Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Short Communication

ENDOPHYTIC BIODIVERSITY IN *OCIMUM SANCTUM*

Faraz Khan*, Rajesh Kumar Tenguria

Division of Microbiology, Department of Botany, Govt. Motilal Vigyan Mahavidhyalaya, Bhopal, Madhya Pradesh, India

**Corresponding author: farazkhan020987@gmail.com*

ABSTRACT

Endophytic fungi are known to possess novel compounds with bioactivity and have a large biodiversity. They are found in almost all plant species. In the present study, an attempt has been made to isolate and screen *Ocimum sanctum* Linn. leaves collected from Ratapani Reserve, Bhopal and adjoining areas for endophytic fungi aseptically in PDA media. Results showed the biodiversity of endophytic fungi living in symbiotic relation with *Ocimum sanctum*. A total of 61 isolates were obtained which had hyphomycetes (78.68%) and ascomycetes (14.75%).

Keywords: Biodiversity, Endophytes, Ascomycetes, *Ocimum sanctum*, Endophytic fungi.

1. INTRODUCTION

Over the years, nature has been playing a key role in biodiversity. They are inspiring source for researchers due to their enormous structural diversity and complexity [1]. The endophytes evolved when the higher plants first appeared on earth, about a hundred million years ago. To prove this, the fossilized tissues of plants and leaves associated with microbes have been deciphered [2]. Endophytes are those microorganisms that inhabit at interior of plants especially leaves, stems, roots shows no apparent harm to host [3]. The endophytes which most often have been isolated are fungi [4]. Approximately 1.5 million fungal species exist [5] of which about 100,000 have been described. There is evidence to suggest that endophytes protect plants against diseases [6] and increase their fitness by enhancing their tolerance to abiotic stress [7, 8]. Only few plants have been investigated for endophytic biodiversity and their potential to produce bioactive secondary metabolites. Currently, endophytes are viewed as an outstanding source of bioactive natural products because many of them occupy unique biological niches growing in unusual environments.

Ratapani Reserve, Bhopal and adjoining forests have rich microbial diversity. The climatic conditions provide suitable moistured environment for growth of endophytic fungi. The occurrence of sub-tropical forest also makes the area unique [9, 10]. Endophytic fungi biodiversity mostly obtained in such climate produces active metabolites [11].

Ocimum sanctum L. (Tulsi) has been used in traditional systems of medicine for curing various ailments due to its therapeutic potential and widespread occurrence. It has medicinal value for various diseases *i.e.,* dysentery, fever and haemorrhage etc. It is widely used in Indian herbals and most promising in treatment of bronchitis and possesses antimicrobial activity [12, 13]. Thus, the study was conducted to identify the diversity and occurrence pattern of fungal endophytes in *O. sanctum* leaves.

2. MATERIAL AND METHODS

2.1. Plant Sample collection

Collection of *O. sanctum* leaves was done from Ratapani Reserve, Bhopal and adjoining areas. Samples were mobbed, sealed in sterile plastic bags after surface cleaning with ethanol; transported in ice, and stored at 4°C. Leaves were processed within 24 hours of collection.

2.2. Isolation of Endophytic fungi

The collected leaves were washed thoroughly in running water, air dried, immersed in ethanol (70% v/v) and then in sodium hypochlorite (3.5 % v/v) for 3 min. Leaf surface was sterilized with 0.01% mercuric chloride solution and washed thoroughly with sterile distilled water to remove contaminants / epiphytes and dried on sterile blotters under laminar airflow to ensure complete drying [9, 14]. From leaves samples, 200 segments were excised into small pieces approximately 0.5cm x 0.5cm in size with the help of a sterilized scalpel and the inner

tissues were placed on potato dextrose agar (PDA) media supplemented with streptomycin (100 mg/lt) to inhibit bacterial growth [10]. The efficacy of sterilization was confirmed through control plate method [14]. The plates were incubated at 25±1°C with 12 h light and dark cycle for 2-3 weeks. Periodically the colonies were examined and each colony that emerged was transferred to antibiotic free PDA media and incubated at room temperature for 10-15 days [15, 16]. Each fungal culture was checked and identified for purity and transferred to agar slants using hyphal tip and single spore isolation method [17-19].

2.3. Identification on basis of cultural and morphological characteristics

Morphological identification was based on fungal culture colony/hyphae, characteristics of spores and reproductive structures if the features were discernible [2, 20, 21]. From an actively growing culture, the hyphae were picked with sterile loop and transferred to 95% ethanol on the sterile glass slide. Then, one drop of lactophenol cotton blue stain was added and cover slip placed immediately on evaporation of ethanol, avoiding air bubble and observed under microscope [22]. Those endophytic fungi which did not sporulate even after 30 days of incubation were grouped as sterile mycelia according to their cultural characteristics [19, 23].

2.4. Analysis

The percent frequency of occurrence or colonization frequency (CF) of endophytic fungi was calculated as the number of leaf segments colonized by a specific fungus divided by total number of segments plated x 100. Dominant endophytic fungi were calculated as percentage colony frequency divided by sum of percentage of colony frequency of all endophytes x 100 [24, 25].

3. RESULTS AND DISCUSSION

Total 61 isolates were obtained from *O. sanctum* leaves samples. Out of the 05 genera recovered, 03 genera belonged to hyphomycetes (78.68%) in which *fusarium moniliforme* (44.26%) showed maximum dominance with minimum of *Alternaria alternata* (9.83%). While, ascomycetes showed *Melanospora fusispora* (14.75%) and coelomycetes was absent (table 1).

