

Comparative evaluation of five *Pleurotus* species for their growth behaviour and yield performance using wheat straw as a substrate

Somnath Kadappa Holkar^{1*} and Ram Chandra²

¹Division of Crop Protection, Indian Institute of Sugarcane Research, Lucknow-226 002, India

²Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221 005, India

*Corresponding Author E-mail : somnathk_holkar@yahoo.com

Publication Info

Paper received:
22 August 2014

Revised received:
22 January 2015

Accepted:
20 March 2015

Abstract

Pleurotus spp. is one of the most important edible mushrooms cultivated in India. The present study was an attempt to compare five *Pleurotus* species in context of actual time required for each growth stage viz., spawn run period, number of days required for initiation of pin heads of sporophores, average weight of fruiting bodies in all the flushes and total yield. The spawn run period in all the five species were recorded between 18 days-21 days, similarly for initiation of pinheads 5 days -7 days were required after spawn run period. A total of 24 days to 27 days, 34 days to 37 days and 47 days to 53 days were required for harvesting the I, II and III flushes respectively. An average number of 41 to 70 sporophores per bag containing 1 kg of dry substrates were obtained from all the *Pleurotus* species. Maximum 14 g weight of single sporophore was recorded from *P. florida*, similarly, an average maximum diameter of 5.3 cm of sporophores of *P. florida* was observed whereas the diameter of sporophores in rest of the species ranged from 3.0 cm to 3.2 cm. The number of sporophores were obtained from *P. sajor-caju* (n=70) and all the species showed significant difference with respect to the number of sporophores in a bunch at probability level of $P=0.05$. Maximum weight of single bunch was recorded (58 g) in *P. florida* and total yield of 740 gkg⁻¹ of dry matter was recorded in *P. florida*.

Key words:

Oyster mushroom, Physiological parameters, *Pleurotus* spp., Wheat straw, Yield performance

Introduction

Mushrooms belong to kingdom fungi, a diverse and distinct group from plants, animals and bacteria. Mushrooms are recognized as saprophytes depending on their mode of survival and having the potential to degrade or decompose complex organic structure of plants or animals. In nature, oyster mushrooms (*Pleurotus* spp.) degrade dead woods are largely cultivated on lingo-cellulose waste materials (Oei and Nieuwenhuijzen, 2005). Oyster mushroom belong to genus *Pleurotus* (Jacq. Fr.) P. Kumm. (Sub-division: Basidiomycotina, Family: *Pleurotaceae*) form a distinct group of edible mushroom species of high economical and nutritional importance. It is one of the second most important edible mushrooms cultivated in India and abroad it is used as a bioremediator (De Boer and Heuvelink, 2000). Oyster

mushrooms are characterized by the production of basidiocarps with an eccentric stalk. The basidiocarps of an oyster mushroom comprise of three distinct parts viz., a fleshy shell or spatula shaped cap called pileus which is short to long and bears lateral to central stalk known as stipe, having ridges and furrows beneath the pileus known as gills or lamellae. The spore print of oyster is whitish, pinkish, lilac or grey in colour and is hyaline, smooth and cylindrical in shape. The basidiospores after germination form non-fertile primary mycelium and later fusion between these two compatible primary mycelia develops into fertile secondary mycelium. It has been cultivated over a wide range of temperature with the use of unfermented, natural and lignino-cellulosic wastes. Mushrooms are recognized as natural and healthy food, originating from an environment friendly organic farming system (Moore and Chiu, 2001).

Although, oyster mushrooms are low in calories and fat but rich in protein, chitin, vitamins and minerals. It contains high amount of glutamic amino butyric acid (GABA) and ornithine. GABA is a non-essential amino acid that functions as a neurotransmitter, whereas ornithine is a precursor in synthesis of arginine (Jayakumar *et al.*, 2006). The moisture content of fresh mushrooms range from 70%–95% depending upon the time of harvest and environmental conditions, whereas dried mushroom contains about 10%–13% moisture. The chemical compounds extracted from various mushrooms have shown pharmaceutical applications e.g., polysaccharide-protein complex (PSPC) shown effective for immune-modulatory and anti-tumour activities (Cohen *et al.*, 2002), likewise type I ribosome inactivation protein and other substances extracted from *Volvariella volvacea* and *Ganoderma lucidum* respectively have shown to possess pharmaceutical importance (Chang and Miles, 2004).

