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Optimization of spawn doses and agroforestry-residues as substrates for *Pleurotus cornucopiae* production

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Abstract
This study was carried out to investigate the effects of different substrates and spawn doses on the yield of *Pleurotus cornucopiae*. In the experiment different spawn doses (1, 2, 3, 4 and 5 %) and five agro-forestry waste viz., wheat straw, paddy straw, sugarcane bagasses, maize straw and saw dust were evaluated for the cultivation of *P. cornucopiae*. The trial was conducted using completely randomized plot design with three replications. Days to spawn run, average number of basidioma and yield as well as biological efficiency of mushroom were observed. The result of this study indicates that maximum yield (517.33 g/2kg substrate) with a biological efficiency of 86.22 per cent was recorded in wheat straw substrate spawned at the rate of 5 per cent spawn. It was found that minimum (14.00 days) time to spawn run and maximum (7.33) number of basidioma was harvested from wheat straw spawned at the rate of 5 per cent.

Keywords: *Pleurotus cornucopiae*, yellow oyster, agro-residues, spawn, branched oyster

Introduction

Mushrooms are recognized as fungi with fruiting bodies that decompose their growth media using biochemical processes in order to obtain energy and growth material. Oyster mushroom is the third largest cultivated mushroom in the world (Adebaya and Martinez-Carrera, 2015) [1] and is commonly called as 'Dhingri' in India because of its oyster like shape (Narain *et al*., 2016) [2]. The *Pleurotus* spp of the class basidiomycetes belongs to a group known as “white rot fungi” (Tsujiyama and Ueno, 2013) [3]. They produce a white mycelium and are generally cultivated on non-composted lignocellulosic substrates (Savoie *et al*., 2007) [4]. Many species of *Pleurotus* including *P. ostreatus* (oyster mushroom), *P. eryngii* (king oyster or Cardoncello), *P. pulmonarius* (phoenix mushroom), *P. djamor* (pink oyster mushroom), *P. sajor-caju* (Indian oyster), *P. cystidiosus* (abalone oyster), *P. citrinopileatus* (golden oyster mushroom) and *P. cornucopiae* (branched oyster mushroom) are commercially cultivated and have considerable economic value (Prez-Martinez *et al*., 2015) [5]. Oyster mushroom cultivation can play an important role in managing organic wastes, whose disposal has become a problem (Das and Mukharjee, 2007) [32]. *P. cornucopiae*, the branched oyster mushroom is one of the oyster mushroom species whose cultivation is not being practiced very commonly in India. This species is characterized by a cap which in the young state is cream colored and then becomes yellowish-ochraceous and at maturity turns darker ochraceous-dark brown. The cap is funnel shaped while the inner flesh is white, thin with a pleasant odour and mild taste (Anonymous, 2012) [7]. *P. cornucopiae* has been widely used in nutritional and medicinal purposes and fruit bodies of *P. cornucopiae* can be used to develop nutraceutical products, which can play significant roles in providing good nutrition and improving human health (Alam *et al*., 2011) [8].

Spawn is the culture of mushroom fungus grown on a suitable substrate and used as a seed of mushroom for cultivating it (Mohammadi and Purjam, 2003) [9]. It is the mushroom mycelium growing on a given substrate and serves as the planting material (seed) in mushroom production (Stanley and Awi-Waadu, 2010) [10]. It is a propagative material comprising of mushroom mycelium and any indispensable substrate for proper fungal growth (Rukhsana *et al*., 2019) [11]. Rate of spawning influences the production of particular mushroom species because, a lower inoculum level may not be sufficient to initiate growth, whereas a higher level may cause competitive inhibition (Sabu *et al*., 2005) [12]. The enhanced spawn rate shortens mycelial colonization time, primordia formation, the time to the first harvest (Yang *et* *al*., 2013) [13] and narrows the scope of competitor invasion (Stamets, 2000) [14]. The increased nutrient level available in spawn at higher rates would provide more energy for mycelial growth and development (Royse *et al*., 2004) [15]. However, the amount of inoculum should not exceed 10% of the weight of the substrate (commercial production 7–10%), because no significant increase in biological efficiency of substrates is recorded, resulting in economic loss (Eira, 2003) [16].

