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Preliminary studies on the domestication of an indigenous strain of *Pleurotus cystidiosus* collected from the living stem of *Lagerstroemia speciosa*

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ABSTRACT

Oyster mushrooms (*Pleurotus* spp.) are popular throughout the world because of their tremendous stability of pileus and stipe, cooking qualities, and longer shelf life. During the present investigations pure culture of *P. cystidiosus* O.K. Mill. was raised through standard tissue culture technique from the fresh young sporophore collected from nature. Subsequently, its spawn was prepared on supplemented wheat grains with different additives and thereafter three locally available lignocellulosic substrates (wheat straw, paddy straw, and sawdust) were used for its cultivation. Among the three substrates used, maximum biological efficiency of 36% was obtained when the mushroom was grown on wheat straw followed by paddy straw (6.3%) and sawdust (2.3%). To enhance the yield of mushroom, wheat straw was further supplemented with rice bran (10%): corn flour (5%), mustard oil seed cake (10%): corn flour (5%), cotton oil seed cake (10%): corn flour (5%) and a mixture of all these four (3:1:1:1) and corn flour (5%). Wheat straw supplemented with rice bran (RB) + mustard oil seed cake (MSC) + cotton oil seed cake (CSC) in the ratio of 3:1:1:1 gave maximum biological efficiency (B.E. 74%) followed by supplementation of wheat straw with rice bran (B.E. 55.15%), mustard oil seed cake (B.E. 50.42 %) and cotton oil seed cake (B.E. 48.58%).

Keywords : *Coremiopleurotus*, ligno-cellulosic substrate, cultivation, sporophores, biological efficiency.

INTRODUCTION

Pleurotus (Fr.) P. Kumm. is an important genus of edible mushrooms which are classified under class *Agaricomycetes*, order *Agaricales* and family *Pleurotaceae* (Kirk *et al*., 2008). Many of its species are cultivated all over the world for a number of associated advantages including nutritional and nutraceutical benefits to the consumers, generation of additional income, recycling of organic residues and employment. The available trend in oyster cultivation is a clear indicator of the fact that the world's production and consumption of *Pleurotus* mushrooms are increasing tremendously. According to Sharma *et al*. (2017), out of the total production of mushrooms, the share of oyster mushroom production is about 16% followed by paddy straw (7%) and milky mushrooms (3%).

Species of the *Pleurotus* possesses acceptable culinary credential being an excellent source of nutritional and nutraceutical constituents (Atri *et al*., 2012; 2013; 2015). Their sporophores are reported to contain a substantial amount of carbohydrates, proteins, low amount of fats, vitamins and some important minerals (K, Na, P, Fe, and Ca). These mushrooms are reported to be high in potassium to sodium ratio, which makes them an ideal food for patients suffering from hypertension and heart diseases (Dehariya *et al*., 2013). Vitamins of A and B-group (thiamine, riboflavin, niacin, pantothenic acid, and biotin), C, D, E, and K are commonly present in these mushrooms which primarily accounts for their nutraceutical potential (FAO, 1970; Mattila *et al*., 1994; Sapers *et al*., 1999; Caglarirmak, 2007; Furlani and Godoy, 2008; Patil *et al*., 2010). The quantity of niacin in *Pleurotus* species is reported to be about 10 times more than the vegetables (Patar *et al*., 2018). 'Pleurotin', a polycyclic aromatic compound, which has been isolated from *Pleurotus* species is reported to have antibacterial properties (Patar *et al*., 2018). Lovastatin is another medicinally important constituent of *Pleurotus* species, which finds application in lowering the bad cholesterol and fats and helps in preventing strokes and heart attacks (The Expert Panel,1998; Mswaka and Magan, 1999; Raghunath *et al*., 2012; Carrie, 2016). Besides, these mushrooms are also reported to possess immense potential for their utility against diseases like cancer, synthesis of important bioactive chemicals, bioremediation, production of ethanol, and in many other biotechnological applications (Madar and Zusman, 1997; Jonsson *et al*., 1998; Rajarathnam *et al*., 1998; Fragoeiro and Magan, 2005). Besides a number of myochemicals are also reported to be produced by edible *Pleurotus* species (Krishnamoorthy and Sankaran, 2014). In India, large quantity of raw materials from agricultural wastes is available, which is quite cheap when compared with international cost. This adds to the prospects of bulk cultivation of oyster mushrooms. Even though in India, the residue straw is commonly used as fodder, yet 50% of the crop residues are still potentially available for the cultivation of edible mushrooms (Pakale, 2004). Further, different substrates used in the cultivation of *Pleurotus* have utility as fertilizers and soil conditioners (Brenneman and Guttman, 1994). In view of the importance and promising future of oyster mushrooms in human food and medicine and its capability to recycle agro wastes, the present investigation was undertaken on the domestication of a locally available wild strain of *P. cystidiosus*. This is the first report on the cultivation of the indigenous strain of *P. cystidiosus* from India.

