

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: - <a href="http://www.journalijar.com">www.journalijar.com</a></p> <p><b>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</b></p> <p>Article DOI: 10.21474/IJAR01/2109 DOI URL: <a href="http://dx.doi.org/10.21474/IJAR01/2109">http://dx.doi.org/10.21474/IJAR01/2109</a></p>	 <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR) ISSN 2320-5407</p> <p>Journal Homepage: <a href="http://www.journalijar.com">http://www.journalijar.com</a> Journal DOI: 10.21474/IJAR01</p>
---	---	---

### RESEARCH ARTICLE

#### SELECTION OF HIGH YIELDING SINGLE SPORE CULTURES OF *VOLVARIELLA VOLVACEA* (BULL.FRIES) SINGER.

\*Mandeep Kaur and S.Dhanda, H.S. Sodhi.

Dpt. Of Microbiology, Punjab Agricultural University Ludhiana-141004.

#### Manuscript Info

##### Manuscript History

Received: 25 September 2016  
Final Accepted: 27 October 2016  
Published: November 2016

##### Key words:-

*Volvariella volvacea*, Mushrooms, enzyme, single spore isolates.

#### Abstract

*Volvariella volvacea* is a tropical mushroom with variable biological efficiency which can be stabilized through selection of single spore cultures. Nineteen single spore cultures, MV-1 to MV19 were isolated from three healthy fruit bodies of the parent, *V. volvacea*, VV-11 and analyzed for growth, enzyme production and yield potential. The growth rate was maximum in single spore culture MV-7 (30mm/day). Maximum biomass production was recorded in MV-9 and MV-14 (12.8g/l in both). The activity of endo-1, 4- $\beta$ -glucanase (EC 3.2.1.4), exo-1, 4- $\beta$ -glucanase (EC 3.2.1.91) was maximum in MV-7 (0.0481 and 0.0369, respectively) while xylanase activity (EC 3.2.1.8) was maximum in MV-5(0.0404). The Laccase activity was maximum for MV-7 (1.61) followed by MV-3 (1.29) and MV-9 (1.16). On the basis of growth characteristics and enzyme activity six *V. volvacea* single spore isolates MV-6, MV-7, MV-9, MV-13, MV-14 and MV-18 were selected for yield trials on paddy straw. In comparison to yield of the parent *V. volvacea*, strain VV-11 (13.28 Kg/q of dry straw, respectively), two single spore strains MV-7 and MV-14 had showed higher yield (15.32 and 16.13 Kg/q of dry straw, respectively) whereas number of fruit bodies was higher for the single spore culture MV-14 (2375 no./q of dry straw). The average weight of fruit body ranged between 5.8-8.3g. During the present study, two *V. volvacea* single spore cultures MV-7 and MV-14 have been selected as high yielding strains.

Copy Right, IJAR, 2016,. All rights reserved.

#### Introduction:-

*Volvariella volvacea*, an edible mushroom of the tropical regions belonging to the family Plutaceae of order Agaricales, ranks sixth among cultivated mushrooms in terms of annual world-wide production. It was first cultivated in China in 1822 (Chang 1969). It can be cultivated in the temperature range of 28° to 32°C and relative humidity of 65-68%. It is a fast growing mushroom to utilize cellulosic materials more effectively than any other cultivated mushroom. Paddy straw is the most common substrate for this mushroom. However this mushroom can also be cultivated on several crop residues like cotton waste, wheat straw and sugarcane industrial waste (Khan et al 1982).

