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Effect of different substrate alone and in combination on the sporophore production of elm oyster mushroom *Hypsizygus ulmarius*

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Abstract
Studies were conducted to assess the efficacy of different substrates, *viz*., banana leaves, casuarina needle, coir pith, ground nut shell, paddy straw, sugarcane trash, sugarcane bagasse, saw dust and water hyacinth and supplements on the sporophore production. Among the different substrate paddy straw (489.6g bed^{-1}) was most efficient in enhancing the yield of *H. ulmarius*. Followed by water hyacinth (474.4g bed^{-1}) and sugarcane trash (472.7g bed^{-1}). Among the six different methods of sterilization tested, the chemical sterilization by soaking in 500 ppm formalin plus 75ppm carbendazim solution and autoclaving for 30 min. was observed to be the most efficient method of substrate sterilization which not only prevented contamination by competitive fungi but also enhanced the yield of the mushrooms (489.5g bed^{-1}). Among the different supplements, horse gram powder recorded the maximum sporophore yield and biological efficiency (498.6g bed^{-1} and 99.7%, respectively). Among the various agro wastes paddy straw plus water hyacinth recorded the maximum sporophore yield (498.9g bed^{-1}) and biological efficiency (99.8%).

Keywords: Substrate pasteurization, organic additives, sporophore yield

Introduction
Oyster mushroom is an efficient lignin-degrading mushroom and can grow well on different types of lingo cellulosic materials. Oyster mushroom can be grown on various substrates including paddy straw, wheat straw, maize stalks/cobs, vegetable plant residues, bagasse etc. It is estimated that about 355 million tons of crop residue is generated annually and about 170 million is left out for burning and incorporating into soil in manure form (Tewari and Pandey, 2002). Even if one per cent of this lignocellulosic agro wastes are diverted to production of mushrooms, India will become a major mushroom producing country in the world. An ideal substrate should contain nitrogen (supplement) and carbohydrates for rapid mushroom growth. The nutrient composition of the substrate is one of the factors limiting the sapro biotic colonization of cultivated mushrooms and particularly the fruiting of *Pleurotus* spp. (Tshinyangu and Hennebert, 1995) [26]. The growth of mushrooms as well as quantitative and qualitative yield of the desired product depends on utilization of nutrients in the medium (Mukhopadhyay *et al*., 2002) [15]. Lignocellulosic materials such as cereal straws, corn cobs, paper, cotton seed hull, bagasse, wood shavings and saw dust as well as food industry wastes are used for mushroom cultivation (Ragunathan *et al*., 1996; Baysal *et al*., 2003; Xing *et al*., 2006) [19, 2, 29] . The supplements or additives supply extra nitrogen and/or easily degradable carbohydrates to increase mushroom yields and hasten the production process (Royse, 2002) [20]. The nutritional content of the substrate can be improved by nitrogen supplementation (Lelley and Jan Ben, 1993) [12]. Supplementing the substrate with controlled liberation of nitrogen and MN, shortens the crop period for *Pleurotus* spp. and also increases mushroom productivity (Curvetto *et al*., 2002) [4]. With this background, this research has been carried out to identify the most suitable substrate, sterilization method, supplements and substrate combination 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), Sloan and Himachal Pradesh. Subcultures were made periodically and maintained on potato dextrose agar (PDA) slants and stored at 25±2°C temp. for further investigations.

Bed preparation

Cultivation of *H. ulmarius* was carried out in transparent polythene bags of 60 × 30cm 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, where 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.

Evaluation of different methods of substrate pasteurization for the cultivation of *H. ulmarius*

The different methods of pasteurization of substrates *viz*., boiling, steaming and autoclaving presoaked paddy straw for 30 minutes. at 15 psi; chemical pasteurization by soaking the substrate in water mixed with 0.1 per cent carbendazim solution for 16 h.; soaking the substrate in 0.1 per cent carbendazim plus 500ppm formalin solution for 18 h. and autoclaving, pre-soaked in 0.01 per cent carbendazim and 500 ppm formalin solution for 16h were evaluated for their suitability and efficacy in reducing the per cent contamination by the competitors. The substrate soaked in cold water for 18 h. (without any pasteurization) served as control. A temp of 28±2°C and relative humidity of 80-85 per cent was maintained in the cropping room. Three replications were maintained for each treatment and the observations namely per cent contamination by competitors, number of days taken for spawn run, button formation, total yield and biological efficiency were recorded.

