IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DIVERSITY OF ENDOPHYTIC FUNGI ISOLATED FROM *TERMINALIA CATAPPA* (LINN).

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Abstract: Present investigation was carried out to find out diversity of endophytic fungi present in the *Terminalia catappa* (Linn.). Healthy plant parts were collected from different regions of Aurangabad (Maharashtra). A total 62 isolates belonging to 09 fungal taxa were obtained from 120 segments observed. Out of total endophytes isolated most of the genera were from hypomycetes. Among isolated fungi *Nigrospora* sp. was the dominant fungi. Simpson's diversity index and Shannon wiener index were calculated to know diversity in different localities.

Index Terms **- Endophytic fungi, *Terminalia catappa* (Linn.), *Nigrospora* sp, diversity index**

I. INTRODUCTION

Term endophytic fungi was used for fungi which are living in the plant tissue without any harm to the plant and they are not developing any diseases symptoms on it. They may develop protective functions or interactions in the host and microbes (Kusari *et.al*. 2012). Recently many researchers worked on potentials of endophytic fungi in various ways and they could found out many beneficial molecules from these microbes (Huang *et.al* 2007,Li *et.al*.2005). An enormous number of different fungi can be isolated from plants growing in their native habitat. Few fungi are widely distributed with the host, suggesting a long standing, close and mutually beneficial interaction. Though many endophytic fungi were isolated from different plants still there is requirement to find out new sources and information about their diversity.

Terminalia catappa Linn. (Combretaceae) is native to Southeast Asia. The generic name originates from the Latin "*terminalis*," referring to the leaves teeming at the ends of the shoots. It is a large tree that grows well in subtropical and tropical climates. *T. catappa* is a well-recognized tree in Ayurveda. The juice of its fresh leaves is used in the preparation of medicinal lotion for leprosy and scabies, and it is taken internally for stomachache and headache. Leaves contain several flavonoids, tannins , saponines and phytosterols. Due to this chemical richness, the leaves (and the bark) are used in different herbal medicines for various purposes. Because of its different uses it is known as "King of Medicine"(Upadhyay *et.al.* 2014).

Endophytic fungi were frequently isolated from other species of *Terminalia* like *T. arjuna, T. chebula, T. bellerica*, but very reraly *T. catappa* was analyzed for presence of endophytic fungi (Kouipou Toghueo *et.al.* 2019).

Despite many endophytic fungi were screened for their usefulness further diversity study is required so that one can use these fungi. Still not much endophytic diversity of *Terminalia catappa* was studied in the Aurangabad region so present investigation was carried out to know diversity of endopytic fungi.

II.MATERIALS AND METHODS

2.1. Isolation of endophytes

Healthy *Terminalia cattapa* plants were selected from five different localities of Aurangabad. These localities are Beed bypass, Shendra, Osmanpura,Bidkin and Khultabad. Leaf with midrib, leaf, stem and petiole explants were collected from each locality and 120 explants were screened for endopyhtic fungi. Explants were surface sterilized with 0.1 % mercuric chloride for two min., followed by 70% ethanol (v/v) for 2 min. and finally washed with sterile distilled water. Segments from samples of 1 x 1cm size were excised and carefully placed on PDA plates. After four days of incubation colonies were observed and photographs were taken. Fungal colonies identified and isolated on PDA. Frequently cultures are maintained by subculture method (Schulz and Boyle, 2005).

2.2Analysis of data

The colonization frequency of endophytic fungi was calculated as the number of segments colonized by a single endophyte divided by the total number of segments observed x 100 (Hata and Futai 1995). The dominant endophytes were calculated as the percentage colony frequency of a given endophyte divided by the sum of the percentage of colony frequencies of all endophytes x 100 (Kumaresan and Suryanarayanan 2002). Utilizing the data of percentage colony frequency in leaves with midrib, leaves without midribs, petiole and stem of different locations, Simpson's Diversity indices and Shannon-Wiener indices were calculated (http://www.countrysideinfo.co.uk /simpsons.htm.).

III. Results and Discussion

Endophytic fungi from leaf, leaf with midrib and stem were isolated, identified and studied for their existence. Plants were collected from five different localities. A total 62 isolates belonging to 09 fungal taxa were obtained from 120 segments observed. Out of total endophytes isolated most of the genera are from hypomycetes. Two isolates detected are from ascomycetes.