Mushrooms are rich source of protein, vitamins, fats, carbohydrates, amino acids and minerals (Jiskani 2001). In mushroom fruiting bodies all essential amino acids, water soluble vitamins and all the essential minerals are present (Buigut 2002). The biological efficiency of a mushroom species is mainly attributed to its hydrolytic enzymes

**Corresponding Author:- Mandeep Kaur.**

Address:- Department of Microbiology, Punjab Agricultural University Ludhiana-141004.

system (Chen et al 2003) and the biological efficiency is very low in paddy straw mushroom (about 10% to 15% on rice straw) in comparison to other popularly cultivated mushrooms (Chang 1974). This mushroom can produce an array of lignocellulolytic enzymes such as endo-1, 4- $\beta$ -glucanase (EC 3.2.1.4), exo-1, 4- $\beta$ -glucanase (EC 3.2.1.91), xylanase (EC 3.2.1.8) and laccase (EC 1.10.3.2). The activities of hydrolytic enzymes responsible for mycelial colonization (cellulases) and sporophore formation (laccase) have been found to vary in different strains of *V. volvacea* (Ahlawat et al 2005, 2008, Chen et al 2003).

Cultivation of *Volvvariella volvacea* in Punjab has been successfully practised but face a lot of variation in its biological efficiency which could be stabilized through selection of stable single spore cultures. Present study was aimed at evaluation of pre-screened parent strains and single spore cultures for their variation and selection in growth rate, enzyme producing capability and yield potential.

## **Methodology:-**

### **The cultures and their maintenance:-**

The pure culture of *Volvvariella volvacea*, strain VV-11 used in the present investigation was procured from Culture Collection Bank, Mushroom Research Complex, Punjab Agricultural University, Ludhiana. Throughout the study the culture was maintained on Potato Dextrose agar (PDA) medium at  $30 \pm 2^\circ\text{C}$  and was sub-cultured at the regular interval of three weeks.

### **Cultivation of *V. volvacea*, VV-11:-**

Cultivation of *V. volvacea*, VV-11 was carried out using paddy straw (Khanna and Kapoor 2007). The spawn of *Volvvariella volvacea*, VV-11 was prepared on wheat grains. Paddy straw bundles (each weighing 500g) were soaked in 1.5%  $\text{CaCO}_3$  mixed with water for 16h to attain a moisture level of 65-68%. Paddy straw bundles were arranged as bed each comprising 22 bundles (5 $\times$ 4+2). Observations on spawn run, first harvest, mushroom yield for 3 weeks and average weight of fruit body and incidence of disease were recorded.

### **Spore print and Single spore cultures:-**

Three healthy mushroom fruit bodies were fixed in pre sterilized spore collection assembly to shed their spores. These spores were collected on white filter paper as off-white coloured powder. Single spore cultures were isolated using serial dilution technique on Complete Yeast Extract (CYM) agar medium plates containing dextrose - 20.0 g; Peptone - 2.0 g;  $\text{MgSO}_4$  - 0.5 g;  $\text{KH}_2\text{PO}_4$  - 0.46 g;  $\text{K}_2\text{HPO}_4$  - 1.0 g; Yeast extract - 2.0 g; Agar - 20.0 g; water - 1000 ml and pH- 6.5. Nineteen single germinating spores were marked and transferred to CYM agar slants.

### **Characterization of Single spore cultures:-**

#### **Growth study:-**

Linear growth rate and biomass production of Single spore cultures along with parent culture VV-11 was estimated on CYM agar plates and broth respectively. To estimate the linear growth, bits of equal size (10 mm) were placed from master plates on to the CYM agar plates, incubated at  $30 \pm 2^\circ\text{C}$ . Linear growth was measured as mm per day. To estimate the biomass production, bits of equal size (10 mm) were inoculated from master plates on to the CYM broth (50ml each flask), at  $30 \pm 2^\circ\text{C}$ . Each flask was filtered after of 10 day of incubation onto preweighed Whatmann No. 1 filter paper. The mycelium was dried in an oven at  $55^\circ\text{C}$ , till it attained constant weight. The weight of dry mycelium obtained was recorded, as the difference of the final and initial values.

