BIODIVERSITY, CONSERVATION AND UTILISATION OF MUSHROOM FLORA FROM THE WESTERN GHATS REGION OF INDIA

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ABSTRACT

The Western Ghats region of Indian subcontinent is one of the globally recognized biodiversity hotspots that has an unestimated wealth of biodiversity. A survey was conducted in the Anaikatti, Attapadi, Palghat, Siruvani, Nilgiris and Kallar regions of the Western Ghats of India during 2008 -10 and about 68 mushroom flora belonging to 19 genera were recorded. The seasonal occurrence of *Volvariella* from June-September; *Calocybe* from February-September; *Pleurotus* from June-July and November-January; *Auricularia, Lentinus, Agaricus* from October-January; *Tricholoma* during June; *Ganoderma*, *Polyporus*, *Trametes* from September-January and June-August; *Lycoperdon* during October; *Termitomyces* from July-October; *Tramella*, *Mycena* and *Rusulla* from January; *Ramaria* from October-November; *Schizophyllum* from June-October and January; *Amanita* from November-December and *Macrolepiota* during December were observed.
Among the mushroom flora a wild strain of *Pleurotus djamor roseus* was found suitable for commercial cultivation with bioefficiency of 132 percent and cost benefit ratio of 1:2.9. The mushroom contained all essential nutrients and could be stored under room temperature for one day and under refrigerated storage for 2 days. The ITS 1 and 2 regions of the mushroom was sequenced and submitted in NCBI (Gen Bank accession No. HM107001). The diethyl ether fraction (10 percent concentration) of *G.lucidum* and *L. edodes* showed the inhibition of mycelial growth by up to 70 percent and 68.2 percent, respectively against *Collectotrichum gloeosporioides*, the fruit rot pathogen of Mango.

Keywords: Biodiversity,Western ghats, Mushroom flora

INTRODUCTION

The Indian sub continent is blessed with diverse agroclimatic zones that harbour a treasure trove of fungal diversity. Though the occurrence of mushrooms is of diverse nature in India, they are not well known. The collections of mushrooms began in India four decades ago [1,2,3]. To date, about 1,200 species of fungi belonging to the order Agaricales, Russulales and Boletales are described in comparison to about 14,000 species of mushrooms reported worldwide that contributes 10 percent of the global mushroom flora. So far, about 1,105 to 1,208 species of mushrooms belonging to 128-130 genera have been documented and among these, 300-315 species belonging to 75-80 genera are considered edible. The Western Ghats region, one of the

four globally recognised biodiversity hotspots forming a long mountainous region along the west coast of India harbor to date consists of 750 species of mushrooms. It has an unestimated wealth of mushroom biodiversity that needs to be tapped properly as there are still several undescribed species yet to be identified. Efforts need to be made to identify and exploit these mushroom flora for utility as their biodiversity and conservation strengthen the food security of a country [4].

Out of 2000 edible species available in the world, about 283 are reported from India. Nearly 6,500 collections of mushroom flora belonging to 223 species, 59 genera and 15 families of Agaricales have been reported from North West Himalayas by Lakhan Pal and his students since 1976 [5]. These data on the occurrence of the mushrooms reveals the richness of mycoprotein in the country. Not only in terms of edibility, there lies enormous applications of these mushrooms for bioremediation, biodegradation, biopesticidal and pharmacological values that could be exploited.

The Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India has contributed to the development and domestication of six species of *Pleurotus*, two isolates of *Agaricus bisporus*, and one species of *Calocybe* for commercial utilization. However, due to increased awareness of the pharmacological values and nutritional values of mushrooms, there is more demand and consumer preference for different varieties of mushrooms among the people and farmers that are urged to exploit the wild mushrooms for utlisation. The mushroom genome stands out as a virtually untapped resource for novel antimicrobials. Recently many antibacterial antifungal and insecticidal properties have been identified from mushrooms. Scientists worldwide are now focusing on the exploitation of biomolecules from mushrooms for pest and disease management which is a challenging field of study. With this aim, surveys were conducted in Western Ghats region so as to exploit the mushrooms for utilization and also to identify their antimicrobial nature.

