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STUDY OF ENDOPHYTIC FUNGAL COMMUNITY FROM BARK OF *VENTILAGO MADRASAPATNA* GAERTN.

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ABSTRACT

Endophytic fungi were isolated from the inner bark of *Ventilago madrasapatna,* a well-known medicinal plant of India. It was investigated for endophytic mycoflora as a possible source of bioactive secondary metabolites. A total 66 isolates of 14 species belongs to 5 classes, were studied adopting a standard isolation protocol. The colonization frequency of the endophytic fungi was reported as 87.84%. Fungus composition included 6.25% Eurotiomycetes, 9.3% Dothideomycetes, 14.26% Soradariomycetes, 11.08% Ascomycetes, 2.6% Leotiomycetes and 1.3% isolates were classified under Mycelia sterilia. The sterile endophytic fungi presently reported are expected to add to the list of new fungal species. Among the endophytic flora, *Fusarium oxysporum was* found to be the core-group fungus with a colonization frequency of 34.22%.*Cladosporium cladosporiodes* and *Botrytis sp*. are present only in bark of plant collected in Belur forest region. These results indicated that distribution of endophytic fungi is mainly influenced by environment factors.

KEY WORDS: ***Ventilago madrasapatna*, Endophytic fungi, Bark**

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INTRODUCTION

Endophytes are microbes that colonize the living internal tissues of plants without producing any symptoms or negative effects[1]. Fungi that are biotrophic mutalists, benign commensals or latent pathogens are included under the broad term 'Endophytes'[2]. During 1940s these fungi were first noticed, but only at the turn of the 21st century the ubiquity of these fungi fully recognized. Endophytes are constantly exposed to inter-generic–genetic exchange with the host plant. Isolation of a potent anticancer agent, taxol from *Pestalotiopsis* microspora[3], *Pestalotiopsis breviseta*[4], an endophyte of the yew tree and the phytohormone-producing fungus from rice plant, *Gibberella fujikuroi*[5] suggests the potential of endophytes as a source of useful metabolites. Medicinal plants are reported to harbor endophytes[6], which in turn provide protection to their host from infectious agents and also provide adaptability to survive in adverse environmental conditions. It is therefore important to determine the endophyte diversity of medicinal plants. Endophytes colonized plants often grow faster than non-colonized ones[7]. Observed biodiversity of endophytes suggests that they can also be aggressive saprophytes or opportunistic pathogens at some stages[8]. At present, endophytes are viewed as outstanding source of novel bioactive natural products because many of them occupying literally millions of unique biological niches (higher plants) growing in so many unusual environments [9]. *Ventilago madrasapatna (*Gaertn.) is a woody liana belonging to the family Rhamnaceae. It is commonly known as Red creeper. This plant is a woody climber. Traditionally, the root bark of *V. madraspatana* is used as a carminative, stomachic, vitiated conditions of kapha, dyspepsia, colic flatulence, erysipelas, leprosy, scabies, and pruritis. The powdered stem bark mixed with gingelly oil is applied externally to treat skin diseases and itch[10]. Apart from these, antifeedant activity of *V. madrasapatna* on insects was also reported[11]. The phytochemical reports on the root bark of *V.madrasapatna* show the presence of various anthraquinones, including ventinone-A.B, chrysophanol, physcion, emodin, islandicin, xanthorin and xanthorin-5-methyl ether[12]. Naphthalene derivatives and naphthoquinones, such as ventilaginone, ventilagol, maderone, cordeauxione and isocordeauxione, are also reported from root bark of this plant[13-14] ventiloquinones E, G & J[15]Ventiloquinones F & iso-ventlone[16] Ventiloquinones L & Elutherin[17]. The plant extracts act as an antibacterial agent[18], Anti-denaturation property and anti-oxidant property[19]. The stem-bark of *V. madrasapatna* was found to possess anti inflammatory and anticancer activities[20]. Based on the recent claims that endophytic microbes may play a key role in therapeutic properties of plants, we postulate that the healing properties may be due to the secretion of metabolites from the endophytes residing in the bark. Hence an attempt has been made to isolate endophytic mycoflora from the bark of the *V. madrasapatna*, a widely used medicinal plant and has not been reported earlier.

MATERIALS AND METHODS

Plant material collection

Bark of *V. madrasapatna* was collected from different locations at Western Ghats forest present in Hassan district Karnataka, India. Healthy and mature plants were carefully chosen for sampling. The samples were brought to the laboratory in sterile bags and processed immediately to reduce the risk of contamination.

Isolation of endophytic fungi

The samples were rinsed gently in running tap water to remove dusts and debris. The bark was cut into segments (1x2 cm).The samples were surface sterilized by modified method[21].