### **Extracellular enzyme activity:-**

#### **Preparation of Extract:-**

Nineteen single spore cultures screened for enzyme activity. Enzyme extract was prepared using mushroom minimal medium (MMM) broth containing L-Asparagine - 1.6 g; Dextrose - 20.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  - 0.5 g;  $\text{KH}_2\text{PO}_4$  - 0.46 g;  $\text{K}_2\text{HPO}_4$  - 1.0 g; Thiamine Hydrochloride - 0.125 g; Agar -20.0 g; water - 1000 ml and pH- 6.5. Basal broth (30 mL) was supplemented with Cellulose (@ 0.5 g/flask) as inducer for extracellular enzyme production. These flasks were then incubated at  $30 \pm 2^\circ\text{C}$  for 10 days. Flasks were shaken daily up to 10 days. Each flask was filtered onto Whatmann No. 1 filter paper. The filtrate was collected in capped vials and stored at  $-4^\circ\text{C}$  to estimate the enzyme activity.

### **Enzyme assays:-**

Cellulases and xylanase were assayed by the method of Sandhu and Kalra (1982). Enzyme extract (0.5ml) was mixed in 0.5 ml of substrate. Substrates used for exoglucanase (FPase, EC 3.2.1.91), endoglucanase (CMCase, EC

3.2.1.4) and xylanase (EC 3.2.1.8) were Whatmann No.1 filter paper, carboxymethyl cellulose (CMC) and xylan, respectively, prepared in 0.1 M acetate buffer separately. For exo-1, 4- $\beta$ -glucanase, 8 discs of 0.6 cm diameter Whatmann filter paper No.1 was used as substrate and 0.5 ml of 0.1 M acetate buffer was mixed. Solutions were kept in the waterbath at 45°C for 6 h and then 1 ml of alkaline CuSO<sub>4</sub> was added in each test tube and again kept in boiling waterbath at 100°C for 20 minutes. Solutions were taken out of waterbath and 1ml of Arsenomolybdate solution was mixed in each test tube. Final volume was made up (10 ml of each test tube) with the help of distilled water. After cooling for 30 minutes at room temperature absorbance was read at 540 nm, using UV- visible spectrophotometer (Elico SL-164). Laccase (EC 1.10.3.2) was assayed following by Dhaliwal et al (1992). 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). Change in absorbance was observed at 495 nm. The units used for cellulases and xylanase is  $\mu$  mole glucose release  $h^{-1}ml^{-1}$  and for laccase change in absorbance by  $0.001 min^{-1} ml^{-1}$ .

#### Protein estimation:-

Mushroom extract for each single spore culture along with parent culture VV-11 was prepared using MMM media. To the 2 ml of culture sample, 2 ml chilled 20% trichloro acetic acid (TCA) was added and mixed thoroughly. After aging for 1hour at 4°C, the contents were centrifuged (14000 rpm x g for 15minutes) and the precipitates were dissolved in 2 ml 0.1 N NaOH. Protein was determined according to the method of Lowry et al (1951).

#### Selection of single spore cultures for yield evaluation:-

On the basis of growth characteristics and enzyme activity of single spore cultures, out of 19 single spore cultures six strains MV-6, MV-7, MV-9, MV-13, MV-14 and MV-18 along with parent culture VV-11 were selected for the yield evaluation trials on paddy straw beds under natural climatic conditions. The spawn of each single spore culture was prepared on wheat grains. Paddy straw bundles were wetted to a moisture content of 66-71%. The beds were laid in growing rooms. The temperature and relative humidity during the crop period was recorded. The observations were made on spawn run period, pin head appearance, number of fruit bodies and weight of fruit bodies.

#### Results and discussion:-

*Volvariella volvacea* strain VV-11 was the high yielding strain evaluated during All India Coordinated Research Project trials (Annual Report 2012-13). The spawn of *Volvariella volvacea*, VV-11 was prepared on wheat grains. Paddy straw bundles (each weighing 500g) were soaked in 1.5% CaCO<sub>3</sub> mixed with water for 16h to attain a moisture level of 65-68%. Paddy straw bundles were arranged as bed each comprising 22 bundles (5 $\times$ 4+2). Observations on spawn run, first harvest, mushroom yield for 3 weeks, average weight of fruit body and incidence of disease were recorded.

