International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 4 | OCT-DEC| 2017 | 46-55
Research Article – Biological Sciences| Open Access| UGC Approved | MCI Approved Journal

DISTRIBUTION AND DIVERSITY OF FUNGAL ENDOPHYTES FROM *CALOTROPIS GIGANTEA* (L.) R. BR. FROM TELANGANA, INDIA

A.Aruna, M.Abhinesh, J.Ramesh and V.Krishna Reddy*
Mycology Laboratory, Department of Botany, Kakatiya University, Warangal - 506009 Telangana.

***Corresponding Author Email:** vkreddyku@gmail.com

ABSTRACT

The present research papers deals with the investigations carried out on distribution and diversity of endophytic fungi associated with Calotropis gigantea (L.) R. Br, a medicinal plant. Diversity and distribution were assessed in terms of colonization frequency (CF), endophytic infection rates (EIR) and relative percentage occurrence (RPO). A total of 42 fungal species representing 27 genera were isolated. These fungi belonged to hyphomycetes, coelomycetes, aganomycetes and mycelia sterilia. Stems were colonized by more number of fungi followed by leaves. Penicillium and Aspergillus species dominated in all parts of the plant. Highest endophytic infection rate was recorded in fruits followed by roots. Relative percentage of occurrence was found to be highest for hyphomycetes followed by coelomycetes and mycelia sterilia. Stem and leaves have shown more RPO for all endophytic fungi. The wide distribution of different endophytic fungal flora with C.gigantea reflects its role in ecophysiology of the host. It is concluded that these endophytes may contribute atleast to some medicinal properties attributed to the host.

KEY WORDS

Calotropis gigantea, endophytic fungi, colonization frequency, endophytic infection rates, relative percentage occurrence

Introduction

Calotropis gigantea (L.) R.Br.(Family: Apocyanaceae) commonly known as milkweed or swallow wort is widely distributed in tropics and sub tropics and rare in cold countries. It is a moderate evergreen shrub found more or less throughout India in warm dry places. The plant secrets a poisonous milky latex. Since ancient times, it is recognized as a medicinal plant [1, 2] with unique properties. Traditionally, *Calotropis* is used alone or in combination with other medicinal [3] to treat common diseases such as fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea [4]. Virtually, every part of the plant was proved to possess medicinal properties. The plant is also a source of reputed Homeopathic drug [5]. Phytochemical analysis of latex revealed the presence of cardiac glycosides, calotropin, uscharin, calotoxin, calactin, uscharidin and gigantin. Latex also contains the protease calotropin DI and DII and calotropin F1, F2 and some poisonous constituents.

The biological diversity of fungal endophytes is enormous especially in tropical countries. Not only species diversity but also with intra species diversity, i.e. genotype diversity, with in small volumes of plant tissues can be high [6]. Fungal endophytes confer many ecophysiological benefits to the host plant [7] and are rich source of novel organic compounds with wide biological activities [8]. They have proved to be potential source for novel drugs [9].

The study of medicinal plants along with their fungal endophytes may yield valuable information on the existence of medicinally important chemical constituents and their biological activities. Against this backdrop, investigations were carried to isolate and study the fungal endophytes of *Calotropis gigantea*.

Material and Methods

Locality of collection

A total of 250 samples of *Calotropis gigantea* plant parts were collected from different ecological locations of Telangana state, India (Warangal, Khammam, Karimnagar, Nalagonda, Hyderabad and Rangareddy) in different seasons. Healthy and 5 to 10 years old mature *Calotropis gigantea* plant parts were carefully chosen for sampling.

Isolation of fungal endophytes

Different parts of the plant, *Calotropis gigantea* such as root, stem, leaves, flower and fruits were collected from the plant growing in different edaphic and environmental conditions. The samples were brought to the laboratory in sterilized polythene bags and were washed thoroughly in tap water followed by sterilized water for few minutes to remove dirt and debris [10]. A total 1250 of segments (root-250, stem-250, leaves-250, flower-250 and fruits-250) were selected for sampling. In root, maturation and elongation zones segments, the were cut in to vertically in 4 to 8 cm segments. The mature internodal parts of stem were collected and cut in to 0.5 -0.8 cm. Healthy and mature leaves lateral parts and midrib were cut into approximately 1cm segments. [11]. The segments of flower were trimmed to 0.2-0.4 cm. The middle-aged fruit samples were cut in to the small pieces of approximate 1 cm.

The samples from leaves were dipped in 70 % ethanol for 5 seconds, and then they were transferred to 4 % sodium hypochlorite for 90 seconds and finally rinsed in sterile distilled water for 10 seconds and then removed excess moisture [12]. Surface sterilization of stem, root, and fruits parts were carried out by [13, 14] method. The segments were immersed first in 75 % ethanol for 60 seconds, followed by 4% sodium hypochlorite for 180 seconds, and then again in 75 % ethanol for 30 seconds and finally rinsed in sterile distilled water for 10 seconds. The flower samples were dipped in 30% ethanol for 5 seconds, then they were immersed in 2% sodium hypochlorite for 60 seconds and rinsed in sterile distilled water for 10 seconds. The samples thus prepared kept were in sterile dry plate to remove excess moisture. The externally sterilized segments were placed on agar plates (Asthana and Hawkers medium and potato dextrose agar medium) supplemented with 150 mg of streptomycin per litre.

