DOI: 10.36036/MR.29.2.2020.116190
***Mushroom Research* 29 (2) : 113-124, 2020**

Study of wild type mushroom community from the district of Amritsar and Gurdaspur (Punjab- India) and some biological aspects

Rajesh Kumar*, Shivnam Rana, Ravneet Kaur, Sukhpal Singh Kang, Harpreet Singh Sodhi and Prem Pal Johl

Department of Microbiology, College of Basic Science and Humanities, Punjab Agricultural University, Ludhiana, Punjab, India-141004
**Corresponding author*, E-mail: navrajguruji@gmail.com

ABSTRACT

Survey of forest areas had been conducted to explore the mushrooms from the districts Amritsar and Gurdaspur during the rainy season (July to September). Six mushrooms (three from each district) were collected. Based on their characters, they were identified as *Schizophyllum commune* (DMRO-580), *Ganoderma lucidum* (a) (DMRO-581), *Collybia* sp. (DMRO-585) from Amritsar and *Lentinus sajor-caju* (DMRO-582), *Ganoderma lucidum* (b) (DMRO-583) and *Psathyrella candolleana* (DMRO-584) from Gurdaspur. The tissue cultures prepared on potato dextrose agar were deposited at the ICAR-Directorate of Mushroom Research, Chambaghat, Solan (HP), India and accessioned as DMRO-580, DMRO-581, DMRO-582, DMRO-583, DMRO-584 including DMRO-585, respectively. *Ganoderma lucidum* (b) showed a maximum linear growth rate of 15 mm/d on both PDA and CYM media. In biomass study, *Collybia* sp. showed maximum biomass on both the 5th and 10th day. The exoglucanase, endoglucanase and xylanase activities were found maximum for DMRO-583. Laccase activity had a maximum for the culture DMRO-582 as 7.5 U/mg proteins. During spawn production, the growth rate on the 8th day and 16th day had a maximum for the culture DMRO-580 as 11.2 mm/d and DMRO-584 as 11.0 mm/d, respectively; while on 24th day the growth rate had maximum for the cultures DMRO-580. Substrate selection showed wheat straw as the best substrate for DMRO-580 and paddy straw for DMRO-583 and compost was a better substrate for DMRO-580 and DMRO-583. These collected mushroom's characteristics studies create a useful reference for the future to produce them commercially and deposited cultures help to increase fungal livestock.

Keywords: Biomass, enzyme activity, substrate selection, survey, wild mushrooms

Mushrooms are cosmopolitan heterotrophic organisms that are specific for their nutritional and ecological requirements. They have been generally identified as humicolous, lignicolous, coprophilous, fungicolous, parasitic or saprophytic or may show some mycorrhizal associations with both broad-leaved forest trees and gymnospermous taxa (Toma *et al.*, 2013). In plains of north India, mushroom growing is predominately a seasonal activity and only one crop is taken in winter months. Indeed, this activity has become popular in Jammu, Punjab, Haryana, western U.P and Delhi, where the product is sold daily in the local market. Based upon the information gathered during collection forays to North Western Himalayas, a total of over 77% taxa have been found distributed in the temperate climate zone, over 10% in the subalpine zone and about 0.4% in the subtropical and alpine zones (Dickinson and Lucas, 1979). About

http://epubs.icar.org.in/ejournal/index.php/MR/article/view/116190

10,000 species within the overall fungal estimates of 1.5 million belong to group macro-fungi. Ascomycota and Basidiomycota are two major phyla from kingdom Mycota containing a wide listed macro-fungi profile. Mushrooms alone are represented by about 41,000 species, of which approximately 850 species are recorded from India (Manoharachary *et al.*, 2005). Thind, (1961) reported the number of groups of larger fungi - *Pezizales*, *Helotialis*, *Clavariaceae*, *Polyporaceae* and *Agaricales*. Sharma and Sidhu (1991) also reported *Geoglossaceae* in eastern Himalaya having 48 species. Sharda (1991) listed a total of 181 taxa (136 species, 36 varieties and 7 forms) in 20 genera which had been reported from Himalaya. From the Kashmir region, 250 species were reported and North Western Himalaya recorded 165 species (Lakhanpal, 1992). Wild mushrooms are generally present in soil having moisture content above 40-45 percent and there is no direct relationship between soil pH and mushroom growth. But the soil of that area could be slightly acidic i.e. 6 to 7 pH (Sharma *et al.*, 2001).