**Table 1:- Cultivation of *Volvariella volvacea*, VV-11**

Replicate	Spawn run (Days)	First harvest (Days)	Yield (Kg/q of dry straw)	NFB (no./q of dry straw)	Average wt. of fruit body (g)
1	5	8	14.83	2213	6.7
2	7	9	13.79	1723	8.0
3	6	7	15.80	2135	7.4
4	5	7	11.85	1669	7.1
5	5	7	12.84	1860	6.9
<b>Mean</b>	<b>5-7</b>	<b>7-9</b>	<b>13.82</b>	<b>1920</b>	<b>7.2</b>
<b>CD at 5%</b>	-	-	<b>1.164</b>	<b>199.72</b>	-

No of replicates: 5

Date of sowing: 18.05.2013

Date of Fragmentation: 25.05.2013

Crop Period: 20 days

Rate of spawn: 300g/bed

NFB: Number of fruit bodies

Spawn run was complete in 5-7 days of spawning and first harvest was made between 7-9 days. Mushrooms were first harvested on 8<sup>th</sup> day of spawning to give 13.82 Kg/q paddy straw, yield and giving 1920 number of fruit bodies in 3 week harvest (Table 1). The average weight of fruit body was 7.2gms. Biological efficiency was 13.82%. Tripathy et al (2011) recorded that biological efficiency was affected by substrate. Wheat and rice bran spawn resulted with the highest biological efficiency in *V. volvacea* (13.6%), *V. diplasia* (12.4%) and the largest number of mushroom production. Wheat and banana leaf spawn yielded the lowest with the B.E. (8%) in *V. volvacea* and

wheat and sugarcane baggase (7.6%) in *V.diplasia*. A significantly higher yield (5.38 kg/ m<sup>2</sup>) was observed in cotton waste medium in indoor method while the lowest value (1.73 kg/m<sup>2</sup>) was observed from paddy straw in outdoor method. The highest number of fruits (49.7 kg/ m<sup>2</sup>) was recorded in cotton waste medium in indoor method while the lowest (17 kg/ m<sup>2</sup>) was given by the paddy straw compost in outdoor method (Rajapakse 2011).

**Table 2: Growth Characteristics of *Volvariella volvacea* Single spore cultures**

Single spore Cultures	Growth in mm diameter (mm per day)				Biomass production (g/L)	Morphological Characteristics On CYM agar medium	
	Day1	Day2	Day3	Day4		Colour	Morphology
MV 1	10.6	40.0	66.0	80.6	6.5	Off-white	Sparse
MV 2	22.0	49.2	78.0	90.0	5.3	Off-white	Aerial
MV 3	20.0	52.6	80.0	90.0	9.3	Greyish	Mat type
MV 4	13.2	42.0	74.0	90.0	6.6	Brownish	Fluffy
MV 5	20.0	46.0	66.0	90.0	8.0	Off-white	Dense
MV 6	17.2	58.0	90.0	90.0	4.6	White	Sparse
MV 7	10.6	42.6	74.0	90.0	9.3	Off-white	Strandy
MV 8	13.2	36.6	56.0	72.6	6.0	Greyish	Aerial
<b>MV 9</b>	17.2	46.0	74.0	90.0	<b>12.8</b>	Off-white	Dense
MV 10	20.0	43.2	66.0	83.2	8.6	White	Mat type
MV 11	22.0	44.0	66.0	82.0	7.3	Off-white	Sparse
MV 12	20.6	52.0	76.0	90.0	9.3	Off-white	Mat type
MV 13	25.2	56.0	77.2	90.0	11.8	Brownish	Mat type
<b>MV 14</b>	23.2	64.6	82.0	90.0	<b>12.8</b>	Off-white	Dense
MV 15	12.6	42.0	72.0	90.0	11.3	Off-white	Dense
MV 16	16.0	36.0	73.2	82.6	8.6	Greyish	Fluffy
MV 17	12.0	29.2	47.2	67.2	11.2	Off-white	Fluffy
MV 18	10.6	26.0	42.0	58.6	12.1	Off-white	Sparse
MV 19	10.0	32.6	50.6	72.6	8.0	Off-white	Aerial
<b>Parent VV-11</b>	13.5	38.7	61.6	83.7	7.6	Off-white	Dense
<b>CD (5%)</b>	<b>3.88</b>	<b>6.28</b>	<b>5.73</b>	<b>3.32</b>	<b>2.53</b>	-	-

