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Analysis and fungal Isolation of some mosses, *Riccia discolor and Targionia hyophylla* from Baramati, district-Pune, Maharashtra, India

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Abstract

The current study focus on fungal isolation of mosses in the Baramati of Maharashtra's District Pune. The serial dilution method and Potato Dextrose Agar (PDA) media were used to isolate soil fungus. Fungi were identified based on their morphological characteristics. Throughout the study, a total of 15 fungal species were identified. The contribution of Deuteromycotina was 53.33% followed by Zygomycotina 26.66%, Ascomycotina 13.33% and Mastigomycotina 6.66 %. During the study., *Rhizopus oryzae*, *Rhizopus Stolonifer*, *Rhizopus* sp., *Mucor mucedu*, *Trichoderma atroviride*, *Trichoderma viride*, *Trichoderma harzianum*, *Aspergillus* sp., *Aspergillus brunneoviolaceus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Penicillium aurantiogriseum.* The predominant genera *Pythium* sp. and *Helminthosporium* sp. were detected less often. The FTIR investigation having presence high concentration of halo compounds, which are resistant to herbicides and insecticides. Other compounds that are present include high amount of vinyl ether, amine, aromatic amine, phenol, primary amide, alkene, sulfonate, fluro compound, alkyne, and aromatic ester, etc.

Keywords: bryophytes, fungal association, FTIR investigation of secondary metabolites

Introduction

Bryophytes are the second largest primitive group, non-vascular land plants and cosmopolitan nature, Crum (2001) [6] reported it is consisting of about 20,000 to 25,000 species all over world. In India Sathish *et al*. (2013) [16] reported that the 482 genera and 2486 species of bryophytes. In Western Ghats of Maharashtra include 128 species of mosses belonging to 11 orders; 26 families and 59 genera Magdum *et at*. (2017) [12]. The bryophytes are divided there phyla: the liverworts or Hepaticae and hornworts or Anthocerotea and the mosses or true Bryophyta. Arbuscular Mycorrhizal fungi, 11 belonged to *Glomus*, two to *Ascolospara*, one to *Gigaspora* and one to *Paruglomus*. These commonly occur Commonly occurs in most mosses and those divers AM fungi particularly *Glomus* species associated with mosses.

The bryophytes are usually considered to paraphytic group of not monophytic group although some studies have produced Contary Result: (Vanderporten Alain; Gottnet Bernard 2009). In particular liverworts and hornworts can form symbiosis with arbuscular mycorrhizal (AM) fungi (Schußlen 2000; Russell and Bulman 2005) [15]. Arbuscular mycorrhiza, formed only by fungi in the division glomeromycota (goffinet 2009) are found in 85% of all plant families (Wang 2006) [23] as per studies by Tapwal *et al*. (2004) Mycoflora rhizosphere influence the growth of bryophyte, still little was paid to bryophytes.

Mycorrhizal hyphae might be an important avenue of phosphorus movement out of the moss carpet and a mean by which the spruce competes with the overlying mosses for nutrients. Mosses also acts as bio indicators at air and soil pollution (Ayrault *et al.*, 2001; Poikolainen *et al.*, 2004) [2, 14]. The phosphorus can inhibit completely limit the formation of symbiosis, causing different responses of AMF in the growth of host plant (Smith & Read 2008) [18].

The Organism and microrganism the low number of plant species limited the diversity (still little known) which is Regulated the flow of energy, balance maintaining the ecosystem (Parniske 2008).

Arbuscular Mycorrhizal (AM) fungi are found in initiate association with the roots of higher plants, since the evolution of land plants. In fact, it was (AM) fungi, which provided nutrition to the early land plants via their hyphase (Dotzler *et al*. 2009; Bontante and Selosse, 2010) [3].

