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EFFICACY OF VARIOUS SPAWN SUBSTRATE, SUBSTRATE PASTEURIZATION AND SUPPLEMENTS IN THE SPAWN BASE ON SPOROPHORE PRODUCTION OF *HYPSIZYGUS ULMARIUS* (BLUE OYSTER MUSHROOM)

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Abstract

Studies were conducted to assess the effect of different spawn substrates on the production of biomass, and their influence on the sporophore production. Among the different spawn substrates *viz*., black gram, cumbu, Ill-filled paddy, maize, paddy, panivaragu, sorghum, thenai and wheat. Among the different spawn substrate paddy grain and Ill-filled paddy recorded the fastest mycelia growth covering the spawn bottle in (11.82 & 12.08) days respectively. Mushroom beds inoculated with Paddy grain and Ill-filled paddy spawn recorded maximum yield (482.08 & 478.72 g), and the best biological efficiency (96.43 & 95.74 %). Among the three different methods of pasteurization tested, the substrates pasteurized by parboiling method recorded the minimum days (11.2) for spawn run and the least contamination percentage. Spawn substrates supplemented with horsegram flour and redgram flour recorded minimum spawn run (10.1 & 10.3) days, maximum yield (489. 8 & 487. 4 g) and biological efficiency (98.0 & 97.3 %).

Introduction

Hypsizygus ulmarius commonly known as blue or elm oyster mushroom, is one of the popular edible mushroom in the global market. Oyster mushroom (*Pleurotus* sp.) is the second largest cultivated mushroom around the world accounting for twenty seven per cent of world production (Oloke and Adebayo, 2015). They are rich in protein, carbohydrates, minerals, unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid and carotenoids that fit the definition of food supplements (Reis *et al*., 2011). In addition to good quantity of protein they contain no cholesterol, high fiber, low sodium, protein polysaccha-ride complexes that impart unique medicinal properties. In India, owing to varied agro -climate and large quantity of farm waste, different types of temperate, tropical and sub-tropical mushrooms are cultivated throughout the country. With ever increasing demand for quality food, mushroom cultivation is now emerging as an important activity in different parts of our country (Ambili and Nithya, 2014).

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Spawn is any form of mycelium that can be dispersed and mixed into a substrate. The first step in mushroom cultivation is the production of good quality spawn which can fulfill the expectation of the growers. The quality spawn may be achieved by selection of a suitable spawn substrate which influences the growth habit of the mycelium and subsequent yield (Balakrishnan and Nair, 1997). At present, spawn on cereal grains are commonly employed for the commercial cultivation of variety of edible mushrooms. Lack of cultivation technology and lack of easily available, accessible and low cost substrates are the major constraints in spawn production in India. With this background, this research has been carried out to identify the most suitable substrate and pasteurization for the cultivation of *Hypsizygus ulmarius* mushrooms

Materials and Methods

Source and maintenance of culture

The pure culture of *H. ulmarius* $Co(OM)_2$ was obtained from National Research Centre for Mushroom (NRCM), Solan, Himachal Pradesh. Subcultures were

made periodically and maintained on potato dextrose agar (PDA) slants at 25 ± 2°C of temp. for further investigations.

Grain spawn preparation

Thoroughly cleaned grains were half-cooked in required water and cooking was stopped before splitting up of the outer husk except for paddy grains where the husk was just allowed to split. Excess water was drained and the grains were air dried and mixed thoroughly with calcium carbonate at three per cent level and filled up to three-fourth volume in 500 ml glucose bottles. The bottles were tightly plugged with non- absorbent cotton and sterilized at 15 psi for two h. The bottles were then aseptically inoculated with the pure culture of *H. ulmarius* and incubated at 25 ± 2°C. The fully colonized spawn bottles were used for bed preparation (Sivaprakasam, 1980).

Bed preparation

Cultivation of *H. ulmarius* was carried out in transparent polythene bags of 60 × 30 cm size with a thickness of 100 gauge and cylindrical beds were prepared using 0.5 kg of paddy straw on dry weight basis following layer spawning method as described by Sivaprakasm (1980) with below mentioned modification. The unchopped whole straw was made into coils and used. A layer of coiled paddy straw was placed at the bottom of polythene bag, over this a twenty g of spawn was sprinkled. In this manner five layers of coiled paddy straw and four layers of spawn were kept in the polythene bag and then bag was tied at the top (modified cylindrical bed method). Eight holes of one cm diameter were made at random in the polythene bags. The mushroom beds were hung from the ceiling by means of ropes (‘uri ‘ method). After spawn running stage, The beds were kept in cropping room, were the temp. was maintained at 23 to 28°C and relative humidity at 80 to 90 per cent. Water was sprinkled regularly as in the standard cylindrical bed preparation method. The following yield parameters were observed in all the experiments