Each Petri dish containing 5 segments was incubated at 27± 2 ºC at 12-h light/dark cycle [15]. After 10-15 days of incubation the fungal colonies developing from the sample fragments were isolated and transferred to fresh tubes. Colony morphology of each fungus was recorded. Slides of fungal colonies were made with lactophenol cottonblue and observed under microscope. Mycelia, spore characteristics were recorded. Photomicrographs were taken under fluorescent microscope and fungi were identified with the help of standard manuals [16, 17, and 18].

Colonization Frequency (CF), Endophytic Infection Rates (EIR) and Relative Percentage Occurrence (RPO) of different groups of fungi were calculated with the help of following formulae.

Colonization Frequency (CF)

$$CF\ (\%) = \frac{\text{Number of species isolated}}{\text{Number segments screened}} \times 100$$

Endophytic Infection Rates (EIR)

$$EIR\ (\%) = \frac{\text{Number of infected segments}}{\text{Total number of segments screened}} \times 100$$

Relative Percentage Occurrence (RPO) of different fungal groups

$$RPO\ (\%) = \frac{\text{Density of colonization of one group}}{\text{Total density of colonization}} \times 100$$

Results and Discussion

The results obtained in the present investigations are presented in Tables 1, 2, 3 and Text Fig-1 and 2.

Table 1. Colonization Frequency of different endophytic fungi associated with *Calotropis gigantean.*

S. No	Name of the fungus	Colonization frequency (CF) (in %)				
		Root	Stem	Leaves	Flower	Fruits
1	*Acremonium strictum*	1.2	2.7	-	-	-
2	*Alternaria alternata*	-	3.1	2.1	-	1.9
3	*A. fasciculate*	-	1.8	-	-	-
4	*A. solani*	-	1.7	7.6	-	-
5	*Arthrinium cuspidatum*	-	1.9	-	-	-
6	*Aspergillus candidus*	-	5.1	6.2	1.9	-
7	*A.flavus*	1.5	6.7	6.2	1.9	2.2
8	*A.nidulans*	4.5	-	3.7	-	-
9	*A.niger*	1.8	5.2	4.6	0.5	-
10	*A.ochraceus*	2.6	3.7	3.7	-	-
11	*A.oryzae*	1.4	4.9	5.3	-	-
12	*A.stellatus*	3.7	-	-	5.6	-
13	*Blastomyces dermatitidis*	-	3.3	-	-	1.7
14	*Cercospora apii*	-	-	2.8	-	-
15	*Cladosporium herbarum*	-	3.1	-	1.5	-
16	*Colletotrichum falcatum*	-	4.5	3.8	0.2	-
17	*C. gloeosporioides*	-	1.9	-	-	-
18	*Curvularia lunata*	1.4	5.6	4.6	1.8	-
19	*Diplodia andamanensis*	1.8	3.7	4.4	-	-
20	*Discosia maculicila*	-	3.5	5.2	2.1	5.2
21	*Fusarium chlamydosporum*	-	4.5	-	-	-
22	*F. equiseti*	-	-	3.7	1.5	-
23	*F. oxysporum*	2.5	5.4	4.1	1.4	-
24	*F. solani*	--	-	4.6	-	4.4
25	*Geotrichum albidum*	-	2.1	2.1	-	-
26	*Gliocladiopsis sagariensis*	-	6.1	4.6	2.5	-
27	*Heterosporium gracile*					
28	*Helminthosporium sativum*	1.4	-	4.7	-	-
29	*Lacellina graminicola*	-	2.9	4.7	-	2.1
30	*Microsporum gypseum*	3.2	-	6.2	-	2.4
31	*Neurospora crassa.*	-	3.4	-	-	-
32	*Nigrospora sphaerica*	-	-	1.6	-	-
33	*Oidiodendron griseum*	-	3.7	3.2	-	1.5
34	*Periconia byssoides*	2.5	-	-	-	1.8
35	*Penicillium citrinum*	1.9	4.8	4.7	1.7	5.1
36	*P. chrysogenum*	2.4	5.5	3.7	-	1.4
37	*P. notatum*	2.5	4.6	-	-	1.5
38	*P. rubrum*	-	5.9	8.6	--	-
39	*Phoma crysanthemicola*	-	4.2	2.6	1.8	-
40	*P.destructiva*	2.3	5.8	4.4	-	-
41	*P. glomerata*	-	-	2.5	1.9	1.9
42	*Rhizoctonia bataticola*	2.5	4.6	-	-	1.7
43	*Stilbum cinnabarinum*	-	-	2.9	-	-
44	*Verticillium dahliae*	-	4..2	-	2.7	-
45	Sterile mycelium 1	2.7	4.2	-	-	-
46	Sterile mycelium 2	-	4.4	3.9	-	-
47	Sterile mycelium 3	-	3.5	-	2.7	-
48	Sterile mycelium 4	-	-	4.2	-	-
49	Sterile mycelium 5	2.1	-	-	3.5	-
	Total: CF %	**45.90**	**142.20**	**137.20**	**35.20**	**34.80**

