Diversity of Myco-flora associated with plants of Dumna Nature Park and their antimicrobial potential

Dubey Sushma[1], Singh Jaya[2], Sinha Chandrashekhar[1] and Gupta Saurabh[2]*
1. Department of Biotechnology, Kalinga University, Raipur, INDIA
2. Biodiversity Conservation and Rural Biotechnology Centre (BCRBC), Jabalpur, INDIA
*gupta.saurabh89@yahoo.com

Abstract
Fungi play an important role in the daily life besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, biogeochemical cycling, as bio-fertilizers, bio-pesticides and many other ways.

Total number of 14 fungal isolates were belonging to different genera. Antibacterial activity was performed by well diffusion method against pathogenic bacteria through cell free culture filtrates of isolated fungi. Sclerotium rolfsii FCN#13 showed maximum antibacterial activity against all selected bacteria.

Keywords: Myco-flora, Antibacterial activity and Diversity.

Introduction

Biodiversity of fungi is essential for anyone collecting and/or monitoring any fungi. Fascinating and beautiful fungi are vital components of nearly all ecosystems and impact human health and our economy in a myriad of ways. Standardized methods for documenting diversity and distribution have been lacking. Dumna Nature Park is an ecotourism site open to the public located in the Jabalpur district of the Indian state of Madhya Pradesh. It includes a dam, forest and wildlife in a 1058-hectare area.

Biodiversity is a term used to describe the enormous variety of life on earth. It can be used more specifically to refer to all the species in one region or ecosystem. More than just the trees, forest biodiversity encompasses the multitude of plants, animals and microorganisms that inhabit forest areas and their associated genetic diversity. Forest biodiversity can be considered at many different levels including the ecosystem, landscapes, species, populations and genetics.

Phytotoxins are compounds produced by a microorganism, These phytotoxins are harmful to plants in very low concentrations and many reproduce atleast some of the symptoms of the relevant fungal disease.[3,7] The exploitation of microrganisms and their metabolic bye-products has become a thrust area of research among scientists involved in weed management.

It is a promising alternative for designing environmentally safe herbicides[9] and to study and investigate the fungal biodiversity of dumna nature park and their antibacterial potential.

Material and Methods

Collection of sample: A systematic and periodical survey of Dumna nature park, Jabalpur was made and infected plants like *Parihenium hysterophorus, Commelina sp., Vinca rosea, Butea monosperma, Lantana camara, Mangifera indica, Hyptis suaveolens, Lycopersicum esculenium, Lathyrus odoratus* were collected in polythene bags and brought to the laboratory for further mycological analysis[8].

Preservation of Samples: For proper drying, diseased plant sample collected during survey was kept between two sheets of blotting paper and placed on a flat surface and kept pressed with light weight material paper, changed regularly after every 24 hrs. till complete dryness. Dried samples were kept in envelopes in mycological herbarium of BCRBC (Biodiversity Conservation and Rural Biotechnology Centre).

Isolation of Fungi: Fungi were isolated from collected infected plant samples. The infected parts of these plants were washed with distilled water and then surface of plant parts was sterilized by 70% alcohol thoroughly under a laminar.[5] Then, plant parts discs of 3mm size were cut and placed in Petri dishes containing potato dextrose agar and incubated at 28±2°c for 5-7 days.[1,10]

Microscopic Studies: Identification of fungi was done after studying the morphological and cultural characteristics with the help of monographs, manuals and research of various workers. Slide culture technique was adopted for identification and slides were prepared with lacto phenol and cotton blue.[4,6,12]

Maintenance of Fungal Cultures: The cultures of fungi were maintained on PDA slant and stored at 4°C.

Determination of Frequency: The frequencies of different fungi were determined by using following formula:

$$\text{Percentage (\%) Frequency of Individual Fungus} = \frac{\text{Total no. of colonies of Individual Fungus in a plate}}{\text{Total no. of different fungi in a plate}} \text{x100}$$

$$\text{Percentage(\%) Frequency} = \frac{\text{T1}}{\text{T2}} \text{x100}$$

Source of Bacterial Strains: Four bacterial cultures were used in screening for antibacterial activities kindly provided by "Research Institute, Biodiversity Conservation and Rural Biotechnology Center, Jabalpur (M.P.)".

Production of Antibacterial Metabolites: Total number of metabolites isolated from 14 Plant phytopathogenic fungi were screened for their antibacterial activity against 4 pathogenic bacteria *Staphylococcus aureus* (BCRBC#B1), *Bacillus subtillis* (BCRBC#B2), *Klebsiella pneumoniae* (BCRBC#B3) and *Escherichia coli* (BCRBC#B4) by well diffusion method.

