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Diversity of Endophytic Fungi from Medicinal Plant *Cynodon Dactylon* (L.) Pers

Narwade P. S.
Designation, Jr. Teacher
Deptt. of Botany,
Deogiri College,
Aurangabad

Taware A. S
Designation, Assist. Prof.
Deptt. of Botany,
Deogiri College,
Aurangabad

Abstract

The present study was done to assess distribution of endophytic fungi from a medicinal plant *Cynodon dactylon* (L) pers from various regions of Aurangabad. Healthy Cynodon plants were collected from five different areas of Aurangabad i.e. Jaibhavaninagar, Parijatnagar, Deogiri campus, Vasantrao Naik campus & University area. Endophytic fungi were isolated from root, stem & leaf. Total 15 fungal isolates were separated. Colonizing frequency, Simpson's Diversity indices and Shannon-Wiener indices and evenness were calculated. Maximum endophytes isolated from Loc-5(33.78%) and minimum endophytes were recovered from Loc 4 (13.51). The dominant endophytic fungus observed was *Fusarium solani* 1. Location 5 has lower Simpson's index which indicates it has great diversity.

Keywords: Endophytes, *Cyanodon dactylon*, Colonizing Frequency, Simpson's Diversity, Shannon-Wiener Indices.

Introduction

Endophytes are the microbes that colonize the living internal tissues of plants without causing any immediate disease symptoms or overt negative effects (Bacon and White 2000). These fungal endophytes which lives within the living tissues of higher plants without producing any apparent symptoms(Bills 1996,Bills *et. al*, 1992.) Endophytic fungi have been reported from various plant species, which contribute to the diversity of microorganisms in natural environments (Nalini *et. al.*2014). Endophytes have proved to be the promising sources of biologically active products which are of interest for specific health care application (Strobel *et. al.* 2001, Strobel 2002.) In recent past, endophytes have received attention of the scientific community due to their capacity to produce novel bioactive compounds. (Aly *et.al.* 2001, Strobel 2003,Schulz *et. al*. 2002, Tan and Zou 2001). Endophytic microorganisms, distinct from mycorrhiza inhabit diverse host plants and become systemic and live asymptomatically in the host system .(Li *et. al.,* 2007; Rodriguez *et al.,* 2009; Botella *et. al*., 2010; Sakalidis *et. al,* 2011) *Cynodon dactylon* (L) pers (Poaceae) which is generally known as durva grass or Bermuda grass. It has been an important component in certain Ayurvedic formulation. (Kumar *et. al.* 2011) for treating diseases relation to oxidative stress (Saroja *et. al*. 2012) It is one of the 'Dasapuspam' used in Ayurveda, is ubiquitous in distribution and is considered as a serious weed, invading the cultivated lands. It has been studied in detail for its chemical constituents and importance in traditional medicine (Sing *et. al*. 2007,& Amita *et. al*. 2011). It is used in ethno medicine for the treatment of respiratory disorders, menstrual disorders, skin injuries, dandruff and diarrhoea (Rajakumar and Shivanna 2009; Shivanna and Rajakumar 2010; Qureshi *et. al*, 2010).

Review of Literature

As'Durva' has many medicinal uses.It is important to study its endophytes.Up till now very few studies has been done on endophytic diversity of *Cynodon dactylon*.According to study done by *Rekha et al* (2014) the endophytes occurring in the aerial part of *cynodon dactylon* were *Alternaria sp. , Aspergillus sp.,cephalosporium sp., Cladosporium sp., penicilliun sp., Trichoderma harzianum, Chaetomium sp., Cochliobolus sp., Phoma sp*. Among them, *Cladosporium sp*. that occurred in high percentage.In another study by *John et al* (2013) where fungal endophytes isolated from *cynodon dactylon* and *Moringa olifera* as *Curvularia sp., Penicillium oxalicum*, *Basidiomycetes sp.,Fusarium graminareum, Aspergillus sp., Helminthosporium sp., and Alternaria brasicola* were

recorded.Most of the studies where done on other plants as *Karmakar et al* (2011) –isolated 106 endophytic fungi from *Artocarpus hirsutus* Lam. And *Vateria indica* Linn. . The frequency dominant genera were *Coniothyrium sp., Trichoderma sp., Mortierella sp., Phyllosticata sp*., and *Acremonium sp.Shankar et al* (2010) studied diversity of some climbers and grass species. The frequently isolated fungi such as *Chaetomium globosum, Colletotrichum gloeosporioides, Phyllosticata sp., Cladosporium sp.*, are generalist species.*Meenatchi et al* (2016) total 179 isolates were obtained from leaf, stem, stem and tissues of *Adenium obeum* collected from Virudhunanagar India. The endophytes were *Aspergillus breviceps, Colletotrichum gloeosporioides, Scorpulariopsis brevicalis*.

