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Isolation and diversity of fungal endopyhte from tuberous rhizome *Curculigo orchioides* Gaertn

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Abstract

Curculigo orchioides Gaertn a tuberous monsoon perennial plants belonging to family Hypoxidaceae. It is commonly or locally known as "Kali Musali". It is well known traditional medicinal plant. The tuberous rhizomes are slightly bitter mucilaginous, tonic and used against fever, diarrhoea, jaundice, asthma etc. *Curculigo orchioides* of family Hypoxidaceae collected at Swami Ramanand Teerth Marathwada University Campus area (Latitude: 19.099469, Longitude: 77.287924). The present investigation deals with diversity of Endophytic fungi in the tuberous rhizome. In all twenty two fungal species were isolated by using PDA and Czepak Dox Agar medium. The dominant genera were *Penicillium*, *Aspergillus*, *Mucor* and *Alternaria*. The Endophytic species have potential for active biomolecules.

Keywords: *Curculigo orchioides*, endophytic fungi, Hypoxidaceae, Kali Musali

1. Introduction

The present research highlights on isolation of endophytic fungi from the tuberous rhizome *Curculigo orchioides* gaertn belonging to family *Hypoxidaceae* is an well known herb in traditional Indian system *orchioides* gaertn of medicine commonly named (kali musali) Golden eye grass or black musali in English or kali musali in Marathi, kalimusali in Hindi or talamuli in Ayurveda and Nilapani in Siddha system of medicine. The plant is rhizomatous, mansoon perennial with plicate leaves flowering in july to august occurs in forest floors of moist deciduous forest as well as in open lateritic rocky plateaus, among grasses up to 2310 meter in various states in India. In Maharashtra it has been reported from Konkan, Western Ghats and Marathawada. The tuberous rhizome is slightly bitter mucilangenous, tonic, alternative demulcent, used in fever, diarrheoa, jaundice, asthma, poultice is applied for skin diseases to reduce itching. The powdered rhizome is applied to stop bleeding to dry up to wounds. The plant is propagated vegetatively by root stock cuttings and through seeds. The rhizome has also been found to be useful in diabetes singly as well as a compound drug. leaves are used in treatment of cancer. In present investigation the endophytic fungi will be isolated from the tuberous rhizome. Fungi will be isolated by using standard techniques. The endophytic fungi will be identified by using pathological literature. The work focus specifically on working towards diversity of endophytic fungi from tuberous rhizome of *Curculigo orchioides*.

Synonyms

English	: Black musale;	Sanskrit	: Bhumitila, Arshoghni;
Marathi	: Kali musali;	Hindi	: Syahmusali, Muslika
Gujrati	: Kali musali,	Tamil	: Nilappanai

Taxonomical hierarchy

Kingdom	-	Plantae
Division	-	Magnoliophyta
Class	-	Monocotyledon
Order	-	Liliales
Family	-	Hypoxidaceae
Genus	-	<i>Curculigo</i>
Species	-	<i>orchioides</i>



Fig 1: Plant sample

Endophytic fungi are known to reside within plant tissues without producing overt symptoms (Bills 1996) [8], being found in almost all plants and several benefits upon their hosts this group of fungi symbolizes a unexplored resource of novel compounds, with application in various fields agriculture, medicine, pharmaceuticals, applied sciences and technology

$$\text{Colonization frequency of endophyte } 0/0 = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

Fungi were grown on specified media under specified culture condition, for identification. The fungi were identified on the basis of their morphological and cultural characteristics (Kenneth *et al.*, 1965 Sutton, 1980) [51, 24].

3. Result

The present research isolation of endophytic fungi occur in *Curculigo orchiooides* of family Hypoxidaceae collected at

2. Materials and methods

Sampling: *Curculigo orchiooides* plants material collected at Swami Ramanand Teerth Marathwada University Campus area in sterial polythin bags;

Surface sterilization and incubation: Isolation of endophytic fungi was done according to the method described by Petrini (1986) [31] Aneja (2010). The plants samples were rinsed gently in running water to remove dust and debris. Tuberos rhizome samples were cut into 0.5-1.0 cm long pieces. Each sample was disinfected with 75% ethanol for 1 min followed by immersion in Sodium hypochlorite (NaOCl 1-13% for 3-10 minutes, depending on the type of samples) and then once again in 75% ethanol for 30 seconds. The segments were then rinsed three times in sterile distilled water and the pieces were blotted-dry on sterile blotting paper. The efficiency of surface sterilization procedure was ascertained for every segment of tissues as per method of Schulz *et al.*, (1993) [40]. About 5-6 segments were placed on Potato dextrose agar (PDA) supplemented with streptomycin (100 µg ml⁻¹) in aseptice condition. The petri dishes were sealed with parafilm and incubated at 27±2oC for 4-6weeks. The Petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments. Most of the fungal growth was initiated within 10 days of inoculation. The fungi that grew out from the segments were periodically isolated and identified by transferring the hyphal tips to fresh PDA plates. Pure cultures were maintained on PDA slants. Endophytic fungi were identified according to their macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore morphology. Colonization Frequency (CF) was calculated as described by Suryanarayanan *et al.*, (2003) [48]. Briefly proper time of incubation was given for CF counting.

