (0.5%), *Sordaria fimicola* (0.5%), *P.conjac* (0.5%), *Myrothecium advena* (0.5%), *M. roridum* (0.25%) and *B. parva* (0.1%) (**Figure 3**).

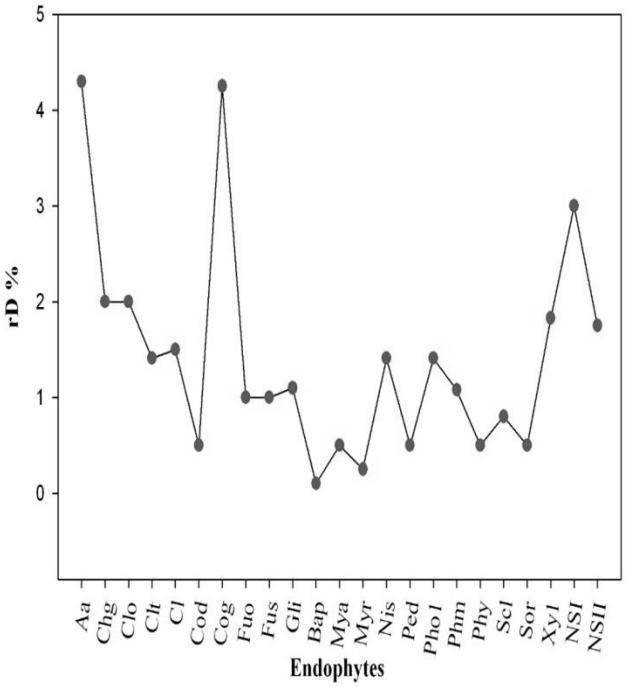

Figure 3: Colonization density (rD%) of fungal endophytes of *Catharanthus roseus*. Aa, *Alternaria alternate;* Chg, *Chaetomium globosum;* Clo, *Cladosporium oxysporum;* Clt, *C. tenuissimum;* Cl, *Curvularia lunata;* Cod, *Colletotrichum dematium;* Cog, *C. gloeosporioides;* Fuo, *Fusarium oxysporum;* Fus, *F. sporotrichioides;* Gli, *Gliocladium* sp.; Bap, *Botryosphaeria parva;* Mya, *Myrothecium advena*; Myr, *Myrothecium roridum;* Nis, *Nigrospora sphaerica;* Ped, *Periconia digitata;* Pho1, *Phoma* sp. 1; Phm, *Phomopsis* sp.*;* Phy, *Phyllosticta conjac;* Scl, *Scytalidium lignicola;* Sor, *Sordaria fimicola;* Xy1, *Xylaria* sp. 1*;* NS1, NS Gr. I*;* NS2, NS Gr. II.

Endophytes isolated from the five different aerial plant organs, namely the leaf lamina, leaf mid-rib, stem, petiole, and inner bark, had a species density of colonisation (rD%) ranging from 0.4-8.0% (**Table 4**). When compared to the other endophytic species, *C. gloeosporioides* (7.9%) and NS Gr. I (7.9%) recovered from leaf lamina had the highest colonization density, followed by *A. alternata* (6.25%) from stem. Except for the petiole, the colonisation density of *A. alternata* was higher in all organs (5.4/5.0/6.25/4.16%) (0.8%). *Chaetomium globosum* colonized the inner bark and stem to a great degree (4.58/4.58%). Petiole had the lowest colonization density, ranging from 0.4-2.5%. The stem and inner bark had more endophytic fungus than the other organs. Every isolated endophytic species colonized the stem, with the exception of *P. digitata.* Similar to this, all of the recovered fungi were discovered in the inner bark with the exception of *B. parva and P. conjac. A. alternata, C. oxysporum, F. oxysporum, C. gloeosporioides, Phoma* sp.1, *Phomopsis* sp., *Xylaria* sp.1, NS Gr. I, and NS Gr. II were the endophytes identified in all organs. Selectively isolated organisms included *Sordaria fimicola, P. digitata, C. dematium, B. parva* and *M. advena,* from one or two organs (**Table 4**).

