VIABILITY OF FUNGAL CULTURES FROM THE GUFCC AND ISOLATION OF WOOD ROT FUNGI FROM THE GOA UNIVERSITY CAMPUS

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BY

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GUIDE

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CERTIFICATE

This is to certify that **Ms. Patricia Alcina Fernandes** has satisfactorily completed the dissertation submitted to Goa University in partial fulfilment of M.Sc. Degree in Botany course in the academic year 2020 – 2022.

Signature of Student

Signature of Guide

Signature and seal of the
Head of department

DECLARATION

This is to certify that this dissertation entitled '**Viability of Fungal Cultures from the GUFCC and Isolation of Wood Rot Fungi from the Goa University Campus'** is an authentic record of the work done by **Ms. Patricia Alcina Fernandes**, student of M.Sc. Botany, Goa University in partial fulfilment of the requirement of M.Sc. Degree and no part there has been presented before for any other Degree or Diploma in any University.

Signature of Student

Signature of Guide

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Identified viable fungal cultures with GUFCC numbers:

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Sr. no.	GUFCC No.	Name of the fungi	Original date
1	5001	*Acremonium* sp.	08/3/2014
2	5013	*Aspergillus* sp. 1	11/3/2013
3	5018	*Aspergillus* sp. 1	11/3/2013
4	5020	*Aspergillus* sp.	11/3/2013
5	5021	*Brettanomyces* sp.	11/3/2013
6	5044	*Gliocladium* sp	11/3/2014
7	5049	*Gliocladium* sp.	11/3/2014
8	5057	*Paecilomyces* sp. 3	11/3/2014
9	5061	*Paecilomyces* sp. 3	11/3/2014
10	5063	*Paecilomyces* sp. 4	11/3/2014
11	5066	*Paecilomyces* sp. 7	11/3/2014
12	5067	*Paecilomyces* sp. 7	11/3/2014
13	5075	*Paecilomyces* sp. 3	11/3/2014
14	5076	*Penicilium* sp. 3	11/3/2014
15	5079	*Penicillium* sp. 4	11/3/2014
16	5082	*Penicillium* sp.4	2/11/2015
17	5084	*Penicillium*	2/11/2015
18	5087	*Penicillium*	2/11/2015
19	5089	*Penicillium* sp.	11/8/2014
20	5102	*Trichoderma* sp.	2/11/2015
21	5130	*Hirsutella* sp.	11/3/2014
22	5135	*Paecilomyces* sp.	11/3/2014
23	5137	*Paecilomyces* sp. 4	11/3/2014
24	5139	*Penicillium* sp.	11/3/2014
25	5349	*Aspergillus* sp. 2	2/11/2015
26	5351	*Aspergillus japonicus*	2/11/2015
27	5354	*Penicillium* sp. I	2/11/2015
28	5376	*Penicillium glaucum*	6/2/2014
29	5378	*Cladosporium*	6/2/2014
30	5380	*Cylindrocarpon*	2/11/2015
31	5381	*Exserohilum rostratum*	6/2/2014
32	5980	*Stachybotrys chartarum*	2/11/2015
33	5988	*Hansfordia*	2/11/2015
34	5990	*Fusarium solani*	2/11/2015
35	5991	*Aspergillus fasciculata*	2/11/2015
36	5992	*Paradict sp. Asthinium diffractum*	2/11/2015
37	5994	*Aspergillus erytrocephalus*	2/11/2015

38	5997	*Helicomyces roseus*	2/11/2015
39	5998	*curvularia geniculata*	2/11/2015
40	5999	*Monodictys fuliginosa*	2/11/2015
41	6010	*Termitomyces fuliginosus*	3/11/2015
42	6102	*Termitomyces medius*	3/11/2015
43	6103	*Termitomyces heimii*	3/11/2015
44	6107	*Termitomyces*	3/11/2015
45	6109	*Pleurotus cystidiosus*	3/11/2015
46	6303	*Volvaniella* sp	3/11/2015
47	6306	*Fistulina*	3/11/2015
48	6313	*Omphalina* sp.	3/11/2015
49	6315	*Inonotus* sp.	3/11/2015
50	6329	*Boletus* sp.	3/11/2015
51	6331	*Boletus* sp.	3/11/2015
52	6332	*Leucoagaricus* sp.	3/11/2015
53	6344	*Stropharia* sp.	3/11/2015
54	6831	*Leucoagaricus variisporus*	3/11/2015
55	6862	*Dictyophora indusiatus*	3/11/2015
56	6867	*Lenzites betulina*	3/11/2015
57	6891	*Termitomyces*	3/11/2015
58	6894	*Clathrus ruber*	28/12/2018
59	6903	*Inonotus* sp.	3/4/2015
60	6905	*Lentinus lepideus*	3/11/2015
61	6907	*Lentinus lepideus*	10/1/2019
62	6912	*Pleurotus ostreatus*	-
63	6936	*Ganoderma*	3/11/2015
64	6942	*Omphalotus sp.*	30/05/2019
65	6945	*Clitocybe*	3/11/2015
66	6950	*Daldinia*	3/11/2015
67	6954	*Termitomyces globulus*	-
68	6957(a)	*Omphalina*	7/11/2012
69	6960	*Ganoderma liucidum*	7/11/2012
70	6965	*R. sambuci*	
71	6967(a)	*Boletus* sp.	7/11/2012
72	6968	*Termitomyces clypeatus*	-
73	6970	*Termitomyces clypeatus*	-
74	6993	*Alternaria alternata*	2/11/2015
75	7001	*Hyphopichia*	4/11/2015
76	7004	*Dekkera* sp.	14/3/2014
77	7005	*Dekkera* sp	14/3/2014
78	7049	*Brettanomyces* sp.	14/3/2014

79	7057	*Hansenula* sp.	14/3/2015
80	7066	*Saccharomyces* sp	14/3/2016
81	7071	*Debaryomyces* sp.	14/3/2017
82	7079	*Brettanomyces* sp.	-
83	9001	*Auricularia polytricha*	30/05/2019
84	9013	*Polyporus* sp.	7/6/2019
85	9017	*Ganoderma boninense*	28/12/2018
86	9037 (b)	*Romania* sp.	10/1/2019
87	9042	*Pannelus* sp.	20/5/2019
88	15022	*Ganoderma liucidum*	10/1/2019
89	15024 NC-243	*Coprinus plicatilis*	-
90	8502-NC-33	*Omphalatus*	7/11/2012

TABLE 3: Viable cultures (unidentified & with GUFCC number)

Sr. no	GUFCC no.	Original date
1	5996	02-11-2015
2	6326	03-11-2015
3	6969	-
4	7047	-
5	7080	-
6	7697	-
7	7702	-
8	7709	15-02-2014
9	7719	15-02-2014
10	7721	-
11	7726	-
12	158-1	-
13	158-3	-
14	158-4	-
15	158-6	-
16	158-8	-
17	158-9	-
18	158-10	-
19	158-11	-
20	158-12	-
21	158-13	-
22	158-14	-
23	158-17	-
24	158-18	-
25	158-21	-

26	158-22	-
27	158-23	-
28	158-24	-
29	158-28	-
30	158-29	-
31	158-30	-
32	158-31	-
33	158-32	-
34	172-6	-
35	172-8	-
36	172-30	-
37	172-33	-
38	172-37	-
39	172-38	-
40	172-40	-
41	172-42	-
42	172-43	-
43	172-44	-
44	172-47	-
45	172-48	-
46	172-50	-
47	172-51	-
48	172-54	-
49	172-56	-
50	172-57	-
51	172-58	-
52	172-59	-

53	172-62	-
54	172-64	-
55	172-65	-
56	172-66	-
57	172-67	-
58	172-68	-
59	A2-27	-
60	NC-74	-
61	NH88	-
62	S2-F1	-
63	S2-F2	-
64	S2-F3	-
65	S2-F5	-
66	S2-F6	-
67	S2-F7	-
68	S2-F8	-
69	S4-F1	-
70	S4-F3	-
71	S4-F5	-
72	S6-F2	-