Evaluation of different substrates for the cultivation of *H. ulmarius*

Nine different locally available substrates *viz*., banana leaves, casuarina needle, coir pith, groundnut shell, paddy straw, sugarcane trash, sugarcane bagasse, saw dust and water hyacinth were evaluated for their ability in promoting the fruit body formation of *H. ulmarius*. Polybag method was followed for the preparation of beds and the substrate was pasteurized by soaking in chemicals *viz*., carbendazim 75 ppm plus formalin 500 ppm. Horse gram powder @ two per cent was used as supplement in all the treatments. A temp. of 28 ± 2°C and a relative humidity of 85 per cent were maintained in the cropping room. The parameters namely number of days taken for spawn run, pinhead formation, number of sporophore, total yield and biological efficiency were assessed and recorded.

Substrate preparation

Fresh banana, Casuarina needle, leaves were collected, shade dried and subjected to chemical pasteurization using 75ppm carbendazim and 500 ppm formalin for 16 h. Then the substrate was shade dried and used for preparation of beds.

Coir pith

Partially decomposed coir pith was chemically pasteurized using 75 ppm carbendazim and 500 ppm Formalin and used for preparation of beds.

Groundnut shells

Well dried groundnut shells crushed into small bits was chemically pasteurized using 75 ppm carbendazim and 500 ppm formalin and used for preparation of beds.

Sugarcane trash

Properly dried sugarcane trash was chemically pasteurized as stated earlier and used for preparation of beds.

Water hyacinth

Water hyacinth plants were collected, chopped to convenient size and sun dried. The dried substrate was chemically pasteurized as stated earlier and used for bed preparation.

Paddy straw

Paddy straw was collected, dried well, pasteurized as stated earlier and used for preparation of beds.

Effect of different bed supplements on the yield of *H. ulmarius*

In the present study, nine different supplements *viz*., calcium carbonate, corn flour, black gram flour, gypsum, horse gram flour, rice flour, red gram flour and sorghum flour @ two per cent were tested for their efficacy in enhancing the yield attributes of *H. ulmarius*. The additives were powdered well and sterilized in an autoclave at 15 psi for one h. Beds without any supplement served as control and each treatment was replicated thrice. Parameters namely spawn run days, pinhead formation, total yield and biological efficiency were assessed and recorded.

Effect of various combinations of bed substrates on the yield of *H. ulmarius*

In the present study along with paddy straw, the substrates like, dried banana leaves, coconut leaves, cotton waste, groundnut shell, sugarcane trash and water hyacinth were combined at the ratio of 1:1 for bed (polybag method) preparation. Beds prepared using paddy straw alone served as control. Each treatment was replicated thrice and the beds were incubated at 28 ± 2°C temp. With a relative humidity of 85 per cent. The parameters namely spawn run days, days for pin head formation, total duration, total yield and biological efficiency were recorded.

Results and discussion

Evaluation of different substrates for the cultivation of *H. ulmarius*

Among the nine different locally available substrates tested for their potentiality in supporting the sporophore formation of *H. ulmarius*, it was found that the paddy straw (489.6g bed^{-1}) was the most efficient in enhancing the yield of *H. ulmarius*, followed by sugarcane trash (474.4 g bed^{-1}), water hyacinth (472.7gbed^{-1}), groundnut shell (458.3gbed^{-1}), banana leaves (437.1g bed^{-1}), sugarcane bagasse (401.5g bed^{-1}) and casuarina needle (375.9g bed^{-1}) in the decreasing order of merit. Minimum fruiting bodies were observed in the beds prepared by using coir pith as substrate (Table 1).