Table 4: Organ wise colonization density of fungal endophytes of *Catharanthus roseus*

Endophytic fungi	Leaf lamina	Leaf mid-rib	Stem	Petiole	Inner bark
Alternaria alternata	5.4	5	6.25	0.8	4.16
Botryosphaeria parva	0	0	0.8	0	0
Chaetomium globosum	0.8	0	4.58	0	4.58
Cladosporium oxysporum	1.25	0.8	3.33	0.41	4.16
C. tenuissimum	2.08	0	2.9	0	2.08
Colletotrichum dematium	0	0	2.08	0	0.41
C. gloeosporioides	7.9	4.5	5.8	0.41	2.5
Curvularia lunata	1.25	1.25	2.9	0	2.08
Fusarium oxysporum	0.8	0.8	1.66	0.41	1.25
F. sporotrichioides	1.66	0	2.9	0	0.41
Gliocladium sp.	0	0	2.5	0.41	2.9
Myrothecium advena	0	0	0.8	0	1.66
M. roridum	0.41	0	0.41	0	0.41
Nigrospora sphaerica	2.5	1.25	2.08	0	1.25
Periconia digitata	0	0.41	0	0	2.08
Phoma sp.1	1.66	0.8	3.75	0.41	0.41
Phomopsis sp.	2.08	1.25	0.8	0.41	0.8
Phyllosticta conjac	2.08	0	0.41	0	0
Scytalidium lignicola	0.41	0.41	0.8	0.8	1.66
Sordaria fimicola	0	0	1.25	0	1.25
Xylaria sp.1	3.75	2.5	0.41	0.41	1.25
NS Gr. I	7.9	1.66	1.66	2.5	2.08
NS Gr. II	2.08	0.8	2.9	1.25	1.66

The overall colonization and isolation rates of endophytic fungi from stem was significantly higher (χ 2 test, g= 4, P<0.001) than other tissues. The colonization rate (%) of endophytic fungi was significantly higher in stem (31.66%) followed by inner bark (25.41%) and leaf lamina (23.33%) whereas, less colonization was observed in leaf mid-rib (9.16%) and petiole (8.33%). The isolation rate

was found maximum for stem (0.51) and leaf lamina (0.44) when compared to other organs, like inner bark (0.39), leaf mid-rib (0.21) and petiole (0.08) (**Table 5**).

Table 5: Colonization and isolation rates of fungal endophytes of *Catharanthus roseus*

	Leaf lamina	Leaf mid-rib	Stem	Petiole	Inner bark
Total no. of plant samples used	240	240	240	240	240
Total no. of samples yielding endophytes	56	22	76	20	61
Total no. of endophytic isolates obtained	106	52	123	20	94
Colonization rate (%)	23.33	9.16	31.66	8.33	25.41
Isolation rate	0.44	0.21	0.51	0.08	0.39

The overall colonization rate of endophytes in monsoon was recorded significantly (χ 2 test, g = 2, P<0.001) higher (33%) when compared to summer (16.25%) and winter (15.25%). The isolation rate was also found significantly higher during monsoon (0.59) than the summer (0.2) and winter (0.18) (**Table 6**).

Table 6: Seasonal variation in colonization and isolation rates of fungal endophytes of *Catharanthus roseus*

	Monsoon	Winter	Summer
Total no. of plant samples	400	400	400
Total no. of samples yielding isolates	132	61	65
Total no. of endophytic isolates obtained	239	75	81
Colonization rate (%)	33	15.25	16.25
Isolation rate	0.59	0.18	0.2

DISCUSSION

The bioprospecting endophytic fungi isolated from medicinal plants is an active area of research, but the presence of endophytic fungi in the host depends on several factors, viz. weather changes, environmental factors of host habitat, geographical locations, etc. Therefore, though the diversity and distribution of endophytic fungi from *C. roseus* was studied number of times even before from other localities, it is an immense need to explore hosts from the new ecological settings.

