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Morphological and Biochemical Characterization of Wild Mushrooms from Sub Mountainous Region of Punjab State

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Abstract—*The diversity of wild mushrooms have been explored from sub mountainous region of Punjab. Five different basidiomycetesnamely: Pleurotusflorida, Pleurotussajor-caju and Macrolepiotaprocera from order Agaricale, Sparassiscrispa and Trameteselegans from order Polyporales were described morphologically and biochemically. These wild mushrooms were explored during rainy season from two Districtsviz Pathankot with GPS coordination 32°26'43" N 75°64'21" E and Hoshiarpur with GPS coordination 31°51'43.178" N, 75°91'14.83"E. These mushrooms were investigated for their capacity to produce lignocellulosic enzymes. It was observed that endo-β-1,4-glucanase was significantly higher in Trameteselegans(160.14 U/ mg) but xylanase and laccase were found to be lowest in case of Trameteselegans. Total cellulases and cellobiase were found to be higher in Pleurotussajor-caju with values 1.96 U/mg and 1.84 U/mg respectively.*

Keywords*: Wild Mushroom, Agaricale, Polyporale, Lignocellulosic Enzymes.*

1. INTRODUCTION

Mushrooms are indispensible part of the ecosystem. Some mushrooms are cultivated but some are directly collected from the wild and consumed. Among 650-700 edible mushroom species, approximately 130 have been domesticated(Agahar-Murugkarand Subbulakshmi 2005). Wild mushrooms are gaining importance due to their high protein and low fat/energy contents. To combat with world's food shortage problem, mushrooms can occupy a place above vegetables and legumes (Baltacioglu *et al* 2015). Therefore, it is necessary to exploit wild mycoflora for their uses in bioremediation, biodegradation, bio-pesticidal and pharmacological qualities. On the top of everything, it is important to conserve biodiversity of macro-fungi to secure it from the danger of extinction.

India represents diversified agro-climatic zones that cherish a stock of fungal diversity. Though mushroom diversity is very rich in India, 50 % of that is yet to describe (Dwivedi *et al* 2012). 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(Kaur*et al* 2015). Punjab harbors in North-east India represents five different agro-climatic zones namely sub-mountain undulating zone, undulating plain zone, central plain zone, western plain zone and western zone which provides a wide range of opportunity to collect diverse mushroom flora. Its humid climatic conditions, plant distributions and field features are very suitable for the growth of edible fungi.

Furthermore, wild mushrooms provide a scope to degrade cellulosic material as it grows on wide range of substrates because of its capacity to produce lingo cellulosic enzymes *i.e.* cellulases, xyalanases and laccases. These wild mushrooms may serve as alternative path for the use of agrowaste. So,present study was aimed to collect new wild mushrooms and calculate their enzyme producing capacity.

2. MATERIAL AND METHODS

2.1 Survey and collection of Mushrooms

The field survey for collection of various fleshy fungi from sub mountain undulating zone was undertaken during July 2015 to September 2015 and July 2016 to September 2016. Required materials and equipments such as isolation kit, typed performa, digital camera for photography, digging equipment, GPS device were carried along during survey. Photographs of wild mushroom were taken in their natural habitat. The map of collection locality was sketched with GPS coordinate and wild mushrooms were carefully handled, packed in brown paper bags and carried to Mushroom Research Complex, Punjab Agricultural University to record macroscopic features used for identification. Standard methods of collection, preservation, macroscopic and microscopic observations were followed. Characteristics of mushrooms were recorded in structured Performa. Mushroom samples were dried in hot air dryer at 60° C. Some collected edible fleshy fungi were also

cultured on Potato dextrose agar (PDA) medium and maintained for further study.

2.2 Morphological Studies of Collected Edible Mushrooms

All the collected edible mushrooms were observed on the basis of morphological and other phenotypic parameters recorded in fresh samples. The diameter of fruit body of collected mushrooms was measured in centimeters. Length of stalk was also recoded. Presence of different part of fruit bodies like, cap, stalk, gills, volva, annulus, shape and scales were observed. Morphology of stalk, e.g. attachment with cap, shape and its base was also documented. Spore print was prepared by placing the pileus downwards in an inverted jar which was kept on a black paper.