Since their origin, AM fungi have travelled through the different ages and they faced different environmental conditions. it is clear that more than 80% of Vascular land plants are associated with AM fungi (smith & Read 2008; Brundrett, 2009) [18, 4]. AM fungi associations are reported from all terrestrial ecosystem including tropical to temperature forests, alpine and dunes deserts grassland, aquatic plants and agro ecosystem as well as metal polluted Soils. The scientists have made several attempts to formulate artificial culture media to support the growth of Arbuscular mycorrhizal fungi (Hildebrandt *et al.*, 2002) [11]

Materials and Methods

Collection of bryophytes

The Mosses sample was collected from of different localities of Baramati area. The rhizosphere soil samples were collected from Mosses of different localities of Baramati area during June, 2020 to April, 2021. The soil samples were collected in sterilized polythene bags and brought in laboratory for isolation of soil fungi. Serial dilution method was adopted for isolation of soil fungi (Aneja, 2003) [1] using Potato Dextrose Agar (PDA) medium supplemented by Streptomycin antibiotic.

Inoculated plates were kept for incubation at room temperature for 7 days. During the incubation period the fungal growth was observed regularly and observation were noted. After 7 days of incubation the photographs of plates were taken. Isolated fungal colonies were used for

preparation of slides. Slides were prepared using cotton blue stain and lactophenol as mounting medium. Slides were observed under light microscope and micro photography was also done. Fungi were identified on the basis of morphological characters of spores by using standard literature. (Nagamani *et al.*, 2006).

Isolation of soil fungi by serial dilution method

In this technique 1 gram (dry weight) of the material to be studied is ground up (if necessary) and dispersed in 9 ml of sterile water. One milliliter of this solution is transferred to a second tube containing 9 ml of sterile water, resulting in a 0.01 dilution of the spore mass in the original material.

The process is repeated to yield dilutions of 0.001, 0.0001, and 0.00001 or even further if necessary. A 1-ml portion from each dilution is pipetted to a separate test tube, and cooled, melted agar medium poured in the petri plate. Alternatively, the solution can be put on the surface of solidified medium and spread evenly throughout. Colony of fungi occurs after 3 to 4 day. After a few days' incubation, colonies will appear in varying densities, depending upon the amount of dilution from the original material. The number of spores present in the original sample can be calculated roughly by selecting the plates showing 15-50 colonies and writing down the colony count. Prepared slide of colonies and identified fungi, and all are pure cultured

Photograph: Culture Growth

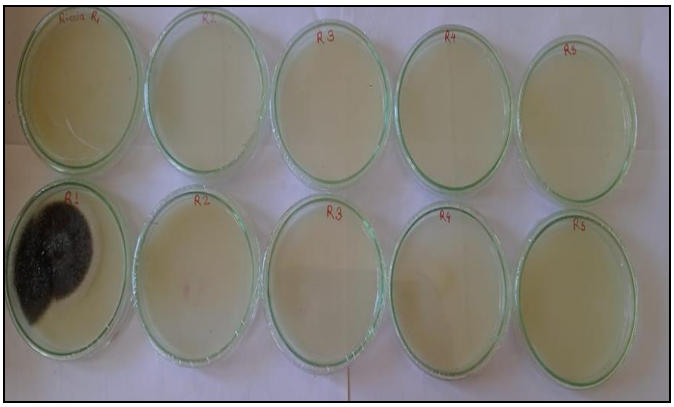


Fig 1: *Riccia discolor* Soil Sample

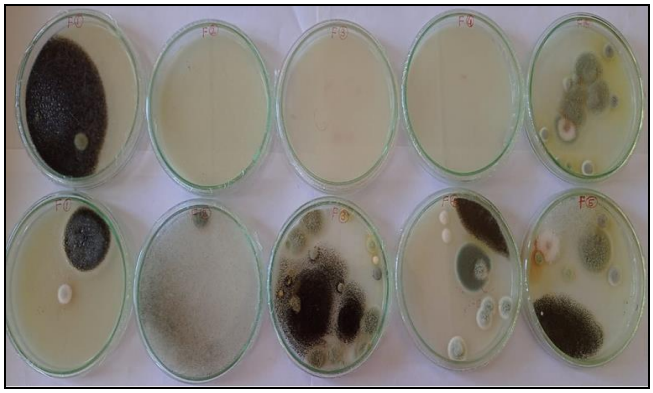


Fig 2: *Targionia hyophylla* Soil Sample

Fig 3: *Riccia discolor*

Fig 4: *Targionia hyophylla*

Observation

Table 1: Soil sample analysis Botanical Garden T.C. College Baramati.