MATERIALS AND METHODS

The material: The sporophores of *P. cystidiosus* were collected from the bark of the living stem of *Lagerstroemia speciosa* (L.) Pers. growing along roadsides on the campus of Punjabi University Patiala having an altitude of 350 m. It has a pleurotoid petaloid to involute sporophore with pileus up to 3-

8 cm broad, pale yellow around the margins and brownish-yellow around the part of attachment, fleshy, white, and smooth with regular margins. Lamellae broad, decurrent, unequal, subdistant, creamish to yellow-white when young, yellow at maturity, gill edges smooth. Stipe lateral, greyish brown, clavate, solid, fibrillose near the base (**Fig. 1**). The dried sporophore was submitted in the Herbarium of Botany Department, Punjabi University, Patiala under PUN number11054 and the gene sequence at NCBI GenBank under accession number Mt705887.

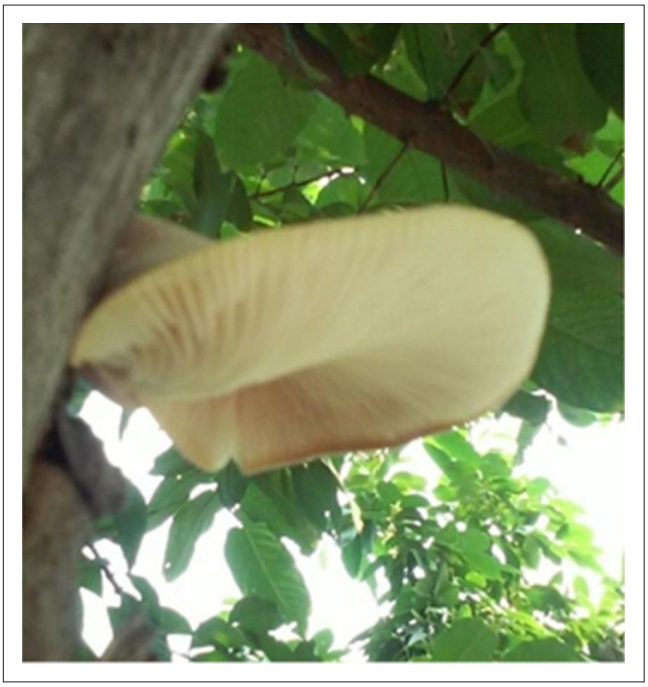

Fig. 1: Basidiocarp of *Pleurotus cystidiosus* in natural habitat

Viable mycelia culture of the collected material was raised through tissue culture technique by taking a portion of the actively growing tissues from the centre of the point of confluence of the stipe with the pileus. The mushroom mycelium was repeatedly sub-cultured on sterile Potato Dextrose Agar (PDA) medium slants so as to purify it. The inoculated slants were incubated at 28 ± 2˚C. The whole procedure of culturing was done aseptically under laminar airflow. Pure culture of this strain of *P. cystidiosus* O.K. Mill. has been deposited in the DMR Culture Bank at ICAR-Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh under accession number DMRP-394.

Spawn preparation: For the preparation of spawn healthy wheat grains supplemented with different additives, i.e. (i) Glucose (1%) + Yeast (1%) + $CaCO_3$ (4%) + $CaSO_4$ (2%) + $FeSO_4$ (0.05%) + $MgSO_4$ (0.05%) (ii) Glucose (1%) + $CaCO_3$ (4%) + $CaSO_4$ (2%) + $FeSO_4$ (0.05 %) + $MgSO_4$ (0.05 %) (iii) Yeast (1%) + $CaCO_3$ (4%) + $CaSO_4$ (2%) + $FeSO_4$ (0.05%) + $MgSO_4$ (0.05 %) and (iv) $CaSO_4$ (2%) + $CaCO_3$ (4%) + $FeSO_4$ (0.05%) + $MgSO_4$ (0.05%) were taken and grain spawn was prepared using the standard methodology (FAO, 1990).