Table 2. Endophytic Infection Rates of different endophytic fungi associated with *Calotropis gigantea*

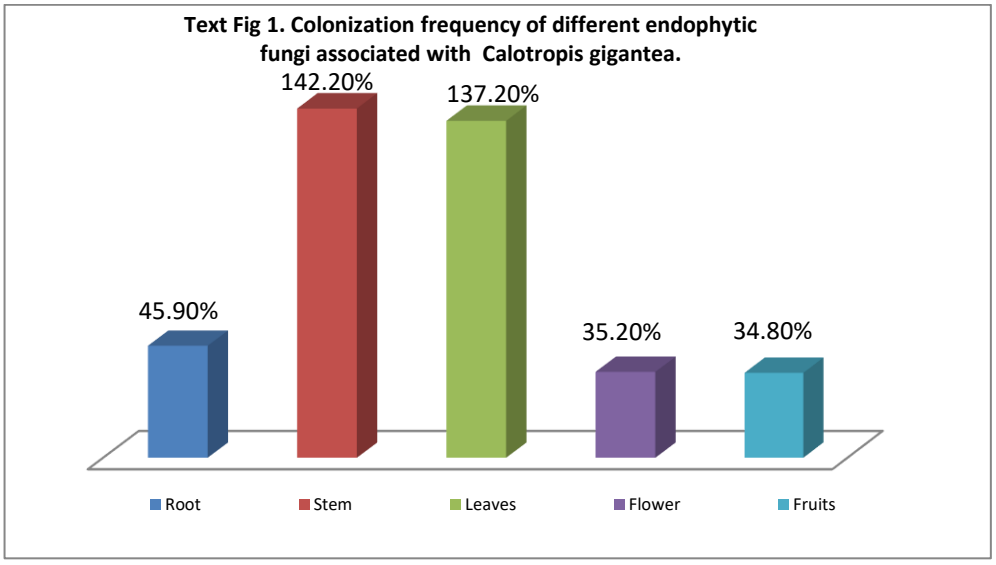
S. No	Name of the fungus	% Endophytic Infection Rates (EIR)														
		Root			Stem			Leaves			Flower			Fruits		
		S	I	EIR (%)	S	I	EIR (%)	S	I	EIR (%)	S	I	EIR (%)	S	I	EIR (%)
1	*Acremonium strictum*	-	-	-	3	1	33	-	-	-	-	-	-	-	-	-
2	*Alternaria alternata*	-	-	-	5	3	60	4	1	25	-	-	-	2	2	100 %
3	*A. fasciculate*	-	-	-	4	1	25	-	-	-	-	-	-	-	-	-
4	*A. solani*	-	-	-	6	2	33	8	5	62	-	-	-	-	-	-
5	*Arthrinium cuspidatum*	-	-	-	1	1	100	-	-	-	-	-	-	-	-	-
6	*Aspergillus candidus*	-	-	-	7	4	57	4	1	25	1	1	100	-	-	-
7	*A.flavus*	2	1	50	-	-	-	6	4	66	-	-	-	2	1	50%
8	*A.nidulens*	5	4	80	-	-	-	-	-	-	-	-	-	-	-	-
9	*A.niger*	2	2	100	4	1	25	3	1	33	1	1	100	-	-	-
10	*A.ochraceus*	-	-	-	-	-	-	5	3	60	-	-	-	-	-	-
11	*A.oryzae*	3	1	33	3	2	66	4	2	50	-	-	-	-	-	-
12	*A.stellatus*	5	2	40	-	-	-	-	-	-	4	3	75	-	-	-
13	*Blastomyces dermatitidis*	-	-	-	-	-	-	-	-	-	-	-	-	3	3	100
14	*Cercospora apii*	-	-	-	-	-	-	2	1	50	-	-	-	-	-	-
15	*Cladosporium herbarum*	-	-	-	-	-	-	-	-	-	1	1	100	-	-	-
16	*Colletotrichum falcatum*	-	-	-	-	-	-	-	-	-	4	3	75	-	-	-
17	*C. gloeosporioides*	-	-	-	-	-	-	-	-	-	2	2	100	-	-	-
18	*Curvularia lunata*	2	2	100	4	2	50	3	2	66	-	-	-	-	-	-
19	*Diplodia andamanensis*	2	2	100	7	3	42	-	-	-	-	-	-	-	-	-
20	*Discosia maculicila*	-	-	-	-	-	-	-	-	-	4	3	75	-	-	-
21	*Fusarium chlamydosporum*	-	-	-	4	2	50	-	-	-	-	-	-	-	-	-
22	*F. equiseti*	-	-	-	-	-	-	3	3	100	-	-	-	-	-	-
23	*F. oxysporum*	-	-	-	4	2	50	-	-	-	-	-	-	-	-	-
24	*F. solani*	-	-	-	-	-	-	6	2	33	-	-	-	-	-	-
25	*Geotrichum albidum*	-	-	-	5	3	60	-	-	-	-	-	-	-	-	-
26	*Gliocladiopsis sagariensis*	-	-	-	7	4	57	-	-	-	-	-	-	2	2	100
27	*Heterosporium gracile*	5	4	80	-	-	-	-	-	-	-	-	-	-	-	-
28	*Helminthosporium sativum*	4	3	75	-	-	-	-	-	-	-	-	-	2	2	100
29	*Lacellina graminicola*	-	-	-	-	-	-	4	1	25	-	-	-	-	-	-
30	*Microsporum gypseum*	4	1	25	-	-	-	-	-	-	-	-	-	2	1	50
31	*Neurospora crassa.*	-	-	-	5	4	80	-	-	-	-	-	-	-	-	-
32	*Nigrospora sphaerica*	-	-	-	-	-	-	6	2	33	-	-	-	-		-
33	*Oidiodendron griseum*	-	-	-	3	2	66	-	-	-	-	-	-	-	-	-
34	*Periconia byssoides*	-	-	-	-	-	-	-	-	-	-	-	-	5	3	60