In addition to spawn doses, the substrate of cultivation also affects the final yield of any oyster mushroom. Large volumes of unused lignocellulosic by-products are available in tropical and subtropical areas. These by-products are usually left to rot in the field or are disposed through burning (Tesfaw *et al*, 2015) [17]. Using locally available lignocellulosic substrates to cultivate oyster mushroom is one solution to transform these inedible wastes into accepted edible biomass of high market and nutrient values (Tesfaw *et al*, 2015) [17]. *Pleurotus* spp. have immense abilities to utilize various lignocellulose substrates with the aid of extracellular enzymes capable of degrading complex organic material (Martinez-Carrera, 2002) [18]. In India, much work has not been done on the cultivation of *P. cornucopiae*. So it was thought worthwhile to evaluate different doses of spawn so as to standardize the appropriate spawn rate for its production on five different agro-forestry substrates.

Materials and methods

Procurement of pure culture

The pure culture of *P. cornucopiae* was procured from Directorate of Mushroom Research, ICAR, Complex, Chambaghat, Solan, (H.P)

Maintenance of Pure culture and spawn preparation

The pure culture obtained was maintained on PDA slants at 4±1 °C in a refrigerator and sub-cultured at regular intervals for further studies. The spawn was prepared on wheat grains by following standard protocol.

Effect of different spawn doses for the production of *P. cornucopiae*

Effect of different doses wheat grain spawn viz., 1, 2, 3, 4 and 5 per cent on the production of *P. cornucopiae* was evaluated while using five different substrates viz wheat straw, paddy straw, sugarcane bagasses, maize straw and saw dust. Standard package of practices for cultivation of oyster mushroom was followed to raise the crop. Each treatment was replicated thrice and data were recorded in terms of time taken for complete spawn run (days), average number of basidioma as well as yield (g/2 kg substrate) and biological efficiency of each substrate was further calculated as per the following formula:

$$\text{Biological Efficiency (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Evaluation of substrates for sporophore production

After optimizing the dose of spawn, all five substrates were evaluated for the cultivation of *P. cornucopiae* in 5 kg capacity growing bags. The selected substrates were chopped into 5 cm long pieces and transferred to gunny bags. The bags were soaked in clean tap water for 12 h. The excess water was drained out and the pre-soaked substrates were sterilized for 30 min at 15 psi pressure. After cooling the substrates, the spawn of *P. cornucopiae* was layer inoculated at the optimized dose. The inoculated bags were incubated in dark for 12-14 days at a humidity range 80-90 per cent and the temperature between 25 to 30 ºC for mycelial growth. When mycelial growth had covered the whole substrate in the bags, the bags were hanged and exposed to light of 2000-3000 lux units intensity for development of fruiting bodies. Data were recorded in terms of time taken for complete spawn run (days), number of basidioma produced and yield (g/5 kg substrate) of *P. cornucopiae*. Biological efficiency (%) of tested agro-forestry wastes was calculated further.

Statistical Analysis

The data recorded in each experiment was subjected to statistical analysis wherever required. The differences exhibited by treatments were tested for their significance by employing the Completely Randomized Design (CRD) as given by (Panse and Sukhatme, 2000) [19].

Results and Discussion

Effect of different spawn doses for the production of *P. cornucopiae*

A perusal of the data presented in Table 1 reveal that irrespective of the substrates used, significantly minimum average time for spawn run (19.86 days) was recorded in substrates spawned at the rate of 5 per cent followed by those spawned at the rate of 4 per cent (21.53 days) and 3 per cent (25.80 days). However, irrespective of the spawn doses evaluated, significantly minimum time for spawn run was recorded in wheat straw substrate (20.80 days) followed by paddy straw substrate (23.46 days) while, maximum time for spawn run was recorded in sawdust substrate (29.13 days). It was further recorded that the minimum (14.00 days) time to spawn run was observed in wheat straw substrate spawned at the rate of 5 per cent which was statistically at par with that of paddy straw (15.33 days) spawned at the same rate. With the increased rate of spawning, mycelium spread in the substrates was fastened resulting in less spawn run period with increased spawn doses in all the substrates tested.