Effect of different substrates on the spawn production of *H. ulmarius*

Nine different commonly available spawn substrates *viz.,* blackgram (*Vigna mungo*), cumbu (*Pennisetum americanum*), ill-filled paddy grain (IFPG), maize, paddy (*Oryza sativa),* ‘*Panivaragu*’ grain (*Panicum millare*), ‘Thenai’ grain (*Settaria italica*), ‘*Varagu*’ grain (*Paspalum scorbiculatum*), sorghum grain (*Sorghum bicolor*) and wheat grains (*Triticum asetivum)* were tested for their efficacy in supporting the mycelia growth of *H. ulmarius*. The spawn substrates were presoaked overnight. The solution was drained completely and dried under shade. At 50 per cent moisture level the substrate was filled in wide mouth glass bottles or heat resistant polypropylene bags to their 3/4th capacity. Then, the mouth was tightly plugged with non absorbent cotton and sterilized at 15 psi pressure for two h. The substrates were allowed to cool after sterilization and inoculated with a nine mm mycelial disc obtained from the actively growing region of eleven days old *H. ulmarius* culture aseptically and incubated at 25 ± 2°C. for complete coverage of mycelium. Observations like spawn run (days), no of sporophore bed^{-1}, yield and biological efficiency were assessed and recorded.

Effect of various supplements on the spawn production of *H. ulmarius*

Different supplements *viz*., blackgram powder, cotton seed powder, fingermillet powder, groundnut cake, horsegram powder, linseed cake powder, redgram powder and sorghum flour were added separately at two per cent level. The supplements were thoroughly mixed with the grains and sterilized. Paddy grain spawn without any supplement served as control. The spawn bottles were incubated and after the incubation period, observations on spawn run days. The supplemented grain spawns thus prepared were used for the preparation of beds. Standard cultivation practices were followed as mentioned earlier and the data on yield attributes *viz*., spawn run days, yield and biological efficiency were recorded.

Results and Discussion

Efficacy of different spawn substrates on spawn production of *H. ulmarius*

The data presented in table 1 revealed that the mycelium of *H. ulmarius* colonized paddy grain substrate in 11.82 days whereas, the ill filled paddy grains followed by sorghum grain and wheat grain took 12.08, 12.92 and 13.40 days, respectively, to colonize the spawn substrate. The growth of mycelia was very slow in ‘*thenai’* (16.35 days) and *‘panivaragu’* (16.58 days). Further, it was observed that the formation and the density of mycelia were found to be less in minor millets when compared to cereals and pulses. In the present study, the paddy grain spawn and the IFPG spawn produced at par results in enhancing the yield attributes of *H. ulmarius*. Further, inoculation of paddy spawn in bed substrates recorded maximum number of sporophores (108.00 nos.), maximum weight of sporophores (482.18 g bed^{-1}) and the best biological efficiency of (96.43%) which was on par with ill-filled paddy grains.

The favorable results with paddy grain and IFPG spawn observed in the present study could be due to

Table 1: Efficacy of different spawn substrates on spawn production of *H. ulmarius.*

Tr. No.	Spawn substrate (grain)	Spawn run (days)	No of sporophore bed^{-1}	Sporophore yield (g)	Biological efficiency (%)
1	Black gram	15.46^{e}	71.85^{g}	296.14^{e}	59.22
2	Cumbu	15.06^{e}	84.16^{e}	390.51^{e}	78.10
3	Ill-filled paddy	12.08^{a}	101.42^{a}	478.72^{a}	95.74
4	Maize	14.74^{d}	98.65^{c}	402.02^{d}	80.40
5	Paddy	11.82^{a}	108.00^{a}	482.18^{a}	96.43
6	Panivaragu	16.58^{f}	79.33^{f}	361.38^{f}	72.20
7	Sorghum	12.92^{b}	101.26^{b}	465. 90^{b}	93.18
8	Thenai	16.35^{f}	68.55^{g}	273.72^{g}	54.74
9	Wheat	13.40^{c}	89.58^{d}	460.63^{c}	92.00

Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

Table 2: Effect of different methods of substrate pasteurization on the spawn run and per cent Contamination.