35	*Penicillium citrinum*	3	3	100	-	-	-	-	-	-	-	-	-	6	5	83
36	*P. chrysogenum*	-	-	-	5	2	40	-	-	-	-	-	-	2	2	100
37	*P. notatum*	4	3	75	-	-	-	-	-	-	-	-	-	-	-	-
38	*P. rubrum*	-	-	-	9	4	44	-	-	-	-	-	-	-	-	-
39	*Phoma crysanthemicola*	-	-	-	-	-	-	5	2	40	-	-	-	-	-	-
40	*P.destructiva*	-	-	-	8	5	62	4	2	50	-	-	-	-	-	-
41	*P. glomerata*	-	-	-	-	-	-	6	4	66	-	-	-	4	3	75
42	*Rhizoctonia bataticola*	-	-	-	6	2	33	-	-	-	-	-	-	-	-	-
43	*Stilbum cinnabarinum*	-	-	-	-	-	-	5	1	20	-	-	-	-	-	-
44	*Verticillium dahliae*	-	-	-	-	-	-	-	-	-	6	2	33	-	-	-
45	Sterile mycelium 1	-	-	-	4	2	50	-	-	-	-	-	-	3	3	100
46	Sterile mycelium 2	-	-	-	2	2	100	3	2	66	-	-	-	-	-	-
47	Sterile mycelium 3	3	3	100	-	-	-	-	-	-	4	2	50	-	-	-
48	Sterile mycelium 4	-	-	-	-	-	-	5	1	20	-	-	-	4	2	50
49	Sterile mycelium 5	2	1	50	-	-	-	-	-	-	3	2	66	-	-	-
	Total- EIR %		69.5			50.90			46.50			66.60			78.30	

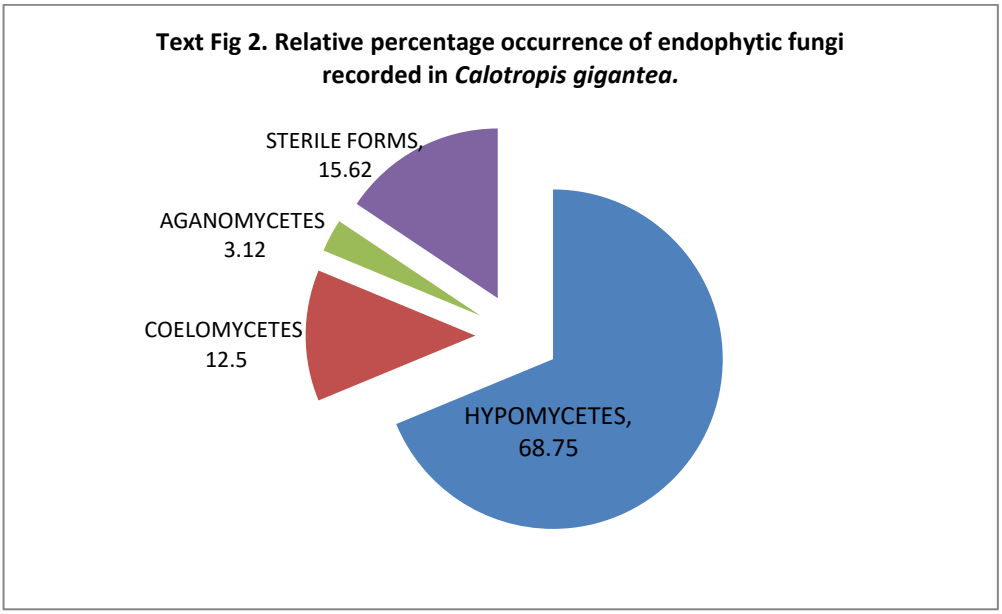
Table 3. Overall relative percentage Occurrence of endophytic fungi recorded in *Calotropis gigantea.*