MATERIALS AND METHODS

Collection and identification of wild mushrooms. Collections of wild mushroom flora were made from the Anaikatti, Attaipadi, Kallar, Nilgiris, Palakkad and Siruvani regions of the Western Ghats of India during the period 2008-2010. The mushrooms collected were identified morphologically as per the keys provided by Directorate of Mushroom Research, Solan, pure cultured and maintained in potato dextrose agar slants and used for further studies.

Molecular characterization

Isolation of DNA from *Pleurotus djamor*. Among the wild mushrooms collected, a new isolate of oyster mushroom, *Pleurotus djamor roseus* was selected. The mushroom was grown in malt extract broth and the mycelial mat was collected and ground with lysis buffer. After maceration the tube was kept in room temperature for 30 min and 150µl of potassium acetate was added, vortexed for 2-3 seconds and kept in freezer for 30 min. The tubes were centrifuged at 15,000 rpm for 5 min. The supernatant was transferred to another tube and equal volume of isopropyl alcohol was added. The tube was mixed by inversion and centrifuged at 15,000 rpm for 2 min and the supernatant was discarded. The DNA pellet was washed in 300 µl of 70 percent ethanol, centrifuged at 10,000 for one min and then the supernatant was discarded. The pellet was air dried and dissolved in 50 µl of 1X TE buffer and used as genomic DNA for PCR reaction [6].

Amplification of the ITS regions with ITS-1 and ITS- 4 primers. The genomic DNA extracted from the pure culture of *Pleurotus djamor* was used for PCR studies. The polymerase chain reaction primers, ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3') were used to amplify the ITS of ribosomal DNA [7]. The PCR reaction was performed in a total reaction mixture of 40 µl containing 20 µl of master mix , 4 µl each of ITS-1 and ITS-2 , 4µl genomic DNA and 8 µl of distilled water. The PCR conditions consisted of 34 cycles of 1 min denaturation at 95°, 30 s annealing at 50˚C, 1 min 20s elongation at 72˚C and ending by 10 min final elongation step at 72˚C with lid heating option at 110˚C [6]. Amplified products were run on 2 percent agarose gel, stained with ethidium bromide and visualized under UV illumination. The sequencing was done using ITS-1 and ITS-4 primers and the nucleotide sequence comparisons were performed using Blast Multiple Alignment Tool (BLAST) network sequences from the National Centre for Biotechnology Information (NCBI) database.

Performance testing of the selected oyster mushroom *Pleurotus djamor roseus.* The yield performance of *P.djamor roseus* was tested at the Mushroom Research and Training Centre, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The new oyster mushroom, *Pleurotus djamor roseus* with pale pinkish white sporophores was selected and tested for its yield performance for commercial cultivation. The spawn of *P. djamor roseus* was prepared in sorghum substrate and used for bed prepaparation. For this, purpose using paddy straw as substrate, cylindrical beds were prepared with sorghum based spawn of *P. djamor roseus* (500 g substrate/bed; 300 g of spawn/bed). The beds were placed in cropping rooms made of thatched shed (using coconut leaves) where temperatures of 23-30˚C with relative humidity of 80 percent was maintained thorough out the cropping period. The beds were held in place with a hanging rope system in the cropping rooms. Five beds were prepared monthly from June, 2009 to May, 2010. The yield characters were days for spawn run (DFSR), days for pinhead formation (DFPF), days for first harvest (DFFH), Total yield (g per kg substrate) and pest and disease levels were recorded. The bioefficiency percentage was calculated. Harvested mushrooms were cleaned and packed (250 g) separately in perforated polythene bags and placed under natural conditions (room temperature). Under refrigerated condition they were placed in non-perforated polythene bags and the observations on keeping quality were recorded.