The present study focused on the isolation and identification of endophytic fungi from different parts of *C. roseus* collected from four different localities. The 1200 plant samples (segments) yielded about 395 endophytic isolates comprising of 21 species in 17 genera. These endophytes were assigned to ascomycetes, mitosporic fungi and agonomycetes. In line with earlier research, it has been found that mitosporic fungi are more common than ascomycetes and agonomycetes in studied host plant collected from respective localities and seasons. (Tejesvi *et al.*, 2005; Gond *et al.*, 2007; Sunayana and Prakash, 2012; Nimbalkar and Singh, 2022). In comparison to the other three localities, the most isolates were recovered from Lonavala, followed by Yawat, Pune, and Pimpari. Maximum annual rainfall (4500 mm) is recorded at Lonavala, which is more favorable for the diversity of endophytic fungi. Singh and Gaikwad (2010) carried out a preliminary survey on endophytic diversity at similar locations (Pune, Yawat, Loanavala, and Pimpri-Chinchwad) and reported similar observations. Barengo *et al.* (2000) studied the diversity of endophytic mycobiota of *Betula pubescens* Ehrh. with site specific parameters, like climate, topography, tree dimensions, age and air pollution. According to them, the frequency of endophytic fungi in leaves and twigs may be reduced due to air pollutants. It is likely that, the air pollutants

affect microenvironment formed around leaf and modify microhabitat of surface directly affecting both spore germination and hyphal penetration (Helander *et al.*, 1996; Kowalski and Gajosek, 1998; Kriel *et al.*, 2000). In the present study, being an urban and industrial area locations like Pune and Pimpari yielded less endophytic isolates possibly because they are exposed to high degree of air pollution and low precipitation as compared to Lonavala. It is apparent that since Lonavala receives high annual rainfall, the recovery of endophytic fungi was high from this region.

The number of endophytic fungi isolated from *C. roseus* was fluctuated seasonally in the current investigation. A higher number of isolates were recovered in the monsoon when compared with the summer and winter. Krishnamurthy *et al*. (2008) studied the seasonal distribution of endophytes of *C. roseus* and other herbaceous medicinal plant hosts and obtained similar results. They recovered maximum endophytes during the wet season, followed by the dry season. Heavy rains along with moderate temperature may result in greater fungal propagule diversity and spore dispersion, and further promote greater tissue colonization success. Suryanarayanan *et al.* (2002) and Tejesvi *et al*. (2005) reported that the plant tissues contain more endophytes during the monsoon season when compared to the dry season. Further, the low temperature could be the cause of the minimal recovery of endophytes (Kowalski, 1993).

The endophyte communities observed in this study differ from that the previously reported endophytic fungi of *C. roseus* grown in India (Krishnamurthy *et al.*, 2008; Kharwar *et al.*, 2008). The present study revealed comparatively higher endophytic diversity from *C. roseus* (21 species in 17 genera) as compared to findings of Kharwar *et al*. (2008) from two different ecosystems in Northern India (19 species in 13 genera). The most ubiquitous endophytic genera obtained in both the studies were *Alternaria*, *Cladosporium*, *Colletotrichum*, *Chaetomium*, *Fusarium, Xylaria*, etc. Krishnamurthy *et al*. (2008) isolated 25 species of endophytic fungi from the leaves of *C. roseus* collected in Malnad region, Southern India. Of these, only five species (*A. alternata, C. globosum, C. gloeosporioides, C. lunata, F. oxysporum*) were common with those recovered in present study. The reasons for differences in overall diversity and type of endophytic taxa observed may be due to the distantly related geographic locations of plant collection and isolation protocols used. Singh and Gaikwad (2010) isolated 23 different endophytic species in 28 genera from leaves, inner bark, and stems of *C. roseus* collected from similar locations, viz., Pune, Lonavala, Yawat, and Pimpri-Chinchwad. The common genera identified in both studies were, including *Alternaria*, *Cladosporium, Cuvularia, Fusarium, Gliocladium, Nigrospora, Periconia*, *Scytalidium*, *Colletotrichum, Phoma*, *Phomopsis*, *Phyllosticta*, *Chaetomium, Sordaria* and *Xylaria*. Differences in protocol, especially sterilization period may account for differences in endophytic fungi diversity across locations. The study's findings point to the management of endophytic fungal communities by a variety of environmental conditions, including climate, water availability, seasons, and geographic locations, which were also reflected in diversity metrics. In a nutshell, endophyte population is dependent on the physiological state of the host plant, which is in turn connected to seasonal weather variation to some extent. This is consistent with the prior research (Mishra *et al.*, 2012; Giauque and Hawkes, 2016; Sreekanth *et al.,* 2017**,** Rampadarath *et al.*, 2018; Costa *et al.*, 2018; Slamet *et al.*, 2021).