S.NO	Name of the fungus	% Relative percentage Occurrence (RPO)									
		Root		Stem		Leaves		Flower		Fruits	
	HYPHOMYCETES	DC	RPO %	DC	RPO %	DC	RPO %	DC	RPO %	DC	RPO %
1	*Acremonium strictum*	-	-	3	3.79	-	-	-	-	-	
2	*Alternaria alternata*	-	-	5	6.32	4	6.34	-	-	2	8.33
3	*A. fasciculate*	-	-	4	5.06	-	-	-	-	-	
4	*A. solani*	-	-	6	7.59	8	12.6	-	-	-	
5	*Arthrinium cuspidatum*	-	-	1	1.26	-	-	-	-	-	
6	*Aspergillus candidus*	-	-	7	8.86	4	6.34	1	7.69	-	
7	*A.flavus*	2	4.87	-	-	6	9.52	-	-	2	8.33
8	*A.nidulans*	5	12.1	-	-	-	-	-	-	-	
9	*A.niger*	2	4.87	4	5.06	3	4.76	1	7.69	-	
10	*A.ochraceus*	-	-	-	-	5	7.93	-	-	-	
11	*A.oryzae*	3	7.31	3	3.79	4	6.34	-	-	-	
12	*A.stellatus*	5	12.19	-	-	-	-	4	30.7	-	
13	*Blastomyces dermatitidis*	-	-	-	-	-	-	-	-	3	12.5
14	*Cercospora apii*	-	-	-	-	2	3.17	-	-	-	
15	*Cladosporium herbarum*	-	-	-	-	-	-	1	7.69	-	
16	*Curvularia lunata*	2	4.87	4	5.06	3	4.76	-	-	-	
17	*Fusarium chlamydosporum*	-	-	4	5.06	-	-	-	-	-	
18	*F. equiseti*	-	-	-	-	3	4.76	-	-	-	
19	*F. oxysporum*	-	-	4	5.06	-	-	-	-	-	
20	*F. solani*	-	-	-	-	6	9.52	-	-	-	
21	*Geotrichum albidum*	-	-	5	6.32	-	-	-	-	-	
22	*Gliocladiopsis sagariensis*	-	-	7	8.86	-	-	-	-	2	8.33
23	*Heterosporium gracile*	5	12.1	-	-	-	-	-	-	-	

24	*Helminthosporium sativum*	4	9.75	-	-	-	-	-	-	2	8.33
25	*Lacellina graminicola*	-	-	-	-	4	6.34	-	-	-	
26	*Microsporum gypseum*	4	9.75	-	-	-	-	-	-	-	
27	*Neurospora crassa.*	-	-	5	6.32	-	-	-	-	-	
28	*Nigrospora sphaerica*	-	-	-		6	9.52	-	-	-	
29	*Oidiodendron griseum*	-	-	3	3.79	-	-	-	-	-	
30	*Periconia byssoides*	-	-	-	-	-	-	-	-	5	20.83
31	*Penicillium citrinum*	3	7.31	-	-	-	-	-	-	6	25
32	*P. chrysogenum*	-	-	5	6.32	-	-	-	-	2	8.33
33	*P. notatum*	4	9.75	-	-	-	-	-	-	-	
34	*P. rubrum*	-	-	9	11.39	-	-	-	-	-	
35	*Stilbum cinnabarinum*	-	-	-	-	5	7.93	-	-	-	
36	*Verticillium dahliae*	-	-	-	-	-	-	6	46.15	-	
	TOTAL	39	94.87	79	99.91	63	99.83	13	99.92	24	99.98
	Total: - RPO %	41.10		79.07		63.10		13.01		24.00	
	COELOMYCETES	DC	RPO %	DC	RPO%	DC	RPO %	DC	RPO %	DC	RPO%
1	*Colletotrichum falcatum*	-	-	-	-	-	-	4	40	-	-
2	*C. gloeosporioides*	-	-	-	-	-	-	2	20	-	-
3	*Diplodia andamanensis*	2	100	7	46.6	-	-	-	-	-	-
4	*Discosia maculicila*	-	-	-	-	-	-	4	40	-	-
5	*Phoma crysanthemicola*	-	-	-	-	5	33.3	-	-	-	-
6	*P.destructiva*	-	-	8	53.3	4	26.6	-	-	-	-
7	*P. glomerata*	-	-	-	-	6	40	-	-	4	100
	TOTAL	2	100	15	99.9	15	99.9	10		4	100
	Total: - RPO %	2		15.01		15.01		10		4	
	AGANOMYCETES										
8	*Rhizoctonia bataticola*	-	-	6	100	-	-	-	-	-	-
	TOTAL	-	-	6	100	-	-	-	-	-	-
	Total: - RPO %	0%		6%		0%		0%		0%	
	STERILE FORM										
9	Sterile mycelium 1	-	-	4	66.6	-		-	-	3	42.8
10	Sterile mycelium 2	-	-	2	33.3	3	37.5	-	-	-	-
11	Sterile mycelium 3	3	60	-	-	-	-	4	57.1	-	-
12	Sterile mycelium 4	-	-	-	-	5	62.5	-	-	4	57.1
13	Sterile mycelium 5	2	40	-	-	-	-	3	42.8	-	-
	TOTAL	5	100	6	99.9	8	100	7	99.9	7	99.9
	Total:- RPO %	5		6		8		7		7	



Text Fig 1. Colonization frequency of different endophytic fungi associated with Calotropis gigantea.



