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UTILIZATION OF AGRO-INDUSTRIAL WASTES AND ORGANIC SUPPLEMENTS FOR CULTIVATION OF BLUE OYSTER MUSHROOM

ADITYA* AND RAJINDER SINGH JARIAL

Department of Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry, College of Horticulture and Forestry, Neri, 177001, Himachal Pradesh, India

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Abstract

Mushroom cultivation is a cost-effective biotechnological approach for converting diverse lignocellulosic wastes into nutritionally rich food. *Hypsizygus ulmarius* is a high yielding mushroom gaining popularity nowadays. In the present study the suitability of various available agro-industrial wastes and supplements for the cultivation of *H. ulmarius* was evaluated. Results indicated that among the seven locally available substrates, wheat straw substrate was found to be the best substrate as the highest yield (744.08 g/0.6 Kg dry substrate) and biological efficiency (124.00 %) were obtained. Number of sporophores was more in wheat straw substrate while stipe length was longest in pine needles and cap diameter was recorded maximum in maize straw. Moreover, yield was also recorded more in wheat straw supplemented with cotton seed hull significantly followed by the same substrate when added with gram flour and rice bran. However, lowest yield was obtained when no supplement was added in wheat straw.

Introduction

World population is growing at an alarming rate and is anticipated to reach about six billion. Agricultural production, on the other hand, is not expanding at a fast enough rate to meet up with rising food demand. Widespread malnutrition with ever increasing protein gaps has necessitated the search for alternative source of protein and food. Mushroom is a special group of fungi with high nutritional value and it is also among the favoured alternatives. This potential is currently under utilised and could turn out to be a vast treasure trove especially for people dependent upon agriculture in marginal areas. At the same time India is rich in agro-wastes and generates on an average 500 Million tons of crop residue per year, out of which more than 90 MT is burned each year causing pollution and health hazards (NPMCR 2019). *Pleurotus* species can effectively degrade agricultural wastes and can be grown at a wide range of temperatures, need a short time and also their fruiting bodies are not attacked by diseases and pests (Tesfaw *et al.* 2015, Aditya *et al.* 2022a, Aditya *et al.* 2023). Biotechnological process through recycling of lignocellulosic organic wastes for cultivation of edible mushrooms leads to production of protein rich food combined with the reduction of environmental pollution. Among *Pleurotus* species, *Hypsizygus ulmarius* is a high yielding mushroom gaining popularity nowadays and introduction of less known mushroom like *H. ulmarius* with excellent properties and qualities has good scope in mushroom world owing to its simple and low-cost technology and higher biological efficiency (Aditya *et al.* 2022c). Mushroom cultivation is one of the best options that can improve and secure environmental, nutritional and economic security. Hence, the present study was aimed to evaluate the suitability of different substrates and supplements for higher biological efficiency of blue oyster mushroom in the sub-tropical zone of Himachal Pradesh, India.

*Author for correspondence: <adityabhatia29@gmail.com>. National Institute of Food Technology Entrepreneurship and Management, Kundli, 131028, Haryana-MoFPI, Government of India.

Materials and Methods

The study was conducted in the Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur under the aegis of Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India. The pure culture of blue oyster mushroom (*Hypsizygus ulmarius*) was procured from Directorate of Mushroom Research, ICAR complex, Chambhaghat, Solan (H.P.). Seven different grains i.e. wheat, paddy, jowar, bajra, oat, maize and barley were evaluated and out of which spawn on bajra grains was found to be the best for spawn production. Similarly, seven different agro/industrial wastes such as wheat straw, maize straw, pine needles (*Pinus roxburghii*), wood chips, saw dust, lantana leaves (*Lantana camara*) and curry leaves (*Murraya koenigii*) were evaluated for production and yield parameters of *H. ulmarius*. Six organic supplements *viz.*, rice bran, wheat bran, maize bran, gram flour, mustard cake and cotton seed hull were evaluated at six different rates i.e. 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 per cent on dry weight of the substrate.