Comparison of *Pleurotus djamor roseus* with other oyster mushrooms. The mushroom *P. djamor roseus* was tested for its yield performance in comparison with the mushroom varieties that are cultivated on a commercial scale in the state. The mushroom varieties *viz.*, *Pleurotus florida* (var. PF), *P. eous* (var. APK1), *P. djamor* (var. MDU1-white), *Hypsizygus ulmarius* (var. CO2) were used for comparison. The beds were prepared with paddy straw as substrate as mentioned earlier and the yield characters were recorded as mentioned earlier with four replications. All experiments were laid out based on completely randomized block design (CRD). Statistical software (AGRES) was used for the analysis of the data.

Studies on the nutritive value of *Pleurotus djamor roseus.* Moisture content of the mushroom was estimated by drying 50 g of fresh mushrooms in an oven at 80^{o}C for three consecutive days. It was later cooled in a desiccator and reweighed. The moisture content was arrived from the differences in the weight [8]. The crude protein content of the mushroom was estimated by Micro Kjeldahl method [9]. The total carbohydrate content was determined by following

Anthrone method [10]. Estimation of ascorbic acid [11], crude fat [8], total phenolic content using the Folin-Ciocalteu method [12], crude fibre [13], total ash content (14), total nitrogen analysed by Diacid extract method-semiautomatic Kjeldhal distillation, total phosphorous by Triacid extractmethod-vanodamolybdate calorimetric method, total potassium and total calcium [15] as described previously. Antioxidant activity was measured using Ferric reducing antioxidant power (FRAP) assay [16]. All nutrients and ingredients were analyzed on fresh weight basis. The experiments on the estimation of nutritive values were performed at the Post harvest Technology Centre, Tamil Nadu Agricultural University, Coimbatore-3.

***In vitro* testing of mushroom flora for antimicrobial properties.** Among the mushroom flora collected *viz., Ganoderma lucidum, Lentinus edodes, Trametes versicolor, Pleurotus djamor roseus* and *Auricularia polytricha* were selected and screened for their antifungal activity against *Colletotrichum gloeopsporioides*, the mango fruit rot pathogen by a dual culture technique [17]. Based on the results obtained from dual culture technique, both *Ganoderma lucidum, Lentinus edodes* were selected for *in vitro* testing of antimicrobial activity against *Colletotrichum gloeosporioides* by a poisoned food technique [18]. For this purpose, the discs (9 mm) of both *Ganoderma lucidum* and *Lentinus edodes* were inoculated in potato dextrose broth and the culture filtrate was collected after seven days and the antimicrobial substances were separated using diethyl ether. The diethyl ether fractions *ie.,* the aqueous phase at different concentrations *viz.,* 1, 5 and 10 percent were prepared in PDA medium separately. The medium containing diethyl ether fractions of *Ganoderma lucidum* and *Lentinus edodes* was plated separately and a mycelial disc of the pathogen *Colletotrichum gloeosporioides* was placed at the center. Three replications were maintained for each treatment. After five days incubation, the mycelial growth of the pathogen was recorded in both treatments as well as in respective controls and the percent inhibition was calculated [19].

RESULTS AND DISCUSSION

Collection of wild mushroom flora. About sixty eight mushroom flora belonging to 19 genera *viz., Agaricus sp., Amanita sp., Auricularia polytricha, Calocybe, Ganoderma lucidum, Lentinus edodes, Lycoperdon esculentum, Mycena sp., Macrolepiota sp., Pleurotus cystidiosus, P.djamor, P. pulmonarius, Polyporusversicolor, Ramaria sp., Russula sp., Ramella sp., Schizophyllum commune, Termitomyces, Trametes versicolor, Tricholoma giganteum,* and *Volvariella volvacea* collected from Anaikatti, Siruvani, Palghat, Kallar and Nilgiris regions of Western ghats during 2008-10 were identified morphologically based on the keys provided by the Directorate of Mushroom Research, Solan, India.