Punjab plains in North-West India have quite favorable agro-climatic conditions for the growth of wild mushrooms. Districts Amritsar and Gurdaspur are well known for their Agri-production, but the wild area of these is with optimized conditions for the growth of macro-fungi during Monsoon season (July to September). This paper reported the survey of wild mushrooms from Amritsar and Gurdaspur. The present study aimed to identify and morphologically characterized the wild mushrooms. Different parameters were done to optimize the linear growth rate, biomass and growth rate during spawn production of wild mushrooms on suitable media. Enzymatic activity of mushroom culture and physio-chemical analysis of soil were also done to analyze the wild mushroom environmental conditions for its growth. Besides the growth parameters, an attempt was also done to find the best and suitable substrate for wild mushroom culture growth for commercial aspects.

MATERIALS AND METHODS

During the survey when new mushrooms were found in their natural habitat, their photograph was taken and a global positioning system (GPS) location was noted down along with nearby village or town name. Mushroom with the unopened cap was preferred and taken in duplicate or triplicate form (if possible). Soil sample of the mushroom growing area was also collected (if it grows on soil) and brought to Mycology Lab and Mushroom Research Complex, Punjab Agricultural University (PAU), Ludhiana, for further analysis.

The morphological characters of wild mushrooms such as pileus, stipe, lamellae morphology, spore print color, annulus, veil, volva and ring present or not were recorded. The spore print of wild mushrooms was prepared on black filter paper for identifying a mushroom. Physio-chemical analysis of soil was done (STI, 2011). Soil moisture was done by the gravimetric method of moisture estimation. The difference in weight is considered to be the water present in the soil sample (Black, 1965). The percent of moisture content was calculated as: $(W_1\text{-}W_2/W_2) \times 100$, Where: W_1 = weight of the sample before drying, W_2 = weights of the sample after drying. Kjeldahl Method was used to estimate the total nitrogen content of soil (OMA, 1995) and phosphorus was determined by Olsen's method (Olsen *et al.*, 1954). The flame photometric method was used for Potassium determination (Toth and Prince, 1949).

To estimate the linear growth (mm/d) of samples petriplates were poured with about 20 ml of melted complete yeast media (CYM) and potato dextrose agar (PDA) media (Singh and Verma, 2000) and allowed to solidify, then bits of equal size (10 mm) of mushroom were placed from master plates on to the CYM and PDA agar plates. These petriplates were incubated at 25 ± 2°C. Linear growth was measured as mm/day up to 10 days. For biomass production complete yeast extract broth (50ml/flask) was

dispensed in 100ml flask, then autoclaved, cooled and inoculated with wild mushroom mycelial agar bit (3 mm diameter) and incubated at 25 ± 2°C. Each flask was filtered onto pre-weighed Whatman No. 1 filter paper after an incubation period of 10 days. The mycelial biomass was dried in an oven at 55°C. The weight of dry mycelia was recorded, as the difference between the final and initial values of filter paper.

Cellulase, xylanase and laccase were estimated as specific enzyme activity (U/mg protein). Cellulase and Xylanase were assayed by the method of Sandhu and Kalra, (1982). Absorbance was read at 540 nm by a UV-visible spectrophotometer (Elico SL 164). The amount of reducing sugars released was estimated using a glucose standard curve. Laccase (EC 1.10.3.2) was assayed by the method of Dhaliwal *et al.* (1991) using a reaction mixture consisting of 1 ml of enzyme filtrate and 3 ml of guaiacol substrate prepared in 0.1M sodium phosphate buffer (pH 6.0). The change in absorbance was observed at 495 nm at 15-sec interval for 2 min. Lowry's method was used for the estimation of total proteins (Lowry *et al.*, 1951). The mycelial run rate (mm/d) was observed on boiled wheat grains in spawn tubes during spawn production. Selection of substrate for growth (mm/day) and fruiting was done on three substrates i.e compost, wheat straw and paddy straw prepared by the standard methodology (Khanna and Kapoor, 2007) used at PAU, Ludhiana.

RESULTS AND DISCUSSION

The forest area of districts Amritsar and Gurdaspur were surveyed to collect wild mushroom during the year 2012-2013. Three mushrooms were collected from the district Amritsar and three from Gurdaspur during the months of July to September each year. Each mushroom was collected, photographed and cultured. The tissue culture of each mushroom was deposited at Directorate of Mushroom Research, Chambaghat, Solan. Accession numbers were given to mushrooms as DMRO-580, DMRO-581, DMRO-582, DMRO-583, DMRO-584 and DMRO-585 (Fig. 1).