TABLE 4: VIABLE CULTURES (without GUFCC number)

Sr. no	Name of the fungi	Original date
1	*T. straita* (microfungi)	6/3/2014
2	Starfruit yeast	-
3	*Xerula* sp.	29/6/2015
4	Actino culture	-
5	*Cashew Aspergillus*	3/6/2014
6	*Pleurotus cystidiosus*	28/12/18
7	*A. niger*	-
8	WS-6 Yeast	-
9	*Pisolithus indicus*	-
10	*Clavaria* sp.	-
11	*Dictyophora orange*	-
12	*T. bulbul* base yeast	6/7/2015
13	*Jasminum malabaricum*	16/8/2014
14	*Jammuna* yeast	-
15	*Xylem microfungi*	3/8/2015

TABLE 5: NON-VIABLE CULTURES

Sr. no	GUFCC no.	Name of the fungi	Date
1	1300	*Pleurotus sajor-caju*	30/5/2013
2	4984	*Aspergillus glaucus*	11/2/2014
3	4997	*Cladosporium cladosporioides*	11/2/2014
4	5003	*Acremonium* sp	11/3/2014
5	5006	*Aschersonia badia*	11/3/2014
6	5010	*Aspergillus niger*	11/3/2014
7	5012	*Aspergillus restrictus*	11/3/2014
8	5016	*Aspergillus* sp. 5	11/3/2014
9	5035	*Fusarium* sp.	11/3/2014
10	5036	*Gliocladium* sp.	11/3/2014
11	5041	*Gliocladium* sp. I	11/3/2014
12	5043	*Gliocladium*	11/3/2014
13	5047	*Gliocladium*	11/3/2014
14	5048	*Gliocladium* sp.	11/3/2014
15	5050	*Gliocladium* sp.	11/3/2014
16	5051	*Hirsutella* sp	11/3/2014
17	5054	*Paceilomyces*	11/3/2014
18	5056	*Paecilomyces* sp. 3	11/3/2014
19	5062	*Paecilomyces* sp. 3	11/3/2014
20	5064	*Paecilomyces* sp. 4	11/3/2014
21	5070	*Paecilomyces* sp.	11/3/2014
22	5071	*Penicillium* sp.	11/3/2014
23	5072	*Penicillium* sp. I	11/3/2014
24	5074	*Penicillium* sp. 2	11/3/2014
25	5078	*Penicillium* sp. 3	11/3/2014
26	5092	*Trichoderma* sp. IV	2/11/2015
27	5092	*Trichoderma* sp. IV	2/12/2015
28	5099	*Trichoderma* sp.	11/3/2014
29	5373	*Aspergillus flavus*	6/2/2014
30	5377	*Rhizopus* sp	6/2/2014
31	5379	*Curvularia lunata*	6/2/2014
32	5383	*Fusarium solani*	6/2/2014
33	5385	*Fusarium* sp.	6/2/2014
34	5386	*Fusarium* sp.	6/2/2014
35	5388	*Phialomyces* sp.	6/2/2014
36	5389	*Trichothecium*	6/2/2014
37	5390	*Trichothecium roseum*	6/2/2014
38	5986	*Collectotrichum dematium*	2/11/2015

39	6307	*Ganoderma lucidium*	3/1/2015
40	6326	*Leucoaagaricus* sp.	3/11/2015
41	6329	*Boletus*	3/11/2015
42	6336	*Agaricus campestris*	-
43	6831	*Leucoaagaricus variisporus*	3/11/2015
44	6834	*Macrolepiota rhacodes*	3/11/2015
45	6872	*Penicillium* sp.	3/11/2015
46	6902	*Inonotus* sp.	3/11/2015
47	6904	*Inonotus* sp.	3/11/2015
48	6916	*Trametes versicolor*	3/11/2015
49	6935	*Omphalotus* sp	28/12/18
50	6941	*Hymenochaetales*	3/11/2015
51	6942	*Omphalotus* sp.	11/3/2014
52	6946	*Boletus* sp.	29/02/2019
53	6947	*Stereum* sp.	3/11/2015
54	6949	*Omphalina* sp.	-
55	6967	*Boletus*	7/11/2012
56	7002	*Hansenula* sp.	14/3/2014
57	7019	*Brettanomyces candida*	14/2/2014
58	7021	*Saccharomyces*	14/3/2014
59	7033	*Brettanomyces* sp.	14/3/2014
60	7040	*Brettanomyces* sp.	14/3/2014
61	7041	*Brettanomyces* sp.	14/3/2014
62	7042	*Brettanomyces* sp.	14/3/2014
63	7044	*Saccharomyces* sp.	-
64	7045	*Saccharomyces* sp.	-
65	7046	*Debaryomyces* sp.	14/3/2014
66	7048	*Brettanomyces* sp.	14/3/2014
67	7048	*Brettanomyces* sp.	14/3/2014
68	7050	*Saccharomyces*	14/3/2014
69	7055	*Hansenula* sp.	14/3/2014
70	7056	*Hansenula* sp.	14/3/2014
71	7058	*Brettanomyces* sp.	14/3/2014
72	7064	*Brettanomyces* sp.	14/3/2014
73	7068	*Saccharomyces* sp.	14/3/2014
74	7069	*Saccharomyces* sp.	14/3/2014
75	7070	*Saccharomyces* sp.	14/3/2014
76	7076	*Brettanomyces* sp.	14/3/2014
77	7077	*Brettanomyces* sp.	14/3/2014
78	7081	*Saccharomyces* sp.	14/3/2014
79	7082	*Brettanomyces* sp.	14/3/14

80	8502	*Omphalotus* sp.	14/3/14
81	13000	*Pleurotus sajor-caju*	30/05/13
82	15021 (a)	*Macrolepiota rhacodes*	7/11/2012
83	-	*Curvularia* L.	-
84	-	Yeast P2	-
85	-	WITS-9	-
86	-	*Lepiota sp.*	-
87	-	*Pleurotus pulmonarius*	-
88	-	*Schizophyllum commune*	-
89	-	*Merasmius sp.*	-
90	-	*Xylaria sp.*	-
91	-	*Xyleria longipes*	-
92	-	*Agaricus silvaticus*	-
93	-	*Xyleria* black/white	-
94	-	W. skirt mushroom	-
95	-	Jamun yeast	-
96	-	Yeast-WT-S9	-
97	5131	-	-
98	5375	-	-
99	5875	-	-
100	5995	-	-
101	6312	-	-
102	6326	-	-
103	7008	-	-
104	7036	-	-
105	7039	-	-
106	7054	-	-
107	7057	-	-
108	7062	-	-
109	7072	-	-
110	7081	-	-
111	7301	-	15/2/14
112	7310	-	-
113	7319	-	15/2/2014
114	7324	-	15/2/14
115	7344	-	15/02/14
116	7681	-	15/2/14
117	7688	-	15/2/14
118	7692	-	15/2/14
119	7694	-	15/2/14
120	7695	-	15/2/14

121	7701	-	15/02/2014
122	7703	-	15/02/2014
123	7706	-	15/02/2014
124	7707	-	15/02/2014
125	7710	-	15/02/2014
126	7711	-	15/02/2014
127	7712	-	15/02/2014
128	7713	-	15/02/2014
129	7715	-	15/02/2014
130	7716	-	15/02/2014
131	7718	-	15/02/2014
132	8421	-	15/02/2014
133	8431	-	15/02/2014
134	15016	-	-
135	158-15	-	-
136	158-26	-	-
137	158-5	-	-
138	172	-	-
139	172-15	-	-
140	172-29	-	-
141	172-32	-	-
142	172-33	-	-
143	172-34	-	-
144	172-35	-	-
145	172-36	-	-
146	172-41	-	-
147	172-46	-	-
148	172-5	-	-
149	172-52	-	-
150	172-55	-	-
151	172-60	-	-
152	6381-NC49	-	-
153	A1-27	-	-
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155	NC-74	-	-
156	NH-88	-	-
157	S2-F10	-	-
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INTRODUCTION

Wood is one of the most durable, rigid, cellulosic materials, but many biotic and abiotic agents can degrade it. These agents often act simultaneously, making it difficult to completely separate causal agents. Since the compounds stored in parenchyma cells can be digestible by different organisms, accessing the more complex polymers is a key challenge. The chemistry and arrangement of the cellulose, hemicellulose, and lignin polymers in the wood cell wall considerably reduces the number of agents which can cause damage to the wood. Many fungi are cellulolytic, but not all can unlock the lignin polymer's chemistry. The fungi that have developed strategies to overcome the recalcitrance of lignin can fully extract the assimilated energy of the lignocellulose cell wall (Goodell *et al.,* 2020).