Text Fig 2. Relative percentage occurrence of endophytic fungi recorded in *Calotropis gigantea.*

In the present study, a total of 42 fungal species representing 27 genera were isolated from different parts of *Calotropis gigantea*. Caruso *et al*. [19] isolated 150 fungal and 71 actinomycete endophytes from internal tissues of woody braches, shoots and leaves of different plants of *Taxus baccata and T. brevifolia*. Arnold *et al*, [20] isolated 418 endophyte morphospecies from 83 healthy leaves of *Histiria concinna and Ouratea lucens*. These fungal genera belonged to diverse groups of fungi, hyphomycetes, coelomycetes, aganomycetes and sterile mycelia. However, a majority of them belonged to hyphomycetes. Among different genera, Aspergillus is represented by seven species followed by *Fusarium and Penicillium* with four species each. Four different types of mycelia sterilia were isolated. Lacap *et al*. [21] reported that sterile mycelia dominated in the findings of most of the endophytic research. Amirita *et al* [12] also reported the dominance of sterile fungi which was similar to the studies conducted earlier in many tropical endophytes by Carrado and Rodrigues, and Khan *et al*. [22,23] while working on endophytic fungi of *Calotropis* procera reported the dominance deuteromycetous fungal species. Similar were the observations of Frohlich and Hyde [24].

Colonization frequency

Colonization frequency (CF) is the expression of ratio between the number of species isolated and number of segments screened. In the present study, CF of different parts of the test plant viz, root, stem, leaves, flowers and fruits was assessed (Table 1 - Text Fig 1). It is evident from the table that out of five parts of the plant, stem was colonized by more number of species (35) followed by leaves (32). Fruits were colonized by least number of species (14). Flowers and roots were also colonized by less number of species. In terms of percentage colonization, the same trend was observed as that of number of fungal species. Analysis of species wise colonization, *Penicillium rubrum* (8.6 %) was highest in leaves that was followed by *Aspergillus flavus* (6.7 %). Highest colonization in root. root, stem, leaves, flowers and fruits was associated with *Aspergillus nidulans* (4.5 %), *Aspergillus flavus* (6.7 %). *Penicillium rubrum* (8.6 %), *Aspergillus stellatus* (5.6 %), and *Discosia maculicila* (5.2 %) respectively. Variation in CF of endophytic fungi in different parts of different plants was also reported by a number of workers [25,26,27].

Endophytic Infection Rates (EIR)

EIR is the percentage of segments that yielded the endophytic fungal colonies. The data presented in table 2 shows that EIR varied with the plant part of *C. gigantea* (78.3%). Highest EIR was recorded in fruits (78.3%) followed by root (69.5%). Stems (50.9%) and leaves (46.5%) have shown almost the same infection rate. Flowers have shown moderate infection rate (66.6%). Huang *et al* [28] opined that tissue specificity plays an important role of fungal assemblage in single or many plant species. The distribution and infection rate of endophytic fungi in a particular plant part is determined by several fungal and host characteristics. Fungal characters include ability to produce enzymes, withstand against the host chemicals. On the other hand, host factors include type of tissue present and other physical stresses [29]. Under certain conditions, endophytes may become parasitic and become pathogenic causing symptomatic infection [30]. Schulz and Boyle [31] proposed that asymptomatic colonization of endophytes is a balanced antagonistic interaction between host plant and endophyte

Relative percentage occurrence (RPO)

RPO indicates the percentage of occurrence of different taxonomic groups of endophytic fungi (Table 3; Text Fig 2). In the present study, four different groups of fungi viz, hyphomycetes, coelomycetes, aganomycetes and mycelia sterilia were isolated from different parts of *Calotropis gigantea*. RPO was found to be highest for hyphomycetes followed by coelomycetes and mycelia sterilia. The number of genera and species of hyphomycetes was far greater than other groups. As far as different parts of the plant are concerned, stems and leaves have shown more RPO for all endophytic groups. Least RPO was recorded for fruits and roots. The difference in endophytes, difference in their metabolic profile and hence difference in their biological activity even in between the same isolates of same species might be related to the chemical difference of host plants [32].

Discussion

Fungal endophytes are diverse group of organisms forming association with almost ubiquitously throughout the plant kingdom. Endophytic fungal strains have been isolated from diverse plants including trees, fodders, vegetables, fruits, cereal grains, commercial crop and medicinal plants [33]. It has been estimated that there may be as many as one million different endophytic fungal taxa, thus endophytes are hyper diverse [34] They are an ecological, polyphyletic group of highly diverse fungi, mostly belonging to ascomycetes and anamorphic fungi [35]. Endophytic fungi are one of the most unexplored and diverse group of organisms that make symbiotic association with plants and may confer beneficial effects on host [36]. These fungi have been widely investigated as source of bioactive compounds. The endophytic fungi from medicinal plants can, therefore, be used for the development of drugs. The endophytic fungal flora, both qualitatively and quantitatively differ with their host and depends on host geographical locations [37,38]. The objective behind the present investigation was to isolate the endophytic fungi from *Calotropis gigantea* and screen them for medicinal properties. *Calotropis gigantea* is a well-known medicinal plant widely distributed in tropical countries. We presume that at least some of the medicinal properties of the

plant would be due to endophytic fungi. Occurrence of a wide range of endophytic fungi with different plant parts lends a support for this assumption.