Extraction of Mycelial Free Culture Filtrate (MFCF): Under aseptic conditions, 5 ml of the fungal culture broth was filtered through a re-weighed Whatsmann filter paper no. 1 and was centrifuged at 6000 rpm for 10 min. The pellet was discarded and the supernatant was used for antibacterial bioassay.

Agar well diffusion method: Nutrient agar media were used for bacteria. 50 µl of the bacterial cultures were spread onto the plates using a sterile spreader. The plates were punched with six millimeter diameter wells and filled with 25 µl of the supernatant (Mycelial Free Culture Filtrate). The bacterial plates were incubated at 37°C. The diameter of the zone of inhibition was measured in millimeters at 24-48 hrs.[2,11]

Results and Discussion

Total number of 14 fungal isolates belonging to different genera with frequencies were identified and they are represented in table 1.

Data recorded in table 2 clearly indicates that antibacterial activities shown by different isolates varied significantly not only in various genus but even strains of a species. In general, maximum broad spectrum activity was recorded in case of cell free culture filtrates obtained from the fermented medium of *Sclerotium rolfsii* (FCN#13).

Table 3 screened for antibacterial activity by well diffusion method against 4 pathogenic bacteria i.e. *Bacillus subtillis* (BCRBC#B2), *Staphylococcus aureus* (BCRBC#B1), *Escherichia coli* (BCRBC#B4) and *Klebsiella pneumoniae* (BCRBC#B3). The present phytopathogenic strain *Sclerotium rolfsii* showed maximum antibacterial activity against *Escherichia coli* (14.7) followed by *Klebsiella pneumonia* (14.3) *Bacillus subtillis* (10.6) and *Staphylococcus aureus* (10.4).

Conclusion

Studies conducted indicate that the cell free culture filtrates obtained from the fermented medium of *Sclerotium rolfsii* (FCN#13) showed maximum antibacterial activity against all bacteria.

Table 1
Plant pathogenic fungi isolated from different plant parts

S.N.	Isolate code no.	Name of fungi	Frequency in %
1	FCN#01	*Alternaria alternata*	46
2.	FCN#02	*Alternaria* sp.	37
3	FCN#03	*Acremonium alternatum*	20
4	FCN#04	*Curvularia lunata*	70
5	FCN#05	*Aspergilus* sp.	50
6	FCN#06	*Colletotrichum gloeosporioides*	42
7	FCN#07	*Colletotrichum* sp.	36
8	FCN#08	*Cladosporium* sp.	20
9	FCN#09	*Dreschlera australiensis*	23
10	FCN#10	*Fusarium oxysporum*	72
11	FCN#11	*Fusarium* sp.	49.2
12	FCN#12	*Helminthosporium microsporum*	69.3
13	FCN#13	*Sclerotium rolfsii*	33.3
14	FCN#14	*Trichoderma viridi*	33

FCN- Fungus Culture Number

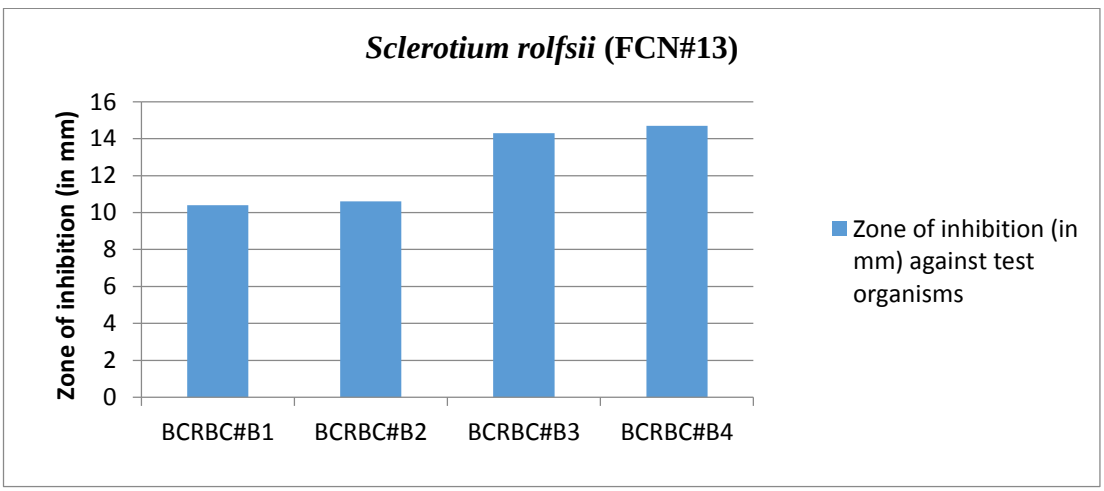
Table 2
Screening the antibacterial activity of plant pathogenic fungi isolated from different Plants