Substrates and substrate preparation for cultivation: Wheat straw, paddy straw, sawdust, and wheat straw supplemented with rice bran (10%) and corn flour (5%), mustard oil seed cake (10%) and corn flour (5%), and cotton oil seed cake and corn flour (5%) were used during the present study for cultivation. The substrates were prepared by following standard methodology given by Upadhyay (1990). After complete colonization, the polypropylene bags were removed from the cylinders. The colonized cylinders and beakers with the colonized substrate as such were dipped in ice-cold water for 5-10 minutes for giving shock treatment. Afterward, these cylinders and beakers were kept in a cropping room at 24 ± 1˚C temperature and high relative humidity of 80-90%. A humidifier was used to maintain relative humidity in the cropping room. The colonized cylinders/beakers were sprinkled with water regularly at least twice a day. The time taken for the emergence of primordia after inoculation of substrates with spawn was noted. The carpophores were harvested, their morphological characteristics, fresh and dry weight, size of largest and smallest carpophores were also recorded. Biological efficiency both on a fresh and dry weight basis was calculated by applying the following formula.

$$\textbf{Biological efficiency} = \frac{\text{Fresh/dry weight of mushrooms}}{\text{Dry weight of substrate}} \times 100$$

RESULTS

Preparation of mother spawn: The wheat grains boiled as per the standard methodology were supplemented with different additives so as to enhance the vegetative growth. The blend of different additives, i.e. glucose (1%), yeast (1%), $CaCO_3$ (4%), $CaSO_4$ (2%), $MgSO_4$ (0.05%) and $FeSO_4$ (0.05%) supported the fastest vegetative growth of mycelium in which wheat grains were fully colonized after 13 days of incubation and the mycelium was thicker and denser. The second best colonization of mycelium in terms of number of days and type of mycelium was observed in wheat grains that were mixed with glucose (1%), $CaCO_3$ (4%), $CaSO_4$ (2%), $MgSO_4$ (0.05%) and $FeSO_4$ (0.05%) and were fully colonized in 15 days. The maximum numbers of days were taken by wheat grains, which were deprived of carbon (glucose) and nitrogen (yeast) sources. It was observed that in control (grains without any treatment), the grains tend to form lumps due to excessive stickiness, which prevented the easy separation of colonized grains from one another in the conical flasks. The combination of all the additives enhanced the vegetative growth of mycelium on the wheat grains and hence it was selected for the preparation of mother spawn for the cultivation of *P. cystidiosus* (**Fig. 2**).

Evaluation of different natural substrates for sporophore production: Cultivation of *P. cystidiosus* was done by using three locally available lignocellulosic substrates, namely wheat straw, paddy straw, and sawdust. The wheat straw substrate was further supplemented and amended to increase the yield of the crop. Each inoculated substrate cylinder took a different time period for complete colonization by the mycelium, appearance of primordia, and further development

into mature sporophores.

Fig. 2: Mother spawn of *P. cystidiosus* with profuse coremia

It took 46 days for complete colonization of mycelium on the wheat straw substrate. The primordia emerged on the colonized cylinder of mycelium after 16 days of the opening of bags in the cropping room. Total of 17 primordia emerged, out of which 10 primordia matured into sporophores and the rest aborted. The colour of harvested sporophores was greyish brown. The range of size for sporophores varied from 5-13 cm in height. On an average 108 g of fresh weight of sporophores were harvested with biological efficiency of 36% (**Fig. 3a**).

On the paddy straw substrate, a large amount of coremial droplets appeared and the time taken for complete spawn run was 48 days while the emergence of primordia was observed after 18 days of the opening of the bags. Out of the 3 primordia that appeared on the colonized cylinder, only 2 matured into sporophores. The colour of the sporophore was greyish brown with a size range of 3-15 cm. The average fresh weight of sporophores was 19 g on an average with biological efficiency of 6.3% (**Fig. 3b**). On sawdust, it took 56 days for complete colonization and only one primordium appeared after 18 days of the opening of bags in the crop room. The biological efficiency of only 2.3% was obtained on this substrate (**Fig. 3c**). Amongst the three substrates used for the cultivation of the indigenous strain of *P. cystidiosus*, wheat straw substrate gave better results than others (**Table 1**).