Aim of the Study

The present study was under taken to investigate the diversity of endophytic fungi and their colonization pattern in *Cynodon dactylon* (L) pers.

Materials and Methods

Isolation of Endophytes

Five different locations were selected for sampling and were denoted as location 1, Jaibhavaninagar (Loc1); location 2, Parijatnagar (Loc2); location 3, Deogiri campus (Loc3); Vasantrao Naik campus (Loc4); location 5 University area (Loc5). Plants were collected from each location separately. All the samples were washed properly in running tap water for half an hour before processing. The samples were cut into small pieces. Root, stem and leaves were cut into 1.0 x 1.0 cm pieces. To eliminate epiphytic microorganisms, all the samples were initially surface treated (Petrini *et. al*., 1992). Segments of each sample were placed on potato dextrose agar (PDA). The parafilm sealed petri plates were then incubated for 72hrs. The endophytic fungi were identified according to their macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore morphology. Standard taxonomic manuals were used to identify the fungal genera (Ainsworth *et. al*., 1973, Barnett and Hunter., 1998). All isolated and identified endophytic fungi were assigned specific code and sub cultured and cultures were kept in deep freeze.

Statistical Analysis

The colonization frequency (%) of fungal isolates was calculated by using the following formula (Suryanarayanan *et al*.2003).

$$CF\ (\%) = \frac{\text{Number of segment colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

The relative frequency (percent CF) of colonization of endophytic species was calculated as the number of segments colonized by a single endophyte divided by the total number of segments observed x 100 .(Hata k, Futai k 1995)This is expressed as %CF = (N*R*col*R*/ N*R*t*R*) x 100; where N*R*col*R*the number of segments colonized by each fungus, and N*R*t*R* = the total number of segments.

The dominant endophytes were calculated as the percentage colony frequency of a given endophyte divided by the sum of the percentage of colony frequencies of all endophytes x 100 (Kumaresan and Suryanarayana, 2002).Utilizing the data of percentage colony Frequency of different locations, Simpson's Diversity indices and Shannon-Wiener indices were calculated. (http://www.countrysideinfo.co.uk/simpsons.htm.).

Result and discussion

Endophytic fungi from root, stem, leaves were isolated, identified and evaluated for their existence. Plant were collected from five different areas. A total 80 isolates from 244 segments observed. Maximum endophytes were isolated from Loc 5 (33.78%) and minimum endophytes were recovered from Loc 3 (13.51%). Among 80 isolates, 15 isolates were separated from Loc 1 (11 from root, 2 from stem, and 2 from leaf). 11 isolates recovered from Loc 2 (9 from root, 2 from stem) while Loc 3 shows 10 isolates(6 from root, 3 from stem, 1 from leaf) while Loc 4 shows 13 isolates (9 from root, 4 from stem) and Loc 5 recovered 25 isolates.(22 from root, 3 from stem)(Table no.1).

The percent colonization at tissue sample by endophytic fungi at Location 5 (University area) was higher than percent colonization of other locations. Generally root samples from Loc 5 exhibited maximum diversity from other root samples. Stem samples from Loc 4(Vasantrao naik campus) harbored higher endophytic % frequency as compared to other stem samples. Leaf samples from Loc 1(Jaibhavaninagar) harbor more endophytic as compared to Loc-3(Deogiri campus). Leaf samples from Loc 2 and Loc 4 and Loc 5 showed zero frequency. Collectotrichum truncatum (Fig-b), 5CRU, Periconia sp., Aspergillus niger these species found at location 5 only.

Fusarium solani 1 was observed as the dominant endophyte fungus in total screened samples.(Fig-a) while *Fusarium* sp. was showing lower dominance (Table no.2).It is isolated from only Loc -1(Jaibhavaninagar). *Fusarium* sp , *Culvularia* sp , *Aspergillus flavus,* are showing higher colonizing frequency. While *Fusarium* sp was showing lowest colonizing percentage.