Table 1: Effect of bed substrate on the growth and sporophore yield of *H. ulmarius*

Tr. No.	Substrates	Spawn run (days)	Pinhead formation (days)	Total duration	No of sporophore bed^{-1}	Total yield (g bed^{-1})	Biological efficiency (%)
1	Banana leaves	18.9^{d}	23.2^{c}	49.5^{c}	109.6^{c}	437.1^{d}	87.4
2	Coir pith	23.4^{h}	28.9^{f}	57.8^{f}	54.3^{g}	247.2^{g}	49.4
3	Casuarina needle	20.2^{f}	24.8^{d}	52.3^{d}	94.9^{e}	375.9^{e}	75.2
4	Groundnut shell	18. 4^{c}	22.8^{c}	48.4^{c}	112.5^{c}	458.3^{c}	91.7
5	Saw dust	20.7^{g}	25.1^{e}	54.7^{e}	82.2^{f}	326.8^{f}	65.4
6	Sugarcane bagasse	19.4^{e}	23.7^{c}	51.2^{d}	101.8^{d}	401.5^{d}	80.3
7	Sugarcane trash	17.9^{b}	22.3^{b}	47.8^{b}	115.4^{b}	472.7^{b}	94.5
8	Water hyacinth	17.8^{b}	21.9^{b}	47.6^{b}	115.6^{b}	474.4^{b}	94.9
9	Paddy straw	17.2^{a}	21.1^{a}	47.1^{a}	119.6^{a}	489.6^{a}	97.9

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

The variations in the growth and yield parameters of the different mushrooms may be due to the biological structure of the substrate (Mahbuba, 2010) [13]. Mandeel *et al*. (2005) [14] reported that mushroom grows in a wide variety of lignocellulosic residues comprising 40-60 per cent cellulose, 20-30 per cent hemicellulose and 15-30 per cent lignin. Cereal straws are rich in cellulose, hemicelluloses and lignin from which the mushroom derives the nutrition (Biswas and Biswas, 2015) [3]. The enhanced yield observed in paddy straw substrates could be due to the presence of favourable nutrients that are better utilized by the fungus. *Pleurotus* spp. has the capacity to degrade cellulose, hemicelluloses and lignin and to produce fruiting bodies. The poor growth and yield observed with coir pith and saw dust substrate could be attributed to the rich lignin content in them and poor ability of *H. ulmarius* to degrade lignin like substances.

Evaluation of different methods of substrate pasteurization for the cultivation of *H. ulmarius*

Among the six different methods of sterilization tested, the chemical sterilization by soaking in 500 ppm formalin plus 75 ppm carbendazim solution and autoclaving for 30 min. was observed to be the most efficient method of substrate sterilization which not only prevented contamination by competitive fungi but also enhanced the yield of the mushrooms (489.5g bed^{-1}) which was on par with chemical sterilization alone by soaking in 500 ppm formalin plus 75 ppm carbendazim solution with nil contamination and an yield of 480.2g bed^{-1}. Autoclaving pre-soaked straw for 30 min. followed by soaking straw substrate in 0.1 per cent carbendazim solution for 16 h. and boiling of presoaked straw for 30 min., recorded a contamination percentage of (6.4%, 9.2%, 12.4%) and an yield of 472.7g, 454.9g and 449.7g bed^{-1}, respectively whereas, the substrate without any sterilization treatment recorded complete contamination by weed moulds (Table 2).

Sterilization of bed substrate is a must for reducing the contamination from competitive fungi and increasing the yield of mushrooms (Zadrazil and Grabbe, 1983) [31]. Singh and Singh (1991) [22] reported that soybean straw was best sterilized by steeping in a solution of formaldehyde at 500 ppm and carbendazim at 75 ppm for 18 h. *Pleurotus* sp. was able to tolerate higher levels of carbendazim (75-200ppm) during chemical sterilization of paddy straw substrate (Nallathambi and Marimuthu, 1992) [17]. Earanna and Shetty (1994) found that when compared to steam sterilization method, the formalin drench method was more effective in sterilizing paddy straw substrate. Likewise, the yields of *Pleurotus* spp. were the highest on paddy straw pre-treated with carbendazim (75 ppm) plus formaldehyde (500ppm) (Nallathambi and Marimuthu, 1994) [18] and soaking of paddy straw for 20 min. in hot water followed by 10 min. dip in 500 ppm solution of carbendazim resulted in the maximum yield and was free from any contamination (Singh *et al.*, 1997) [23] whereas, Eswaran and Ramabadran (1997) obtained maximum yield of *Pleurotus* sp. from beds treated with carbendazim @ 25ppm and formalin @ 200ppm.