Table 1: Effect of spawn doses on spawn run period of *Pleurotus cornucopiae* in different substrates

Spawn Dose (%)	Average time taken for spawn run in different substrates (days)					Mean
	Wheat Straw	Paddy Straw	Sugarcane Bagasse	Maize Straw	Saw Dust	
1	28.00	29.00	31.00	33.00	34.33	31.06
2	25.00	27.66	29.00	30.00	31.00	28.53
3	21.00	25.00	26.00	28.00	29.00	25.80
4	16.00	20.33	22.66	23.33	25.33	21.53
5	14.00	15.33	21.00	23.00	26.00	19.86
Mean	20.80	23.46	25.93	27.46	29.13	
	CD	SE				
Spawn Dose	0.72	0.25				
Substrate	0.72	0.25				
Interaction	1.62	0.57				

The results presented in Table 2 depict that irrespective of the substrate used, average maximum number (5.13) of basidioma was recorded when the substrates were spawned at the rate of 5 per cent significantly followed by those spawned at the rate of 4 per cent (4.06). Among the different substrtaes evaluated, wheat straw produced maximum number of basidioma (4.46) which was statistically at par with that in paddy straw (3.93) irrespective of the spawn doses used. Maximum (7.33) number of basidioma was harvested from wheat straw spawned at the rate of 5 per cent which was statistically at par with the number of basidioma harvested from paddy straw spawned at the same rate (6.33).

Table 2: Effect of spawn doses on fruit body production of *Pleurotus cornucopiae* in different substrates

Spawn dose (%)	Average number of basidioma in different substrates					Mean
	Wheat straw	**Paddy straw**	**Sugarcane bagasse**	**Maize straw**	**Saw dust**	
1	2.66	2.33	2.00	1.66	1.33	2.00
2	3.33	3.00	2.00	2.33	2.00	2.53
3	3.66	3.00	2.66	3.00	2.33	2.93
4	5.33	5.00	4.33	3.00	2.66	4.06
5	7.33	6.33	4.66	4.33	3.00	5.13
Mean	4.46	3.93	3.13	2.86	2.26	
	CD	SE				
Spawn Dose	0.55	0.19				
Substrate	0.55	0.19				
Interaction	1.24	0.43				

Yield of oyster mushroom under study and biological efficiency of substrates at different spawn doses under investigation has been presented in Table 3. It is clear from the table that irrespective of the substrate used, maximum average yield (428.73 g/ 2kg substrate) was recorded at 5 per cent rate of spawning followed by 4, 3, 2 and 1 per cent rate of spawning. However, among the five substrates evaluated, wheat straw yielded highest quantity (444.40 g/2kg subastrate) of mushroom followed by paddy straw, sugarcane baggasse, maize straw and sawdust irrespective of the spawn doses under study. Individually, maximum yield (517.33 g/2kg substrate) with a biological efficiency of 86.22 per cent was recorded in wheat straw substrate spawned at the rate of 5 per cent, which was statistically at par with the yield and biological efficiency recorded at the rate of 4 per cent spawning (507.33 g/2kg; 84.55%) in the same substrate. These studies indicated that the test mushroom can be cultivated best on wheat straw spawned with wheat grain spawn @ 4 per cent. After standardizing rate of spawning, a final yield trial was conducted using five different substrates so as to optimize the best substrate for the cultivation of *P. cornucopiae*.

Table 3: Influence of different spawn doses on the yield of *Pleurotus cornucopiae* in different substrates

Spawn dose (%)	Average Yield (g/2kg substrates) in different substrates					Mean
	Wheat straw	**Paddy straw**	**Sugarcane bagasse**	**Maize straw**	**Saw dust**	
1	241.00 (40.16)	233.33 (38.88)	214.00 (35.66)	199.00 (33.16)	167.00 (27.83)	210.86
2	464.33 (77.38)	397.66 (66.27)	384.33 (64.05)	336.66 (56.11)	178.66 (29.77)	352.33
3	492.00 (82.00)	479.33 (79.88)	438.66 (73.11)	413.00 (68.83)	184.33 (30.72)	401.46
4	507.33 (84.55)	493.00 (82.16)	457.33 (76.22)	430.00 (71.66)	197.00 (32.83)	416.93
5	517.33 (86.22)	498.33 (83.05)	475.00 (79.16)	447.66 (74.61)	205.33 (34.22)	428.73
Mean	444.40	420.33	393.86	365.26	186.46	
	CD	SE				
Spawn dose	4.69	1.64				
Substrate	4.69	1.64				
Interaction	10.49	3.68				

Figures in parentheses represent respective biological efficiency (%)