Production and productivity of oyster mushroom is the result of various successive steps evolved and modifications made in several technologies throughout the world. More than 20 species are commercially cultivated in different parts of the world. A very primitive practice of growing *Pleurotus* spp. was adopted by Lumberman in Europe during the 19th century that showed wood logs and stumps bearing fructification in cool and moist places in nature. In the recent past enormous work has been done on the use of different substrates and supplements for cultivation of oyster mushrooms to increase yield and quality improvement as described by Kadam *et al.* (2008), Rajak *et al.* (2011), Oseni *et al.* (2012), Pala *et al.* (2013) and Pokhrel *et al.* (2013). Present study was an attempt to evaluate five *Pleurotus* spp. commercially grown in India for their yield potential and biological efficiencies (BE) using wheat straw as a potential substrate.

Materials and Methods

Collection and maintenance of mushroom cultures:

Two species of *Pleurotus* viz., *Pleurotus ostreatus* (Jacq.: Fr) Kumm and *Pleurotus flabellatus* (Berk and Br.) Sacc., were obtained from Rajendra Agriculture University, Samastipur, (Bihar). Two *Pleurotus* spp. viz., *Pleurotus florida* (Mont.) Singer and *Pleurotus sajor – caju* (Fries) Singer., were maintained in the Mushroom Laboratory, Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi. Whereas pure culture of *Pleurotus eryngii* (DC. Ex. FY.) was obtained from Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi. Cultures of all these collected species were sub-cultured and maintained on PDA medium by incubating at 25°C for further studies.

Mushroom spawn production: The spawn called seed of mushrooms was prepared from five *Pleurotus* species viz., *Pleurotus eryngii*, (DC. Ex. FY.), *Pleurotus sajor – caju* (Fries). Singer, *Pleurotus florida* (Mont.) Singer, *Pleurotus flabellatus* (Berk and Br.) Sacc., *Pleurotus ostreatus* (Jacq.: Fr) Kumm, using wheat grains (*Triticum aestivum* Linn) (Sainos *et al.*, 2006). Well cleaned wheat grains were boiled for 30 min or until the grain became soft and firm without bursting. Excess water was drained off from the grains and cooled on a cheese cloth and turned up and down several times with the help of a spoon for quick cooling. These cooled grains were mixed with 2% chalk (calcium carbonate) and 2% gypsum (Calcium Sulphate). Gypsum and chalk powder were added to prevent the grains from sticking together and keeping pH optimum for growth of the mycelium (Ram and Pant, 2002). Prepared grains (250 g/bottle) were filled into 500 ml sterile glass bottles and plugged with non-absorbent cotton plugs. These grain filled bottles were sterilized in an autoclave for 15 min at 121°C (15 lbs pressure per square inch). After sterilization, the bottles were allowed to cool at room temperature and inoculated under laminar air flow using bits of mushroom mycelia from 7-8 days old mother cultures of *Pleurotus* spp. In order to develop spawn, inoculated sterilized grains were kept for incubation at 25°C temperature in BOD incubator for 14 days-15 days (Chang and Miles 1989). These bottles were well mixed thoroughly at 4 day interval for uniform growth of the mycelium and to get completely impregnated grains.

Substrate preparation, inoculation and incubation:

Well dried four-year-old wheat straw was soaked in water for 18 hrs. Excess water was drained off from the substrate and moist wheat straw was sterilized in an autoclave at 121°C at 15 lbs pressure/inch for 15 min. Substrate was then allowed to cool at room temperature and then filled in polythene bags of 40 cmx40 cm size. Previously prepared spawn in glass bottles was used to inoculate the substrate. Wet wheat straw filled in polythene bags was inoculated with mushroom spawn @ 50 g kg⁻¹ substrate. Substrate was filled by gently pressing with hands so that it could give cylindrical shape to the bags. one fourth space of the bags was kept free to tie the bags with the help of thick thread. Small holes of about 2-3 mm diameter were made randomly on the filled bags for proper aeration. Substrate filled bags were kept vertically in dark room with average temperature was 24°C-28°C and relative humidity of 65% to 85%. Polythene bags were removed after completion of colonized mycelial growth. Compact mushroom beds were sprayed regularly with water to provide adequate moisture. Three flushes of mushrooms were harvested during the total cropping period.

