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Diversity of Endophytic Fungal Community in The Medicinal Plant Tridax Procumbens L.

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Abstract:

Considering that all plants appear to house endophytes with unique bioactive components and behaviors, the study of endophytic fungi from medicinal plants is a new topic. To isolate fungus endophytes, Tridax procumbens L., a popular medicinal plant in India, was used. Throughout the academic year 2020-2021, the plant was collected from several locations of the Amravati district, Maharashtra (India), and its endophytic fungi were isolated from different plant parts. Ten different fungal species including Alternaria alternata, Aspergillus fumigatus, Colletotrichum gleosporoides, Colletotrichum dematium,Curvularia lunata,Fusarium solani,Fusarium oxysporum, Helminthosporium sp., Penicillium sp. and Phoma sp. were isolated. This study reveals that Aspergillus fumigatus shows maximum colonizing frequency (20 %) in roots. Alternaria alternata shows a maximum colonizing frequency (12 %) in the stem, and Fusarium solani shows a maximum colonizing frequency (14 %) in the leaf. This methodical research showed that the traditional medicinal plant Tridax procumbens L. is an abundant source of endophytic fungi, which may be further researched for some novel bioactive chemicals.

Keywords: Endophytic Fungi, Tridax procumbens, Frequency Distribution.

Introduction:

The study of endophytes distribution, biodiversity and their biochemical characterization is of immense importance in plant biology to understand and also to improve plant fitness. The studies of endophytic fungi are of great importance as they are the source of secondary metabolites, agents of biological control, and for the development of new pharmaceutical products (Srimathi Selvanathan, et al., 2011). Almost all plant species harbor one or more endophytic organisms (Tan Zou, et al., 2001). The fungal survey of the higher plants during the last couple of decades established the assumption that all host plants harbor their own endophytic fungal community and that the endophytic colonization of the internal living tissues of the higher plant by fungi is ubiquitous (Elizabeth Arnold, et al.2001). Medicinal plants are reported to harbor endophytes 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 endophytic biodiversity of medicinal plants (Gary Strobel et al.,2003). Tridax procumbens also known as “coat buttons” is a perennial plant from the Asteraceae family, native to Central and South America (Vilwanathan Ravikumar, et al., 2005b). Since ancient times, this species has been used in Ayurveda in India (Shiva Kethamakka, et al., 2014). Different substances such as oils, teas and skin poultices, among others, have been manufactured using this species (Rosalee de la Foret,2012) Tridax procumbens L. has diverse pharmacological properties including immunomodulatory, anti-oxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities. (Vilwanathan Ravikumar et al., 2005b; Vilwanathan Ravikumar et al., 2005a; Durgacharan Bhagwat et al. 2008, Hitesh Joshi 2006, Ujawala Deogade et al.2012). The diversity of fungal endophytes has been investigated in this ethnomedicinal plant and the same is reported in this research article.

2. Material and Methods

2.1 Sampling area and collection of plant materials

Mature healthy plant materials such as leaves, stem, root, and flowers were collected from Tridax procumbens L. from different localities of the Amravati district of Maharashtra state (India). The samples

were collected during pre-monsoon (Feb-May), monsoon (June-Sept), and post-monsoon (Oct-Jan) seasons. Samples were collected from symptomless plants, placed in zip-lock plastic bags, and carefully brought to the laboratory, and processed immediately for surface sterilization. thereafter processed or stored at 4^0C so as to reduce the chances of contamination.

2.2 Processing of sample for isolation of endophytes

Collected plant samples were washed in running tap water to remove soil particles and adhered debris, and finally washed with distilled water. The stem, leaves, root, and flowers were cut into segments (0.5 – sterilized by agitating in 70% ethanol (5 s), followed by treatment with 4% NaOCl (90 s), and then rinsed in sterile distilled water (10 s). 50 segments (leaf, stem, and root samples) from the Tridax procumbens L. plant were processed for the isolation of endophytic fungi. Leaf, stem, and root segments were then placed in Petri dishes containing potato dextrose agar (PDA) media, amended with Streptomycin 150 mg/l. The Petri dishes were sealed using parafilm and incubated at 20-28°C. The efficiency of surface sterilization was ascertained for every segment of tissue following the imprint method.