Sr. no.	Parameter	Measurement
1.	pH	8.89
2.	Electrical Conductivity ds/m^2	0.38
3.	Organic carbon %	0.6
4.	Available N Kg/ha	144
5.	Available P Kg/ha	6.3
6.	Available K Kg/ha	430
7.	Fe ppm	0.38
8.	Zn ppm	0.04
9.	S ppm	11.83
10.	Bo ppm	0.07

Observation

Table 2: Water sample analysis: Botanical Garden T.C. College Baramati.

Sr. no.	Parameter	Measurement
1.	pH	7.24
2.	Electrical Conductivity ds/m^2	1.2

3.	Ca++	2
4.	Mg++	2
5.	Na++	6.2
6.	Hco_3	4.4
7.	Cl^-	3.6

Riccia discolor: Rhizosphere soil sample

Table 3

Sr. no	No. of Petri Plate	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7st Day	Total No. of colonies
1.	R1+R1	0	0	0	0	1	1	1	3
2.	R2+R2	0	0	0	0	0	0	0	0
3.	R3+R3	0	0	0	0	1	1	1	3
4.	R4+R4	0	0	0	0	0	0	0	0
5.	R5+R5	0	0	0	0	0	0	0	0

Targionia hyophylla Rhizosphere Soil Sample

Table 4

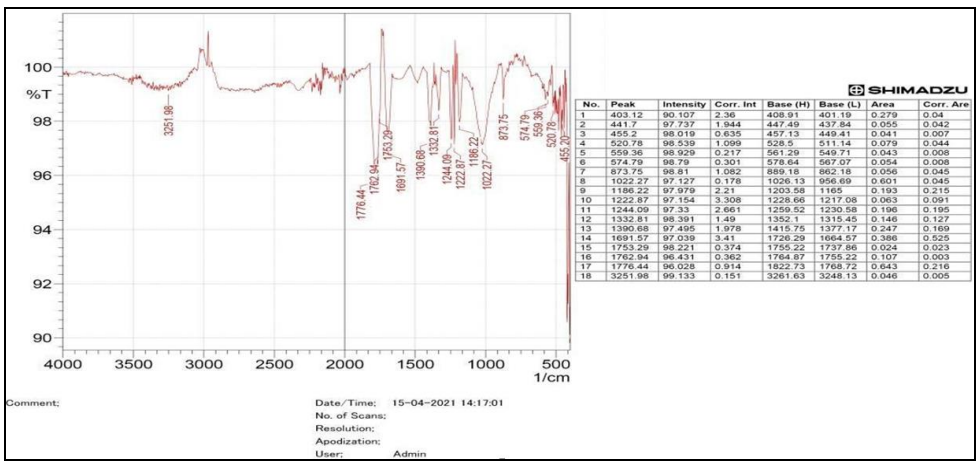
Sr. no.	No. of Petri plate	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7st Day	Total no. of colonies
1.	T1+T1	0	0	1	1	1	1	3	13
2.	T2+T2	0	0	0	0	0	0	2	6
3.	T3+T3	0	0	13	14	1	1	16	75
4.	F4+F4	0	0	0	0	9	9	9	26
5.	T5+T5	0	0	15	19	19	19	19	91