Harvesting, yield and biological efficiency: Matured fruiting bodies of all these five species were harvested by



Fig. 1.: Comparative evaluation of five oyster mushroom species (*Pleurotus* spp.) showing morphological differentiation of sporophores of (a) *Pleurotus ostreatus*, (b) *Pleurotus florida*, (c) *Pleurotus sajor-caju*, (d) *Pleurotus eryngii* and (e) *Pleurotus flabellatus*

gentle twisting from the beds. Total yield of all the species were pooled from the harvesting of all the three flushes, whereas biological efficiency was calculated using the formula described by Chang *et al.* (1981).

Statistical analysis: Data were analyzed using analysis of variance (ANNOVA) for the experiment arranged in a completely randomized design having three replications along with the control. Standard error (SE) and critical difference (CD) were calculated at 5% level of probability (Gomez and Gomez, 1984).

Results and Discussion

The spawn run period of all the *Pleurotus* spp. viz., *Pleurotus sajor-caju*, *Pleurotus florida*, *Pleurotus eryngii*, *Pleurotus ostreatus* and *Pleurotus flabellatus* ranged between 18 days - 21 days. Spawn run period was significantly vigorous and rapid in *P. eryngii* and it required 18 days, followed by *P. flabellatus* which required 19 days, *P. florida* and *P. ostreatus* required 20 days and *P. sajor-caju* required 21 days to complete the total spawn run. Time required for initiation of pin heads ranged from 5 days-7 days. It was evidenced that 6 to 7 days were required by *P. sajor-caju* and *P. ostreatus*, for initiation of pin heads and showed significant difference at 5% probability level whereas, *P. eryngii*, *P. flabellatus*, *P. florida* and *P. eryngii* required a minimum time of 5 days for initiation of pin heads (Table 1).

The actual time required for harvesting of first flush for all the *Pleurotus* spp. ranged from 23 to 27 days. *P. sajor-caju* and *P. ostreatus* required 27 days for harvesting of the first flush, whereas *P. eryngii* and *P. florida* were vigorous and rapid in growth as compared to other species and required 24 days to harvest their first flush. The time required to harvest the second flush from all the five species was 11 to 13 days from the harvesting of the first flush. *P. eryngii* and *P. ostreatus* were harvested on 34th days, whereas *P. sajor-caju*

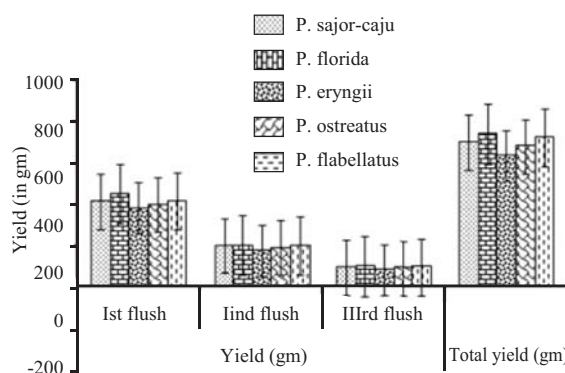


Fig. 2.: Comparison of yields (g) of Ist, IInd and IIIrd flushes and cumulative total yield from five *Pleurotus* species

Table 1.: Comparative evaluation of actual time required (days) for different growth stages of five *Pleurotus* species

<i>Pleurotus</i> species	Spawn run period	Time required for different growth stages (days)				
		Initiation of pin heads	Harvesting of 1st flush	Harvesting of 11nd flush	Harvesting of III rd flush	
<i>P. sajor-caju</i>	21	6.3	26.7	37.3	50.3	
<i>P. florida</i>	19	5.0	24.3	35.3	47.0	
<i>P. eryngii</i>	18	5.3	23.7	34.0	46.7	
<i>P. ostreatus</i>	20	6.7	26.7	34.0	52.7	
<i>P. flabellatus</i>	18.7	5.3	24.0	37.3	49.3	
SEM _s	0.44	0.24	3.52	0.61	0.83	
C.D. (P=0.05)	0.98	0.54	7.85	1.36	1.84	

Table 2.: Comparative performance of five oyster mushroom species (*Pleurotus* spp.) for yield, biological efficiencies and yield contributing traits