2.3 Isolation of endophytic fungi (scheme for isolation only)

Endophytic fungi usually began to produce hyphal filaments after 5-6 days of incubation at 20-28^0C. The hyphal tips that appeared were carefully transferred to potato dextrose agar plates for further growth.

2.4 Colonization frequency

(CF) The colonizing frequency of each endophytic fungus was calculated as according to the formula Suryanarayanan et al. (2003).

$$CF\% = \frac{\text{Total number of isolate endophytes}}{\text{Total number of segment analysed}} \times 100$$

2.5 Fungal identification

The endophytic fungal isolates were stained with lactophenol cotton blue and were morphologically identified based on spore morphology with the help of a standard manuals (Ellis, 1971; Sutton, 1980; Onions et al., 1981; Udhayaprakash, 2004).

3. Result and Discussion

About 200 segments (50 segments of each part) of the medicinal plant Tridax procumbens were screened for the isolation of endophytic fungi. A maximum of 10 isolates of endophytic fungi were isolated from the leaf while 6 endophytic fungi were isolated from the flower (Table 1). This study reveals that Aspergillus fumigatus shows maximum colonization frequency (20%) in the root. On leaf segments maximum colonization, the frequency was shown by the endophytic fungus Aspergillus fumigatus (16%), on the stem by Alternaria alternata, Aspergillus fumigatus and Colletotrichum dematium (6%) each. The flowers were maximumly colonized by the endophytic fungus Fusarium oxysporum (6%). A maximum of 43 colonies of 10 different isolates were reported from leaf segments only while a minimum of 20 colonies of 6 isolates were shown in flower segments. Five endophytic fungi namely Aspergillus japonicus, Fusarium sp., Aspergillus niger, and Penicillium sp., were also reported in Tridax procumbens (Ravindra P.A. et al. 2018).

Seasonal distribution of endophytic fungi isolated from Tridax procumbens L. was also reported and it seems that more endophytic fungi were prevalent in the rainy and winter season than in summer (Table 5). The maximum number of isolates were found in the rainy season (Anand S. et al. 2017). Colletotrichum gleosporiodes, Colletotrichum dematium,Curvularia lunata, Helminthosporium sp., and Penicillium sp. were not reported during the summer season.

In the current study, the colonization frequency of endophytic fungi was observed in all parts (leaves, stem, root, and flower) of the plant. Aspergillus fumigatus was found in all parts of the plant. The colonization frequency of endophytic fungi was found to be more in leaves followed by stem, root, and flower. The frequency of isolation was maximum in the rainy season. Observations in our study are in conformity with the findings reported in Tridax procumbens L. (Ravindra P.A. et al. 2018, Anand S. et al. 2017).

Table 1: Isolated Endophytic Fungal Species from Tridax procumbens L.

Sr. No.	Endophytic Fungi	No. of samples inoculated (50 each)			
		Total no. of colonies isolated in leaf	Total no. of colonies isolated in stem	Total no. of colonies isolated in root	Total no. of colonies isolated in flower
1	Alternaria alternata	6	3	-	-
2	Aspergillus fumigatus	8	3	10	4
3	Colletotrichum gloeosporioides	5	2	3	2
4	Colletotrichum dematium	3	3	1	2
5	Curvularia lunata	2	1	-	-
6	Fusarium oxysporum	2	1	-	6
7	Fusarium solani	7	2	2	4
8	Helminthosporium sp.	2	1	1	-
9	Penicillium sp.	5	1	2	-
10	Phoma sp.	3	2	2	2
Total		**43**	**19**	**21**	**20**

Table 2: Colonization Frequency of Endophytic Fungi Isolated from Leaf Segments of Tridax procumbens L.

