



## Isolation, frequency distribution and diversity of novel endophytic fungal communities of *Fusarium* species in *Rhus mysorensis* L. from Sanganer region of Rajasthan

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### ABSTRACT

A total of ten plant samples of *Rhus mysorensis* (Darsan) from unique locations of Sanganer region of Rajasthan were collected for isolation of fungal endophytes. Of these, maximum frequency of *Fusarium* species (26.33 %) were recorded which are morphologically similar but ecologically variant. The present paper discusses the diversity of *Fusarium* species (*Fusarium oxysporum* & *Fusarium roseum*) within the *Rhus mysorensis* plant samples of unique location of sanganer region of rajasthan on the basis of frequency distribution and occurrence.

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### Introduction

Fungal endophytes are one of the largely untapped resources and poorly investigated group of microorganisms that represents abundant and dependable high value bioactive metabolites and chemically novel compounds with potential for exploitation in a wide variety of medicinal, agricultural and industry (Banerjee, 2011; Strobel, 2003; Tan & Zou, 2001). Interest on fungal endophytes has recently surged, which has led to a considerable amount of research regarding the role of these fungi in host plants (Ananda & Sridhar 2002). Endophytic infection enhances the defense of the host plants, this has led to the hypothesis that plants might have accommodated endophytes to improve their fitness in a given environment under certain stress conditions (Arnold *et al.*, 2003). Due to extra ordinary role of these fungi in ecosystem as well as in production of various bioactive molecules, it is necessary to explore these fungi on the basis of morphological, cultural and ecological level (Strobel, 2003; Tan & Zou, 2001). This type of investigation in this plant has been done for the first time.

Rajasthan is one of twenty five hot spots of global biodiversity with approximately 5,000 species of flowering plants. Sanganer region of Rajasthan has large number of plant diversity. *Rhus mysorensis* is common plant (locally known as Darsan) in rocky habitats of deciduous forests belongs to the family Anacardiaceae. It is small tree, leaves 3-foliate, leaflets obovate, cuneate, sinuate-toothed or lobed, softly pubescent; terinal leaflet shortly petiolulate, 2.5-4.0 cm long; lateral ones sessile, sometimes very small. Flowers small, polygamous, in terminal and axillary panicles. Drupes subglobose, small, glabrous, brown [Figure 1 (a)]. It is a common plant present in hilly area and forest region of Rajasthan. Deepak *et al.*, (2010) reported the medicinal potential of the plant. Endophytic fungal study has been carried out first time in this plant to explore the potential and medicinal value.

### Materials and Methods

#### Collection of plant samples

Plants samples (*Rhus mysorensis* L.) from unique environmental niches of sanganer region, especially those with an unusual biology and possessing novel strategies for survival were selected for study (Frohlich *et al.*, 2000; Raviraja *et al.*, 2006). The samples were collected in sterilized polythene bags from the different locations of Sanganer region of Jaipur, during the months of July-November.

#### Isolation of fungal endophytes

The leaves and nodes were used as explants for isolation of fungal endophytes (Raviraja, 2005). All explants were surface-sterilized by dipping in 75% ethanol for 1 minute, 4 % sodium hypochlorite for 5 minutes followed by rinsing three times in sterilized distill water. In each petri dish (9 cm diameter), a total of five-six processed explants were evenly spaced onto the surface of Potato Dextrose Agar (PDA) media supplemented with 200 µg /ml tetracycline incubated at 28°C and daily observation was recorded (Raviraja, 2005). The sporulating mycelia of fungi appeared on the plates were carefully isolated, sub-cultured and maintained the pure culture on PDA slants (Raviraja, 2005).

#### Identification of fungal endophytes

The isolated endophytic fungi have been described and identified on the basis of morphological features like colony characterization, growth of fungi on different media, colour of colony (front and reserve), conidial development, size, shape, conidia, attachment of conidia and shape of conidial head (Hawksworth, 1991; Nagmani *et al.*, 2006). Then the fungus is grown in a slide culture, sporulation characteristics and the spores of the fungus remain undisturbed and attached to the sporophores thus facilitating in identification. This technique was performed for various stages of conidia formation and proper identification of the sporulating fungi (Anthony & Walkes 1962).