Tr. No.	Substrates	Spawn run (days)	Contamination (%)
1	Paddy - cooked in water	13.0^{f}	9.6
2	Paddy - cooked in steam	11.7^{b}	6.1
3	Paddy - par boiling	11.2^{a}	4. 2
4	Sorghum grain cooked in water	13.8^{e}	10.7
5	Sorghum grain cooked in steam	13.0^{d}	6.4
6	Sorghum grain - par boiling	11.9^{b}	7.0
7	IFPg - cooked in water	12.8^{c}	9.5
8	IFPg - cooked in steam	12.5^{c}	6.8
9	IFPg - par boiling	11.3^{a}	4.6
10	Wheat grain (cooked in water)	13.3^{f}	12.2

IFPg – ill filled paddy grain

Values not sharing a common supers

Table 3: Effect of various supplements in the spawn base on the weight of sporophores and biological efficiency of *H. ulmarius.*

Tr. No.	Supplements (@ 2 %)	Spawn run (days)	Weight of sporophore (g/ bed)	Biological efficiency (%)
1	Blackgram flour	12.9^{e}	460.9^{e}	92.2
2	Cotton seed powder	11.3^{c}	476.7^{c}	95.3
3	Finger millet flour	13.6^{f}	395.5^{f}	79.1
4	Groundnut cake	10.9^{b}	483.0^{b}	96.6
5	Horse gram flour	10.1^{a}	489. 8^{a}	98.0
6	Linseed cake	12.4^{e}	468.6^{d}	93.7
7	Redgram flour	10.3^{a}	487. 4^{a}	97.3
8	Sorghum flour	10.6^{b}	483.6^{b}	96.7
9	Control (without supplement)	11.9^{d}	476.3^{c}	95.3

Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

increased surface area, better aeration and more space for the mycelial extension and colonization. Moreover, the use of IFPG is comparatively cheaper and gives more number of spawn bottles per unit weight (Eswaran *et al.*, 1998). The net profit for the production of ill-filled paddy spawn was about Rs. 10-15 as against Rs. 5 from sorghum grain spawn production. In the present study, the poor performance of millet seeds as spawn substrate could have been due to less moisture absorbance, lack of aeration and less space for the mycelial extension and colonization.

Effect of different methods of substrate pasteurization on the spawn production of *H. ulmarius*

Among the three different methods of pasteurization tested *viz*., cooked in water, cooked in steam, par boiling. the substrates pasteurized by parboiling method recorded the minimum days for spawn run and the least contamination percentage. With regard to the substrates tested, parboiled paddy recorded the minimum number of spawn run days (11.2 days) followed by parboiled ill filled paddy grains (11.3 days) and parboiled sorghum grain (11.9 days) when compared to the respective grains cooked in water and steam (Table 2). The maximum contamination percentage was observed in the grain substrate cooked in water.

According to Rathaiah and Shill (1999) the parboiled paddy was the most suitable spawn substrate for both oyster and paddy straw mushroom. In the present study, the growth of the mycelium in the parboiled substrates might have been due to the reason that, the nutrients in the parboiled substrates could easily be absorbed by the mycelium due to breaking down of carbohydrate and protein chains during parboiling process (Kannan and Eswaran, 2010). Also, Darwin Christdhas Henry (2007) suggested that there was a drastic reduction in the moisture content of parboiled grains than in the cooked grains, which might have reduced the chances of bacterial and fungal contamination percentage in the parboiled substrates.

Effect of various supplements in the spawn base on the weight of sporophores and biological efficiency of *H. ulmarius*

The data from the table 3 revealed that the spawn substrates supplemented with horsegram and redgram flour exerted a stimulatory effect to the maximum on the growth of *H. ulmarius.* The mycelium ramified rapidly in the spawn bag supplemented with horsegram flour as well as redgram flour and formed a white mat within 10.1 and 10.3 days respectively, when compared to control. Blackgram flour showed maximum number of spawn run days. Spawn

substrates supplemented with horsegram and redgram recorded (489. 8 g bed^{-1} and 487.4 g bed^{-1} respectively) followed by the sorghum flour (483.6 g bed^{-1}) which was on par with groundnut cake (483.0 g bed^{-1}) when compared to control (476.3 g bed^{-1}). Also, the biological efficiency of the beds prepared by using the spawn supplemented with horsegram and redgram flour was found to be significantly higher (98.0 and 97.3 per cent, respectively) when compared to the control. The fast mycelial growth and higher yield observed in the present study may be due to the reason that the additives horsegram and redgram flours would have increased the protein content of the substrate and necessary minerals for the growth of the oyster mushroom (Kattan *et al.*, 1991; Lakshmanan, 2004). The present study has brought out that ill filed paddy grains supplemented with horsegram or redgram powder at two per cent level can be used successfully as a substrate for the spawn preparation of *H. ulmarius.*

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