Wood rot fungi are an essential component of the forest ecosystem (Wang *et al.*, 2011). The rotting of wood is a crucial process in nutrient recycling, soil formation, and the carbon budget of forest ecosystems (Lonsdale *et al.,* 2008). It is a vital functional component of all ecosystems as it has two main significant aspects. Firstly, it is the principal process whereby essential nutrients are made available to the primary producers. Secondly, the decomposition process plays an integral part in the formation of humus molecules (Mulchand, 2016).

The three main constituents of wood include cellulose, hemicelluloses, and lignin. Most wood constitutes 40-44% of cellulose and provides the wood with many of its unique material properties. (Côté *et al.,* 1968). Hemicellulose is a branching heteropolymer made up of pentose and hexose sugar monomers, representing 15-32% of the wood cell wall. Hemicellulose is a connector between cellulose and lignin that permits the three polymers to behave as an integrated matrix. Hemicellulose is an integral part of the wood structure. Still, it is often considered the weak-linked component in the wood since its degradation

exceptionally affects the capability of lignocellulose to act as a rigid structural system. It is usually the first major component of wood which is attacked and deconstructed by fungi. (Curling *et al.,* 2002).

Lignins are highly resistant to biological and chemical degradation and offer mechanical resistance to wood (Matrinez *et al.,* 2005). The plant material constitutes 25-30% of lignin, which helps the plant with structural integrity and protects pests and pathogens. (Orth *et al.,* 1993).

The fungus, mainly basidiomycetes, are the most efficient lignin decomposers (Eriksson *et al.,* 1990). Polypores are wood-rotting fungi that play a significant role in the wood attack and decay processes. Several species are lignicolous and grow on bark or wood.

The Importance of the Wood–Water Relationship:

Water is a vital constituent of wood materials and biomass in the natural environment. Its intake is an integral component correlated with wood's ingenuity to be attacked and decay by fungi. Still, moisture also affects wood by its movement into the cellular structure related to cellulose which causes dimensional changes. Wood surfaces are subjected to a continual dusting of fungal propagules that, once moisture levels and environmental conditions are correct, can germinate and exploit the micro-cracks that can develop in coatings.

For new fungal colonization to initiate on other pieces, its spores or other small fragments must be produced and be transported either in the air, water, or *via* other organisms (such as insects). Many other different requirements must also be met for the colonization of fungi to occur, these include:

a. **Wood substrate:** a primary requirement for decay is a nutrient source, typically the timber itself. These wood or surrounding supporting materials/soils must also contain nutrients, including nitrogen (N), essential to fungal growth and the decay process.

b. Water and Air: Typically, wood must be at or near the Fiber Saturation Point (FSP), which denotes the point in the drying process at which only water bound in the wood cell wall remains. FSP is required for the fungal spores or fragments to germinate and initiate to grow into new fungal colonies. However, because the hyphae are needed to be surrounded by a moist, aqueous Extracellular Polysaccharide Matrix (ECM) when the fungi attack the wood, some amount of moisture in the cell lumen of the wood is required to assist the fungal growth. In many cases, fungal decay can initiate at approximately 30% moisture content (oven-dry basis), with an optimum between 40 and 80%, then decreases with increasing moisture levels above 100% as cell lumens start being filled up with water and oxygen becomes circumscribed. Water is usually the most vital limiting factor in decay. Nevertheless, liquid water is essential for the fungi to begin the secretion of metabolites needed for decay, even in the deficit of wood or other satisfactory substrates. For the growth of fungus, the moisture content of wood must also not be too high to preclude sufficient oxygen levels.

c. Temperature: Fungi may grow as temperatures decline to levels near freezing (Miller *et al.*, 1981), but reaction rates fall, and the decay process ultimately stops at freezing temperatures. Metabolic reactions increase with increasing temperature, with most fungi having growth optima between 24^{o}C and 32^{o}C (Zabel *et al.*, 2020). The fungal metabolism becomes more constrained for most decay fungi as temperatures exceed 39 - 40^{o}C. However, some thermophilic fungi survive and have been active at temperatures exceeding 50^{o}C in specialized environments such as pulp chip piles (Smith *et al.*, 1972; Horvath *et*

al., 2012). Exposure to temperatures about 56^{o}C results in permanent denaturing of proteins and DNA, effectively killing most non-thermophilic organisms (Barry, 2020). Comparatively, few fungi have evolved their potential to degrade and utilize the three cell wall polymers.

Growth and Infection of Wood

Under suitable conditions, wood rot fungi can colonize timber from felling and seasoning to processing and final use. Wood rot fungi are predominantly from the subdivision Basidiomycotina. They invade wood cells and decompose cell wall components, resulting in harmful effects on strength and other wood properties (Rayner and Boddy, 1988).

Wood rot fungi fundamentally initiate as fungal spores or mycelial fragments. Under favourable conditions, spores germinate to give rise to delicate hair-like structures known as fungal hyphae. Unicellular fungal growth is not common in wood-rotting fungi except during the formation of spores. As the fungal hyphae grow on the substratum, some species may form a mat of many interwoven hyphae, known as mycelium or a mycelial mat. During the initial growth phase, all wood-inhabiting fungi derive their nutrition from the stored products in the parenchyma as a source of energy and also to build up fungal biomass within or on the surface of the wood substratum.

Three kinds of wood decay are distinguished *viz.*, the white-rot, where lignin is degraded, and cellulose is partially degraded, and thus wood is bleached; the brown rot, where cellulose is degraded and lignin is left as a brown residue; and the soft rot. The potential of white-rot and brown-rot fungi to decompose all principal components of wood is crucial for carbon flux in ecosystems (Leonowiez *et al.*, 1999; Baldrian and Gabriel, 2003). Due to the extraordinary capacity of breaking down cellulose, these fungi have a wide range of applications, including in food, animal feed, textile, fuel, detergents, and chemical

industries, while other environment-friendly applications include waste management and in development of eco-safe technologies for paper and pulp industries (Mulchand, 2016),

Brown Rot Fungi

Although they affect all three polymers, brown rot fungi primarily utilize the carbohydrate polymers of the wood cell wall after the initial colonization stages. These fungi attack softwoods. At the cellular level, the brown rot decay produces degradation far in advance of the point of hyphal growth and depolymerizes the carbohydrates much faster than it is utilized. This results in rapid losses in properties such as tension or bending at very low mass losses (Wilcox, 1978; Curling, 2001). As compared to white-rot fungi, the brown rot fungi have a more limited suite of enzymes that are involved in the depolymerization and deconstruction of cellulose, and these fungi do not possess peroxidase enzymes for lignin depolymerization. However, brown rot fungi speedily attack and degrade cellulose by using a non-enzymatic mechanism to depolymerize lignin to permit access to the cellulosic components. The lignin then speedily depolymerizes, leaving modified lignin that long led researchers to conclude that brown rot fungi had little effect on this polymer (Goodell et al; 2020).