2.3 Raising biomass for production of enzymes

Wild mushroom cultures were grown on mushroom minimal medium (MMM) (*Raper et al* 1972) in 250 ml Erlenmeyer flasks containing 50ml MMM medium for enzyme production. MMM broth was supplemented with wheat straw (@ 0.5 g/flask) as inducers for extracellular enzyme production. All the cultures were grown in triplicate and incubated at 30^{o} C for 10 days. Cultures broth was filtered using whatman paper no. 1 and filtrates were collected for the estimation of enzyme activity.

2.4 Estimation of enzyme activity and total protein

Total cellulases were determined at 50 ^{o}C using whatman No.1 filter paper strips (1-6 cm) according to the method of Toyama and Ogawa 1977. Endoglucanase (CMCase) activity was estimated by the method of Mandels*et al* (1976).The xylanase activity was determined at 50 °C for 10 minutes using beechwoodxylan (1%, w/v, Sigma, USA) as the substrate. The reducing sugar released by the enzymatic reaction was estimated using the DNS method with glucose or xylose as the standard.Assay of laccase was done by the method of Turner (1974). For estimation of total protein, standard method of Lowry *et al* (1951) was followed. Specific enzyme activity is calculated for each enzyme, as the number of enzyme units per mldivided by the concentration of protein in mg/ml. Specific activity values are therefore quoted as units/mg or nmol/min/mg.

3. RESULT AND DISCUSSION

3.1 Morphology of wild mushroom

***3.1.1 Pleurotusflorida* and *Pleurotus Sajor-caju*:** These mushrooms aresaprobic in nature and belongs to the class Basidiomycetes, order Agaricales and family Tricholomataceae (Table 1). The fruiting bodies were observed on tree trunk (*P.florida*) and wooden log (*P. sajor-caju)*grown as a group stacked one over another. The pileus ranges from 6 to 12 cm in diameter. *P. florida* (Fig.1) and *P.sajor-caju* (Fig.3) arefan shaped, white in colour. But, at younger stage *P.sajor-caju*is spoon shaped and gives coral like appearance. Stipe is very short and ranges from 1 to 2 cm in length. Gills are running down the stem and spore print is creamy white for P. florida and white for P. sajor-caju. According to Rajarathnam et al (1987) and Ali et al (2012) morphological characters of Pleurotus spp. was described.

Table 1: Morphological study of wild edible mushrooms

Mushroom	Pleurotusflorida	Macrolepiotaprocera	Pleurotussajor-caju	Sparassiscrispa	Trameteselegans
Collection date	15-08-15	3-07-16	4-07-16	20-07-16	2-08-16
Location, GPS data	Gho, Dstt. Pathankot 32o19’45.4”N 75° 40’14.7” E	Akhwana,Dstt. Pathankot32o 16’54” N 75 o43’54” E	Gidarpur, Dstt. Pathankot 32o11’38.1”N 75° 30’15.2” E	Dharkalan, Dstt. Pathankot 32o24’18” N 75o47’21” E	Bhangala, Dstt. Hoshiarpur 32o1’14”N 75° 36’37” E
Habitat, Vegetation community	Tree trunk	Humus	Dead wooden log	Roots of Malabar tree	Wooden log
Smell, taste	Typical mushroom like	Typical mushroom like	Typical mushroom like	Not distinctive	Not distinctive
Spore print	Cream	White	White	Off white to brown	White
Pileus color	Cream	White	White	Off white to brown	White
Pileus dimension	6-8 cm	6-12 cm	1-3 cm	10-34 cm	6-10 cm
Pileus shape, Margin	Fan shaped	Hemispherical to convex type	Spoon shaped	Fan shaped, inrolled margin	semicircular shape
Pileus surface	Smooth	Rough	Smooth	Smooth	tough surface
Scales	No	Present	No	No	No
Stipe attachment	Central	Central	Central	Sessile	Sessile
Stipe size, color	Cream,1-2 cm	Off-white, 10-12 cm	White, 4-5 cm	-	-
Stipe shape, base	Non-bulbous	Bulbous	Non-bulbous	-	-
Ring	No	Present	No	-	-
Veil	No	No	No	-	-

Volva	No	No	No	-	-
Basal association	No	No	No	-	-
Gill attachment	Shortly decurrent	Free	Shortly decurrent	-	-
Gill color	Creamish yellow	White	White	-	-
Edibility	Edible	Edible	Edible	Edible	-

3.1.2 ***Macrolepiotaprocera***: Mushroom is tall; large in size with pileus dimensions ranges between 6-12 cm (Table 1& Fig. 2). Stipe is longer, 10-12 cm in size, off-white in colour and centrally attached. Base of stipe is bulbous and ring is present. Brown color scales are present on pileus surface. This mushroom is a member of order Agaricale and family Agaricaceae. Gills are crowded, white in colour and attached freely with the cap. Spore print is white in colour. The genus Microlepiota was well described by Ge*et al* (2010).