Conclusion

A variety of relationships can coexist between endophytes and their host plants, ranging from mutualism or symbiosis to antagonism or slightly pathogenic [39]. The host endophytic relationships can be described in terms of host specificity, host-recurrence, host selectivity and host preference [40,41]. The diversity, distribution of endophytic fungi in different parts of C.gigantea can be understood against this background. Distribution, occurrence of a large number of fungi indicates a significant role of endophytic fungi in host metabolism, ecology and survival. The metabolites of different fungi encountered in the present study need to be isolated, characterized and screened for pharmacological properties.

Acknowledgements

Our sincere thanks are due to Head, Department of Botany, Kakatiya University for encouragement and facilities. Two of us (AA and MA) owe their sincere gratitude to UGC, New Delhi for awarding Rajiv Gandhi National Fellowship.

References

[1] Deshmukh, S.K., Mishra, P.D., Almeida, A.K., Veerekar, S., Sahoo, M.R., Periyasamy, G., Goswami, H., Khanna, A., Balakrishna, A. & Vishwakarma, R. (2009): Anti-inflammatory and anticancer activity of ergoflavin isolated from an endophytic fungus. Chemistry and Biodiversity 6, 784 - 78

[2] Babu, A.R. & Karki, S.S. (2011): Anti-convulsant activity of various extracts of leaves of *Calotropis gigantea* Linn against seizure induced models. International Journal of pharmaceutical Sciences 3, 200 -203.

[3] Caius, J.F. (1986): The medicinal and poisonous plants of India. J. Bomby. Natural hist. Soc, XLI Scientific Publ. Jodhpur, India. 270 - 271.

[4] Das, M.K., Mazumder, P.M. & Das, S. (1996): Effect of Methanolic Extract of flowers of *Calotropis* procera on leukocyte and neutrophil migration. International Bulletin of Drug Research 1, 41-46.

[5] Ferrington, E.A. (1990): Clinical Materia Medica (reprint Ed.) B. Jain Publ. Pvt. Ltd., New Delhi, Ganapathm. Kalyani Publishers, Ludhiana, India. P. 347 -353.

[6] Muller, M.M., Valjakka, R., suokko, A. & Huntula, J. (2001): Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. Molecular Ecology 10, 1801 - 1810.

[7] Arechavaleta, M., Bacon, C.W., Hoveland, C.S. & Radcliffe, D.E. (1989): Effect of the tall fescue endophytes on plant response to environmental stress. Agronomy Journal 81, 83 - 90.

[8] Giridharan, P., Shilpa, A.V., Amit, K., Mishra, P.D. & Sunil Kumar, D. (2012): Anticancer activity of sclerotiorin isolated from an endophytic fungus Cephalotheca faveolata Yaguchi, Nishim. & Udagawa Indian Journal of Experimental Biology 50, 464 - 468.

[9] Wang, Z., Wang, M., Mei, W., Han, Z. & Dai, H. (2008): A New cytotoxic pregnanone from *Calotropis gigantea*. Molecules 13, 3033 - 3039.

[10] Selvanathan, S., Indrakumar, I. & Johnpaul, M. (2011): Biodiversity of the endophytic fungi Isolated from *Calotropis gigantea* (L.) R.Br. Recent Research in Science and Technology 3, 94-100.

[11] Dobranic, J.K., Johnson, L.A. & Alikhan, Q.R. (1995): Isolation of endophytic fungi from eastern larch (Larix laricina) leaves from New Brunswich, Canadian Journal of Botany. 41, 194-198.

[12] Amirita, A., Sindhu, P., Swetha, J., Vasanthi, N.S. & Kannan, K.P. (2012): Enumeration of endophytic fungi from medicinal plants. Journal of Science & Technology, 2, 13-19.

[13] Fisher, P.J., Petrini, O. & Sutton, B.C. (1993): A comparative study of fungal endophytes in leaves xylem and bark of Eucalyptus nitens in Australia and England. Sydowia, 45, 338-345.

[14] Fisher, P.J., Petrini, O. Petrini, L.E. & Sutton, B.C. (1994): Fungal endophytes from the leaves and twigs of Quercus ilex L. from England Majorea and Switzerland. New Phytol. 127, 133-137.

[15] Suryanarayanan, T.S., Venkatesan, G. & Murali, T. S. (2003): Endophytic fungal communities in leaves of tropical forest trees: Diversity and distribution patterns, Current Science 85 (4):486 - 492.

[16] Klich, M.A. & Pitt, J.I. (1988): A laboratory guide to the common Aspergillus species and their teleomorphs Commonwealth Scientific and Indusrial Research Organization, Division of Food processing, New South Wales, Australia.p.116.

[17] Singh, K., Frisvad, J.C., Thrane, U. & Mathur, S. (1991): An illustrated manual on identification of some seed-borne Aspergilli, Fusaria, Penicillia.pp. 31-69.

[18] Barnett, H. & Hunter, B. (1998): Descriptions and illustrations of genera. Illustrated genera of imperfect

fungi (4th ed.) American Phytopathological Society, St. Paul, MN.