Chowdhary and Kaushik, (2015) reported *alternaria alternata* and *Fusarium* sp. dominance in *Ocimum sanctum* [13]. *Ocimum sanctum*, *Ocimum bacilicum* and *Leucas aspera* were screened to study endophytic diversity of the plants showed 103 fungal endophytes belonging to fourteen genera [26].

Table 1: Biodiversity of endophytic fungi in *Ocimum sanctum*

Endophytic fungi	No. of endophytes	Colonization frequency (%)	Dominance (%)	Total (%)
Ascomycetes				
Melanospora fusispora	9	4.5	14.75	**14.75**
Coelomycetes				
No Isolates	-	-	-	0.0
Hyphomycetes				
Fusarium moniliforme	27	13.5	**44.26**	**78.68**
Cladosporium cladosporioides	15	7.5	24.59	
Alternaria alternata	6	3	**9.83**	
Sterile mycelia	4	2	6.55	**6.55**
Total No. of isolates	**61**	**30.5**		

4. CONCLUSION

It was observed that the hyphomycetes dominated during the monsoon and post monsoon time in leaves of the *Ocimum sanctum* at Ratapani Reserve, Bhopal. The presence of ascomycetes was suppressed by Hyphomycetes, moreover coelomycetes was completely inhibited. These fungi survive accordingly due to the presence of different compound which provide them stability and helps to maintain symbiotic relation and afford protection to the host plant.

5. ACKNOWLEDGEMENT

The authors are thankful to Division of Microbiology, Department of Botany, Govt. MVM, Bhopal to provide laboratory facilities to carry out the research work.

Conflict of interest

None declared

6. REFERENCES

1. Tenguria RK, Khan FN, Quereshi S. *World Jour Sci Tech,* 2011; **1(5):**127-149.
2. Taylor JE, Hyde KD, Jones EBG. *New Phytologist,* 1999; **142:**335-346.
3. Azevedo JL. *Prog Microbial Ecol,* 2001; 279-287.
4. Strobel G, Daisy B. *Microbiol Mol Bio Reviews*, 2003; **67(4):**491-502.
5. Hawksworth DL. *Mycol Res,* 2001; **105:** 1422-31.
6. Arnold AE, Herre EA. *Mycologia,* 2003; **95(3):**388-98.
7. Redman RS, Sheehan KG, Rodriguez RJ, Henson JM. *Science,* 2002; **298:**181.
8. Bae H, Kim S, Sicher Jr RC, Kim MS, Strem MD, Bailey BA. *Biol Cont,* 2008; **46:**24-35.
9. Tenguria RK, Khan FN, Quereshi S. *World J. Sci. Technol*., 2013; **1:**127-149.
10. Tenguria RK, Firodiya AD, Khan FN. *Internatl J Appl Biol Pharm Technol.,* 2012; **3(4):**376-380.
11. Bills G, Dombrowski A, Pelaez F, Polishook J, An Z. Recent and future discoveries of pharmacologically active metabolites from tropical fungi, 2002: p. 165-94. In R. Watling, J C Frankland, A M Ainsworth, S Issac, and C H Robinson. (eds.), Tropical Mycology: Micromycetes, 2. CABI Publishing, New York, N.Y.
12. Desale MG, Bodhankar MG. *AIJCSR,* **1(3):**17-22.
13. Chowdhary K, Kaushik N. *PLoS One,* 2015; **10(11):**e0141444.
14. Ahmad I, Mehmood Z, Mohammad P, Ahmed S. *J Med Arom Plant Sci,* 2000; **22:**173-176.
15. Schulz B, Boyle C, Draeger S. *Mycol Res*, 2002; **106:** 996-1004.
16. Janardhan KK, Khaliq A, Naushin F, Ramaswamy K. *Curr Sci,* 1994; **67(6):**465-469.
17. Suryanarayanan TS, Murali T, Venkatesan G. *Can J Bot,* 2002; **80:**818-826.
18. Tenguria RK, Khan FN. *Jour. Inno. Pharm. Bio. Sci.* 2015; **2(2):**222-228.
19. Tenguria RK, Khan FN. *Curr Bot*, 2011; **2(2):**27-29.
20. Promputtha I, Lumyong S, Dhanasekaran V, McKenzie EHC, Hyde KD, Jeewon R. *Microbial Ecol,* 2007; **53:**579-590.
21. Seifert KA, Morgan-Jones G, Gams W, Kendrick WB. The genera of Hyphomycetes. [CBS Biodiversity Series no. 9.] Utrecht: CBS-KNAW Fungal Biodiversity Centre.
22. De Hoog GS, Guarro J, Gené J, Figueras MJ. Atlas of Clinical Fungi, ed. 2. Central bureau voor Schimmelcultures/Universitat Rovira i Virgili, Utrecht/Reus, 2000.
23. Gamboa MA, Bayman P. *Biotropica,* 2001; **33:**352-360.
24. Fisher PJ, Petrini O. *Sydowia,* 1987; **40:**46-50.
25. Kumaresan V, Suryanarayanan TS. *Fungal Diver,* 2002; **9:**81-91.
26. Banerjee D, Manna S, Mahapatra S, Pati BR. *Acta Microbial Immunol Hung,* 2009; **56(3):**243-250.