<i>Pleurotus</i> species	Sporophores (n)	Average wt. of sporophores (g)		Average Length of stalk (cm)		Average diameter of sporophores (cm)		Average No. of sporophores /bunch		Average Wt. of bunch (g)			Average yield of different flushes (g)			Average total yield (g)	Biological efficiency (%)
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Ist	11nd	111rd			
<i>P. sajor-caju</i>	70	5.5	12	1.9	5.4	3.0	6.1	3.7	13.3	20	34.1	411.7	200	91.7	698.3	69.8	
<i>P. florida</i>	41	8.5	14	2.8	6.0	5.3	9.6	2.7	8.0	24.3	58.0	451.7	198.3	98.3	740	74.0	
<i>P. eryngii</i>	61	5.4	10.8	2.0	5.4	3.0	6.0	4.3	11.0	21.2	32.9	381.7	173.0	83.3	633.7	63.4	
<i>P. ostreatus</i>	68	4.8	9.2	1.7	5.2	3.1	6.1	4.7	11.3	17.3	30.3	397.7	186.7	90.7	681.0	68.1	
<i>P. flabellatus</i>	63	5.3	11.0	1.9	5.3	3.2	6.0	4.3	10.3	19.00	31.7	416.3	198.3	96.7	722.7	72.27	
SEM _s	2.14	0.37	0.69	0.12	0.11	0.17	0.14	0.27	0.60	0.89	0.93	8.24	4.09	1.87	11.45	-	
C.D. (P=0.05)	4.77	0.83	1.53	0.28	0.24	0.38	0.31	0.61	1.33	1.9	2.07	18.35	9.12	4.17	25.51	-	

and *P. flabellatus* was harvested at 37 day, respectively, followed by *P. florida* (35 days). Data pertaining to harvesting of third flush revealed that, *P. ostreatus* required maximum time (52.7 days) followed by *P. sajor-caju* (50 days), *P. florida* and *P. eryngii* (47 days) (Table 1).

Critical perusal of Table 2 showed that maximum number of sporophores/bag (n=70) was found in *P. sajor-caju* followed by *P. ostreatus* (n=68), *P. flabellatus* (n=63), *P. eryngii* (n=61) and *P. florida* (n=41) (Fig. 1). The results showed significant increase in sporophore production in *P. flabellatus* and *P. ostreatus*. The results of the present study superseded the findings of Shah *et al.* (2004) where an experiment was carried out to investigate the cultivation of oyster mushroom on different substrates : 50% sawdust + 50% wheat straw, 75% sawdust + 25% leaves, 50% wheat straw + 50% leaves, 100% sawdust, 100% wheat straw and 100% leaves. The result showed that 100% use of sawdust as a substrate produced highest yield (646.9 g), biological efficiency (64.69%) and number of fruiting bodies (22.11).

Data with respect to minimum and maximum average weight of sporophores were recorded for each species. Minimum and maximum average weight of sporophores of *P. florida* was recorded as 8.5 g and 14 g, respectively, followed by *P. ostreatus* (4.8 g and 9.2 g). The results revealed that minimum average stalk length of 2.8 cm was recorded in *P. florida* followed by 2.0 cm in *P. eryngii*, 1.9 cm in both *P. sajor-caju* and *P. flabellatus* and 1.7 cm in *P. ostreatus* (Table 2). Significant differences in the weight of sporophores were obtained between *P. ostreatus* and *P. sajor-caju* as well as between *P. ostreatus* and *P. flabellatus* and *P. sajor-caju* and *P. eryngii*.

Data revealed that maximum stalk length of 6.0 cm was recorded in *P. florida*, whereas minimum of 5.2 cm was recorded in *P. ostreatus* followed by *P. sajor-caju* and *P. eryngii* showed similar stalk length of about 5.4 cm. The results of the present study, using only wheat straw as the sole substrate supersede the findings of Tupatkor and Jadhao (2006) who conducted an experiment to correlate yield performance and morphological parameters of oyster mushroom with different substrates *viz.*, wheat straw, rice straw, bajra straw, soyabean straw + wheat straw and groundnut creepers. Highest stipe length (3.56) and pileus size (29.40 cm²) was obtained using soyabean straw + wheat straw and bajra stalks and leaves. The results showed that minimum diameter of 5.3 cm was measured in *P. florida* followed by *P. flabellatus* (3.2 cm), *P. ostreatus* (3.1 cm), *P. sajor-caju* and *P. eryngii* (3.0 cm). Maximum diameter of 9.6 cm was measured in *P. Florida* followed by *P. sajor-caju* and *P. ostreatus* (6.1 cm) and *P. eryngii* and *P. flabellatus* (6.0 cm). Maximum number of sporophores/bunch (n=13) was observed in *P. sajor-caju* followed by *P. florida* (n=8). Data