Fungal Observation: Fungal Diversity**.**

Table 5

Sr. no.	Name of fungi	Sub -division	Percentage of contribution
1.	*Pythium* sp.	Mastigomycotina	6.66 %
2.	*Rhizopus oryzae*	Zygomycotina	26.66%
3.	*Rhizopus stolonifer*	Zygomycotina	
4.	*Rhizopus* sp	Zygomycotina	
5.	*Mucor mucedo*	Zygomycotina	
6.	*Trichoderma atroviride*	Ascomycotina	13.33 %
7.	*Trichoderma viride*	Ascomycotina	
8.	*Trichoderma harzianum*	Deuteromycotina	53.33 %
9.	*Aspergillus* sp.	Deuteromycotina	
10.	*Aspergillus brunneoviolaceus*	Deuteromycotina	
11.	*Aspergillus ochraceus*	Deuteromycotina	
12.	*Aspergillus niger*	Deuteromycotina	
13.	*Aspergillus fumigatus*	Deuteromycotina	
14.	*Helminthosporium*sp.	Deuteromycotina	
15.	*Penicillium aurantiogriseum*	Deuteromycotina	

Sample No 1. *Riccia discolor*



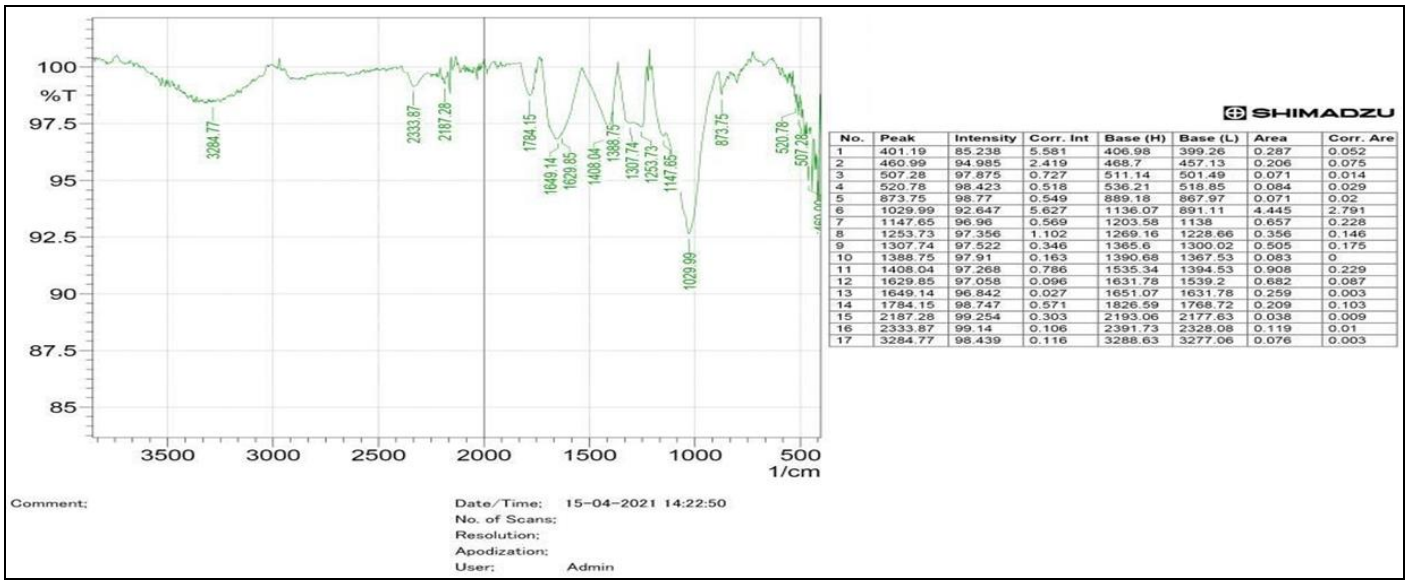
No.	Peak	Intensity	Corr. Int	Base (H)	Base (L)	Area	Corr. Are
1	403.12	90.107	2.36	408.91	401.19	0.279	0.04
2	441.7	97.737	1.944	447.49	437.84	0.055	0.042
3	455.2	98.019	0.635	457.13	449.41	0.041	0.007
4	520.78	98.539	1.099	528.5	511.14	0.079	0.044
5	559.36	98.929	0.217	561.29	549.71	0.043	0.008
6	574.79	98.79	0.301	578.64	567.07	0.054	0.008
7	873.75	98.81	1.082	889.18	862.18	0.056	0.045
8	1022.27	97.127	0.178	1026.13	956.69	0.601	0.045
9	1186.22	97.979	2.21	1203.58	1165	0.193	0.215
10	1222.87	97.154	3.308	1228.66	1217.08	0.063	0.091
11	1244.09	97.33	2.661	1259.52	1230.58	0.196	0.195
12	1332.81	98.391	1.49	1352.1	1315.45	0.146	0.127
13	1390.68	97.495	1.978	1415.75	1377.17	0.247	0.169
14	1691.57	97.039	3.41	1726.29	1664.57	0.386	0.525
15	1753.29	98.221	0.374	1755.22	1737.86	0.024	0.023
16	1762.94	96.431	0.362	1764.87	1755.22	0.107	0.003
17	1776.44	96.028	0.914	1822.73	1768.72	0.643	0.216
18	3251.98	99.133	0.151	3261.63	3248.13	0.046	0.005

