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Research Article

BIODIVERSITY OF ENDOPHYTIC FUNGI IN LEAVES OF *CARISSA CARANDAS* LINN. FROM CENTRAL REGION OF MADHYA PRADESH

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ABSTRACT: *Carissa carandas* Linn, a widely used medicinal plant in India, were exploited for endophytes as a possible source of bioactive secondary metabolites. All isolates were identified based on colony morphology and examination of spores and fruiting bodies using stereo and light microscopes. Total 126 endophytic fungi of 12 genera were isolated from 300 segments of fresh *Carissa caranda* leaves collected from central region of Madhya Pradesh, India. The endophytic fungi recovered belong to ascomycetes (6.34%), coelomycetes (15.07%), hyphomycetes (51.58%) and sterile mycelium (26.98%) each. The most dominant endophytes observed were *Aspergillus flavus* (12.69%), *Trichoderma spp.* (11.11%) and *Pestalotiopsis* spp. (10.31%).

Keywords: *Carissa carandas*, Endophyte, Endophytic fungi, Natural product, Secondary metabolites

INTRODUCTION

Carissa carandas Linn. (syn. *Carissa congesta* Wight) is a large dichotomously branched evergreen, indigenous shrub with short stem and strong thorns in pairs, belonging to the family Apocynaceae. The plant is precious for *Ayurveda*, the Indian system of medicine. It is native and common throughout much of India and Africa, called karaunda in India; namdaeng in Thailand; caramba, caranda, caraunda and perunkila in the Philippines **(**Jain, 1991**)**. It is grown commonly as a hedge plant. This species is woody, straggly, climbing shrub, typically growing on height of 3-5 m from the surface; and rich in white, sticky latex. The branches are abundant and spreading, forming thick masses situate with pointed thorns. *C. carandas* is an evergreen tree growing in tropical to subtropical regions, semiarid to wet tropical regions with anti-microbial, antiviral, cardiotonic, anti-convulsant, antipyretic, and against cancer **(**Jigna *et al*., 2005; Robins *et al*., 1996; Vohra *et al*., 1963; Hegde *et al*., 2009; Taylor *et al*., 1995; Sulaiman *et al*., 2008).

Endophytic fungi are under-explored group of microorganisms that live within a plant's tissue without causing any symptoms or apparent injury to host. Dreyfuss and Chapela (1994) predicted that there may be at least one million species of endophytic fungi residing in plants. A large number of endophytic fungi pertain to Ascomycetes and Fungi Imperfecti (Huang *et al*., 2001). They are of biotechnological importance as new useful characteristics which may be found by further exploration. A number of endophytic organisms harbour on all plant species (Tan and Zou, 2001).The colonization of endophytic fungi in plant tissues occurs in a manner similar to those of plant pathogens and mycorrhizae (Lumyong *et al*., 2004). Colonization comprises a series of steps involving host recognition by the fungus, spore germination, penetration of the epidermis and tissue colonization.

Mostly, endophytic fungi had been studied in temperate regions and are found extensively still fungal biodiversity remains ambiguous (Rodrigues, 1994; Wilson and Carroll, 1994; Fröhlich and Hyde, 1999). Tropical endophytic fungi are most likely conservative and much less understood.

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Central region of Madhya Pradesh lies in between latitude 22º 27' to 23º 53' N and 77º 08' to 79º 43' E longitude and falls under tropic region. It has a rich microbial diversity as of different climatic factors at various altitudes giving rise to well-off and luxuriant vegetation which is amongst the richest in Central India. The occurrence of biodiversity makes the area unique and the association of endophytic fungi with tropical plant species of this region has not yet been explored. Fungi from this unique habitat have been shown to be the sources of new species, possibly containing new bioactive compounds. The various practical applications of these endophytes; potent anticancer agent, paclitaxel from *Metarhizium anisopliae*, an endophyte of the *Taxus chinensis* and anti-microbial agent cephalosol from *Cephalosporium acremonium* isolated from *Trachelospermum jasminoides* suggests the potential of endophytes as a source of useful metabolites (Liu, 2009; Zhang, 2008), as biocontrol agents in plant protection, sources of novel metabolites for therapeutics and as model systems for studying the host parasite interactions in natural ecosystems (Strobel, 2004; Deshmukh, 2009; Deshmukh, 2009). The present study was carried out to determine the endophytic flora from leaf tissues of *C. carandas* from central region of Madhya Pradesh, India.