The samples were immersed in 70% ethanol for 5 s, followed by 4% sodium hypochlorite for 90 s and then rinsed in sterile distilled water for 10 s. The excess moisture was dried on a sterile filter paper. The surface sterilized segments were placed in petridishes containing PDA medium. The petridishes were sealed using parafilm and incubated at 26 ± 1°C at 12-h light/dark cycle. The Petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments. The endophytic fungi were identified according to their macro and microscopic structures. Species level identification was done with the fungi identification manuals .[22-26].

Statistical analysis

The colonization frequency (%CF) of endophytic fungi was calculated using the formula given by Hata and Futai [27]. Samples were incubated and growth was examined.

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

Where, N_{col} = Number of segments colonized by each fungus

N_t = Total number of segments studied

RESULTS AND DISCUSSION

Current study was carried out on *V.madrasapatna*, an important medicinal plant. There is no data on the diversity of fungal endophytic communities associated with the *V. madrasapatna*. In this present investigation a total of 66 fungal isolates were obtained from stem bark of *V.madrasapatna,* representing 14 endophytic fungal taxa. The colonization frequency was 87.8%. The stem bark collected from showed variation in their endophyte fungal colonization different region differed in their endophytic fungal colonization (Table 1). The colonization of the endophytic fungi is ubiquitous yet selective in nature and this selective colonization of the endophytic may lead to the production of special compounds within the host plant[28].The endophytic colonization presented in this experiment was found in Monsoon season (July to October) in the plant. The colonization frequency of endophytic fungi may vary according to seasons. The environmental conditions under which the host is growing also affect the endophytic population[29]. An excellent example for this is the anticancer drug taxol, which was previously supposed to occur only in the plant tissues[8]. Endophytes colonizing inside plant tissues usually get nutrition and protection from the host plant, and in response they produce some functional metabolites, which enhance the host fitness, anti-feedant activities, resistance against stresses as well as quality of the products[30]. The isolation of fungal endophytes in different region was not uniform. This variation of fungal endophytes is mainly influenced by environment factors and type of host tissue[31]. The plant was highly colonized by *Fusarium oxysporum* showing 34.2% colonization frequency, and stood number one among all fungal taxa recovered from this host. In this study, members of Soradariomycetes were found more abundant than those of Eurotiomycetes and others. The numbers of fungal isolates recovered from different region bark were 72, 67, and 59 respectively. Isolate recovery from different fungal groups was maximum 14.26% of Soradariomycetes, followed by 11.08% Ascomycetes, 9.3% Dothideomycetes, 6.25% Eurotiomycetes and 2.6% Leotiomycetes respectively (Fig 1). However, 1.3% isolates remained unidentified and could not produce spores, which provide the bases for identification; therefore, they have been listed under Mycelia-Sterilia. In this plant bark, *Fusarium, Aspergillus*, and *Alternaria* sp. were the dominating genera. *Fusarium* sp followed by *Aspergillus* sp and Alternaria sp. which were dominant in all bark samples, Isolates of *Cladosporium cladosporioides* and *Botrytis sp* were recovered from only in the Belur forest area. Whereas another genera *Trichoderma* sp. was confined to Gundya forest area and Charmudi

ghat region, *Fusarium campactum* confined Gundya forest area and Belur forest area. Apart from these sterile mycelia also shows the ubiquitous distribution, sterile mycelia is present in all the samples, except sterile mycelium 1 and sterile mycelium (hyaline) in Belur forest area. Four sterile mycelia that differ in shape and size of chlamydospore, breadth and color of mycelium, branching of hyphae etc. Characteristically they do not reproduce either by asexual method or through conidial formation even cultured in well defined media, there by represent a new phylogenetic lineage of fungi and also contribute to broad spectrum of exploring fungal biodiversity. The phytochemical reports on the root bark of *V.madrasapatna* show the presence of various anthraquinone, including ventinone-A.B, chrysophanol, physcion, emodin, islandicin, xanthorin and xanthorin-5-methyl ether[12]. Emodin is known to occur in at least 17 plant families worldwide, and has been investigated for antibacterial[32-34], antifungal[35], antiparasitic[36],antiviral[37-38],anti-inflammatory[39], analgesic[40], antioxidant[41-42],immunosuppressive[43],antiulcer[44], hepatoprotective[45]. Emodin is an anticancer pro-drug [46-47]. Some of the endophytic fungus *Thielavia subthermophila* have the capacity to convert hypercin to emodin in the plant *Hypericum perforatum* [46] (Kusari et al 2008), hence we are trying to identify the endophytic fungus responsible for the emodin synthesis in the *V.madrasapatna.*

Figure-1
Percentage recovery of Endophytic isolates from different fungal groups in different regions