Maximum endophytes isolated from Loc 5 followed by Loc 2 and minimum endophytes were recovered from Loc 4 .Among 62 isolates, 11 isolates (2 from leaf with midrib, 3 from leaf, 1 from stem and 5 from petiole) were separated from Loc 1, 16 isolates (4 from leaf with midrib, 3 from leaf, 5 from stem and 4 from petiole) recovered from Loc 2 , While Loc 3 shows 10 isolates (2 from leaf with midrib, 3 from leaf, 3 from stem and 2 from petiole), Loc 4 exhibited only 3isolates (2 from leaf with midrib, 1from leaf) and Loc 5 recovered 22 isolates (7 from leaf with midrib, 7 from leaf, 5 from stem and 3 from petiole) (Table No. I.1, Fig 1.A).

In one of the study done by Varma *et. al.* (2007), leaf samples harboring higher colonizing percentage, but the present study indicate that stem samples as well as leaf samples are showing higher percentage of colonizing frequency. *Nigrospora* sp. was observed as the dominant endophytes followed by fungus *Colletotrichum truncatum* in total screened samples. *Aspergillus* sp. were isolated almost from all the locations. (Table No.I.2, Fig.1.B,Fig.1.C). *Phoma putaminum* and *Daldinia eschscholtzi*, *Penicillium* sp.were recorded from very few locations.

According to studies done by Khan *et.al*. 2007, most of the endophytic isolates were belonging to duteromycetes and prominent isolate was *Phoma* sp. followed by *Aspergillus* and *Penicillum*. Observations in the present study supports it as *Phoma putaminum* was recorded as one of endophytic fungi. Results from present study are in accordance with Aharwal *et al.* (2014)where they also recorded *Aspergillus*, *Alternaria* and *Curvularia* as endophytic fungi. But in this work *Curvularia* was not isolated as endophyte.

Very few studies were done occurrence and distribution of an endophytic fungi from *Terminalia catappa*. Isolation studies were done on endophytic assemblage of *Terminalia arjuna* were researchers recorded *Chaetomium , Pestalotiopsis sp., Cladosporium* sterile hyphae as endophytic isolates (Tenguria & Firodiya 2015).

According to Toghueo *et,al.*(2016) *Trichoderma* sp. isolated from *Terminalia catapa* exhibited antagonistic activity against *Fusarium* sp. In the present work *Trichoderma* was not isolated from *Terminalia catapa*.

Diveristy of endophytes vary according to host species and geographical areas. Sampling was done from different five locations around Aurangabad and it was observed that some of the fungal strains were present in nearby parallel locations. High Simpson's diversity index and low Shannon wiener index in location 1 i.e. Beed bypass region indicated higher diversity of endophytic fungi. In accordance with Simpson's diversity index and Shannon wiener index different types of endophytic fungi were obtained from Shendra region (loc 2)(Table No.I.3).

Isolated endophytic strains further can be screened for their biological activities and for secondary metabolites.

Table No.I.1: Occurrence and identification of endophytic fungi from leaf with midrib and leaf without midrib, stem and petiole samples of *Terminalia catappa* growing at five different locations

	Beed bypass				Shendra				Osmanpura				Bidkin				Khultabad			
Terminalia cattapa	LW	L	S	P	LW	L	S	P	LW	L	S	P	LW	L	S	P	LW	L	S	P
Colletotrichum truncatum	2	2		4														2		
A.flavus		1	1	1						2	1	1							2	
Alternaria						1	3		1	1	1									
A.nidulans					2	2		1			1	1								
Trichoderma atroviride					1								1							
Nigrospora													1				7	3		
Sterile hyphae					1		2		1											
Phoma putaminum								1										2	3	3
Pen chrysogenum								2												
Daldinia eschscholtzi														1						

Table No.I.2: Colonizing frequency and dominance of fungi isolated from *Terminalia catappa*

Name	Total isolates	CF	Dominance of fungi
Colletotrichum	10.0	8.3	16.1
A.flavus	9.0	7.5	14.5
Alternaria	7.0	5.8	11.3
A.nidulans	7.0	5.8	11.3
Trichoderma atroviride	2.0	1.7	3.2
Nigrospora	11.0	9.2	17.7
Sterile hyphae	4.0	3.3	6.5
Phoma putaminum	9.0	7.5	14.5
Pen chrysogenum	2.0	1.7	3.2
Daldinia eschscholtzi	1.0	0.8	1.6

Terminalia cattapa	Loc1	Loc2	Loc3	Loc5
Simpson's diversity index	0.56	0.16	0.22	0.32
Shannon wiener index	0.25	0.7	0.55	0.5
Eveness	0.84	0.9	0.92	0.83