White Rot Fungi

White rot fungi belonging to the order basidiomycetes, which biodegrades lignin in nature, required for global carbon recycling (Siripon *et al.*, 2009 and K.Selvam, 2012). Using enzymes secreted by the hyphae, these fungi can depolymerize all three cell wall polymers, often leaving a whitened mass of fibers at the latter stages of decay.

Most white-rot fungi that tend to digest and utilize the polymeric wood components as they are depolymerized are known as "simultaneous" white rots. However, some white-rot fungi

can preferentially digest the hemicellulose and lignin components leaving much of the crystalline cellulose relatively undegraded are, known as "selective" white-rot fungi. In both, types of white rot, the rate of structural losses is much slower than in brown rots, and depolymerization of the cell wall of the wood is more directly interconnected to enzymatic erosion of the cell wall layers and also to the fungal metabolic processes. White rot fungi usually attack hardwoods but can also attack softwoods at times (Krah, 2018). This coupling of degradation with utilization results in declines in properties such as tension or bending those parallel mass losses caused by decay. Enzymes of specific importance in lignin degradation by white-rot fungi are primarily oxidative and include laccase, lignin peroxidase, manganese peroxidase, versatile peroxidase, and the dye-decolorizing peroxidases. Many of these enzymes are unique to white-rot fungi, with only a relatively few dye-decolorizing peroxidases, for example, found in select lignin-degrading bacteria (Goodell et al; 2020).

Since the chief components of the lignin-degrading system of white-rot are extracellular, these fungi can degrade a highly varied range of very persistent or toxic environmental pollutants, and hence these fungi possess several advantages that can be exploited in bioremediation systems. The capability of fungi to degrade lignocellulose materials is because of their highly efficient enzymatic system efficiency (Selvam *et al.,* 2012).

Soft Rot Fungi

Most soft rot fungi belong to Ascomycota. There are two types of soft rot attack *viz.,* Type 1 soft rot results in the formation of diamond-shaped cavities aligned with the cellulose microfibril angle within the S-2 cell wall layer, and Type 2 is a more generalized erosion of the S-2 cell wall layer from the lumen outward (Zabel, 2020).

Soft rot fungi are primarily found in more extreme and wetter conditions, less suited for traditional white and brown rot fungi (Goodell et al; 2020). Their damage is limited to a few mm of the external wood exposed to the environment, possibly due to low oxygen levels in interior wood below ground so that it can support more aggressive members of Basidiomycota. Both white and brown rot characteristics are shown by soft root damages, where these fungi utilize cellulose and hemicellulose. Still, they can degrade lignin, as evidenced by the cavities and erosion they cause.

As these fungi have the extraordinary capacity to degrade cellulose, they have a wide range of benefits. The chief potential applications are food, animal feed, textile, fuel, detergents, and chemical industries. At the same time, other eco-friendly applications include those in the environmental waste management and development of environmentally safe technologies for paper and pulp industries (Mulchand, 2016).

VIABILITY:

Culture collections containing fungi are vital to biologists, microbiologists, epidemiologists and others involved in health and natural sciences. The importance of fungi and other microorganisms in the medical sector has led to the enhancement of techniques and methods for their isolation and preservation. The continuous isolation of new strains and the need to maintain such strains to conduct pathological and taxonomical studies and industrial application have motivated various scientific and industrial research centers to maintain large collections of living microorganisms (Roseli, 2011).

Two of the simplest procedures for maintaining wood rot fungi cultures for research are continuous transfer on agar slants and storage under sterile water (Burdsall and Dorworth, 1994). The advantages and disadvantages of the different methods of maintaining fungus cultures over long periods have been thoroughly discussed by Smith and Onions (1983)

and Dana *et al.,* (2010). The method of preserving fungus on agar slant is a classical method. It consists of transferring the culture at frequent intervals to a suitable solid substrate depending on the microorganisms and the room conditions (de Moraes Borba and Rodrigues, 2000).

The preservation of fungal strains, as type material, as reference stocks for diagnostic or teaching purposes, and in national culture collections is an essential aspect of mycology. Ideally, stored strains should remain viable over long periods but be dormant to prevent the accumulation of mutations resulting in morphological and biochemical alterations (Borman *et al.*, 2006).

Varieties of fungi are commercially used in the industries to produce different products like acetic acid, antibiotics, food processing, bio-pest control, *etc.* So, their preservation for a long time use is essential for the industrial economy. The preservation of fungal culture is very tedious for a long-term period. Usually, this can be done by continuous subculture method and storage at 4°C (Kitamoto *et al.,* 2002; Yang and Rossignol, 1998). Nowadays, most of the fungal cultures are stored in liquid N by cryopreservation technique at -196°C for long term preservation (Hwang, 1966, 1968; Hwang and Howells, 1968; Corbery and Le Tacon, 1997; Espinel-Ingroff *et al.,* 2004; Borman *et al.,* 2006). The disadvantage of cryopreservation includes the damaged caused by the cells and tissues of cultures due to ultra-freezing shock, and the cultures cannot be revived (Corbery and Le Tacon, 1997). Some cryoprotectants, *viz.*, glycerol, DMSO, ethylene glycol, *etc.,* are used to solve this problem (Hwang, 1966, 1968; Hwang and Howells, 1968; Corbery and Le Tacon, 1997; Nagpal *et al.,* 2012). However, this does not protect the culture completely. The risk of damage to the user while handling liquid N is a big problem. The availability, facility, and cost of liquid N for small laboratories situated in interior areas pose a big problem (Stalpers *et al.,* 1987; Ryan *et al.,* 2000).

AIMS AND OBJECTIVES

The present study was aimed to study the following objectives:

1. To collect, culture, and identify different litter degrading fungal species from the Goa University Campus.
2. To assess the viability of the fungal cultures presently available in the Goa University Fungal Collection Centre (GUFCC) by sub-culturing them on Malt Extract Agar (MEA) and to study their macro-morphological characteristics.

REVIEW OF LITERTURE

Borba and Rodrigues (2000) evaluated the viability and sporulating capability of 45 Coelomycetes. They maintained the subcultures under mineral oil, in soil and on agar slant for varying periods of time *viz.,* 50 years, 39 years and 2 years, respectively. Out of the 34 strains maintained under mineral oil, 20 strains were viable, and one strain of *Pestalotiopsis guepinii* sporulated after 3 years covered by 0.3-cm mineral oil. Of the 16 strains in soil only *Septoria lycopersici* strain showed viability but failed to sporulate. Also, 11 endophytic strains preserved on corn meal agar slants were viable and showed sporulation. One endophytic strain of *Phomopsis* sp. recorded viability after 8 years of storage under mineral oil but failed to sporulate after several attempts.

Dana et al., (2010) maintained and stored fourteen isolates of 12 species of wood rot fungi belonging to basidiomycete for 18 years on agar slants and transferred without subculturing annually onto mycelium-agar under cold sterile water. Then using a standard soil-block decay test and measured by mean percent mass loss of wood blocks the effectiveness of their decay was evaluated. No major changes in effectiveness of the decay for 12 of the fungus isolates were observed. With respect to storage method two fungi showed a difference in the amount of decay 1 fungus (*Fomitopsis lilacinogilva*) as it produced more decay maintained on agar slant and the 2nd fungus (*Trametes versicolor*) produced more decay stored in sterile water. This indicates that the fungi did not change enzymatically or over time as a result of the storage method. The result suggested that for decay fungi isolates, storage under sterile water is an easy and effective method for long periods.