[19] Caruso, M., Colombo, A.L., Fedeli, L., Pavesi, A., Quaroni, S., Saracch, M. & Ventrella, G. (2000): Isolation of endophytic fungi and actionomycetes taxane producers. Annals of Microbial 50, 3-13.

[20] Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. & Kursar, T.A. (2000): Are tropical fungal endophytes hyperdivers Ecology Letters 3, 267-274.

[21] Lacap, D.C., Hyde, K.D. & Liew, E.Y. (2003): An evaluation of the fungal "morphotype" concepts based on ribosomal DNA sequence. Fungal Diversity 12: 53 -66.

[22] Corrado, M. & Rodrigues, K.F. (2004): Antimicrobial evaluation of fungal exacts produced by endophytic strains of Phomopsis sp. Journal of Basic Microbiology 44, 157-160.

[23] Khan, R., Shahzad, S., Choudhary, M.I., Khan, S.A. & Ahmad, A. (2007): Biodiversity of the endophytic fungi isolated from *Calotropis* procera (ait.) R.Br. Pakistan Journal of Bot any 39, 2233 - 2239.

[24] Frohlich, J. & Hyde, K.D. (1999): Biodiversity of palm fungi in the tropics are global fungal diversity estimated realistic Biodiversity and Conservation, 8, 977-1004.

[25] Suryanarayanan, T.S., Murali, T.S. & Venkatesan, G. (2002): Occurrence and distribution of fungal endophytes in tropical forests across a rainfall gradient. Canadian Journal of. Botany 80, 818-826.

[26] Shekhawat, K.K., Rao, D.V. & Batra, A. (2010): Morphological study of endophytic fungi inhabiting leaves of Melia azadirachta. International Journal of Pharmaceutical Sciences Review and Research 5, 177 - 180.

[27] Goveas, W.S., Royston, M., Shashi, K.N., Leo, D.S. (2011): Isolation of endophytic fungi from Coscinium fenestratum a red listed endangered medicinal plant Eurasia Journal of Biosciences 5 ,48 - 53, DOI :10. 5053 ejobios. 5.0.6.

[28] Huang, W.Y., Cai, Y. Z., Hyde, K.D., Corke, H. & Sun, M. (2008): Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Diversity 33: 61 -75.

[29] Salim, K.A., E1 - Beih, A.A., Abd, E., Rahman, T.M. & Diwany, A. (2012): Biology of endophytic fungi Current Research in Environmental & Applied Mycology 2 (1); 31- 82, Doi 10. 5943 creams 2/1/3.

[30] Brown, K.B., Hyde, K.D. & Guest, D.I. (1998): Preliminary studies on endophytic fungal communities of Musa acuminata species complex in Hong Kong and Australia. Fungal Divers 1, 27-58.

[31] Schulz, B. & Boyle, C. (2005): The endophytic continuum. Mycological Research 109: 661 - 686.

[32] Paulus, B., Kanowski, J., Gadek, P. & Hyde, K.D. (2006): Diversity and Distribution of saprobic microfungi in leaf litter of on Australian tropical rainforest. Mycological Research 110, 1441 -1454.

[33] Lu, Y., Chuan, C., Hong, C., Jianfen, Z. & Weiqin, C. (2012): Isolation and Identification of Endophytic Fungi from Actinidia macrosperima and investigation on their bioactivities ID 382742, 8 doi - 10.1155/ 2012/382742.

[34] Huang, W.Y., Cai, Y.Z., Xing, J., Corke, H. & Sun, M. (2007): Potential antioxidant resource endophytic fungi isolated from traditional Chinese medicinal plants. Economic Botany 61, 14-30.

[35] Arnold, A.E. (2007): Understanding the diversity of foliar endophytic fungi progress, challenges and frontiers. Fungal Biology Reviews 21, 51 - 66.

[36] Shiomi, H.F., Silva, I.S., Demelo, I.S., Nunes, F.V. & Bettiol, W. (2006): Bioprospecting endophytic Bacteria for Biological control of Coffee leaf rust Sci. Agric., 63 (1):32-39.

[37] Arnold, A.E. & Herre, E.A. (2003): Canopy cover and leaf age affect colonization by tropical fungal endophytes ecological pattern and process in Theobroma cacao (Malvaceae) Mycologia 95, 388 -398.

[38] Gange, A.C., Dey, S., Currie, A.F., Sutton, B.V. (2007): Site - and species -species differences in endophyte occurrence in two herbaceous plants. Journal of. Ecology 95, 614 - 622.

[39] Purahong, W. & Hyde, K.D. (2011): Effects of fungal endophytes on grass and non-grass litter decomposition rates. Fungal Divers 47, 1 - 7.

[40] Zhou, D. & Hyde, K.D. (2001): Host - specificity, host - exclusivity, and host - recurrence in saprobic fungi. Mycological Research 105: 1449 - 1457.

[41] Cohen, S.D., (2006): Host selectivity and genetic variation of Discula umbrinella isolates from two oak species: analyses of intergenic spaces region sequences of ribosomal DNA. Microbial Ecology 52, 463- 469.

***Corresponding Author:**
V. Krishna Reddy*
Email: vkreddyku@gmail.com