Table 2: Evaluation of different methods of substrate pasteurization for the cultivation of *H. ulmarius*

Tr. No.	Methods of pasteurization	Contamination (%)	Spawn run (days)	Pinhead formation (days)	Total yield (g bed^{-1})	Biological efficiency (%)
1	Boiling pre- soaked straw for 30 min.	12.4	18.5^{c}	5.3^{b}	449.7^{d}	89.9
2	Steaming pre- soaked straw for 30 min.	18.4	18.9^{d}	6.1^{c}	368.2^{e}	73.6
3	Autoclaving pre- soaked straw for 30 min.	6.4	17.6^{b}	4.2^{a}	472.7^{c}	94.5
4	Soaking straw in carbendazim 0.1% for 16 h.	2.1	24.1^{e}	8.2^{d}	354.9^{e}	70.9
5	Soaking straw in carbendazim 0.01% 75 ppm + formalin 500 ppm for 16 h.	0.0	17.2^{a}	4.4^{a}	480.2^{a}	96.0
6	Soaking straw in carbendazim 0.01% 75 ppm + formalin 500 ppm for 16 h. + autoclaving for 30 min.	0.0	17.3^{a}	4.3^{a}	489.5^{a}	96.3
7	Control	81.3	21.4^{e}	5.4^{b}	116.3^{f}	23.3

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

Effect of various organic additives to bed substrate on sporophore production of *H. ulmarius*

From the data presented in table 3, all other supplements tested except gypsum and black gram flour recorded increased yield when compared to control. The supplements *viz.*, horse gram, red gram powder and calcium carbonate Significantly enhanced the yield than all other treatments. Among the supplements, horse gram powder recorded the maximum sporophore yield and biological efficiency (498.6g bed^{-1} and 99.7%, respectively) followed by red gram powder (492.2 g bed^{-1} and 98.4%, respectively) and calcium carbonate (486.1 g bed^{-1} and 97.2% respectively). Paddy straw devoid of any supplements served as control and recorded a sporophore yield of 481.3g bed^{-1} and 96.3 per cent biological efficiency.

Estrada *et al*. (2009) [6] opined that supplementation is an important step in enhancing oyster mushroom production.

However, the supplement ratio should not be high due to the possibility of yield reduction (Fanadzo *et al*., 2010) [8], possibility of contamination (Yildiz *et al*., 2002) [30], increase in the bed temp. and possibility of mycelium inhibition (Upadhyay *et al*., 2002) [28]. In the present study, supplementation of horse gram and red gram powder recorded a significant increase in the yield. Efficient utilization of lignocellulolytic substrates by mushroom fungi largely depends on the activity of extracellular enzymes. In the beds supplemented with gram powder, an increased rate of decomposition was observed in the present study. Jaganathan (1972) [10] also observed increased rate of decomposition of straw beds that were treated with amendments. This revealed that the enzymes are possible weapons for substrate decomposition and yield enhancement.

There is a direct correlation between the enhanced production of different enzymes *viz*., cellulose(s), hemicellulose(s), and laccase, which degrades cellulose, hemicelluloses and lignin, respectively which is induced by supplementing gram powder. The various organic and inorganic additives mostly influence the production of enzymes by the mushroom fungi (Arunprasad, 2004) [1]. Enzyme production by the fungal mycelium is of paramount importance in the colonization process and also an important determinant of mushroom yield (Ferdinandi *et al*., 2014) [9]. It is appropriate that the enhanced yield observed in the present study might be attributed to the increased enzyme activity due to the supplementation of gram powder (Munoz *et al*., 1997) [16] which would have triggered quicker degradation of substrates lead to the release of nutrients which were utilized by the fungus resulting in yield enhancement.