Crahay et al., (2012) developed an efficient cryopreservation procedure for the long-term storage of ECM fungi. Two cryopreservation protocols were compared. The first one was the conventional straw protocol (SP), where the mycelium are grown in on agar followed by collection of the mycelium by punching into sterile straw before cryopreservation. In the second, the cryovial protocol (CP), the mycelium was grown directly in cryovials with agar and then cryopreserved. The survival was then evaluated before and immediately after freezing and after 1 week, 1 m, and 6 m of storage at 130 C. Survival rate (80 % for the CP as compared to 25 % for the SP) and faster re-growth (within 10 d for the CP compared to the 4 weeks for the SP) were observed for most isolates with the CP. This suggested that the preparation of the cultures before freezing had a huge impact on the survival of the isolates. On a set of 98 ECM isolates the suitability of the CP for cryopreservation was confirmed which displayed 88% survival rate. Only few isolates belonging to *Suillus luteus, Hebeloma crustuliniforme*, *Paxillus involutus* and *Thelephora terrestris* did not survive. This suggested that for large set of ECM fungi the CP is a suitable method for the ultra-low cryopreservation and that further studies are required for the more recalcitrant ones.

Selvam et al., (2012) collected and identified fifty six samples from Western Ghats area, of Tamil nadu and Karnataka. Obtained *Phanerochaete chrysosporium*-787 from Microbial Type Culture Collection, Chandigarh, and used as the reference fungus. Based on their ability to oxidize dyes, poly R-478 and remazol brilliant blue, to degrade native lignin the screening of the fungi was done for their ligninolytic activity and further confirmation was carried out by the liberation of ethylene from KTBA (2-keto-4-thiomethyl butyric acid).

The colour removals in 57 samples were in the range of 11.5 to 38.4% in poly- R dye and 11.1 to 72.0 % in remazol brilliant blue. The rate of mycelial growth was in the range of 1.24 to 3.67 mg/day and lignin degradation percent was found to be between 20.4 to 68.8. The ligninolytic activity were further confirmed by the ability of the fungi to release ethylene from KTBA and the results wherein the range of 1.210 to 3.121 ppm. From the screening results three best wood rot fungi namely, *Polyporus hirsutus, Daedalea flavida, Phellinus* sp1 were selected for further studies.

Ling-Ping Xiao (2013) recovered water-soluble hemicelluloses from birch wood (*Betula alnoides*) decayed by white-rot fungi (*Ganoderma lucidum* C7016) for 16 weeks. For comparison dimethyl sulfoxide (DMSO)-soluble hemicelluloses were isolated from the untreated birch wood. It was observed that the fungal-degraded polysaccharides had high content of uronic acids (20.6 to 22.5 %) indicating acidic hemicelluloses. The analysis of neutral sugars in the DMSO-soluble hemicelluloses H0 revealed a reasonable purity of the isolated crude xylan (58.8 %) though being contaminated by glucan (26.6 %), which was ascribed to no cellulosic glucan. After the fungal degradation, HSQC NMR spectra of the hemicellulose preparations showed high presence of galactan and 4-O-methyl-α-D-glucuronic acid signals and a clear decrease of anomeric and aliphatic xylan signals. This observation indicated side-chain degradation mechanism of hemicelluloses by white-rot fungi. The water-soluble polysaccharides from the fungal degraded birch wood with a Mw value than those of the DMSO soluble hemicelluloses which suggested that white-rot fungi played an important role in releasing the low-molecular-weight hemicelluloses. The analysis of gel permeation chromatography demonstrated that the recovered water-soluble hemicelluloses had a lower average molecular weight than that of the DMSO-soluble hemicelluloses. Fourier transform infrared spectroscopy, scanning electron microscopy,

one- and two-dimensional nuclear magnetic resonance spectroscopy also showed significant changes between those of fungal degraded and DMSO-soluble hemicelluloses. This research revealed the changes of hemicelluloses in fungal degradation in the natural environment.

Lyngdoh and Dkhar (2014) carried out field surveys and collected basidiocarps of wood-rotting fungi in eight forest stands of East Khasi Hills district of Meghalaya, India. Seventy eight wood-decaying fungi belonging to 23 families were identified. The undisturbed Mawphlang sacred grove showed greater diversity of rotting fungi (33.54 %) as compared to the other forest sites. Also logs also harbored the maximum number of wood-rotting fungi (59.7 %) as compared to living trees (7.8%). *Microporus xanthopus* had the occurrence percentage of 87.5 %, followed by *Cyclomyces tabacinus, Microporus affinis* and *Trametes versicolor* with 62.5 %. 89.61%) of the wood-rotting fungi were white-rot fungi and very few were brown-rots. The rare wood-rotting fungus, *Heterobasidion perplexa* reported there for the first time in India.

Robert *et al.,* (2014) compared the genomes of 33 basidiomycetes fungi including four newly sequenced wood rot fungi. The newly sequenced genomes, *Botryobasidium botryosum* and *Jaapia argillacea* genomes lacked PODs but showed diverse enzymes acting on crystalline cellulose and group close to the model white-rot species *Phanerochaete chrysosporium* in the PCA. Laboratory assays performed showed that both *B. botryosum* and *J. argillacea* can break down polymeric components of woody plant cell walls, which is a characteristic of white rot. Also, expansions in reduction of polyketide

synthase genes specific to the brown-rot fungi was observed. The result suggests a continuum rather than a dichotomy between the white-rot and brown-rot wood decay.

Paul et al., (2015) preserved 15 different species of fungi at 4°C in different concentration of glycerol by employing two methods, culturing on PDA slants and Slice cut method. After every six months of sub-culture, all the cultures with control showed viability and regenerated up to 12 months. Most of the fungi which was kept in the distilled water were viable only up to 18 months, except for 5 cultures. In 5% of glycerol all the fungi were viable and regenerate for 18 months in both the methods. In 15% of glycerol most of the fungi were viable up to 24 months, only Aspergillus oryzae, A. niger and Penicillium rugulosum was not recover in slant culture method and in slice cut method some more fungi were not regenerated. All the fungal cultures were viable and regenerated in both the methods up to 24 months in case of 50% glycerol. The maximum viability of 100% was found in 50% of glycerol up to 24 months and 86.66% up to 30 months in slant cultured method. For 30 months, in 50% of glycerol all the fungi were regenerated in slant methods except Aspergillus niger and A. fumigatus and in slice cut method five cultures were not regenerated. In crude glycerol fungal cultures, most of the fungi were viable up to 24 months and for 30 months some of them were not viable in both of the methods. According to the result it clearly showed that 50% of glycerol was better for long term preservation up to 30 months in slant method. Through this technique it was clearly showed that fungi were preserved up to 24-30 months without any contamination. In slant culture method at 50% of glycerol, 100 and 86.66% of fungi are viable up to 24 and 30 months of preservation, respectively. In slice cut culture method 100% of fungi are having regeneration capacity up to 24 months of preservation. This study helps to preserve the fungi with easy and low cost for long term period at 4°C under refrigerator.

Mulchand, (2016) collected and assessed 65 samples of fungi from different sites of forests of Western Maharashtra. They studied the macro- and micro-morphological characters and the cultural behaviour. Out of 15 species of wood decaying polypores identified, descriptions on taxonomy, morphology and culture characters and chemical tests were performed for two species of brown rot *i.e., Daedalea africana* and *Daedalea quercina*.

Tahir *et al.,* (2016), collected more than 20 species of brown rot fungi from rotten wood out of which five were cultured on Malt Extract Agar medium at 28°C with pH 5.5 and identified based on their morphology. Two of them were common brown rot fungi belonging to Basidiomycota while three species were un-common brown rot fungi belonging to Ascomycota.

Susanna *et al.,* (2019) studies the growth and micromorphological characteristics of bracket fungus *Ganoderma adspersum* on Malt-Extract Agar (MEA) and Potato-Dextrose Agar (PDA) media for 6 days and submerged culture for 14 days at varied temperatures (25, 30, 35, 38 °C). The optimum temperature for growth of *G. adspersum* was found to be 25-30 °C. Temperature above 30°C suppressed mycelial growth of *G. Adspersum*.