Table 1: Evaluation of different substrates for the cultivation of *P. cystidiosus*

Name of substrate	Net weight of dry substrate/bag	Fresh weight of mushroom (g)			Average fresh weight of sporophores /bag (g)	Dry weight of mushroom (g)			Average dry weight of sporophores /bag (g)	Biological efficiency on fresh weight basis	Biological efficiency on dry weight basis
		Bag I	Bag II	Bag III		Bag I	Bag II	Bag III			
Wheat Straw	300 g	176	40	--	108	40.0	4.0	--	22	36%	7.3%
Paddy Straw	300 g	32	6.0	--	19	3.68	0.8	--	2.24	6.3%	0.7%
Saw Dust	300 g	7.0	--	--	7.0	0.9	--	--	0.9	2.33%	0.3%

Sporophore production on wheat straw supplemented with different additives: When wheat straw was supplemented with rice bran (10%) and corn flour (5%), primordia appeared after 23 days of the opening of beakers in the crop room. The total number of primordia was 8, out of which 5 matured into sporophores. The size of the mature sporophore was 9.5 cm in height with a pileus diameter of 7 cm and stipe size of 5.6 x 1 cm. An average of 27.57 g of fresh mushroom was harvested from this substrate with biological efficiency of 55.15% and 3.96% on fresh and dry weight basis, respectively (**Table 2**).

Upon supplementation of wheat straw with mustard oil cake (10%) and corn flour (5%) primordia appeared after 25 days of the opening of beakers in the crop room. The total primordia were 8 in number of which 5 matured into sporophores. The size of sporophores was 8 cm in height, while the diameter of pileus and length and breadth of the stipe was 5.2 cm and 4 x 0.9 cm, respectively. An average of

Fig. 3: Sporophore production on (a) wheat straw;(b) paddy straw; (c) sawdust

25.12 g of fresh weight of sporophores were harvested from this substrate with 50.42% biological efficiency whereas 3.08% biological efficiency was obtained by taking into account dry weight of the harvested mushrooms.

When wheat straw was supplemented with cotton oil seed cake (10%) and corn flour, primordia formation took 27 days after opening the beakers. In all only 3 primordia appeared, out of which 2 matured into sporophores. The colour of the mature sporophore was greyish brown with a sporophore height of 8.5 cm. The pileus diameter was 6 cm and the stipe was 4.25 x 0.8 cm in size. The biological efficiency of 48.5% on fresh and 2.1% on a dry weight basis was recorded.

Sporophore production on the mixture (wheat straw + rice bran + mustard oil seed cake + cotton oil seed cake-3:1:1:1) and corn flour (5%): The total number of days for the emergence of primordia was 23 after opening of the beakers in the cropping room. In all 6 primordia appeared and all turned into mature sporophores. The size of the sporophore was 9.7 cm with a pileus diameter of 6.7 cm and stipe size of 5.6-1.1 cm. The fresh weight of mushrooms harvested from this substrate was 37.0 g with biological efficiency of 74%.

Biological efficiency: The biological efficiency was calculated on both fresh and dry weight basis of harvested sporophores in relation to the net dry weight of the substrate used for cultivation. It was observed that wheat straw alone gave biological efficiency of 36% followed in decreasing order by paddy straw (6.3%) on a fresh weight basis out of the three natural lignocellulosic substrates used for cultivation. However, better results were achieved when wheat straw was supplemented with a mixture of rice bran (RB), mustard oil seed cake (MSC), and cotton oil seed cake (CSC). In this combination, the mushroom gave maximum biological efficiency of 74%. Also when wheat straw was supplemented with rice bran (B.E. 55.15%), mustard oil seed cake (B.E. 50.42%), and cotton oil seed cake (B.E. 48.58%),the biological efficiency of *P. cystidiosus* increased in comparison to when different lignocellulosic substrates were used alone without any supplementation.

Table 2: Evaluation of sporophore production on wheat straw substrate supplemented with different additives.