3.1.3 ***Sparassiscrispa*:** This mushroom was found on roots of Malabar neem, saprobic in nature (Table 1& Fig. 4). Fruiting bodies are larger in size having diameter 10-34 cm. Pileus is wide composed of tightly packed branches, off-white to yellow in colour. Spore print is white. *Sparassiscrispa* belongs to order Polyporales, family Sparassidaceae. Dictionary of fungi by Kirk *et al* (2010) was referred to describe the collected wild mushrooms.

3.1.4 ***Trameteselegans*:** This mushroom is tough, white in colour and belongs to order Polyporale family Polyporaceae (Table 1 &Fig. 5). This saprophytic mushroom is semicircular have concentric zone of texture. Cap is 6-10 cm in diameter, found on wooden log andstipe is absent. Pileus has round to angular pores on its surface. Fruit body is very hard and woody, so it is non edible mushroom. Spore print is white in colour. Dictionary of fungi by Kirk *et al* (2010) was referred to describe the collected wild mushrooms.

3.2 Biochemical characterization of wild mushrooms:

It was observed that endo-β-1,4-glucanase was significantly higher in *Trameteselegans*(160.14 U/ mg) but specific activity of xylanaseand laccase was found to be lowest in case of *Trameteselegans*(Table 2).Production of endo-β-1,4-glucanase was lowest in case of *Pleurotus* spp. with specific activity of 14.65 and 7.39 U/mg in *P.florida* and *P. sajor-caju* respectively. Total cellulases and cellobiase were found to be higher in *Pleurotussajor-caju*with values 1.96 U/mg and 1.84 U/mg respectively (Table 2).Goyal and Soni (2011) recorded that activity of endo-β-1,4-glucanase was higher in case of *Pleurotus*species as compare to other enzymes. Laccase activity of *S. crispa* was very low and comparable to the findings of Sou*et al* (2017).

Table 2: Specific activity (U/mg) of lingo-cellulosic enzymes in wild mushroom culture filtrates.

S. No.	Mushroom	Endo-β-1,4-Glucanase (U/mg)	Cellobiase (U/mg)	Total Cellulases (U/mg)	Xylanase (U/mg)	Laccasse (U/mg)
1	P. florida	14.65	0.089	0.154	0.724	2.14
2	M. procera	42.91	0.91	0.87	12.53	1.38
3	P. sajor-caju	7.39	1.84	1.96	3.03	0.87
4	S. crispa	83.84	0.320	0.228	2.08	0.273
5	T. elegans	160.14	0.058	0.144	0.07	0.08
LSD = 0.05 %		**1.74**	**0.257**	**0.113**	**0.148**	**0.304**

4. ACKNOWLEDGEMENTS

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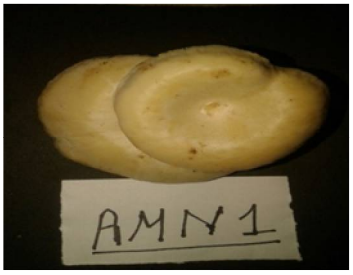

Figure 1: Pleurotusflorida

Figure 2: Macrolepiotaprocera

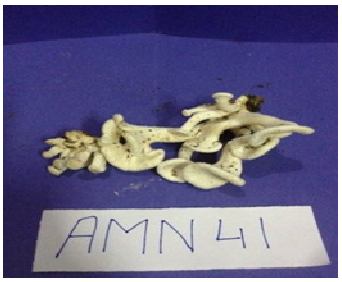

Figure 3: Pleurotussajor-caju

Figure 4: Sparassiscrispa

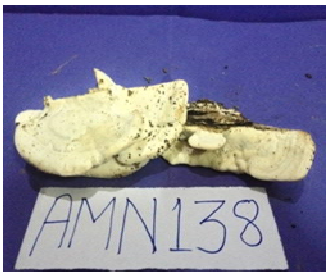

Figure 5: *Tarmeteselegans*