## Results

### Statistical Analysis

Colonization rate (%) of fungal endophytes was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed  $\times 100$  [Table-1, figure-4]. In *Rhus mysorensis* L. a total of eight different endophytic fungal genera along with six different mycelia sterilia were found in explants (leaves and nodes) [Table-3, figure-3]. Among these *Fusarium* sp. showed the highest colonizing frequency (26.33 %), followed by *Colletotrichum* sp. (11.83 %), and *Alternaria* sp. (10.49 %). In addition, *Helminthosporium* sp. (5.35 %), *Curvularia* sp. (4.46 %) and *Aspergillus* sp. (4.24 %), were isolated with low frequency of colonization. *Cladosporium* sp. (2 %) and *Phomopsis* sp. (2 %) were isolated with very low frequency of colonization. *Helminthosporium* sp. was found absent in site VII. *Phomopsis* sp. was found absent in site I, II, IV and VII, where as *Cladosporium* sp. was found absent in site I, II IX and X respectively [Table-2, figure-2] .

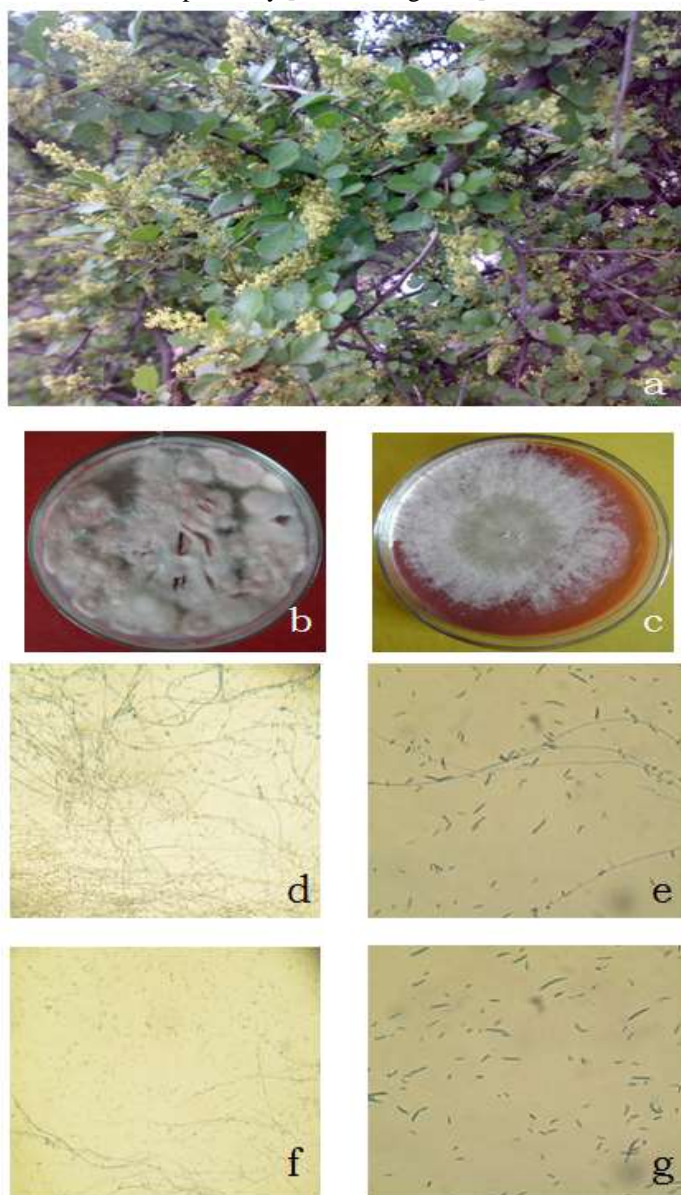


Figure: 1

- (a) Mature plant of *Rhus mysorensis* L. collected from Sanganer region of Rajasthan  
 (b) Pure culture of *Fusarium oxysporum* on PDA media  
 (c) Pure culture of *Fusarium roseum* on PDA media

(d) Conidiophores (*Fusarium oxysporum*) variable, slender, short, branched irregularly bearing a whorl of phialides, single or grouped into sporodochia (10X)

(e) Mature conidia of *Fusarium oxysporum* slightly curved or bent at pointed ends (40X)

(f) Various developmental stages *Fusarium roseum* conidia (Microconidia one celled ovoid) (10X)

(g) Mature conidia of *Fusarium roseum* slightly curved or bent at pointed ends (40X)