**Media used: CYM agar for linear growth and CYM broth for biomass**

**Temperature for incubation: 32°C**

**Incubation Period: 3-4 days for linear growth and 9-10 days for biomass production**

Nineteen single spore cultures MV-1 to MV-19 along with the parent culture VV-11 were subjected to growth on CYM media (both agar and broth). On CYM agar the growth rate was maximum for MV-2, MV-13 and MV-14 on day 1, while it was maximum for MV-3, MV-6, MV-12 and MV-14 on day 2. On the 3rd day the growth (mm/day) was maximum for MV-2, MV-6, MV-7, MV-9, MV-12, MV-13, MV-14 and MV-16. On day 4 the growth rate was minimum for MV-8, MV-17, MV-18 and MV-19 (Table 3). In broth culture the biomass harvested was between 5.3 to 12.8g/l with maximum for MV-9, MV-3, MV-14, MV-15, MV-17 and MV-18 (Table 3).

26 strains of *V. volvacea* were grown on malt extract agar medium. The strains were characterized based upon the mycelial growth rate, aerial hyphae, type of growth and intensity of chlamydo spores formed. Fourteen strains showed radial growth of 90 to 100 mm in 8 days, while 8 strains grew up to 60-70 mm. Rest of the 4 strains grew very slowly and showed growth of 11 to 35 mm. Most of the fast growing strains formed thin or thick fluffy growth with sufficient aerial hyphae and no chlamydo spores on 8th day of growth. However, some exceptions were also noticed where in the fast growing strains, OE-272 and OE-274 with radial growth of 90 mm formed quite low level of aerial hyphae (Ahlawat 2011).

The enzyme assay of single spore cultures indicated maximum enzyme activity of endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.91) and xylanase (EC 3.2.1.8) for the culture MV-7. The endoglucanase activity for MV-1, MV-2, MV-3 and MV-9 was in between 0.0312 to 0.0378. While the endoglucanase activity was lower for culture

MV-5 (0.0104). The exoglucanase activity for culture MV-4 (0.0120) was the lowest. Maximum xylanase activity was found in MV-5(0.0404) (Table4, Fig 4.4). The Laccase activity was maximum for MV-7 (1.61) followed by MV-3 (1.29) and MV-9 (1.16) with the lowest in MV-11 (0.23) (as shown in Table 3).

Enzyme activity was recorded in strain, OE-143, followed by OE-214, OE-139 and OE-215. Highest endoglucanase activity was recorded in the strain, OE-143, followed by 1.49U in OE-145 and 1.24U in OE-210. Highest  $\beta$ -glucosidase activity was recorded in the strain, OE-145, followed by 2.55U in OE-117 and 2.50U in OE-210. Xylanase activity was highest in strain, OE-215, followed by strains, OE-272, OE-213, OE-209, OE-117 and OE-214. Highest activity of laccase was recorded in strain OE-143, followed by strains, OE-29, OE-145, OE-210 and OE-272. Variations in activities of extracellular enzymes in *V. volvacea* strains along with role of cellulases in mycelial colonization and laccase in sporophore formation have also been reported (Ahlawat et al 2008).