Another study done by Rekha and Shivanaa (2014) *Cladosporiu*m sp. occurred in high percentage in *Cynodon*. But in present study *Fusarium solani* 1 is showing higher percentage of colonizing percentage. *Nigrospora* sp. was found by Chanda Patel *et.al.*(2013) but in this study do not find this sp. In another study done by Ravindra and Kharwar (2011), they found *Aspergillus fumigatus* from stem of *Cynodon* but in the present study this species was not found.

Despite significant variations in the specific recovery of the endohytic community from plant tissue in each location, the inter-site comparison is significant. For instance, location 5(University area) has lower Simpson's index which indicates it has greater diversity, while location 4(Vasantrao Naik campus) has higher Simpson's index reflects lower diversity. Shannon Wiener Index is higher for Loc 5(University area) indicating maximum number of fungal isolates. Location 3(Deogiri campus) has higher evenness showing less diversity(Table No.3).

Location 5(University area) which is showing greater diversity and maximum number of fungal isolates (25) as compared to other location. Location 5 is the University area which is less polluted as compared to other which is within city area.

Table No.1: Distribution of Endophytic Fungi in *Cynodon dactylon*

Name of Fungi	Location 1			Location 2			Location 3			Location 4			Location 5		
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
Fusarium solani 1	1	-	2	1	-	-	3	-	-	3	-	-	2	-	-
Penicillium Sp	2	-	-	-	-	-	1	-	-	-	-	-	2	-	-
Aspergillus flavus	2	-	-	4	2	-	-	-	1	1	-	-	-	-	-
Curvularia Sp	2	-	-	2	-	-	1	1	-	-	-	-	4	1	-
Cephalosporium Sp	2	2	-	1	-	-	-	-	-	-	-	-	-	-	-
Fusarium solani	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-
Dreschsleria Sp	-	-	-	-	-	-	1	2	-	-	-	-	-	-	-
Fusarium sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cladosporium Sp	-	-	-	-	-	-	-	-	-	-	-	-	3	2	-
Trichoderma Sp	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
CrVn	-	-	-	-	-	-	-	-	-	5	2				
Colletotrichum truncatum	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
5CRU	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-
Periconia Sp	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-

R-Root; S-Stem; L-Leaf., Loc-1:- Jaibhavaninagar ; Loc- 2:- Parijatnagar ; Loc- 3:- Deogiri campusLoc-4:- Vasantrao Naik campus ; loc-5:- University area.

Table No. 2: Colonizing Frequency and Dominance of Fungi Isolated From *Cynodon dactylon*

Sr. No.	Name of Endophyte	Total	CF	Dominance
1	*Fusarium solani*1	12	20.00	15.00
2	*Penicillium* Sp	5	8.33	6.25
3	*Aspergillus flavus*	10	16.67	12.50
4	*Curvularia* Sp	11	18.33	13.75
5	*Cephalosporium* Sp	5	8.33	6.25
6	*Fusarium solani*	2	3.33	2.50
7	*Dreschsleria* Sp	3	5.00	3.75
8	*Fusarium* sp.	1	1.67	1.25
9	*Cladosporium* Sp	5	8.33	6.25
10	*Trichoderma* Sp	2	3.33	2.50
11	CrVn	7	11.67	8.75
12	*Colletotrichum truncatum*	2	3.33	2.50
13	5CRU	3	5.00	3.75
14	*Periconia* Sp	6	10.00	7.50
15	*Aspergillus niger*	6	10.00	7.50

Table No.3: Different Diversity Indices for Each Location

Sr. No.	Data Analysis	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5
1	Simpson's diversity Index	0.12	0.31	0.16	0.32	0.11
2	Shannon Wiener Index	0.8	0.53	0.71	0.46	0.84
3	Evenness	0.94	0.76	1.19	0.77	0.93

Fig. A-- Fusarium Solani 1

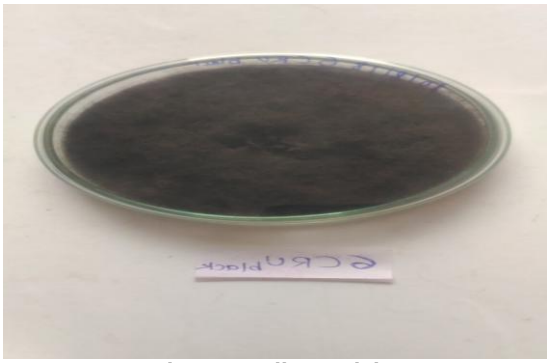

Fig. B—Collectotrichum Truncatum

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