Collections revealed the occurrence of *Volvariella* from June-September; *Calocybe* from February-September; *Pleurotus* from June-July and November-January; *Auricularia, Lentinus, Agaricus* from October-January; *Tricholoma* during June; *Ganoderma, Polyporus, Trametes* from September-January and June-August; *Lycoperdon* during October; *Termitomyces* from July-October; *Tramella, Mycena* and *Rusulla* from January; *Ramaria* from October-November; *Schizophyllum* from June-October and January; *Amanita* from November-December *Macrolepiota* during December (Table 1).

Table1. Wild mushroom flora of the Western Ghats region of India

S. No.	Mushroom flora	Place	2008-09	2009-10
1.	*Volvariella volvacea*	Palghat, Anaikatti	June August July	September August -
2.	*Pleurotus djamor* *P. cystidiosus* *P. pulmonarius* *Pleurotus* sp.	Kallar Anaikatti Nilgiris Siruvani	June November January -	- July - June
3.	*Lentinus edodes* *L. crinitus*	Nilgiris Anaikatti	October November	January -
4.	*Ganoderma lucidum*	Anaikatti Coimbatore	September, October September	January September
5.	*Calocybe indica*	Anaikatti Coimbatore	February May, July	June, July September
6.	*Agaricus sp.*	Nilgiris	October,December	January
7.	*Polyporus versicolor*	Siruvani	January	November
8.	*Trametes versicolor*	Siruvani Kallar Attaipadi	September October, December November	September October November
9.	*Auricularia polytricha*	Attaipadi Kallar	November	November
10.	*Termitomyces sp.*	Anaikatti Attaipadi	July, September September, August October	July , August October
11.	*Tricholoma giganteum*	Coimbatore	June	-
12.	*Schizophyllum commune*	Coimbatore	June, August , September	July, October, January
13.	*Lycoperdon esculentum*	Anaikatti Siruvani Kallar	October October, November November	October
14.	*Ramaria sp.*	Anaikatti	November	October November
15.	*Russula sp.*	Nilgiris	-	January
16.	*Tramella sp.*	Nilgiris	January	January
17.	*Amanita sp.*	Nilgiris	November	December
18.	*Mycena sp.*	Nilgiris	January	January
19.	*Macrolepiota sp.*	Nilgiris	December	-

Hot season: March–May/June
Rainy seasons: June–October /November
Cool weather conditions: November–February

It was observed that the climatic conditions prevailing in the areas of Anaikatti, Attaipadi, Kallar, Nilgiris, Palakkad and Siruvani regions of the Western Ghats favored the occurrence of diverse mushrooms. The mushrooms occurred through out the year and collections were grouped into three seasons: hot season from March–May/June, rainy seasons from June–October/November and cool weather conditions from November-February. The *V. volavcea* was collected during periods coinciding with rainy seasons followed by a hot and humid climate. *Pleurotus* sp. was mostly observed during rainy seasons. *Lentinus edodes* and *L. crinitus.*, *Agaricus* sp., *Amanita* sp., *Mycena* sp., *Tramella* sp., *Ramaria sp.,Russula sp., Macrolepiota sp., A.polytricha*, *P.versicolor* during cool seasons prevailing after rains. The occurrence of *L. esculentum* coincided with the initiation of rains from North West monsoon during that season . However, *T.versicolor, Termitomyces sp*., and S. *commune* were collected from both rainy and cool periods. Collections of *T. giganteum* were made during the period when the weather is warm just before the onset of southwest monsoon. *C. indica* and *G. lucidum* was collected during the period before and after the southwest monsoon. Distribution of 134 species of mushrooms representing 45 genera was recorded in Kerala during monsoon seasons. Best collection of fleshy agarics especially the popular edible mushrooms, the species of the termitophilic genera *Termitomyces, Podabrella*, the wood decomposer *Pleurotus*, and the ectomycorrhizhal fungi belonging to Boletaceae was dominant in the Western Ghats [20]. About, 90 mushroom accessions in 35 genera were documented in the Nilgiris biosphere reserve (21).