S. N.	Name of pathogenic fungi	Antibacterial activity against some human pathogenic bacteria, Zone of inhibition in mm			
		BCRBC #B1	BCRBC #B2	BCRBC #B3	BCRBC #B4
1	*Alternaria alternata*	-	+	+	-
2	*Alternaria* sp.	+		++	+
3	*Acremonium alternatum*	+	+	++	++
4	*Curvularia lunata*		+	-	-
5	*Aspergilus* sp.	+	+	-	-
6	*Colletotrichum gloeosporioides*	+	+	++	-
7	*Colletotrichum* sp.	-	+	+	-
8	*Cladosporium* sp.	-	+	+	-
9	*Dreschlera australiensis*	-	-	-	-
10	*Fusarium oxysporum*	+	++	+	-
11	*Fusarium* sp.	-	-	+	-
12	*Helminthosporium microsporum*	-	+	+	++
13	*Sclerotium rolfsii*	++	++	+++	+++
14	*Trichoderma viridi*	-	+	-	+

BCRBC#B1- *Stayphylococcus aureus,* BCRBC#B2-*Bacillus subtilis,*
BCRBC#B3- *Klebsiella pneumoniae,* BCRBC#B4- *Escherichia coli*
- = No zone of inhibition
+ = Zone of inhibition more than 7 mm
++ = zone of inhibition more than 10 mm
+++ = zone of inhibition more than 14 mm

Table 3
Antibacterial activity of *Sclerotium rolfsii* (FCN#13) against test organism

Sclerotium rolfsii (FCDN#13)	Zone of inhibition (in mm) against test organisms			
	BCRBC#B1	BCRBC#B2	BCRBC#B3	BCRBC#B4
	10.4 mm	10.6 mm	14.3 mm	14.7 mm



References

1. Agarwal G.P. and Hasuja S.K., Microorgaisms in the laboratory: A laboratory guide of mycology, Microbiology and plant pathology, Print House, Lucknow (India), 155 **(1986)**

2. Alzoreky N.S. and Nakahara K., Antibacterial activity of extracts from some edible plants commonly consumed in Asia, *Int J Food Microbiol*., **80**, 223–30 **(2003)**

3. Berestetskiy A., review of fungal phytotoxins: From basic studies to practical use, *Applied Biochemistry and Microbiology*, **44(5)**, 453-465 **(2008)**

4. Barnett H.L. and Hunter B.B., Illustrated genera of Imperfect Fungi, Burgess Publishing Company, Minneapolis, Minnesota, 241 **(1972)**

5. Chopada G.B., Singh P. and Korat C., Pathogenic variation among Fusarium oxysporum f. sp. lycopersici isolates and varietal screening of tomato against wilt under South Gujarat, *Bioscan*, **9**, 351–354 **(2014)**

6. Ellis M.B., Dematiaceous Hyphomycetes, Common Wealth Institude, Kew, Surrey, England, 608 **(1971)**

7. Jain P., Sharma H.P., Basri F., Priya K. and Singh P., Phytochemical analysis of Bacopa monnieri (L.) Wettst. and their anti-fungal activities, *Indian Journal of Traditional Knowledge (NISCAIR)*, **16(2)**, 310-318 **(2017)**

8. Jain P., Sharma H.P. and Vishal A., Extraction, purification and characterization of peroxidase form Bacopa monnieri from Ranchi, *Vegetos*, **31(4)**, 123-125 **(2018)**

9. Jain P., Sharma H.P. and Ravi A., Comparative biofungicidal activities of methanolic extract and silver nanoparticles of *Annona reticulate*, *Vegetos, An International Journal of Plant Research & Biotechnology*, **31(2)**, 60-66 **(2018 B)**

10. Marak T.R., Ambesh B.S. and Das S., Cultural, morphological and biochemical variations of Alternaria solani causing diseases on solanaceous crops, *The Bioscan*., **9(3)**, 1295-1300 **(2014)**

11. Rios J.L., Recio M.C. and Villar A., Screening methods for natural products with antimicrobial activity: A review of the literature, *J Ethnopharmacol*., **23**, 127–49 **(1988)**

12. Subramaniam C.V., Hyphomycetes, I.C.A.R., New Delhi, 930 **(1971)**

13. Saxena S. and Pandey A.K., Microbial metabolites as eco-friendly agrochemicals for the next millennium, *Appl Microbiol Biotechnol*., **55**, 395–403 **(2001)**

14. Sutton B.C., The Coelomycetes, Commonwealth Mycological Institute, Kew, London **(1980)**.

(Received 04th May 2021, accepted 18th July 2021)