The diversity index is a quantitative metric that sheds light on the number of various species and the degree of distribution of individuals among those species. Shannon-Wiener index, Simpson's diversity index and evenness index varied among all the four localities. In the current investigation, it was found that the diversity and evenness of species did not correspond with the number of endophytic fungal isolates. The majority of endophytic fungus isolates were found in Lonavala, although the species diversity was noticeably higher in Pune. Highest species diversity and evenness were recorded in Pune as compared to other locations.

In the present investigation, the highest colonization rate was recorded in the stem followed by inner bark, leaf lamina, leaf mid rib and petiole. In the previous studies, it was observed that most of the fungi were isolated from leaves followed by stem (Kumar and Hyde, 2004; Kharwar *et al.*, 2008; Sreekanth *et al.*, 2017). The qualitative and quantitative differences in colonization frequencies of endophytes in plant organs of different host species have been reported (Suryanarayanan and Vijaykrishna, 2001). The isolation of fungus from very young leaves provided evidence that fungal endophytes were common in leaves. The endophytic fungi have developed the unique substrate usage patterns that could be the cause of the variations in colonisation frequencies. It has been noted that there are differences in the rates of colonization and isolation depending on the organs. Rainfall and humidity, which encourage the germination of adhering spores and their penetration into host tissues, were major determinants of the density of the endophytic infection. In turn, this

causes endophytes' rates of infection and colonization to rise. Additionally, it has been found that the precipitation encourages endophyte colonization rates (Rodrigues, 1994; Suryanarayanan *et al.,* 1998; Wilson, 2000; Wang and Guo, 2007).

The hyphomycetes *Cladosporium, Alternaria, and Fusarium* species were frequently described as endophytes from various host plants (Ragazzi *et al.,* 2001; Suryanarayanan *et al.,* 2002; Takeda *et al.,* 2003; Raviraja *et al.,* 2005; Tejesvi *et al.,* 2006; Wang and Guo, 2007; Kharwar *et al.,* 2008; Huang *et al.,* 2008). Coelomycete species of *Phomopsis, Phyllosticta, Phoma,* and *Colletotrichum* were most frequently isolated from a variety of distantly related host species in addition to the same host (Brown *et al.,* 1998; Barengo *et al.,* 2000; Suryanarayanan and Vijaykrishna, 2001; Ragazzi *et al.,* 2001; Toofanee and Dulymamode, 2002; Suryanarayanan *et al.,* 2003; Raviraja *et al.,* 2005; Tejesvi *et al.,* 2007; Gond *et al.,* 2007; Huang *et al.,* 2008). In the present study, *C. oxysporum, C. tenuissimum, F. oxysporum, A. alternata, Phomopsis* sp., *Phyllosticta conjac* and *Colletotrichum* sp. were isolated irrespective of seasons and locations. This shows that these endophytes, which are typically thought of as being ubiquitous and predominating the endophyte assemblages of many hosts, have a wide spectrum of host adaptations. This suggests that some fungal genera have evolved to live an endophytic lifestyle.

CONCLUSION

In conclusion, the findings of this study demonstrate significant fungal diversity found in *C. roseus* from selected four distinct locations in Maharashtra, which are understudied and require more investigation from different geographical locations. Different endophytes that were isolated from *C. roseus* throughout this inquiry may one day serve as a source of secondary metabolites.

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