Molecular characterization. Amplification of the ITS regions of *Pleurotus djamor* with ITS-1 and ITS-4 primers shared 99 per cent homology with *P.djamor* . The sequences were submitted to NCBI and given Gen bank accession number HM107001.

Performance of the oyster mushroom *P. djamor roseus.* The mushroom required a maximum of 8.6 days for complete spawn run, 10.2 days for pin head formation, and 12.6 days for first harvest. The pin heads were pale pink in color and mature mushrooms were pale pinkish white in color with pileus measuring 5 cm and stipe length of 2 cm (Figure 1). Maximum yield of 660 g of mushroom/500 g substrate (132 percent bioefficiency) was recorded during the month of June. However, the bioefficiency ranged from 124 to 132 percent through out the year (Table 2). This mushroom can be best cultivated in thatched sheds with temperature range of 23-30°C and relative humidity of 70-80 percent through out the year.

Figure1. Morphology of *P.djamor roseus*

Table 2. Performance of the oyster mushroom *P.djamor roseus*

Month	DFSR	DFPF	DFFH	Total yield-g/ bed)	Bioefficiency (%)
June,2009	7.4	8.0	10.0	660.0	132.0
July	7.8	8.8	10.6	610.0	122.0
August	7.4	8.4	10.4	620.0	124.0
September	7.8	8.8	10.0	632.0	126.4
October	8.0	9.4	11.6	650.0	130.0
November	7.4	8.4	11.4	634.0	126.8
December	7.6	8.2	10.6	620.0	124.0
January. 2010	7.2	8.0	11.0	620.0	124.0
February.	7.0	8.0	10.6	635.0	127.0
March.	7.0	8.0	11.2	655.0	131.0
April	7.6	8.6	10.6	630.0	126.0
May	8.6	10.2	12.6	645.0	129.0
CD(P=0.05)				29.2	

Mean of five replications . g: Grams; % : Per cent

The mushroom when compared with the existing commercially grown oyster mushroom species revealed that *P. djamor roseus* performed on par with *P. eous* (var. APK-1) with bioefficiency of 132 percent (Table 3). Among the oyster mushrooms, *P. djamor roseus* recorded an earlier harvest and the cropping period was completed by 17-20 days which is very short when compared to *P. eous* (var. APK1) and other mushrooms. The mushrooms could be stored under room temperature for one day and under refrigerated storage for 2 days without any microbial spoilage, color change and liquefaction.

Table 3. Comparison of *P. djamor roseus* with commercially grown oyster mushroom

Mushrooms	DFSR	DFPF	DFFH	Yield g/500 g substrate			Yield g/ bed	BE (%)	C: B ratio
				I H	II H	III H			
P. florida var. PF	17.3	20.8	24.8	400	250	100	750	150.0	1:3.6
P. sajorcaju var. M2	19.3	22.5	25.0	350	250	100	700	140.0	1:3.4
P. eous var. APK-1	12.0	13.2	14.8	350	220	105	675	135.0	1:3.2
P. djamor var. MDU1	20.0	23.0	26.3	350	250	100	700	140.0	1:3.5
H. ulmarius var. CO2	22.0	23.0	24.6	400	200	100	705	141.0	1:3.5
P. djamor roseus	8.3	10.3	11.3	360	250.0	50.0	660	132.0	1:3.2
CD (P=0.05)							42.23		

Mean of four replications; H : Harvest; C:B: Cost: Benefit Ratio

The nutritive values analyzed for 100 g fresh mushroom (*P. djamor roseus*) showed the presence of all essential nutrients viz., carbohydrates (3.8%), protein (2.6%), fat (0.2%), crude fibre (0.94%), total ash (1.12%), ascorbic acid (15.43 mg/g), total nitrogen (1.28%), total phosphorous (0.445 %), total potassium (2.56 %), calcium (1.2 %), sodium (0.38 %) with total antioxidant

activity of 186.3 (µg/g) and a calorific value of 19.8. No pest and disease was recorded during the cropping period.