Sr. No.	Endophytic Fungi	No. of colonies grown	% Colonization frequency
1	Alternaria alternata	6	12
2	Aspergillus fumigatus	8	16
3	Colletotrichum gloeosporioides	5	10
4	Colletotrichum dematium.	3	6
5	Curvularia lunata	2	4
6	Fusarium solani	7	14
7	Fusarium oxysporum	2	4
8	Helminthosporium sp.	2	4
9	Penicillium sp.	5	10
10	Phoma sp.	3	6

Table 3: Colonization Frequency of Endophytic Fungi Isolated from Stem Segments of Tridax procumbens L.

Sr. No.	Endophytic Fungi	No. of colonies grown	% Colonization frequency
1	Alternaria alternata	3	6
2	Aspergillus fumigatus	3	6
3	Colletotrichum gloeosporioides	2	4
4	Colletotrichum dematium	3	6
5	Curvularia lunata	1	2
6	Fusarium solani	2	4
7	Fusarium oxysporum	1	2
8	Helminthosporium sp.	1	2
9	Penicillium sp.	1	2
10	Phoma sp.	2	4

Table 4: Colonization Frequency of Endophytic Fungi Isolated from Root Segments of Tridax procumbens L

Sr. No.	Endophytic Fungi	No. of colonies grown	% Colonization frequency
1	Alternaria alternata	-	-
2	Aspergillus fumigatus	10	20
3	Colletotrichum gloeosporioides	3	6
4	Colletotrichum dematium	1	2
5	Curvularia lunata	-	-
6	Fusarium solani	2	4
7	Fusarium oxysporum	3	6
8	Helminthosporium sp.	1	2
9	Penicillium sp.	2	4
10	Phoma sp.	2	4

Table 5: Colonization Frequency of Endophytic Fungi Isolated from Flower Segments of Tridax procumbens L.

Sr.No.	Endophytic Fungi	No. of colonies grown	% Colonization frequency
1	Alternaria alternata	-	-
2	Aspergillus fumigatus	2	4
3	Colletotrichum gloeosporioides	1	2
4	Colletotrichum dematium	1	2
5	Curvularia lunata	-	-
6	Fusarium solani	2	4
7	Fusarium oxysporum	3	6
8	Helminthosporium sp.	-	-
9	Penicillium sp.	-	-
10	Phoma sp.	1	2

Table 6: Seasonal Distribution of Endophytic Fungi Isolated from Tridax procumbens L

Sr.No.	Endophytic Fungi	Seasonal Variation		
		Summer	Rainy	Winter
1	Alternaria alternata	+	+	+
2	Aspergillus fumigatus	+	+	+
3	Colletotrichum gloeosporioides	-	+	+
4	Colletotrichum dematium	-	+	+
5	Curvularia lunata	-	+	+
6	Fusarium solani	+	+	+
7	Fusarium oxysporum	+	+	+
8	Helminthosporium sp.	+	+	+
9	Penicillium sp.	-	+	+
10	Phoma sp.	+	+	+

4. Conclusion

The diversity of the endophytic fungal community in the medicinal plant Tridax procumbens L. was studied in the different plant parts and a maximum number of species were reported in leaves and stems. Aspergillus fumigatus shows maximum colonization frequency in roots while Alternaria alternata in stem and Fusarium solani in leaves.

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6. Author's Biography
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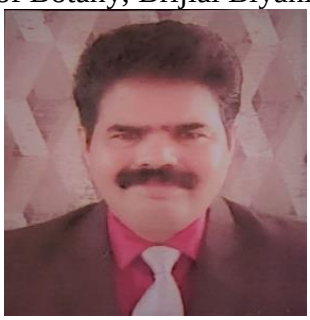

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