**Table 3:- Enzyme Assay In Single spore Cultures of *Volvariella volvacea*.**

<i>Volvariella volvacea</i> single sporecultures	Protein Content (Mg/L)	Enzyme Activity (U h <sup>-1</sup> ml <sup>-1</sup> )			Laccases (U min <sup>-1</sup> mol <sup>-1</sup> )
		Endoglucanase	Exoglucanase	Xylanase	
MV 1	2.1	0.0315	0.0176	0.0187	0.94
MV 2	2.3	0.0326	0.0274	0.0249	1.03
MV 3	1.9	0.0312	0.0214	0.0186	1.29
MV 4	2.1	0.0118	0.0120	0.0179	0.63
MV 5	2.2	0.0104	0.0267	0.0404	0.43
MV 6	2.4	0.0179	0.0214	0.0190	0.39
MV 7	2.1	0.0481	0.0369	0.0356	1.61
MV 8	2.0	0.0119	0.0181	0.0171	0.97
MV 9	2.4	0.0378	0.0271	0.0336	1.16
MV 10	2.3	0.0219	0.0173	0.0191	0.62
MV 11	2.2	0.0245	0.0174	0.0225	0.23
MV 12	1.8	0.0238	0.0198	0.0215	0.66
MV 13	2.3	0.0333	0.0294	0.0264	1.05
MV 14	2.2	0.0199	0.0095	0.0174	0.54
MV 15	1.9	0.0213	0.0216	0.0194	0.80
MV 16	1.6	0.0264	0.0312	0.0205	0.79
MV 17	2.1	0.0209	0.0182	0.0174	0.45
MV 18	2.3	0.0188	0.0190	0.0159	0.77
MV 19	2.4	0.0229	0.0213	0.0183	0.76
<b>Parent VV-11</b>	2.4	0.0194	0.0166	0.0143	0.86
<b>CD 5%</b>	-	<b>0.0032</b>	<b>0.0019</b>	<b>NS</b>	<b>0.35</b>

**Exoglucanase, Endoglucanase and Xylanase activity**- $\mu\text{mol}$  glucose released h<sup>-1</sup>mol<sup>-1</sup>

**Laccase**- Change in absorbance by 0.001 min<sup>-1</sup>mL<sup>-1</sup>.

Data is mean of three replicates

**Incubation temperature:** 30 $\pm$ 2<sup>0</sup>C

CD at 5% level

Days of incubation- 12

**Inducer:** Cellulose 0.5g each flask

**Medium:** Mushroom Minimal Media

On the basis of growth characteristics and enzyme activity six single spore cultures MV-6, MV-7, MV-9, MV-13, MV-14 and MV-18 were selected for yield trials. Six single spore cultures of *Volvariella volvacea* were grown on paddy straw under natural climatic conditions. In comparison to the parent strain *Volvariella volvacea*, strain VV-11, two single spore strains MV-7 and MV-14 had shown higher yield whereas number of fruit bodies was higher for the single spore culture MV-14. The yield potential for two single spore strains MV-6 and MV-18 was minimum. The average weight of fruit bodies ranged between 5.8-8.3g.

**Table 4:- Cultivation of *Volvariella volvacea*, single spore cultures**

Strain number	Spawn run (Days)	First harvest (Days)	Yield (Kg/q of dry straw)	NFB (no./q dry straw)	Average wt. of fruit body (g)
MV-6	8-11	10-12	3.18	475	6.7
MV-7	6-8	10-12	15.32	1845	8.3
MV-9	7-9	10-12	13.05	1717	7.6
MV-13	9-11	13-15	12.23	1675	7.3
MV-14	5-7	8-10	16.13	2375	6.8
MV-18	8-10	12-13	5.96	1027	5.8
<b>Parent VV-11</b>	6-8	7-9	13.28	1870	7.1
<b>CD at 5%</b>	-	-			-

No of single spore cultures: 6

Crop Period: 20 days

Date of sowing: 14.08.2013

Rate of spawn: 300g/bed

NFB: Number of fruit bodies

Out of 20 strains of screened for enzyme assays, 12 strains with varied enzyme activities were used for the yield evaluation trials on pasteurized paddy straw bundles. On pasteurized paddy straw bundles, strains, OE-274, OE-215, OE-272 and OE-55 showed very thick and fluffy growth, while strains, OE-210, OE-213 and OE-139 completely colonized the substrate but with very light (thin) mycelial growth. Mushrooms were harvested earliest in the strain, OE-274 after 20 d of spawning, followed by strains, OE-272, OE-139 and OE-140. Highest mushroom yield of 12.36 kg q<sup>-1</sup> dry substrate was recorded in the strain, OE-274, followed by 10.49 kg in OE-272 and 10.45 kg in OE-210 (Ahlawat et al 2008).