pertaining to maximum weight of a bunch of 58g was recorded in *P. florida* followed by 34.1 g in *P. sajor-caju*, 32.9 g in *P. eryngii*, 31.7 g in *P. flabellatus* and 30.3 g in *P. ostreatus* of (Table 2), respectively. The results showed significant difference in bunch weight of *P. flabellatus*, *P. sajor-caju*, *P. ostreatus* and *P. eryngii* at (P=0.05).

Yield of all the five species was compared and it was highest yield was obtained from the first flush in all the species and subsequently decreased in second and third flushes. Total yield was measured using the sum of three flushes. The results revealed that highest yield of 451.7 g from first flush was obtained from *P. Florida*, whereas lowest yield of 381.7 g was recorded from the first flush of *P. eryngii*. A significant difference in yield between *P. ostreatus* (397.7g) and *P. flabellatus* (416.3 g) was noted. However, yield of second flush was found highest in *P. sajor-caju* followed by *P. florida*, *P. flabellatus*, *P. ostreatus* and *P. eryngii*. Similarly, maximum yield from third flush was recorded in *P. Florida* (98.3 g) and lowest in *P. eryngii* (83.3 g). The results showed that maximum total yield was recorded from *P. Florida* (740 g) followed by *P. flabellatus* (722.7 g), *P. sajor-caju* (698.3 g), *P. ostreatus* (681 g) and *P. eryngii* (633.7 g) (Fig. 2; Table 2). Total yield of *P. sajor-caju* was significantly different from *P. flabellatus*. These results are superlatives with respect to biological efficiencies and yield Abdurrahman *et al.*, (2008) conducted an experiment of three species of oyster mushroom *viz.*, *P. eryngii*, *P. ostreatus* and *P. sajor-caju* cultivated on wheat straw and the total fresh mushroom yield was obtained with 100 g material. Maximum yield of 20.2 g /100 g of substrate was obtained from *P. sajor-caju*. Results of the present study compared with Garcha *et al.*, (1984) where in cultivated *P. florida* and *Pleurotus sajor-caju* on wheat straw, maize, bajra, groundnut stalks, berseem, sugarcane bagasse, chilli, bhindi and potato wastes. Results revealed that the maximum yield of *P. florida* and *P. sajor-caju* (48-60% BE) was obtained using wheat and paddy straw as substrate. Ram and Pant (2004) reported the effect of different amount of spawn on spawn run period of *P. sajor-caju* and *P. flabellatus*. The results revealed that spawn run was faster when 5% spawn was used in substrate for these two species followed by 4% and 3% spawn. In the present study 5% spawn was used for rapid growth and yield of all these five *Pleurotus* species.

Results of the present study confirmed biological efficiencies in the range of 63.4 to 74.0. Data suggested that maximum biological efficiency was obtained from *P. florida* (74%) followed by *P. flabellatus* (72.27%), *P. sajor-caju* (69.83%), *P. ostreatus* (68.1%) and *P. eryngii* (63.37%). In the present study a comparative evaluation of different *Pleurotus* species was made to have a clear picture of growth behaviour, yield contributing traits for high yield and quality for mushroom growers and researchers.

Acknowledgments

Authors are highly thankful to the Head, Department of Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi for providing all the facilities required to perform proposed research work. Help rendered by the Head of Department of Plant Pathology, RAU, Samastipur, Bihar and Division of Plant Pathology, IARI, New Delhi for providing mushroom cultures is highly acknowledged.