Antimicrobial activity of mushroom fungi against plant pathogen. Among the mushrooms tested by dual culture technique, *G. lucidum* and *L. edodes* inhibited mycelial growth of *Colletotrichum gloeosoprioides* to 68 percent and 65.3 percent, respectively (Table 4). The diethyl ether extract (10 percent) of *L. edodes* and *G. lucidum* was tested by a poisoned food technique and the results showed that the mycelial growth was inhibited by 70 percent and 68.2 percent, respectively (Figure 2). Perusal of reports show that the shiitake mycelial leachate contained an antibiotic substance that exhibited clear zones of inhibition against plant pathogenic bacteria *Pseudomonas syringae pv. glycinea, P. syringae pv. tabaci, X. campestris pv. campestris* and *Ralstonia solanacearum* [22]. In addition, we found that *Ganoderma lucidum* exhibited a broad spectrum of antifungal, antibacterial and antiviral activities [23]. The ethanolic and methanolic extracts of *Agaricus bisoprus* and *P. sajor-caju* exhibited antimicrobial activity against human pathogens *viz*., *Escherichia coli, Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Klebsiella pseudomonaie* [24]. Though there are several reports on the novel biomolecules from higher basidiomycetes, the scientific community is now focusing on the exploitation of the antimicrobial compounds against plant pathogens as well.

Table 4 .*Invitro* testing of mushroom fungi against *C.gloeosporioides* by dual culture technique

S. No.	Mushroom Species	Mycelial growth of *C. gloeosporioides* (mm)	Percent inhibition
1.	*Ganoderma lucidum*	24	68.0
2.	*Trametes versicolor*	50	33.3
3.	*Lentinus edodes*	26	65.3
4.	*Pleurotus djamor roseus*	59	21.3
5.	*Auricularia polytricha*	44	41.3
6.	Pathogen control	75	-

Means of three replications

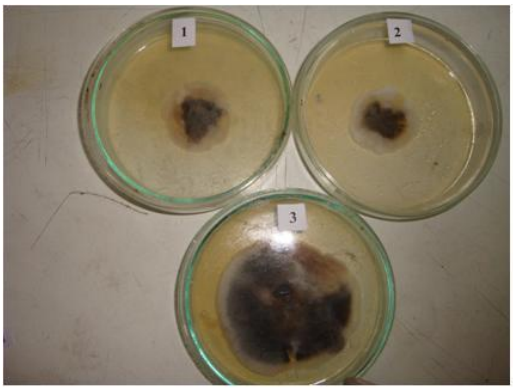

Figure 2. Antimicrobial activity of diethyl ether extract of *G. lucidum (1)* and *L. edodes (2)* against *C. gloeosporioides; 3= pathogen control*.

CONCLUSION

Perusal of literature shows there is no doubt that the diverse climatic condition in India made this country a natural habitat for many mushrooms. Currently due to deforestation activities several mushroom species are endangered and hence it is crucial to conserve the mushroom flora to strengthen the food security of a country. In that way, this paper clearly depicts the biodiversity of mushroom flora occurring in the Western Ghats region of India and their conservation in order to identify the potential mushroom species for domestication also to exploit the biomolecules. The oyster mushroom *Pleurotus djamor roseus* is highly suitable for commercial cultivation in subtropical regions of the world. This study on the antimicrobial nature of *L.edodes and G. lucidum* paves way for developing biopesticidal molecules from mushrooms against plant pathogens.

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