Muhammad and Kasim, (2019) isolated and identified the fungi associated with post-harvest decay of banana sold in local markets in Sokoto. 6 clusters of rotted fruits samples were collected, small pieces of lesions were inoculated, sub-cultured and identified. A total of seven species were identified belonging to five genera and classified as agents

associated with the post-harvest decay of banana. The occurrence percentage of the isolates was determined. The most frequently occurring isolates were *Colletotrichum musae* from Kasuwar Daji while *C. musae, A. niger* and *Fusarium sp*. was found to be the most frequently occurring isolates from Tsohuwar Kasuwa Market.

Goodell et al., (2020) reviewed the fundamentals of fungal decay, staining and mold processes and then used these fundamentals as the basis for a discussion of wood rot fungal attack. Biotechnological applications of decay fungi were also reviewed, and an overview is presented on how fungi overcome the protective barriers that coatings provide on surfaces. Advances in biochemical analyses have, in some cases, radically altered the perceptions of how wood is degraded, and even the relationships between fungal species, while other new findings have reinforced traditional perspectives.

Lina et al., (2021) collected fungi and analyzed the production of lignocellulolytic enzymes from their growth on lignocellulosic material. From the three sites SJEP, LVEP, and TecNM-ITTG, identified by taxonomic and molecular techniques, where 11 different families were identified. Of the 33 specimens collected, 20 strains were isolated and propagated in PDA solid culture medium at 28 ± 1 ◦C. The potency index (PI) of cellulase, xylanase, and manganese peroxidase (MnP) activities, and the quantification of lignin peroxidase (LiP), was determined with the strains *Phlebiopsis flavidoalba* TecNM-ITTG L20-19 and *Phanerochaete sordida* TecNM-ITTG L32-1-19 being the ones with the highest PI of hydrolase activities with 2.01 and 1.83 cellulase PI and 1.95 and 2.24 xylanase PI, respectively, while *Phlebiopsis flavidoalba* TecNM-ITTG L20-19 and

Trametes sanguinea TecNM-ITTG L14-19 with 7115 U/L LiP activity had the highest oxidase activities, indicating their ability to oxidize complex molecules such as lignin.

MATERIALS AND METHODS

Collection, culturing, sub-culturing, and identification of wood-rotting fungus from Goa University campus:

1. **Three step sterilization technique:**

The rotting wood samples were collected from the Goa University campus. The samples were chosen randomly, collected carefully in *Zip-lock* bags, and processed within a few hours of collection.

The wood samples were separately surface sterilized using 75% ethanol for 1 minute, 4% NaOCl for 3 minutes, and later rinsed in sterile distilled water. The samples were then inoculated on solidified Potato Dextrose Agar (PDA) plates amended with antibiotics streptomycin sulfate (0.4 mg/mL) and ampicillin (0.4 mg/mL) (Bills, 1996).

The sterilized wood samples were carefully cut 1-2cm and inoculated on the media. The plates were incubated at 25°C and observed for fungal growth. The pure fungal cultures were transferred periodically on the slants using the same media and maintained in GUFCC.

Preparation of Potato Dextrose Agar medium:

39.0 g of potato dextrose agar powder was dissolved in 1 litre of water. (de Farias et al; 2010)

Add streptomycin sulphate (0.4 mg/ml) and ampicillin (0.4 mg/ml). (Bills, G.F;1996)

Adjust the pH to 5.6

Autoclave at 121℃ for 15 minutes.

Preparation of Malt Extract Agar Medium:

Weigh 18 g of agar, 5g of peptone and suspend in 1 litre of distilled water.

Adjust the pH to 6.5

Boil to dissolve completely.

Add 15g of malt extract.

Mix well.

Autoclave at 121℃ for 10 minutes.

The media and the glass vials were autoclaved at 15psi for 20 minutes, and the slants were prepared under sterile conditions.

2. **Moist chamber incubation method:** (Rossman et al.,1998)

The method is explained using a flowchart (Fig. 1).

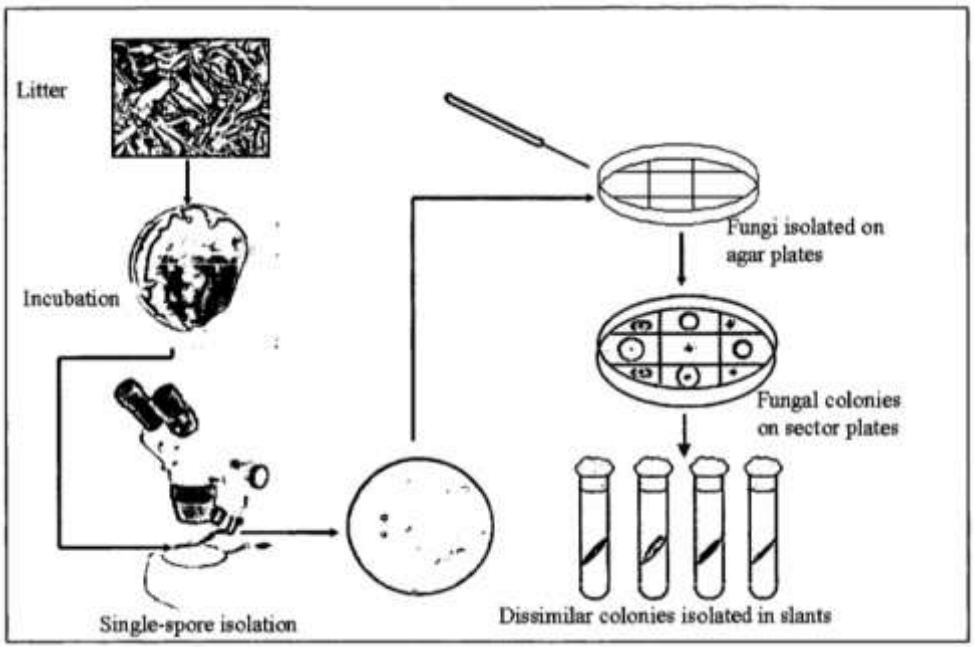

Fig. 1: Moist chamber method

The litter sample was incubated at room temperature (26-30 °C) in sterile Petri plates lined with moist cotton and filter paper for varied duration of time.

The incubated samples were examined under a stereoscope on alternate days.

The sporulated fungi were carefully picked up using a sterile needle and mounted on a glass slide with a drop of lactophenol mountant.

The slides were further observed under transmitted light microscope.

The sporulating fungi were cultured using single spore isolation technique of Onions et al. (1981). A small spore mass was carefully picked up using a sterile, finetipped needle and suspended on a drop of sterile distilled water placed on a slide.

The spore suspension was spread on malt extract agar medium and incubated at 25 °C. After 24 h, germinating spores were individually transferred to a fresh Malt extract agar (MEA) plate or slant.

Slide preparation:

To identify the fungi, slides were prepared using lactophenol cotton blue stain (Leck A.; 1999) and observed under a compound microscope for the presence of hyphae, conidia, and other characters.

Viability of the fungal species from the Goa University Fungus Culture Collection (GUFCC):

The fungal cultures from GUFCC were sub-cultured on Malt Extract Agar (MEA) and incubated at room temperature for 15 days to 3 months (depending on the growth rate of different fungi). The viability of each culture was assessed.

The fungal cultures were successfully sub-cultured in triplicate on MEA and incubated at room temperature. The cultures were then checked periodically for fungal growth. Both macroscopic and microscopic features of the viable cultures were studied.

RESULT AND DISCUSSION

Decayed wooden pieces fallen on the ground and which showed macroscopic fungal-like growth were collected as these are associated with wood rotting. In all 34 different samples were collected from the Goa University campus. Using the above-mentioned techniques, culturing of the fungi was carried out on petri plates. Once the fungal colonization took place, mycelial growth was observed along with sporulation of few samples. Later, these each fungal samples were sub-cultured on 3 malt extract slants, for slide preparation and to preserve it in GUFCC.