References

- Abdurrahman, D., H. Acay and A. Yildiz: Yield performance and nutritional contents of three oyster mushroom species cultivated on wheat stalk. *Afr. J. Biotechnol.*, **7**, 3497-3501 (2008).
- Chang, S.T. and P.G Miles: Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact, 2nd Edn. CRC Press, Boca Raton, 451 (2004).
- Chang, S.T. and P.G. Miles: Edible mushrooms and their cultivation. CRC, Boca Raton, Fla, **1**, 345 (1989).
- Chang, S.T., O.W. Lau and K.Y. Cho: The cultivation and nutritional value of *Pleurotus sajor-caju*. *Eur. J. Appl. Microbiol. Biotechnol.*, **12**, 58-62, (1981).
- Cohen, R., L. Persky and Y. Hadar: Biotechnology applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. *Appl. Microbiol. Biotechnol.*, **58**, 582-594 (2002).
- De Boer, E. and A.E. Heuvelink: Methods for the detection and isolation of Shiga toxin-producing *Escherichia coli*. *Symp. Ser. Soc. Appl. Microbiol.*, **29**, 133-143 (2000).
- Garcha, H.S., S. Dhanda and P.K. Khanna: Evaluation of various organic wastes for the cultivation of (*Pleurotus*) species (Dhingri). *Mush. News Lett. Trop.*, **5**, 13-16 (1984).
- Gomez, K.A. and A.A. Gomez: Statistical procedures for agricultural research. Philippines. John wiley and Sons. 2nd Edn. (1984).
- Jayakumar, T., E. Ramesh and P. Geraldine: Antioxidant activity of the oyster mushroom, *Pleurotus ostreatus*, on CCl₄-induced liver injury in rats. *Food Chem. Toxicol.*, **44**, 1989-96 (2006).
- Kadam, R.M., B.S. Jadhav, S.S. Patil, B.S. Kadam and J.A. Kadam: Evaluation of yield performance and nutritional value of *Pleurotus sajor-caju* cultivated on different lingo cellulosic wastes. *Int. J. Plant Sci.*, **3**, 443-445 (2008).
- Moore, D. and S.W. Chiu: Filamentous fungi as food. In: (Eds. S.B, Hyde D) Exploitation of filamentous fungi. Fungal Diversity Press, Hong Kong (2001).
- Oei, P. and B.V Nieuwenhuijzen: Agrodok 40-small-scale mushroom cultivation (oyster, shiitake and wood ear mushrooms). 1st Edn. Agromisa Foundation and CTA: Digigrafi, Wageningen, Netherlands ISBN Agromisa: 90-8573-038-4 ISBN CTA: 92-9081-303 (2005).
- Oseni, T.O., S.S. Dube, K.P. Wahome, M.T. Masarirambi and D.M. Earnshaw.: Experimental Agriculture and Horticulture ID: 1929-0861-2012-12-4 (2012).
- Pala, S.A., A.H. Wani and R.A. Mir: Evaluation of yield performance of *Pleurotus sajor-caju* on different agro-based wastes. *Afr. J. Agric. Res.*, **8**, 3025-3028 (2013).
- Pokhrel, C.P., N. Kalyan, U. Budathoki and R.K.P. Yadav: Cultivation of *Pleurotus sajor-caju* using different agricultural residues. *Int. J. Agric. Pol. Res.*, **1**, 19-23 (2013).
- Rajak, S. and M. Basu: Yield, fruit body diameter and cropping duration of Oyster mushroom (*Pleurotus sajor-caju*) grown on different grasses and paddy straw as substrates. *European J. Medicinal Plants*, **1**, 10-17 (2011).
- Ram, R.C and D.C Pant: Effect of temperature and pH on mycelial growth of *Pleurotus* species. *Indian J. Plant Pathology.*, **19**, 58-60 (2002).
- Ram, R.C. and D.C. Pant: Effect of different amounts of spawn run period. *Indian J. Plant Pathology*, **22**, 131 (2004).
- Sainos, E., G. Diaz-Godinez, O. Loera, A.M. Montiel-Gonzalez and C. Sanchez: Growth of *Pleurotus ostreatus* on wheat straw and wheat-grain-based media: biochemical aspects and preparation of mushroom inoculums. *Appl. Microbiol. Biotechnol.*, **72**, 812-815 (2006).
- Shah, Z.A., M. Ashraf and Ch. Ishtiaq: Comparative study on cultivation and yield performance of Oyster mushroom (*Pleurotus ostreatus*) on different substrates wheat straw, leaves, saw dust. *Pakistan J. Nutr.*, **3**, 158-160 (2004).
- Tupatkar, P.N. and S.M. Jadhao: Effect of different substrates on yield of oyster mushroom (*Pleurotus sajor-caju*). *Agric. Sci. Dig.*, **26**, 224-226 (2006).