### Conclusion:-

Nineteen single spore cultures of *Volvariella volvacea* strain VV-11 were isolated from three different fruit bodies. On the basis of linear growth, biomass production, and enzyme activity six of the nineteen single spore cultures were selected for cultivation. In comparison to the parent strain *Volvariella volvacea*, strain VV-11, two single spore strains MV-7 and MV-14 had shown higher yield whereas number of fruit bodies was higher for the single spore culture MV-14.

### References:-

- Ahlawat O P, Ahlawat K and Dhar B L (2005) Influence of lignocellulolytic enzymes on substrate colonization and yield in monosporous isolates and parent strains of *Volvariella volovacea*. Ind. J. of Microbiol. **45**(3): 205-10.
- Ahlawat O P, Gupta P, Kamal S and Dhar B L (2005) High yielding strains of paddy straw mushroom, *Volvariella volvacea*. Mush. Research letters. **11**(1):2.
- Ahlawat O P, Gupta P, Kamal S and Dhar B L (2008) The development of molecular and biochemical markers for selecting a potential high yielding strain of paddy straw mushroom, *Volvariella volvacea*. J of Plant Biochem and Biotech (**1**): 57-63.
- Ahlawat O P, Gupta P, Dhar B L, Sagar T G, Rajendranath R, Rathnam K (2008) Profile of the extracellular lignocellulolytic enzymes activities as a tool to select the promising strains of *Volvariella volvacea*. Indian J Microbiol **48**(3):389-96.
- Ahlawat O P, Singh R and Kumar S (2011) Evaluation of *Volvariella volvacea* Strains for Yield and Diseases/Insect-Pests Resistance Using Composted Substrate of Paddy Straw and Cotton Mill Wastes. Indian J Microbiol **51**:200-05.
- Buigut S.K. (2002) Mushroom production in sustainable small-scale farming system-opportunities and constraints: a survey of Uasin Gishu district. In: Proceedings of the Horticulture seminar on Sustainable Horticultural Production in the Tropics at Jomo Kenyatta, University of Agriculture & Technology, Juja, Kenya, October 3-6, 2001, Kenya.
- Chang S T (1969) A cytological study of spore germination of *Volvariella volvacea*. Bot Mag **82**:102-09.
- Chang ST (1974) Production of straw mushroom (*Volvariella volvacea*) from cotton wastes. Mush J **21**: 348-54.

9. Chen SC, Ma DB, Ge W and Buswell JA (2003) Induction of laccase activity in the edible straw mushroom, *Volvariella volvacea*. FEMS Microbiol Letters **218**(1):143–48.
10. Dhaliwal R P S, Garcha H S and Khanna P K (1991) Regulation of lignocellulytic enzyme system in *P. ostreatus* Ind. J. of Microbiol.**31**: 181-84.
11. Jiskani M M (2001) Energy potential of mushrooms. The DAWN, Economic and Business Review **(4)**:15-21.
12. Khan S M, Qadir A and Ramzan M (1982) Fungal mycelium as a source of protein for animal feed. Agric (Urdu) 21st National Sci Conf Sci Soci Pakistan (Abstr).
13. Khanna P K and Kapoor S (2007) Manual on Mushroom Production, Punjab Agricultural University, Ludhiana, Punjab, India.
14. Lowry O H, Rosenbrough N J, Farr A L and Randall R J (1951) Protein measurement with folin phenol reagent. J Boil Chem **193**: 265-75.
15. Sandhu D K and Kalra M K (1982) production of Cellulases, Xylanases and Pectinases by *Trich-oderma longibrachiatum* on different substrates. Trans British mycol soc **79**: 409-13.
16. Tripathi A, Patel A K, Sahoo T K (2011) Yield evaluation of paddy straw mushrooms various lignocellulosic wastes. Asian J Plant Sci **4**:566-69.