Table No.I.3 Diversity indices of fungi isolated from *Terminalia catappa*

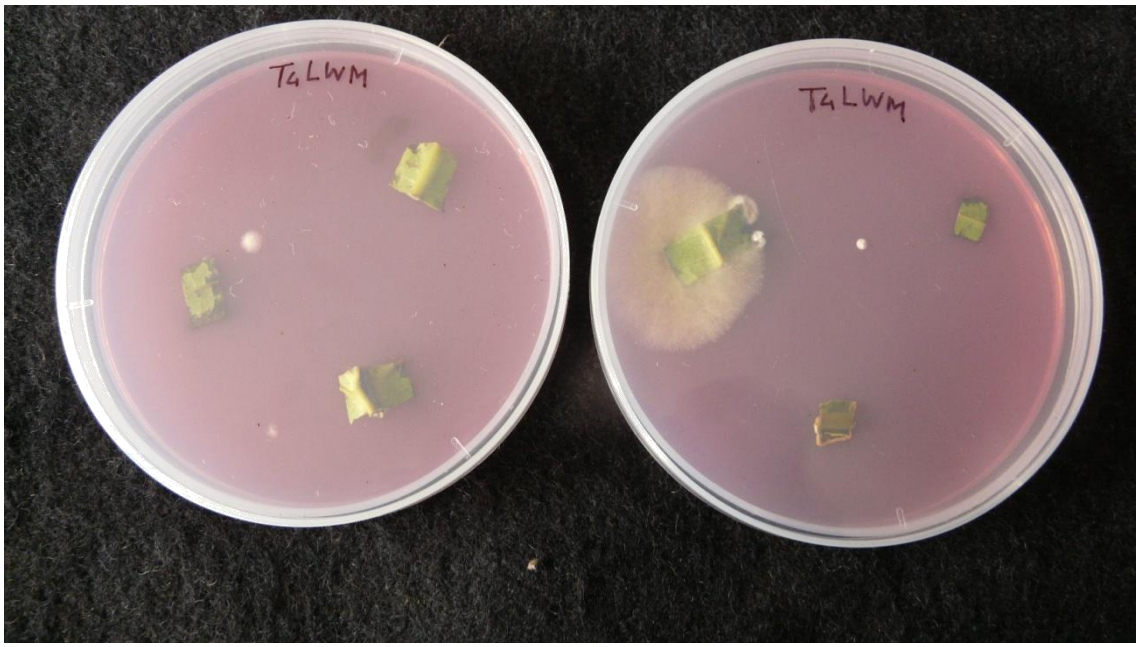

Fig.1.A.: Plates showing growth of endophytes on explants of ***Terminalia catappa***

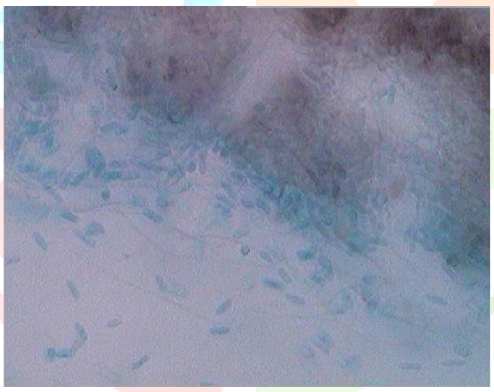

Fig 1.B. *Phoma putaminum*

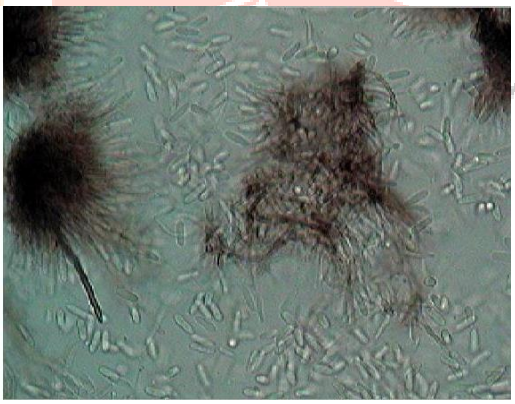

Fig.1.C.*Colletotrichum truncatum*

IV.Acknowledgement

The author is very much thankful to the Principal, Deogiri College and Marathwada Shikshan Prasark Mandal for providing the all necessary facilities. Author also extend her thanks to University Grant Commission, New Delhi for providing financial assistance as Major Research Project.

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