Fig 5

Table 6

Sr. no.	Frequency	Appearance	Group	Compound Class
1	403.12cm	Strong	C-I Stretching	Halo Compound
2	4041.7cm	Strong	C-I Stretching	Halo Compound
3	455.2cm	Strong	C-I Stretching	Halo Compound
4	520.78cm	Strong	C-I Stretching	Halo Compound
5	559.36cm	Strong	C-I Stretching	Halo Compound
6	574.79cm	Strong	C-I Stretching	Halo Compound
7	873.75cm	Strong	C-I Stretching	Halo Compound
8	1022.27cm	Strong	C-I Stretching	Halo Compound
9	1186.22cm	Strong	C-OStretching	Tertiary Alcohol
10	1222.87cm	Strong	C-O Stretchig	Vinyl Ether
11	1244.09cm	Medium	C-N tretching	Amine
12	1332.81cm	Strong	C-N tretching	Aromatic Amine
13	1390.68cm	Medium	O-H Bending	Phenol
14	1691.56cm	Strong	C=O tretching	Pramiry Amide
15	1753.29cm	Strong	C=O tretching	Esters
16	1762.94cm	Strong	C=O tretching	Carboxylic Acid
17	1776.44cm	Strong	C=O tretching	Vinyl I Phenyl Ester
18	3251.98cm	Weak Broad	O-H Bending	Alcohol

Sample No. 2 *Targionia hyophylla*



No.	Peak	Intensity	Corr. Int	Base (H)	Base (L)	Area	Corr. Are
1	401.19	85.238	5.581	406.98	399.26	0.287	0.052
2	460.99	94.985	2.419	468.7	457.13	0.206	0.075
3	507.28	97.875	0.727	511.14	501.49	0.071	0.014
4	520.78	98.423	0.518	536.21	518.85	0.084	0.029
5	873.75	98.77	0.549	889.18	867.97	0.071	0.02
6	1029.99	92.647	5.627	1136.07	891.11	4.445	2.791
7	1147.65	96.96	0.569	1203.58	1138	0.657	0.228
8	1253.73	97.356	1.102	1269.16	1228.66	0.356	0.146
9	1307.74	97.522	0.346	1365.6	1300.02	0.505	0.175
10	1388.75	97.91	0.163	1390.68	1367.53	0.083	0
11	1408.04	97.268	0.786	1535.34	1394.53	0.908	0.229
12	1629.85	97.058	0.096	1631.78	1539.2	0.682	0.087
13	1649.14	96.842	0.027	1651.07	1631.78	0.259	0.003
14	1784.15	98.747	0.571	1826.59	1768.72	0.209	0.103
15	2187.28	99.254	0.303	2193.06	2177.63	0.038	0.009
16	2333.87	99.14	0.106	2391.73	2328.08	0.119	0.01
17	3284.77	98.439	0.116	3288.63	3277.06	0.076	0.003