Name of substrate	Net weight of dry substrate/beaker	Fresh weight of Sporophores per beaker (g)			Average weight of sporophores /beaker (g)	Dry weight of Sporophores per beaker (g)			Average dry weight (g)	Biological efficiency on fresh weight basis	Biological efficiency on dry weight basis
		B I	B 1I	B 11I		B I	B 1I	B 11I			
Wheat Straw (10% RB)	50 g	30.657	28.137	23.935	27.576	2.44	1.957	1.563	1.98	55.15%	3.96%
Wheat Straw (10% MSC)	50 g	28.191	15.163	32.283	25.212	1.607	1.293	1.724	1.54	50.42%	3.08%
Wheat Straw (10 % CSC)	50 g	24.293	--	--	24.293	1.068	--	--	1.068	48.58%	2.13%
Wheat Straw (Mixture-RB+MSC+CSC)	50 g	40.0	43.0	28.0	37.0	3.468	4.136	1.108	2.904	74%	5.80%

(Abbreviations: RB: Rice Bran; MSC: Musturd oil seed cake; CSC: Cotton oil deed cake)

DISCUSSION

Different species of *Pleurotus* forms a heterogeneous group of commercially important taxa some of which produce synnematoid fructifications taxonomically classified under subgenus *Coremiopleurotus* Hilber (Zervakis *et al*., 2004). *P. cystidiosus* on which the present investigation has been undertaken is one such synnematoid species. Despite wide occurrence of this edible mushroom in Punjab state so far no attempt has been made for its domestication. Presently, pure culture of this mushroom was raised through tissue culture as was done by Asghar *et al*. (2007) while working with *P. sajor caju*. Just like other cultivated mushrooms *P. cystidiosus* also gave best quality of spawn on wheat grains with some additives. In line with the present investigations number of investigators has also recommended the use of wheat grains for the production of quality spawn in different *Pleurotus* spp. (Holkar and Chandra, 2016; Ita *et al*., 2018; Tewari, 2004). While evaluating wheat grains, saw dust and rice bran for spawn production of oyster mushroom, Islaam *et al*. (2007) also reported wheat grains to be the best substrate in this regard which is in conformity with the present findings. Lechner and Alberto (2011) while working with *P. albidus*, and Singh *et al*. (2012) with *P. florida* and *P. sajor-caju* also

reported wheat grains as the best substrate for spawn production which is in conformity with the present investigations.

Supplementation of substrates is a well established practice to increase the productivity in oyster mushrooms (Dhanda *et al*., 1996; Peng *et al*., 2000; Khan *et al*., 2019). In conformity with this, presently also, maximum biological efficiency (74%) on fresh weight basis was obtained when wheat straw was supplemented with rice bran, mustard oil seed cake and cotton oil seed cake and corn flour. A number of similar such reports are available suggesting increased biological efficiency of mushrooms upon supplementation of substrates with rice or wheat bran @ 10-50% (Peng *et al*., 2000; Atikpo *et al*., 2008; Pathmashini *et al*., 2008). Similarly Mudakir *et al*. (2014) recorded biological efficiency of 64.49% when *P. cystidiosus* was grown on supplemented saw dust with cocoa powder waste in the ratio of 75:25%. While working with *P. cystidiosus*, Petcharat and Tongwised (2004) also obtained maximum biological efficiency of 60.79% when grown on saw dust supplemented with oil palm kernel meal and $Ca(OH)_2$ in a specified proportion (89%:10%:1%). As compared, in *P. cystidiosus* and *P. ostreatus*, Hoa *et al*. (2015) recorded almost comparable biological efficiency of 50.14% and 65.65%, respectively when cultivated on 100% corn cobs without any supplementation.

During the present investigation, using local strain of *P. cystidiosus*, numbers of days required from complete mycelium run to harvesting were found to range from 90-120 on an average basis. This observation is also in conformity with the findings of Petcharat and Tongwised (2004) who also recorded total of 120 days for complete cycle of crop run in this mushroom. As compared to other species of oyster mushrooms comparatively longer time is taken by *P. cystidiosus* to complete the life cycle which needs to be shortened by somehow restricting the transformation of vegetative mycelium into coremia.

CONCLUSION

Pleurotus cystidiosus is one such species, which is although equally rich in various nutritional and nutraceutical components as are other species of edible mushrooms. Supplementation with extra nitrogen not only increased the yield but it also reduced the number of days for fruiting. Cold shock is essential for fruiting. Not much work has been done on this mushroom as far its evaluation, domestication and popularization is concerned. The most common problem posed by this mushroom is in the initiation of fruit body formation and poor conversion of substrate into utility product. There is a need to understand its requirements for bulk production with a view to increase its biological efficiency.

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