Swami Ramanand Teerth Marathwada University Campus area. The study of endophyte distribution, biodiversity and their biochemical characteristics are of immense importance in plant biology to understand and to improve plant fitness. Twenty two species belonging to 12 genera of fungi were isolated during the present studies. Most dominant endophyte genera were *Penicillium*, *Aspergillus*, *Mucor* and *Alternaria*.



Fig 2: Fungal endophytes isolated from tuberous rhizomes of *Curculigo orchiooides* Gaertn

Table 1: Fungal endophytes isolated from tuberous rhizomes of *Curculigo orchoides* Gaertn. And their colonization frequency.

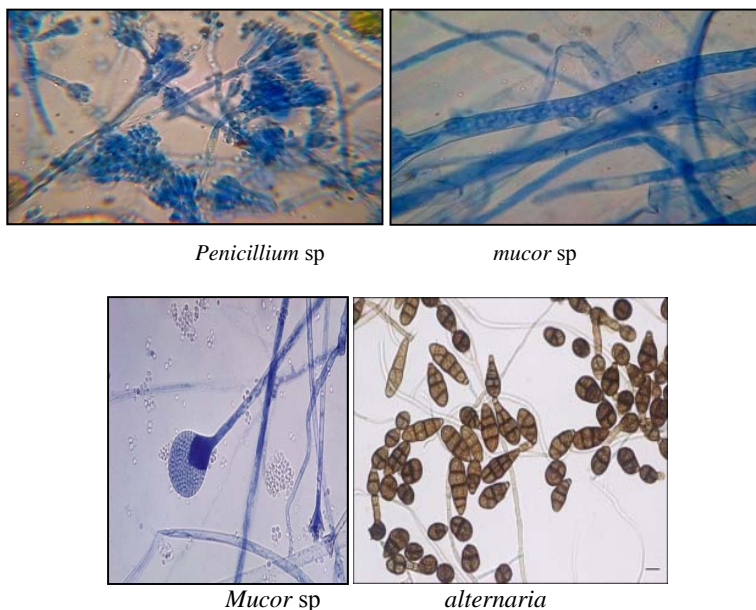
Sr. No	Fungal Endophytes	Number of isolates	Frequency of Colonization %
1	<i>Aspergillus flavas</i>	4	6.6
2	<i>A. fumigatus</i>	3	5
3	<i>A. niger</i>	6	10
4	<i>A. nidulans</i>	5	8.3
5	<i>A. oryzae</i>	3	5
6	<i>A. tamaraii</i>	4	6.6
7	<i>Alternaria alternata</i>	4	6.6
8	<i>Alternaria solani</i>	5	8.3
9	<i>Mucor sp</i>	3	5
10	<i>Mucor hiemalis</i>	3	5
11	<i>Penicillium sp</i>	3	5
12	<i>P. chrysogenoum</i>	4	6.6
13	<i>Phoma sp</i>	3	5
14	<i>P. hedericola</i>	3	5
15	R M (Sterile mycelium)	2	3.3
16	R M (Sterile mycelium)	2	3.3
17	R M (Sterile mycelium)	1	1.6
18	R M (Sterile mycelium)	1	1.6
19	RM (Sterile mycelium)	1	1.6
20	RM (Sterile mycelium)	1	1.6
21	RM (Sterile mycelium)	1	1.6
22	RM (Sterile mycelium)	1	1.6

60 segments were plated for analyzing the frequency in tuberous rhizome respectively.

4. Discussion

Endophytic organisms have received considerable attention as they are found to protect their host against pest, pathogens and even domestic herbivorous (Weber, 1981) [56]. Only a few plants have been investigated for their endophytic flora and their potential to produce bioactive compounds. Some studies have been conducted about the endophytic biodiversity, taxonomy, reproduction, host ecology and their effects on host (Faeth *et al.*, 2004; Petrini, 1986; Dayle *et al.*, 2001; Redman *et al.*, 2002; Clay & Schardl, 2002) [16, 32, 13, 34, 12]. Currently, endophytes are considered as unexplored source of bioactive natural compounds. They have been found to play a crucial role in the production of beneficial chemical compounds. Diverse endophytic population was detected to colonize this plant.

fungal strains of 22 different species were isolated. The endophytic fungal communities found at three different sites were different area of campus. Herbal medicine is one of the oldest forms of health care known, every plant on earth is known to harbor at least one endophytic microbe. Endophytic fungi are one of the most unexplored and diverse group of organisms having symbiotic associations with higher life forms and may produce beneficial substances for host (Weber, 1981, Rezwana *et al* 2007) [56, 33]. *Alternaria sp*, *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *penicillium citrinum*, *mucor* (Blodgett *et al.*, 2000; Suryanarayanan *et al* 1998, 2002) [9, 46] were isolated as endophytes in the present study which have been previously reported as endophytes. Previous studies reported distinct endophyte community compositions in different host plants suggesting host preference (Cannon and Simmons, 2002) [11].



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