Fungal slides were prepared using lactophenol cotton blue stain and identified at genus level on basis of spore shape, size, texture and shape of fungal hyphae using the identification key (Seifert, et al, 2011) (The genera of Hyphomycetes, Netherlands) with the help of mycologist.

Out of 34 samples cultured, 11 samples didn't produce spore making it not possible with identification. 12 samples were identified as *Aspergillus* sp., 6 samples were *Cladosporium* sp., 2 samples were *Paecilomyces* sp., 1 fungal culture each belongs to the genus *Corynespora*, *Fusarium* and *Acremonium*. Table 1 shows the culture number along with collection date and identification.

Culture number	Name of fungus	Date of collection
1	*Aspergillus* sp. 1	06/01/2022
2	*Aspergillus* sp. 2	26/11/2021
3	*Aspergillus* sp. 3	06/01/2022
4	*Aspergillus* sp. 4	18/11/2021
5	*Aspergillus* sp. 5	17/11/2021

6	*Cladosporium* sp. 1	17/11/2021
7	*Cladosporium* sp. 2	06/10/2021
8	*Aspergillus* sp. 6	18/11/2021
9	(No sporulation)	10/12/2021
10	(No sporulation)	10/12/2021
11	*Aspergillus* sp. 7	26/11/2021
12	*Aspergillus* sp. 8	06/01/2022
13	*Aspergillus* sp. 9	06/01/2022
14	(No sporulation)	10/12/2021
15	(No sporulation)	10/12/2022
16	*Cladosporium* sp. 3	10/12/2022
17	*Cladosporium* sp. 4	06/01/2022
18	*Cladosporium* sp. 5	18/11/2021
19	(No sporulation)	12/12/2021
20	(No sporulation)	06/01/2022
21	(No sporulation)	10/12/2021
22	*Paecilomyces* sp. 1	03/12/2021
23	(No sporulation)	03/12/2021
24	(No sporulation)	10/12/2021
25	*Cladosporium* sp. 6	03/12/2021
26	(No sporulation)	14/01/2022
27	*Aspergillus* sp. 10	14/01/2022
28	(No sporulation)	24/01/2022
29	*Corynesporium* sp.	14/01/2022
30	*Fusarium* sp.	14/01/2022
31	*Acremonium* sp.	14/01/2022
32	*Aspergillus* sp. 11	14/01/2022

33	*Aspergillus* sp. 12	10/12/2021
34	*Paecilomyces* sp. 2	14/01/2022

Out 34 samples, 12 fungi belong to genus *Aspergillus*. This tentatively indicates that the diversity of *Aspergillus* associated with wood decay is maximum in the studied area.

These cultures were then added to the GUFCC.

In the second objective of the study, the old, micro and macro fungal cultures from the GUFCC were grown onto the 2% malt extract agar slants. In this study, to check the viability of the few selected cultures from the GUFCC in the past few years from 2012 to 2019, were sub-cultured thrice separately on 2% malt extract agar media and the mycelial and conidial growth were observed.

In all, 338 fungal isolates were assessed for their viability. After many observations, over few days to months, it was observed that most of the cultures took a long time to grow whereas few cultures started to show mycelial growth after few days of sub-culture. It is presumed that this could be due to the age of the culture along with other factors in consideration.

The climatic and ecological conditions favor growth of different types of fungi in nature (Persoon, 1801; Fries, 1821), such as annual rainfall, temperature, habitat or soil properties or small scale eco-physiological interactions. Temperature, moisture or pH, are commonly applied to induce fungal growth. (Teppo and C. Alisha 2021).

For the germination of spores, the oxygen requirements vary from species to species and also between spores from different individuals of a single species. Oxygen requirements dependent on environmental or incubation conditions of the spore and physical and chemical factors. Different external conditions may be required in different phases of the

spore germination, such as swelling or germ-tube production (Goddard, 1935; Wood-Baker, 1955; Sussman and Halvorson, 1966). Internal conditions of the spores (maturity, longevity, dormancy, and vitality) also play a role (Tabak and Cooke, 1968). Concentration of the spore per volume of medium that may be related to O2/CO2 requirements or physicochemical features are vital. Gas requirements are connected to the germination vs. spore density phenomenon: higher concentrations of spores inside a medium germinate poorer compared to lower concentrations at the edge of the medium. This seems to be the general rule (Coons, 1916; Weimer, 1917).

Carbon dioxide also stimulates spore germination in wood rot fungi in moderately elevated concentrations (Hintikka, 1970). This may be an ecological adaptation to life in or on wood which releases CO2 when decomposed. CO2 has an acidifying effect on culture medium and potential spore germination induction may be due to decreasing pH, rather than CO2 itself. CO2 acts as stimulant for spore germination in some fungi, whereas others require high CO2 concentration and complete absence of oxygen for the formation of viable spores. (Orpin, 1975; Gruninger et al., 2014).

Out of 338, a total of 177 different fungal isolates showed viability and 161 isolates were non-viable. Of the 177 viable isolates, 90 fungal isolates have a unique GUFCC number and are identified (Table 2). 72 isolates with GUFCC number were non-identified (Table 3) and only a few i.e., 15 isolates were identified and wasn't labelled with any GUFCC number (Table 4).

Table 5 shows the inventory of all the 161 non-viable isolates.

Viability (in %) = (177/338) x 100 = 52.36%

Non viability (in %) = (161/338) x 100 = 47.63%

The macro-morphological characters of the viable genera were recorded:

1. *Acremonium*

Colonies hyaline, slow growing, pink, usually moist or smooth; mycelium fine; sporogenous cells phialidic, mostly simple, awl-shaped, erect, arising from submerged or slightly fasciculated aerial hyphae; conidia cylindrical.

2. *Alternaria*

Colonies effuse, gray, dark, blackish brown or thick brown, or olive black; mycelium immersed or partly superficial, hyphae pale brown, stroma rarely formed; conidiophores short.

3. *Aspergillus*

Colonies restricted to fast growing, white blue, blue-green, green, yellowish green, brown, fawn, ochraceous to black, zonate or azonate, velvety, floccose, lanose or funiculose; mycelium mostly submerged.

4. *Auricularia*

Gelatinous, ear shaped fruiting body, slightly downy to conspicuously hirsute upper surface that is sometimes, wrinkled or veined.

5. *Boletus*

Dense network of branched hyphae, colourless or white; mostly submerged into the substratum.

6. *Brettanomyces*

Colonies of different sizes; off white, milky, raised, even and glossy; superficial or submerged in the media.

7. *Cladosporium*

Colonies effuse, olive green; mycelium submerged, velvety, floccose, hairy; reverse olivaceous black; conidiophores macronematous.

8. *Clathrus*

Usually white with a yellow shared in the center, has distinct mycelium cords and aerial hyphae; mycelium growth is patchy; reverse white, with yellow or brown tinge in the center and noticeable mycelium cords.

9. *Clitocybe*

Fungal colonies grow in patches, hyphae hyaline white in colour, reverse off white to yellowish; colony margin wavy.

10. *Coprinus*

Hyphae grows as mashy, sticky, butyrous mass, white when young becoming yellow or brown in later stages.

11. *Curvularia*

Colonies effuse, brown, gray, or black, hairy, cottony, or velvety; mycelium immersed; stromata often large, erect, black, cylindrical, sometimes branched.

12. *Cylindrocarpon*

Colony texture cottony or felty with aerial mycelium; colour white to cinnamon; conidiophores arising from the mycelium and from the agar surface, produced terminal or phialides.

13. *Daldinia*

White felty colonies, blackish gray colouration on the reverse side; blackish or coloured stromata that are 3-5cm in diameter and varying shapes.