The data pertaining to production trial on different substrates have been presented in Table 4. It is clear from the table that that minimum average time taken for spawn run (16.33 days) was recorded on wheat straw which was significantly followed by paddy straw (20.00 days) and sugarcane bagasse (22.33 days). However, maximum average time taken for spawn run (25.00 days) was observed on saw dust followed significantly by maize straw (23.66 days). Maximum number (20.49) of basidioma was recorded on wheat straw which was significantly followed by paddy straw (19.93) and sugarcane bagasse (11.53). Significantly minimum number (4.52) of basidioma was observed on saw dust followed by maize straw (11.71). As far as yield of *P. cornucopiae* was concerned, it was recorded to be maximum (1276.00 g/5kg substrate) on wheat straw which was significantly followed by paddy straw (1250.00 g/5kg substrate) and sugarcane bagasse (1150.00 g/5kg substrate). Significantly minimum average yield (504.00 g/5kg substrate) was recorded on saw dust followed by that in maize straw (1110 g/5kg substrate). As far as biological efficiency of the substrates was concerned, it was found to be maximum (85.06%) on wheat straw followed by paddy straw (83.33%), sugarcane bagasse (76.66%), maize straw (74.00%) and saw dust (33.60%).

Table 4: Evaluation of different substrates for yield performance of *P. cornucopiae*

Substrates	Time taken for spawn run (days)	No of basidioma	Yield (g/5kg substrate)	Biological Efficiency (%)
Wheat straw	16.33	20.49	1276	85.06
Paddy straw	20.00	19.93	1250	83.33
Sugarcane bagasse	22.33	11.53	1150	76.66
Maize straw	23.66	11.71	1110	74.00
Saw dust	25.00	4.52	504	33.60
CD	1.42	0.03	7.35	
SE	0.44	0.01	2.30	

During present studies, spawn run period was found to reduce with the increase in spawn dose. Among different substrates, wheat straw proved to be the best at all the spawn doses in terms of number of basidioma produced and mushroom yield. These results are in conformity with the findings of Pal *et al.* (2017) [20] who also recorded shortest spawn run period with increased spawn doses as well as higher yield and biological efficiency with the increased rate of spawning in case of *P. pulmonarius*. Ram and Pant (2004) [21] have also reported rapid spawn run at higher dose of spawn i.e at 5 per cent as compared to 4 and 3 per cent in case of *P. sajor-caju* and *P. flabellatus* grown on wheat straw, rice straw and sugarcane bagasses which further support our results. The findings of Shukla and Jaitly (2011) [22] also support these findings as they reported maximum number of *P sajor-caju* basidioma on wheat straw.

During final experiment, minimum spawn run period of 16.33 days was recorded on wheat straw substrate followed by paddy straw while, saw dust took maximum time (25.00 days) for its colonization with mycelium of *P. cornucopiae*. As far as yield was concerned, it was found to be maximum in wheat straw with maximum number of basidioma and a biological efficiency of 85.06 per cent. Paddy straw proved to be the second best substrate with a biological efficiency of 83.33 per cent whereas saw dust proved to be least supportive for the production of test mushroom with a biological efficiency of 33.60 per cent only. The variation in number of days taken for a spawn to complete colonization of a given substrate is a function of a fungal strain, growth conditions and substrate type (Chang and Miles, 2004) [23]. This variation could in turn be attributed to the variation in chemical composition and carbon to nitrogen ratio of substrates used (Bhatti *et al*., 1987) [24]. Pant *et al*.(2006) [25] have also reported higher yield and biological efficiency of *Pleurotus* species on wheat straw as compared to other substrates including sugarcane bagasse used. Wheat straw is the commonly used substrate for the cultivation of oyster mushroom (Jarial *et al*., 2013) [26]. In general, the biological efficiency of wheat straw comes out to be 99 to 100 per cent (Suman and Jarial, 2006 and Gupta *et al*., 2011) [27, 28]. However, during present investigation, a biological efficiency of only 85.06 per cent was recorded on wheat straw which could be possibly due to slower conversion of lignocellulosic material by this particular species of the oyster mushroom. As there are no such reports available in the literature with regards to *P. cornucopiae*, so these results cannot be compared with any available data. However, Owaid *et al*. (2014) [29] reported that saw dust extract exhibited poor mycelial growth of *P. ostreatus in vitro*. Onuoha (2007) [30] reported reduced yield of *P. ostreatus* on saw dust. Kalpana *et al*. (2011) [31] attributed reduced yield on saw dust possibly due to pre-treatment of wood by fungicides and antibacterial chemicals in wood factories.

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