Wild mushrooms from the district Amritsar

Schizophyllum commune (DMRO-580)

The wild mushroom DMRO-580 was collected in August 2012 from the village Malluwall, district Amritsar. The mushroom was growing on dead tree bark. The mushroom had no taste and no smell. It was fan-shaped with white pileus (2-5cm). The pileus was non- sticky, non-hygrophanous with no scale. The stipe was short with no ring, veil and volva. The gills were white and freely attached underneath the pileus. The color of the spore print was cream-white. The mushroom showed morphological similarity with *Schizophyllum commune*, which known for its medicinal properties as well as genetic, studies (Table 1). *Schizophyllum commune* could grow on dead spent wood (Cooke, 1961), with the irregular fan-shaped pileus, 1-5 cm in diameter, white-colored, dry. Lamellae are white to greyish colored, split type and spore print color obtained was white (Kuo, 2003) which was similar to DMRO-580.

Ganoderma lucidum (a) (DMRO-581)

The wild mushroom DMRO-581 was also collected in August 2012 from the village Bhknakhurd, District Amritsar. The GPS location was 31°35'2.4" N 74°42'43.7" E (Table. 1). The mushroom was growing on an oak tree (*Quercus*) stem. The mushroom had a bitter taste and a typical smell of mushroom. It was fan-shaped with white pileus (6-11cm). The pileus was nonsticky, hygrophanous with no scales. The margin of this mushroom was light yellow. The stipe was laterally attached, 5-9cm long, flat and brown colored with no ring, veil and volva. This mushroom had pores instead of gills underneath the pileus. The color of the spore print was brown (Table 1). The mushroom showed similar morphological characteristic as *Ganoderma lucidum*,

Fig. 1. Photographs of collected wild mushroom cultures

which is well known for its medicinal properties and characters (Binion *et al.*, 2008) *Ganoderma* use in the treatment of fatigue, coughing, asthma, insomnia, indigestion, hypertension, high cholesterol and neurosis (Zhou *et al.*, 2007).

***Collybia* sp. (DMRO-585)**

The wild mushroom DMRO-585 was collected in September 2013 from the village Verka, District Amritsar on GPS location was 31°39’50"N 74°55’54"E (Table. 1). The mushroom was collected from the

Table 1. Wild mushrooms collected from the districts Amritsar and Gurdaspur

Culture no	**DMRO580**	**DMRO581**	**DMRO582**	**DMRO583**	**DMRO584**	**DMRO585**
Date of collection	15/8/12	20/8/12	2/7/13	17/9/12	2/7/13	19/8/13
Locality & GPS data	31^{0}34’2.4"N 74^{0}43’54.2"E (vill-Malluwall Amritsar)	31^{0}35’2.4"N 74^{0}42’43.7"E (Bhknakhurd Amritsar)	32°2’27"N 75°22’43"E (Desh Bhagat nagar Gurdaspur)	32°3’7"N 75°22’27"E (Bypass Gurdaspur)	32°3’36"N 75°30’13"E (talibpur Pandori Gurdaspur)	31°39’50"N 74°55’54"E (Verka Amritsar)
Habitat	Lignicolous	Lignocolous	Lignocolous	Lignocolous	Leaf litter	Leaf litter
Smell	no	Mushroomy	No	Mushroomy	—-	no
Taste	—-	Bitter	—-	Bitter	—-	
Vegetation community	no	Oak	Tree	sheesham	No	Grass
Pileus dimention	2-5cm	6-11cm	4-6cm	7-14cm	3-5cm	3-6cm
Pileus colour	White	Shiny Brown	White	Brown	Greyish white	Reddishbrown
Pileus shape, Margin	Fan shaped, rolled inward	Fan shaped, Cream, smooth	Depressed, decurved	Fan shaped	Hemisperical, smooth	Depressed, decurved
Pileus sticky/ non sticky	Non sticky	Non sticky	Non sticky	Non sticky	sticky	Non sticky
Hygrophanous/ non hygrophanous	Non hygrophanous	Hygrophanous	Non hygrophanous	Hygrophannous	Hygrophanous	Non Hygrophanous
Scales	No	No	No	No	No	No
Stipe Size, color	sessile	5-9cm , Brown	2-4cm, white	10-17cm, Brown	4-7cm, white	3-5cm
Stipe attachment, shape, base	——	Lateral, flat	Central,round	Lateral, flat	Central,round	Central, round
Ring	No	No	No	No	No	No
Veil	No	No	No	No	No	No
Volva	No	No	No	No	No	No
Basal association		Mycorhiza		Mycorhiza		—-
Lamellae attachment	Free	Pores	Decurrent	pores	Free	Adnexed
Gills color, edges	White, split	Brown	White, enrolled	Brown	Brown, Smooth	Creamish, free
Spore print colour	Creamish white	Brown	Grayish brown	Brown	Brown	Light Brown
Edible/non edible/ Med.	Medicinal	Medicinal	Edible	Medicinal	May be edible	May be edible
Similarity with	*Schizophyllum commune*	*Ganoderma lucidum*	*Lentinus sajor-kaju*	*Ganoderma lucidum*	*Psathyrella candolleona*	*Collybia* sp.