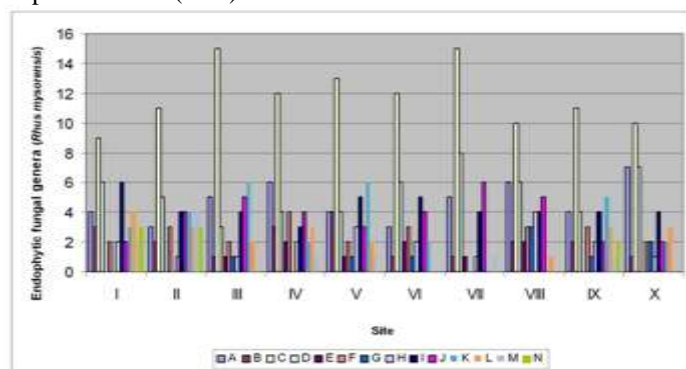


Figure 2: Associated endophytic fungal genera in the plant *Rhus mysorensis* L. A - *Alternaria* sp.; B - *Curvularia* sp.; C - *Fusarium* sp.; D - *Colletotrichum* sp.; E - *Cladosporium* sp.; F - *Helminthosporium* sp.; G - *Phomopsis* sp.; H - *Aspergillus* sp.; I - Mycelia Sterilia 1; J - Mycelia Sterilia 2; K - Mycelia Sterilia 3; L - Mycelia Sterilia 4; M - Mycelia Sterilia 5; N - Mycelia Sterilia 6 [Table- 2]

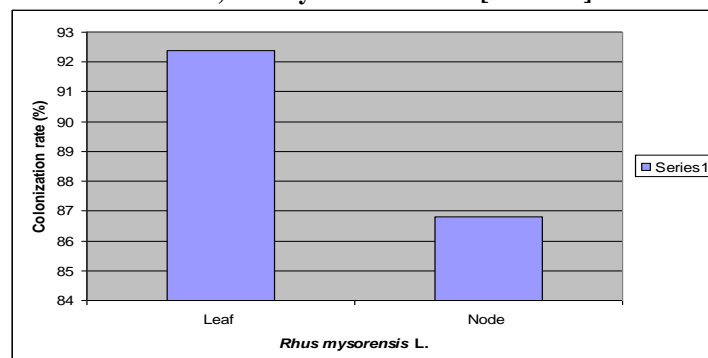


Figure 3: Colonization Rate (CR) of endophytic fungi in different explants of *Rhus mysorensis* L. (Leaf and Node) [Table- 3]

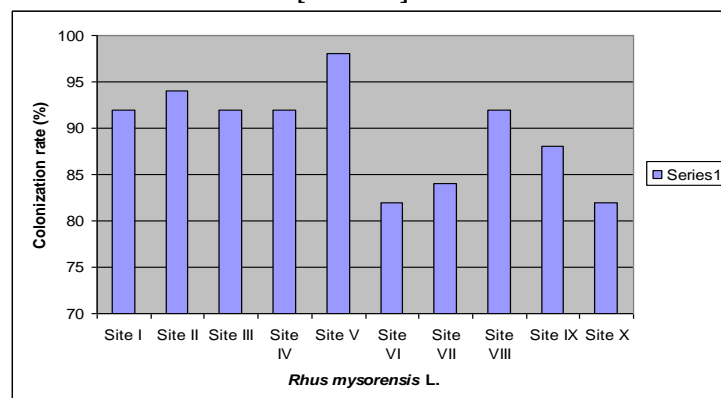


Figure: 4 Colonization Rate (CR) of fungal endophytes in different sites of *Rhus mysorensis* L. [Table- 1]

### Discussion

Host plants and endophytic fungi are symbionts, in which hosts and endophytes benefit from each other. Plants provide nutrition and protection to their endophytes in return, endophytes excrete functional products and increase in their host resistance to biotic and abiotic stresses.

**Table: 1 The overall colonization rate (%) of endophytic fungi of different locations from the plant *Rhus mysorensis* L.**

Location	Type of Tissues examined	No. of Samples	Samples yielding Isolates	Colonization Rate (%)
Site I	Leaf/ Node	50(25/25)	46 (23/23)	92
Site II	Leaf/ Node	50(25/25)	47 (24/23)	94
Site III	Leaf/ Node	50(25/25)	46 (22/24)	92
Site IV	Leaf/ Node	50(25/25)	46 (24/22)	92
Site V	Leaf/ Node	50(25/25)	49 (25/24)	98
Site VI	Leaf/ Node	50(25/25)	41 (21/20)	82
Site VII	Leaf/ Node	50(25/25)	42 (22/20)	84
Site VIII	Leaf/ Node	50(25/25)	46 (24/22)	92
Site IX	Leaf/ Node	50(25/25)	44 (24/20)	88
Site X	Leaf/ Node	50(25/25)	41 (22/19)	82
Total	Leaf/ Node	500(250/250)	448(231/217)	89.6