Fig 6

Table 7

Sr. No.	Frequency	Appearance	Group	Compound Class
1.	401.19cm	Strong	C-IStretching	Halo Compound
2.	460.99cm	Strong	C-IStretching	Halo Compound
3.	507.28cm	Strong	C-IStretching	Halo Compound
4.	520.78cm	Strong	C-IStretching	Halo Compound
5.	873.75cm	Strong	C-IStretching	Halo Compound
6.	1029.99cm	Strong	C-IStretching	Alkene
7.	1147.45cm	Strong	C-OStretching	Ester
8.	1253.73cm	Strong	C-IStretching	Aromatic Amine
9.	1307.74cm	Strong	C-NStretching	Aromatic Ester
10.	1388.75cm	Strong	C-OStretching	Sulfonate
11.	1408.04cm	Strong	S-OStretching	FluoroCompound
12.	1649.14cm	Strong	C-FStretching	Alkene
13.	1784.15cm	Strong	C-CStretching	Acid Halide
14.	2187.28cm	Weak	C=OStretching	Alkyne
15.	2333.87cm	Strong	O=C=O Stretching	Carboxylic Acid
16.	3284.77cm	Weak Broad	O-HStretching	Alcohol

Results and Discussions

The current study includes 15 different fungus species. It was found in fungal associations with mosses. *Pythium* sp., *Rhizopus oryzae*, *Rhizopus Stolonifer*, *Rhizopus* sp., *Mucor mucedu*, *Trichoderma atroviride*, *Trichoderma viride*, *Trichoderma harzianum*, *Aspergillus* sp., *Aspergillus brunneoviolaceus*, *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Helminthosporium* sp. and *Penicillium aurantiogriseum.* Deuteromycotina contributed the most, followed by Mastigomycotina, Zygomycotina, and Ascomycotina**.** *Rhizopus* is a genus of common saprophytic fungi on plant and specialized parasites animals. They are found in a wild variety of organic substances, including "matuse fruits and vegetable. *Rhizopus stolonifer*, a Zygomycetes has a filamentous growth habit its filaments are coenocytic, that is they are non- septate. It is the only fungus yet known to produce rhizoids which penetrate the substratum in order obtain nutrients.other member of the zygomycetes, especially species of the genus *Mucor* have been shown to undergo fungal dimorphism Lubberhusen *et al.* (2003). *Mucor* is microbial genus of approximately 40 species of moulds commonly found in soil digestive system. Colonies of this fungal genus are typically white to beige or grey and fast growing. Older colonies become gray to brown in Colour due to the development is spores mycosis a large genus within the Mucorales comprising mainly sprotrophs occurring in soil and dung but also entophytes and parasitic plant and other fungi (Wather *et al*., 2013) The moss species collected from Baramati tehsil contains a high concentration of halo compounds, which are resistant to herbicides and insecticides. Other compounds that are present include vinyl ether, amine, aromatic amine, phenol, primary amide, alkene, sulfonate, fluro compound, alkyne, and aromatic ester.

Conclusion

Fungi are the most essential organism in our earth it continue the nutrient cycle it both beneficial and harmful for human and plant. Mycorrhizal association is very essential of the plant because it has several benefit like absorption of nutrients, increase drought resistance, enhance.

Plant is efficiency in absorbing water and nutrients from soil. The association is closely interested and mutually beneficial. In each case, the fungus appears to be gaining access to organic energy and in a few systems the animal ensures close association by dispersing with the symbionts attached.

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