decomposed leaf litter mixed with soil. The substrate surrounding the mushroom was collected and analyzed for physicochemical properties. The soil was loamy sandy with clay, silt and sand as 7.35%, 22.0%, 47.3% soil, respectively. The pH and moisture content of the soil was 7.4 and 58%. The C, N, P, K were estimated (Table. 2). The pileus was light brown, 3-6cm in diameter, smooth, non-sticky, non-hygrophanous surface with no scales and curved margin. The stipe was centrally attached, thin, round with no ring, veil, and volva. Gills were adnexed, cream white. Spore print color was cream white. The pileus, stem, lamellae color, shape, size were similar in morphological character to *Collybia* sp. Other features like veil, volva, ring and spore print also resembled *Collybia* sp. (Kirk *et al.*, 2008).

Wild mushrooms collected from the district Gurdaspur

Lentinus sajor-caju (DMRO-582)

The wild mushroom DMRO-582 was collected in July 2013 from the Desh Bhagatnagar, District Gurdaspur. GPS location was 32°2'27"N 75°22'43"E (Table 1). The mushroom was growing on dead tree bark (Table 2). The mushroom had no taste and no smell. The pileus of this mushroom was 4-6 cm in diameter, depressed, non-sticky, non-hygrophanous with no scale including curved margin. The stipe was centrally attached, white-colored thick and round with no ring, veil and volva. The length of the stipe was 3-5cm. The gills were white and decurrent similarity in characteristics with *Lentinus sajor-caju*, which is an edible species (Corner, 1981)

Ganoderma lucidum (b) (DMRO-583)

The wild mushroom DMRO 583 was collected in September 2012 from the bypass of the district Gurdaspur. The GPS location for this mushroom was 32°3'7"N 75°22'27"E (Table. 1). The mushroom was growing on dead tree bark. The mushroom had a bitter taste and a typical smell of mushroom. It was fan-shaped with a white pileus (7-14cm). The pileus was non-sticky, hygrophanous with no scales. The stipe was laterally attached, 10-17cm long, shaped flat and brown colored with no ring, veil and volva. This mushroom had pores instead of gills underneath the pileus. The color of the spore print was Dark-brown. The mushroom showed similar morphological characteristics with *Ganoderma lucidum*, which is well known for its medicinal properties and characters (Binion *et al.*, 2008).

Table 2. Physico-chemical analysis of soil

Mushroom	Physio-chemical properties of soil									
	Concentration in mg/100gm of soil									
	Soil type	Clay	Silt	Sand	Moisture	pH	C	N	P	K
Schizophyllum commune	Wooden substrate									
Ganoderma lucidum(a)	Tree bark									
Lentinus sajor-caju	Wooden substrate									
Ganoderma lucidum(b)	Tree bark									
Psathyrella candolleana	Loamy clay	10.6	33.0	52.4	72	7.1	1.8	1.3	5.1	95.0
Collybia sp	Loamy sandy	7.35	22.0	47.3	58	7.4	5.1	1.9	4.6	107
CD(5%)		.321	3.58	.358	4.53	NS	.358	.226	.358	2.26

a) Average of triplicates; b) Sample collected in sterile polybags; c) Season- Rainy (July, August, September 2013); d) Temperature at that time-25-32 °C; e) pH measured with standard pH-meter

Psathyrella candolleana (DMRO-584)

The wild mushroom DMRO-584 was collected from the forest area of district Gurdaspur during July, 2013, GPS location was 32°3'36"N 75°30'13"E (Table 1) (village Talibpur Pandori). The mushroom was collected from the decomposed leaf litter mixed with soil. The substrate surrounding the mushroom was collected and analyzed for physicochemical properties. The soil was loamy clay with clay, silt and sand as 10.6%, 33.0%, 52.4% soil, respectively. The pH and moisture content of the soil was 7.1 and 72%. The C, N, P, K were also estimated (Table 2). The pileus was greyish white, 3-5cm in diameter. It was smooth but sticky showing hygrophanous surfaces with no scales. The stipe was also white 4-7 cm long, round and attached centrally with no ring, veil and volva. The lamellae underneath the pileus were free, smooth also brownish. Spore print color was brown. The mushrooms were collected showed morphological characteristics; pileus, stem, lamellae color, shape, size, veil, volva, ring and spore print similar to *Psathyrella candolleana*, which is an edible mushroom (Kuo, 2011).