14. *Debaryomyces*

Cells single or in short chains, some cells have lobes, spherical to short oval, multilateral budding; slow growing fungi, creamy white colour, circular, dull, margin smooth, slightly crenate, entire.

15. *Dekkera*

Round, dome shaped, dark green colonies retain the bromocresol green, with maturity grows a white centre point with many tiny white growths around the centre point.

16. *Exserohilum*

Colonies range from grey to blackish brown; texture pseudo-like to floccose; olivaceous to black reverse.

17. *Fistulina*

Fungus grows into large colonies, dark brown to black in colour, margin irregular.

18. *Fusarium*

Colonies usually fast growing, pale to bright coloured; aerial mycelium, felty or diffuse, fruit body when present a sporodochium; sporogenous cells arise directly from vegetative hyphae to form conidiophores.

19. *Ganoderma*

Mycelia forms as white cottony mass, developing into stable, spherical aggregates consisting of dense, branched and partially intertwined network of hyphae.

20. *Gliocladium*

Colonies white, greenish or shades of salmon pink or rose; hyphae hyaline, branched, becomes darker with age.

21. *Hansenula*

Colony butyrous, tan to cream in colour, flat, smooth and creamy in texture; pseudo hyphae form as chains of ovoid or cylindrical cells.

22. *Hansfordia*

Colonies effuse, velvety, orange grey to brownish grey; reverse brownish grey; mycelium immersed, smooth, roughened or verruculose, hyaline to greyish brown; conidiophores macronematous, mononematous, erect or repent, straight or flexuous, smooth, roughened to verrucose, greyish brown.

23. *Helicomyces*

Colonies effused on substrate, brown to dark brown, often inconspicuous, composed of dry and compressed conidia on surface of woody substrate. Mycelium partly superficial, partly immersed in the substrate, branched septate, hyaline and smooth-walled hyphae. Conidia become brown to dark brown when mature.

24. *Hirsutella*

Mycelium dark-brown to black, Stroma cylindrical, black, with a cream-white swollen terminal part; initially dark brown becoming black with age, Perithecia immersed to slightly erumpent, globose to flask-shaped.

25. *Hyphopichia*

Fungus grows as big patch in the center of the media, Hyphae light brown to cinnamon brown in colour, butrous surface, margin irregular, hyphae superficial.

26. *Inonotus*

Colonies forms in large patches which eventually grows into large patch of fungus, dark brown to black in colour, superficial on the substratum, margin irregular.

27. *Lentinus*

Hyphae thick, initially white then turns into cinnamon brown to chestnut brown, creamy butyrous at the surface; hyphae submerged into the medium; reverse dark brown.

28. *Lenzites*

Colonies small, globose, light yellow in colour, creamy mucilaginous at the surface.

29. *Leucoagaricus*

Hyphae yellowish brown in colour, light yellow in the center and turning dark brown to blackish at the periphery, mycelia mostly superficial.

30. *Monodictys*

Colonies effuse green, greenish-blue, lavender, dark gray-blackish brown or black; mycelium mostly superficial; stroma, setae and hyphopodia absent.

31. *Omphalina*

Mycelia white to off-white in colour; Two types of hyaline hyphae were seen- narrow and wide; special modification of hyphae such as knots rings, and various types of hyphal junctions. Central portion of the mycelium mat showed pink colour pigmentation.

32. *Omphalotus*

Fast growing fungi, hyphae yellowish to red brown but becoming dark brown to blackish towards the base; surface turns in butyrous slimy sticky hyphal mass; reverse brown to black.

33. *Paecilomyces*

Colonies growing fast; aerial mycelium never typically green, funiculus, conidiophore well developed, erect; mostly sporogenous cells are borne more or less directly on the vegetative hyphae.

34. *Pannelus*

Forms dark brown colonies in the center and light brown colonies in the periphery**.**

35. *Penicilium*

Grows as compact dense colonies, colonies variously coloured, usually blue-green, texture variable, zonate or azonate; vegetative hyphae creeping; reverse uncoloured or variously coloured; conidiophores usually conspicuous, more or less erect, hyaline.

36. *Pleurotus*

Fast growing mycelial growth, hyphae hyaline, submerged into the substratum; sporogenous cells are sometimes borne more or less directly on the vegetative hyphae.

37. *Polyporus*

fleshy, stipitate basidiome with dimitic hyphal system; hyaline, smooth, cylindrical to sub-ellipsoid basidiospores with no reaction to Melzer's reagent; terrestrial growing on exposed or buried wood causing white rot in wood and having wide global distribution.

38. *R. sambuci*

Mycelia black, through the outer layer of the host tissue, to near superficial, solitary or scattered, globose to sub globose, cells of asco-stromata dark brown colour.

39. *Romania*

Mycelia fast growing, hyphae white, mycelia immersed into the substratum; reverse yellow to gray, often with complex discoloured zones.

40. *Saccharomyces*

Minute Colonies are spheroidal to ellipsoidal, occur singly or in cluster. Butyrous, cream coloured, smooth; margin entire, undulating or somewhat lobate.

41. *Stachybotrys*

Colonies effuse, usually blackish or blackish green; mycelium superficial, immersed; hyphae sometimes forming ropes; conidiophores macronematous, mononematous, colourless grey, brown, olivaceous brown or black, smooth or verrucose.

42. *Stropharia*

Hyphae hyaline, white to off white in colour, formed as a dense mycelial mat, mycelia superficial; reverse yellow, exudate formed rarely.

43. *Termitomyces*

Initially watery white colonies, after few days changes to grayish, reverse red in colour; pileus convexo-mucronate, surface rusty ochraceous to orange.

44. *Trichoderma*

Colonies with watery translucent smooth surface to floccose to compactly tufted pustules; conidiation effuse or tufted or forming compact pustules, green or sometimes white, grey or brown; reverse sometimes yellow, amber brownish; mycelium hyaline; exudate formed rarely, hyaline to yellow on surface of the mycelium.

45. *Volvaniella*

Longitudinally linear soon aerial and disorganized, greyish white at first, then dingy yellowish and brown, eventually becoming light grey brown to reddish brown, often with complex discoloured zones with musty fragrance.

This assessment gives the data for the easy finding and long-term maintenance and storage of the cultures for further use for various studies.

CONCLUSION

The present study was initiated to check the viability of various fungal cultures available in the Goa University Fungal Culture Collection (GUFCC) and also to isolate different wood rotting fungal species from the Goa University campus and to maintain their pure cultures in GUFCC.

The present study included the isolation, culture, sub-culture and identification of the wood rot fungal samples collected from the Goa University campus. Out of the 34 samples investigated, 12 samples belonged to the genus *Aspergillus* which includes soft rot fungi belonging to Ascomycota. Six samples were identified as *Cladosporium* species which are members of Ascomycota, commonly found on living and dead plant materials. Two fungal cultures were identified as *Paecilomyces* species which are ubiquitous, decay, filamentous fungi usually found on decaying vegetation. One each was identified as *Corynespora* sp., a member of Ascomycota, *Fusarium* sp. which has been reported as wood-penetrating agents of several plant species, and *Actemonium* sp. where most of the species are saprophytic, isolated from dead decaying plant materials and soil.

It's concluded that all the six different fungal genera isolated from dead wood samples are wood litter fungi inhibiting moist soil, dead and decaying plant materials involved in degradation. Out of the 34 collected samples, 11 fungal cultures did not show sporulation and hence could not be identified.

In the second objective of the study, previously isolated and maintained, micro- and macro- fungal cultures from the GUFCC were sub cultured to assess their viability. It was concluded that out of the selected 338 fungal isolates, 177 isolates were viable and 161 isolates were non-viable.

The isolation and maintenance of fungal cultures serves a base to conduct taxonomical, medical, and pathological studies. Besides they have industrial applications that include

solvents, antibiotics, enzymes, vitamins, amino acids, polymers, and many other useful compounds by the process of fermentation.

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