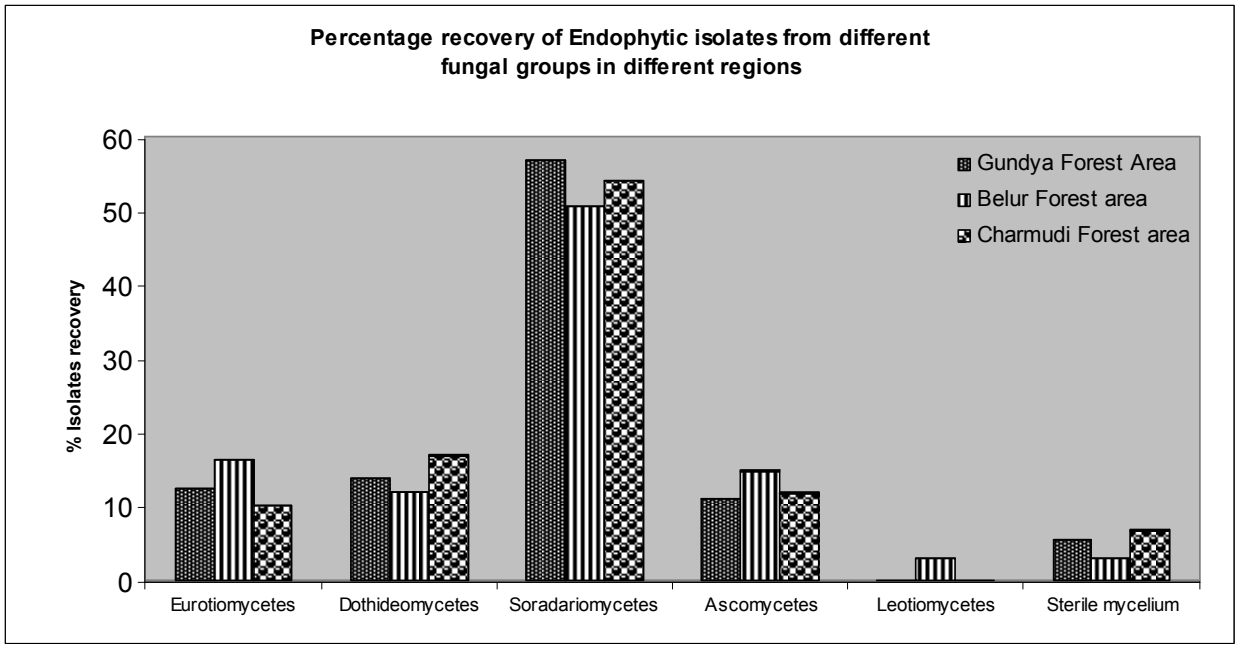

Table-1
Endophytic fungi isolated from different places of Western Ghats of Karnataka

S.n	Class	Endophytes	Gundya forest area			Belur forest area			Charmudi area		
			No. of Isolates	CF	Dominant fungi	No. of Isolates	CF	Dominant fungi	No. of Isolates	CF	Dominant fungi
1	Eurotiomycetes	*Aspergillus niger*	4	5.3	5.5	6	8	8.9	3	4	5.08
2	Eurotiomycetes	*Aspergilus oryzae*	5	6.6	6.9	5	6.6	8.9	3	4	5.08
3	Dothideomycetes	*Alternaria sp.*	10	13.3	13.8	3	4	4.4	10	13.33	16.9
4	Dothideomycetes	*Cladosporium cladosporioides*	-	-	-	5	6.6	7.4	-	-	-
5	Soradariomycetes	*Fusarium campactum*	8	10.6	11.1	4	5.3	5.9	-	-	-
6	Soradariomycetes	*Fusarium oxysporum*	25	33.3	34.7	27	36	40.29	25	33.3	42.37
7	Soradariomycetes	*Fusarium solani*	3	4	4.16	3	4	4.47	3	4	5.08
8	Soradariomycetes	*Trichoderma sp.*	5	6.6	6.9	-	-	-	4	5.3	6.7
9	Ascomycetes	*Trichothecium roseum*	8	10.6	11.1	10	13.3	14.9	7	9.3	11.8
10	Leotiomycetes	*Botrytis sp.*	-	-	-	2	2.6	2.9	-	-	-
11		*Sterile mycelium 1*	1	1.3	1.38	-	-	-	1	1.3	1.69
12		*Sterile mycelium 2*	1	1.3	1.38	1	1.3	1.49	1	1.3	1.69
13		*Sterile mycelium (hyaline)*	1	1.3	1.38	-	-	-	1	1.3	1.69
14		*Sterile mycelium* (brown)	1	1.3	1.38	1	1.3	1.49	1	1.3	1.69
		Total	**72**	95.7		67	89.24		59	78.5	

CONCLUSION

It is evident from available literature that, most endophytic fungi isolated from plants of medicinal value, often produced or induced either novel bioactive compounds or antimicrobials. Therefore, we selected this host (*V. madrasapatna*) for the present study and isolated a good number of fungi which were distributed in uneven fashion of this medicinal plant. Currently, we are involved in the screening of these fungi in the hope of obtaining novel bioactive compounds.

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