Characterization of mushroom cultures

Linear growth study

The cultures were grown on complete yeast extract media and potato dextrose agar media to study the linear growth rate (mm/day) at 25± 2 °C up to 10 days (Table. 3). The growth of culture *Ganoderma lucidum* (b) was maximum to cover the Petri-plates in both media within six days of incubation. On the 6th day, the growth of *Psathyrella candolleana* was 13.7 and 14.1 mm/day on CYM and PDA media, respectively which was higher than *Ganoderma lucidum* (a).

The growth rate of *Lentinus sajor-caju* was consistently low from the colony diameter point of view but the mycelial mat was thick and fibrous (Table 3). This study was closely related to the results of James and Gilbertson, (1986) and Sharma and Atri, (2013).

Biomass study

After 5 days of incubation, maximum biomass was obtained from the cultures *Schizophyllum*

Table 3. Linear growth of wild mushroom cultures

Mushroom	Linear growth (mm/day)							
	Incubation period (d)							
	4d		6d		8d		10d	
	CYM	PDA	CYM	PDA	CYM	PDA	CYM	PDA
Schizophyllum commune	8.5	7.37	8.25	7.66	11.0	9.43	9.0	9.0
Ganoderma lucidum(a)	7.0	6.37	6.66	6.16	6.31	5.68	9.0	9.0
Lentinus sajor-caju	6.25	5.25	7.16	6.0	6.93	5.87	8.85	7.3
Ganoderma lucidum(b)	12.5	12.5	15	15	—-	—-	—-	—-
Psathyrella candolleana	11.5	12.3	13.7	14.1	11.2	11.2	—-	—-
Collybia sp	7.25	5.62	7.41	8.0	8	8.06	9.0	8.6
CD (5%)	Factor A-0.343, Factor B-0.283, Factor C-0.200, A×B-0.696, A×C-0.495,B×C-0.401, A×B×C-0.981							

a) Average of three replicates; b) Incubation Temperature – 25±2°C; c) Petri plate diameter – 90 mm; d) Incubation Time – 10 days; e) Medium- Potato Dextrose Agar, pH-6.5

commune, *Ganoderma lucidum* (a), *Psathyrella candolleana* and *Collybia* sp., Maximum biomass after 10 days of incubation was obtained from *Collybia* sp followed by *Ganoderma lucidum* (a) and *Psathyrella candolleana* on CYM (Table 4). These observations correlated with the findings of Kim *et al.* (2002) on mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media and Nasreen *et al*. (2005) on study of different growth parameters in *Ganoderma lucidum*.

Table 4. Biomass of wild mushroom cultures

Mushroom	Dry weight (in gm/L/day)		CD(5%)
	Incubation period (d)		
	5d	10d	
Schizophyllum commune	1.4	2.3	0.4
Ganoderma lucidum(a)	1.6	2.7	0.4
Lentinus sajor-caju	1.3	2.2	0.2
Ganoderma lucidum(b)	1.1	2.1	0.2
Psathyrella candolleana	1.5	2.8	0.4
Collybia sp	1.6	3.1	0.4
CD (5%)	0.2	0.2	

a) Average of three replicates; b) Incubation Temperature – 25±2°C' c) Weight of Dry filter paper– 1.1gm; d) Incubation Time – 10 days; e) Medium- Complete Yeast Extract Medium (CYM); f) Drying temp. and time - 55°C, 4 hours, pH-6.5

From these parameters, it was observed that these medicinal and edible mushrooms have a higher linear growth rate on respective media. It concluded that suitable media could use for the cultivation of mushroom mycelium on large scale. Similarly, higher biomass yield of these six mushrooms was observed after 10 days on CYM, which is the evidence of high yield. The cultivation of mushrooms for fruit body production is a long-term process, taking one to several months for the first fruit bodies to appear, depending on species and substrate. By contrast, the growth of pure mushroom cultures in the submerged condition in liquid culture media permits the acceleration of the growth, resulting in a biomass yield in several days (Wasser *et al.*, 2000).

Enzyme activity of mushroom cultures

The exoglucanase activity was found maximum for *Schizophyllum commune* and *Ganoderma lucidum* (b) whereas endoglucanase activity was maximum for *Lentinus sajor-caju*, *Ganoderma lucidum* (b) and *Collybia* sp. The xylanase activity was at par in all cultures except *Psathyrella*