**Table: 2 Occurrence and distribution of fungal endophytes isolated from the plant *Rhus mysorensis* L.**

Fungal genera / Morphospecies	Location										Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	
<i>Alternaria</i> sp.	4	3	5	6	4	3	5	6	4	7	47
<i>Curvularia</i> sp.	3	2	1	3	4	1	1	2	2	1	20
<i>Fusarium</i> sp.	9	11	15	12	13	12	15	10	11	10	118
<i>Colletotrichum</i> sp.	6	5	3	4	4	6	8	6	4	7	53
<i>Cladosporium</i> sp.	0	0	1	2	1	2	1	2	0	0	9
<i>Helminthosporium</i> sp.	2	3	2	4	2	3	0	3	3	2	24
<i>Phomopsis</i> sp.	0	0	1	0	1	1	0	3	1	2	9
<i>Aspergillus</i> sp.	2	1	1	2	3	2	1	4	2	1	19
Mycelia Sterilia 1	6	4	4	3	5	5	4	4	4	4	43
Mycelia Sterilia 2	2	4	5	4	3	4	6	5	2	2	37
Mycelia Sterilia 3	3	4	6	2	6	2	0	0	5	2	30
Mycelia Sterilia 4	4	3	2	3	2	0	0	1	3	3	21
Mycelia Sterilia 5	2	4	0	1	1	0	1	0	1	0	10
Mycelia Sterilia 6	3	3	0	0	0	0	0	0	2	0	8
Total	46	47	46	46	49	41	42	46	44	41	448

**Table 3: The maximum endophytic colonization in specific tissue of the plant *Rhus mysorensis* L.**

Plant Sample	Type of tissue with maximum Colonization	Colonization Rate (%)
<i>Rhus mysorensis</i> L.	Node	86.8
<i>Rhus mysorensis</i> L.	Leaf	92.4

**Table 4: The number and taxonomic identification of endophytic fungi isolated from different locations (*Rhus mysorensis* L.)  
Al: *Alternaria* sp.; Co: *Colletotrichum* sp.; Fu: *Fusarium* sp.; As: *Aspergillus* sp.; Ph: *Phomopsis* sp.; He: *Helminthosporium* sp.; Cl: *Cladosporium* sp.; Cu: *Curvularia* sp.; MS: *Mycelia Sterilia*.**

Location	Al	Co	Fu	As	Ph	He	Cl	Cu	MS	Total
Site I	04	06	09	02	-	02	-	03	20	46
Site II	03	05	11	01	-	03	-	02	22	47
Site III	05	03	15	01	01	02	01	01	17	46
Site IV	06	04	12	02	-	04	02	03	13	46
Site V	04	04	13	03	01	02	01	04	17	49
Site VI	03	06	12	02	01	03	02	01	11	41
Site VII	05	08	15	01	-	-	01	01	11	42
Site VIII	06	06	10	04	03	03	02	02	10	46
Site IX	04	04	11	02	01	03	-	02	17	44
Site X	07	07	10	01	02	02	-	01	11	41
Total	47 (10.49 %)	53 (11.8%)	118 (26.3%)	19 (4.24 %)	09 (2 %)	24 (5.35%)	09 (2 %)	20 (4.46 %)	149 (33.25 %)	448

The living aerial parts of all plants may have mutualistic internal endophytic fungi, which exhibit momentous part of fungal diversity. Endophytic fungi of the plant *Rhus mysorensis* from different locations of sanganer region exhibit high diversity (Tiwari, 2012; Singh *et al.*, 2009; Zou *et al.*, 2000; Murali *et al.*, 2006; Tiwari & Chittora, 2013; Tiwari, 2013).

A total of 448 endophytic fungal isolates were obtained from ten different locations of sanganer region. These endophytic fungal isolates were classified into eight fungal genera and six types of mycelia sterilia [Table-2, figure-2]. Most of these fungi belonged to *Alternaria* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., *Phomopsis* sp., *Curvularia* sp., *Cladosporium* sp. and *Helminthosporium* species.

The maximum colonization rate obtained was of *Fusarium* species (*Fusarium oxysporum* & *Fusarium roseum*) [Figure 1 (b-g)]. Ecologically as a fungal endophyte *Fusarium* species was diversified in most of the locations of sanganer region of rajasthan in this plant (Tiwari, 2012; Singh *et al.*, 2009). However, these endophytic fungi exhibited various colonization rate (CR). The evidence of host preference, tissue specificity and spatial heterogeneity was found in endophyte distribution based on fungal community compositions and colonizing frequencies (Zou *et al.*, 2000; Murali *et al.*, 2006; Tiwari & Chittora, 2013; Tiwari, 2013). Mycelia sterilia was a large group of fungi which failed to sporulate and was ubiquitous in plant endophytic isolation.

### Conclusion

Endophytic fungal communities from different habitats are indeed different: communities associated with Sanganer region of Rajasthan are poorly investigated group of microorganisms that represents an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agriculture and industrial arenas. In the above investigation, the most frequently isolated endophytic fungal communities were *Fusarium* species which are dominantly associated with the darsan plants.

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