Table 3; Effect of various organic additives to bed substrate on sporophore production of *H. ulmarius*

Tr. No.	Additives (@ 2 % bed^{-1})	Spawn run (days)	Pinhead formation (days)	Total duration	Sporophore yield (g bed^{-1})	Biological efficiency (%)
1	Calcium carbonate	17.2^{c}	5.4^{c}	48.0^{d}	486.1^{c}	97.2
2	Corn flour	17.0^{c}	4.9^{c}	47.8^{b}	482.4^{c}	96.5
3	Black gram flour	18.1^{d}	6.2^{e}	48.7^{c}	447.5^{d}	89.5
4	Gypsum	18.9^{e}	7.4^{f}	49.2^{e}	426.8^{e}	85.4
5	Horse gram flour	15.8^{a}	3.9^{a}	46.2^{a}	498.6^{a}	99.7
6	Rice flour	17.2^{c}	5.7^{d}	48.2^{c}	482.9^{c}	96.6
7	Red gram flour	16.3^{b}	4.0^{b}	46.5^{a}	492.2^{b}	98.4
8	Sorghum flour	16.6^{b}	4.3^{b}	47.3^{b}	484.8^{c}	96.9
9	Control	17.4^{c}	5.6^{d}	47.2^{b}	481.3^{c}	96.3

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

Effect of different combination of bed substrates on the yield of *H. ulmarius*

Among the various agro wastes *viz*., banana leaves, coconut leaves, cotton wastes, water hyacinth, groundnut shell and sugarcane trash, in combination with paddy straw, paddy straw plus water hyacinth recorded the maximum sporophore yield (498.9g bed^{-1}) and biological efficiency (99.8%) followed by paddy straw plus Sugarcane trash combination recording (489.3g bed^{-1} and 97.9% respectively) while paddy straw alone recorded 482.6g bed^{-1} of sporophore yield and 96.5 per cent biological efficiency. The minimum biological efficiency (77.3%) and sporophore yield (386.4g bed^{-1}) was recorded with the combination of paddy straw plus coconut leaves (Table 4).

Table 4: Effect of different combination of bed substrates on the sporophore yield of *H. ulmarius*

Tr. No.	Substrate combination (1:1)	Spawn run (days)	Pinhead formation (days)	Total duration	Yield (g bed^{-1})	Biological efficiency (%)
1	Paddy straw + Banana leaves	17.3^{b}	6.1^{d}	48.9^{e}	432.7^{d}	86.5
2	Paddy straw + Coconut leaves	19.2^{e}	7.7^{e}	49.9^{f}	386.4^{e}	77.3
3	Paddy straw + Cotton waste	18.3^{d}	5.3^{c}	48.1^{d}	480.9^{c}	96.2
4	Paddy straw + Groundnut shell	17.9^{c}	4.8^{b}	47.6^{c}	483.9^{c}	96.8
5	Paddy straw + Sugarcane trash	17.0^{b}	4.2^{a}	46.0^{b}	489.3^{b}	97.9
6	Paddy straw + Water hyacinth	16.7^{a}	4.0^{a}	45.7^{a}	498.9^{a}	99.8
7	Paddy straw	17.4^{b}	4.7^{b}	46.5^{b}	482.6^{c}	96.5

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

Paddy straw substrate was reported to be superior for cultivation of oyster mushroom (Karthika and Murugesan, 2015) [11], whereas in the present study maximum yield could be obtained by using water hyacinth (WH) in combination with the paddy straw. The significant enhancement in the yield observed in the present study with paddy straw in combination with water hyacinth might be due to the supplementation of nutritional requirement of *H. ulmarius* being mitigated through water hyacinth and paddy straw. Moreover, the spongy tissues of water hyacinth might have also provided better aeration and water retention capacity to the beds which in combination with paddy straw substrate facilitated increased sporophore production. Besides, rapid degradation of complex lignocellulosic components in water hyacinth leads to earlier mobilization of simple substances for initial mycelia growth (Shah *et al*., 2011) [21]. Besides, addition of water hyacinth along with paddy straw (1:1) will therefore, reduce the mushroom production cost and would also help in recycle the nuisance weed (water hyacinth) in an eco-friendly way.

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