MATERIAL AND METHODS

During the monsoon of 2011 (July to September), leaf samples of *C. carandas* Linn. were collected from the area of Central region of Madhya Pradesh. The samples placed in labeled sterile polyethylene bags, transported in icebox to the laboratory and placed in a refrigerator at 4°C. All samples were processed within 24 hours of collection. They washed thoroughly in running water and air dried in laminar airflow. Leaf samples were first immersed in sodium hypochlorite (3.5 % v/v) for 3 min followed by second immersion in 70% ethanol (v/v) for 1 min. The samples rinsed three times in sterile distilled water and dried on sterile blotters under laminar airflow to ensure complete drying. With the help of a sterile scalpel, about 300 segments from leaf samples of 2.5x2.5 mm size were excised and the inner tissues are carefully placed on to Potato Dextrose Agar (PDA) supplemented with streptomycin (100 mg/l) to suppress bacterial growth. The absence of growth of any fungi on the medium, by pressing the sterilized leaf on to the surface of PDA medium, confirms that the sterilization procedure was effective in removing the epiphytes (Strobel, 2002; Schulz, 2002). The plates were incubated at 25 ± 1°C up to 6 weeks (Suryanarayan, 2002; Bills, 2002). The colonies were periodically examined and for identification each colony that emerge was transferred to antibiotic-free Potato Dextrose Agar medium (PDA). Endophytic isolates were identified on the basis of culture characteristics, morphology of fruiting body and spores. The percent frequency of occurrence was calculated as described by Suryanarayanan *et al.* (2003), the number of leaf segments colonized by a specific fungus divided by total number of segments plated x 100 and dominant endophytes were calculated as percentage colony frequency divided by sum of percentage of colony frequency of all endophytes x 100 (Fisher, 1987; Kumaresan, 2002).

RESULTS AND DISCUSSION

A total of 126 endophytic fungi from the leaf tissue of *C. carandas* belonging to 12 genera were isolated and identified by their morphology and spore characteristics from central region of Madhya Pradesh, India. The colonization frequency was calculated 42.4% (Table 1). The fungal composition included to ascomycetes (6.34%), coelomycetes (15.07%), hyphomycetes (51.58%) and sterile mycelium (26.98%) each. In the tropics, only a few studies have been carried out on endophytes of tree species (Fröhlich and Hyde, 2000). We have recovered endophytic genera like *Alternaria* spp., *Fusarium* spp., *Phoma* sp. which are reported as endophytes. In this study *Aspergillus* spp., *Trichoderma* spp. and *Pestalotiopsis* spp. were the most dominant endophytes. *Aspergillus flavus* obtained as an endophyte from four mangrove plants *Avicennia officinalis* L., *Kandelia candel* (L.) Druce, *Excoecaria agallocha* L. showed good antioxidant potency (Chinnarajan Ravindran, 2012). An endophytic fungus, *Pestalotiopsis terminaliae* was isolated from *Terminalia arjuna* produce taxol, an important chemotherapeutic drug used in the treatment of breast, lung and ovarian cancers (Gangadevi, 2009).

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Table 1 Frequency of colonization of endophytic fungi isolated from *Carissa carandas*.

Endophytic Fungi	No. of Endophytes	Colonization Frequency* (%)	Dominance (%)
Ascomycetes			
Chaetomium globosum	3	1.0	2.4
Colletotrichum gloeosporioides	5	1.7	4.0
Coelomycetes			
Pestalotiopsis spp.	13	4.4	10.4
Phoma sp.	6	2.0	4.7
Hyphomycetes			
Acremonium spp.	8	2.7	6.4
Aspergillus flavus	16	5.4	12.7
Aspergillus terreus	4	1.4	3.3
Alternaria alternata	9	3.0	7.1
Fusarium spp.	8	2.7	6.4
Penicillium spp.	6	2.0	4.7
Trichoderma spp.	14	4.7	11.1
Sterile mycelia	34	11.4	26.9
Total No. of isolates	**126**	**42.4%**	

* Based on 300 segments for frequency analysis.

But, the host tissue type, seasonal variation and environment are mainly influencing factors on endophyte occurrence (Rodrigues, 1994; Halmschlager, 1993). Presently, we are pursuing fermentation of the endophytes recovered to obtain the secondary metabolites to facilitate screening against therapeutic targets.

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