Table 5. Specific enzyme activity of wild mushroom cultures

Mushroom	Enzyme activity(U/mg protein)			
	Exoglucanase	Endoglucanase	Xylanase	Laccase
Schizophyllum commune	0.710	0.680	0.319	1.50
Ganoderma lucidum(a)	0.495	0.623	0.265	2.50
Lentinus sajor-caju	0.612	0.828	0.327	7.50
Ganoderma lucidum(b)	0.765	0.853	0.366	2.00
Psathyrella candolleana	0.356	0.489	0.221	3.35
Collybia sp	0.540	0.705	0.347	2.40
CD (5%)	0.116	0.217	0.146	0.905

a) Average of three replicates; b) Incubation temperature-25±2°C, Incubation time-10 days; c) Medium used- Mushroom minimal medium, pH-6.5; d) Wavelength- Exoglucanase, endoglucanase and xylanase was 540nm (Sandhu and Kalra, 1982); e) For Laccase 495nm (Dhaliwal *et al.,* 1991); f) Reference-For Endoglucanase, Exoglucanase and Laccase standard glucose solution used; g) For xylanase standard xylose solution used

candolleana showing 0.221 U/mg proteins. The laccase activity was maximum for the culture *Lentinus sajor-caju* showed 7.5 U/mg protein followed by the cultures *Ganoderma lucidum* (a) and *Psathyrella candolleana* (Table 5). Steiner *et al*. (1987) showed maximum enzyme production at 25±2°C for a wild strain of *Schizophyllum commune* for cellulase and xylanase production also showed similar results during present finding. These six wild mushrooms are capable to grow on the different substrate due to its enzymatic activity. An enzyme produced by mushrooms hydrolysis lignin, cellulose, hemicellulose of the substrate and utilizes it as a main source of carbon.

The growth rate during spawn production

The mycelial run rate on 8th day and 16th day was maximum for the cultures *Schizophyllum commune* and *Psathyrella candolleana*, while on 24th day the growth rate was maximum for the cultures *Schizophyllum commune*, *Psathyrella candolleana* and *Collybia* sp. (Table 6). A similar experiment setup was used by Stanley and Awi-waadu (2010) by taking a different type of grains to check mycelial extension rate of fungi, where the growth of mycelia of *P. tuber-ragium* was observed as 1.57 mm/day on wheat grains. All six species of wild mushroom had optimum growth rate during spawn production on wheat grain which provides these wild mushroom cultivation easy.

Table 6. Spawn production from wild mushroom cultures on wheat grains

Mushroom no.	Mycelial run rate (in mm/day)			CD(5%)
	Incubation period (in days)			
	8d	16d	24d	
Schizophyllum commune	11.2	7.62	6.25	0.43
Ganoderma lucidum(a)	2.50	3.32	5.08	0.37
Lentinus sajor-caju	6.25	5.62	6.04	0.37
Ganoderma lucidum(b)	2.25	3.62	5.33	0.43
Psathyrella candolleana	7.50	11.0	6.25	0.70
Collybia sp	3.12	5.57	6.25	0.29
CD (5%)	0.14	0.15	0.26	

a) Average of three replicates; b) Incubation Temperature – 25±2°C; c) Test tube size- (25mm×198mm); d) Incubation Time – 24 days; e) Substrate- Boiled wheat grains

Substrate selection

On compost, the culture *Schizophyllum commune* showed maximum growth on the 8th and 16th day along

Table 7. Substrate selection to study growth for possible fruiting of wild mushroom cultures

Mushroom	Growth rate (in mm/day)								
	Substrates								
	Compost			Wheat straw			Paddy straw		
	8d	16d	24d	8d	16d	24d	8d	16d	24d
Schizophyllum commune	4.18	3.76	3.06	3.25	4.03	3.75	3.25	2.81	3.75
Ganoderma lucidum(a)	2.37	1.43	2.22	2.31	1.84	3.27	2.68	3.31	3.75
Lentinus sajor-caju	2.50	2.00	2.39	2.43	2.34	3.75	1.68	1.37	1.08
Ganoderma lucidum(b)	2.50	1.40	3.75	2.18	1.18	3.75	2.87	3.75	3.75
Psathyrella candolleana	3.25	3.84	2.95	2.93	5.18	3.75	3.37	3.75	3.75
Collybia sp	2.25	2.37	3.75	2.62	1.71	1.60	2.62	1.75	1.35
CD (5%)	Factor A-0.090, Factor B-0.064, Factor C-0.064, A×B-0.157, A×C-0.157,B×C-0.111, A×B×C-0.271								

a) Average of three replicates; b) Incubation Temperature – 25±2°C; c) Petri plate diameter – 90mm; d) Incubation Time – 24 days; e) Substrates-Compost, wheat straw, paddy straw

with the culture *Psathyrella candolleana* on the 16th day. On the 24th day, the maximum growth rate was observed in *Ganoderma lucidum* (b) and *collybia*. On wheat straw, the growth rate was consistently high up to 24th day for the culture *Schizophyllum commune* whereas the maximum growth rate on the 24th day was also recorded for *Lentinus sajor-caju*, *Ganoderma lucidum* (b), and *Psathyrella candolleana*. On paddy straw, the maximum growth rate was consistent for the culture *Ganoderma lucidum* (b); similar observation was also made on the 24th day for the cultures *Schizophyllum commune*, *Ganoderma lucidum* (b) and *Psathyrella candolleana* (Table 7). The wild mushroom culture showed the same range of growth rate on compost, wheat and paddy straw (Table. 7) as reported by Lakshmi (2013), Philippoussis *et al.* (2002) and Kumara and Achal (2008). From this parameter, it was concluded that these mushrooms can utilize a wide range of substrates as an energy source for their optimum growth. This made mushroom cosmopolitan and their cultivation on the waste substrate is an eco-friendly and cheap concept to clean the environment.

CONCLUSION

The present study indicated that from the six wild mushrooms (Fig. 1), the mushroom DMRO-582 was *Lentinus sajor-caju*, an edible species which is a new record from Gurdaspur. The Mushrooms DMRO-584 (*Psathyrella candolleana*) and DMRO-585 (*Collybia* sp) may also be edible species. Mushrooms DMRO-580 (*Schizophyllum commune*), DMRO-581 [*Ganoderma lucidum*(a)] and DMRO583 [*Ganoderma lucidum* (b)] are of medicinal importance. This study proved that a rich mycoflora is present in the districts Amritsar and Gurdaspur. Linear growth and biomass production studies were done for optimizing the growth of wild fungal culture to grow on semi-synthetic media. The exoglucanase and endoglucanase activities were found maximum for *Schizophyllum commune* (0.710 U/mg proteins) and *Ganoderma lucidum* (b) (0.765 U/mg proteins). The xylanase activity was at par in all cultures except *Psathyrella candolleana* (.221 U/mg proteins) and laccase activity had maximum for the culture *Lentinus sajor-caju* showed 7.5 U/mg proteins. Spawn production and substrate selection of wild strains of collected edible (*Lentinus sajor-caju*) and medicinal (*Schizophyllum* and both *Ganoderma* strains) could be an important record for future reference for further studies. The mycoflora collected during the present study could further be exploited for commercial cultivation in Punjab and adjoining areas.

ACKNOWLEDGEMENT

The authors are grateful to the In charge, Mushroom Farm, Department of Microbiology and Punjab Agricultural University, Ludhiana-141004, Punjab, India for providing the necessary facilities to carry out the experiments.

REFERENCES

1. Binion, D.E., S.L. Stephenson, W.C. Roody, H.H. Burdsall, L.N. Vasilyeva and O.K. Miller jr. 2008. Macrofungi of Oak. Pp. 467Morgantown, WV: West Virginia University Press.

2. Black, C.A. 1965. Methods of Soil Analysis: Part I Physical and mineralogical properties. American Society of Agronomy, Madison, Wisconsin, USA.

3. Cooke, W.B. 1961. The genus *Schizophyllum. Mycologia* **53:** 575-99.

4. Corner, E.J.H. 1981. The Agaric genera *Lentinus*, *Panus* and *Pleurotus*. *Beihefte Zur Nova Hedwigia* **69:** 1-169.

5. Dhaliwal, R.P.S., H.S. Garcha and P.K. Khanna. 1991. Regulation of lignocellulytic enzyme system in *P. ostreatus*. *Ind J Microbiol* **31:** 181-84.

6. Dickinson, C. and J. Lucas. 1979. The Encyclopedia of Mushrooms, GP Putnam's Sons, New York.

7. James, E.A. and R.L. Gilbertson. 1986. Cultural studies and genetic of sexuality of *Ganoderma lucidum* and *G. tsugae* in Relation to The taxonomy o*f G. lucidum* complex. *Mycologia* **78**: 694-705.

8. Khanna, P.K. and S. Kapoor. 2007. A manual on mushroom production, p.26-31.

9. Kim, S.W., H.J. Hwang, J.P. Park, Y.J. Cho, C.H. Song and J.W. Yun. 2002. Mycelial growth and exo-biopolymer production by submerged culture of various edible mushrooms under different media. *Lett Appl Microbiol* **34:** 56-61.

10. Kirk, P.M., P.F. Cannon, D.W. Minter and J.A. Stalpers. 2008. Dictionary of the Fungi Wallingford: CABI. ISBN978-0-85199-826-8, 10:160.

11. Kumari, D. and V. Achal. 2008. Effect of different substrates on the production and non-enzymatic antioxidant activity of *Pleurotus ostreatus* (Oyster mushroom). *Life Sci J* **5:** 3.

12. Kuo, M. 2003. *Schizophyllum commune,* Retrieved from the *MushroomExpert.Com* Web site: http://www.mushroomexpert.com/ schizophyllumcommune.html.

13. Kuo, M. 2011. *Psathyrella candolleana*. Retrieved from the *MushroomExpert.Com* Web site: http://www.mushroomexpert.com/ psathyrella candolleana.html.

14. Lakhanpal, T.N. 1992. In conservation of mushroom resources of India-Introduction and action plan. World wide fund for nature- India, New Delhi.

15. Lakshmi, S.S. 2013. Comparative Study on Mycelia growth rate of G*anoderma lucidum* and *Pleurotus flabellatus* on Agro-wastes. *Inter J Adv Res* **1:** 199-203.

16. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin-phenol reagent. *J Biol Chem* **193:** 265-75.

17. Manoharachary, C., K. Sridhar, R. Singh, Adholeya, T.S. Suryanarayanan, S. Rawat and B.N. Johri. 2005. Fungal Biodiversity: Distribution, Conservation and Prospecting of Fungi from India. *Current Sci* **89:** 58-71.

18. Nasreen, Z., T. Kausar, M. Nadeema and R. Bajwa. 2005. Study of different growth parameters in *Ganoderma lucidum. Micología Aplicada Inter* **17:** 5-8.

19. Official Methods of Analysis (OMA). 1995.16 ed., AOAC international, Gaithersburg, MD, sec. 33.2.11, method 991.20.

20. Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean. 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate, U. S. Department of Agriculture Circular No. 939.

21. Philippoussis, A., P. Diamantopoulou and Zervakis. 2002. Monitoring of mycelia growth and fructification of *Lentinula edodes* on several agricultural residues. *Mush Biol Sci Products* **279:** 87.

22. Sandhu, D.K. and M.K. Kalra. 1982. Production of cellulases, xylanases and pectinases by *Trichoderma longibrachiatum* on different substrates. *Trans Brit Mycol Soc* **79:** 409-13.

23. Sharda, R.M. 1991. In Himalayan Botanical Researches. p.1-60. Ashish pub house, New Delhi.

24. Sharma, B.M., J. Pal and S.K. Sharma. 2001. Physico-Chemical Properties of Soils supporting growth of *Morchella esculenta*. *Mush Res J* **10:** 47-50.

25. Sharma, P.M. and D. Sidhu. 1991. Himalayan Botanical Researches. p. 13-29. Ashish pub house, New Delhi.

26. Sharma, S.K. and N.S. Atri. 2013. Study on Mycelial Growth Pattern of Five Wild *Lentinus* Species. *Europ J App Sci* **5:** 71-75.

27. Singh S.K. and R.N. Verma. 2000. Effect of nutrient on mycilial growth and sclerotia formation in M*orchella esculenta*. National Research centre foe Mushroom Chambghat, Solan H.P, India. Science and cultivation of edible fungi, ISBN 905809 1430.

28. Soil Testing in India (STI). 2011. Methods Manual Department of Agriculture & Cooperation, p.70. Ministry of Agriculture Government of India, New Delhi.

29. Stanley and O. Herbert. 2010. Effect of Substrates of Spawn Production on Mycelial Growth of Oyster Mushroom Species. *Res J Appl Sci* **5:** 161-64.

30. Steiner, W., R.M. Lafferty, I. Gomes and H. Esterbauer. 1987. Studies on a wild type strain of *Schizophyllum commune*: Cellulase and xylanase production and formation of the extracellular polysaccharide *Schizophyllam*. *Biotechnol Bioeng* **30:** 169-70.

31. Thind, K.S. 1961. The *Clavariaceae* of India, p.197. Indian Council of Agricultural Research. New Delhi.

32. Toma, F.M., H.M. Ismael and N.Q.F. Abdulla. 2013. Survey and Identification of Mushrooms in Erbil Governorate. *Res J Environ Earth Sci* **5:** 262-66.

33. Toth, S.J. and A.L. Prince 1949. Estimation of cation exchange capacity and exchangeable Ca, K and Na content of soils by flame photometer techniques. *Soil Sci* **67:** 435-39.

34. Wasser, S.P., E. Nevo, D. Sokolov, T. Reshetnikov and M. Tismenetsky. 2000. Dietary supplements from medicinal mushrooms: diversity of types and variety of regulations. *Int Jour Med Mush* **2:** 1-19.

35. Zhou, X., L. Juan, Y. Yizhou, Z. Jingya, S. Xiaofen and T. Kexuan. 2007. Ganodermataceae: Natural products and their related pharmacological functions. *Americ J Chine Med* **35:** 559-74.