The observations on mycelium running time, time for first flush, sporocarps produced, cap diameter, stipe length, yield and biological efficiency of mushroom were recorded. The experiments were conducted in the completely randomized block design with three replications and analyzed by using the statistical package program OPSTAT.

Results and Discussion

Results presented in Table 1 indicate that minimum average time (14.66 days) taken for spawn run was recorded in wheat straw which was significantly followed by maize straw (16.33 days) and pine needles (19.33 days) as well as on saw dust and lantana leaves which took equal time for mycelial colonization (19.33 days). However, maximum average time (22.33 days) taken for spawn run was observed in wood chips significantly followed by curry leaves (20.66 days). Minimum time (20.66 days) taken for first flush was recorded in wheat straw significantly followed by maize straw (23.33 days) while, maximum time (34.00 days) for first flush was taken by wood chips significantly followed by pine needles (30.33 days) which was statistically at par with saw dust (30.00 days). Significantly maximum number (37.33) of sporocarps were recorded in wheat straw followed by maize straw (28.33) while, minimum number (16.66) of sporocarps were harvested from wood chips which was statistically at par with curry leaves (18.00), lantana leaves (20.33) and pine needles (20.66). Longest stipe length (6.46 cm) was recorded in pine needles which was statistically at par with maize straw (5.77 cm) and wheat straw (5.66 cm) while, shortest stipe length was recorded on wood chips substrate (3.36 cm) which was statistically at par with curry leaves (3.90 cm) and lantana leaves (4.06 cm) which was further statistically at par with saw dust (4.26 cm). As far as the cap diameter was concerned, it was found to be maximum (10.13 cm) in maize straw significantly followed by wheat straw (7.90 cm) which further did not differ significantly with pine needles (6.76 cm). However, minimum cap diameter (4.83 cm) of *H. ulmarius* was recorded in saw dust which was statistically at par with wood chips (5.10 cm) and curry leaves (5.93 cm).

Yield of *H. ulmarius* was recorded to be significantly maximum (744.00 g/0.6 Kg substrate) in wheat straw which was followed by maize straw (679.50 g/0.6 Kg substrate) and pine needles (576.00 g/0.6 Kg substrate) being statistically at par with lantana leaves (566.80 g/0.6 Kg substrate). However, significantly minimum yield (436.79 g/0.6 Kg substrate) was recorded on wood chips followed by curry leaves (462.45 g/0.6 Kg substrate) and saw dust (517.62 g/0.6 Kg substrate). As far as the biological efficiency of different substrates was concerned, it was found to be maximum (124.00 %) in wheat straw followed by maize straw (113.20 %) while, lowest (72.80 %) biological efficiency was recorded on wood chips followed by curry leaves (77.07 %).

Results on substrate supplementation revealed that, irrespective of the different doses of various supplements used, significantly mean minimum time (11.17 days) for spawn run was recorded when wheat straw was supplemented with cotton seed hull followed by same substrate amended with gram flour (12.05 days) and rice bran (12.16 days) which were statistically at par with each other (Table 2). Significantly, mean maximum time (15.68 days) for spawn run was recorded in standard check (wheat straw) without any amendments. However, when wheat straw amended with mustard cake even at the lowest rate (5.0 %) of supplementation resulted in the inhibition of mycelia colonization leading to the development of different types of competitive moulds. Irrespective of the different supplements used, the average minimum time (8.76 days) taken for spawn run was recorded in substrate supplemented at the rate of 10.0 per cent significantly followed by those supplemented at the rate of 9.0 per cent (9.38 days). However, average maximum time (13.09 days) taken for spawn run was observed in substrate supplemented at the rate of 5.0 per cent which was significantly followed by those supplemented at the rate of 6.0 per cent (12.48 days).

Table 1. Effect of different substrates on production and yield parameters of *Hypsizygus ulmarius.*

Substrates	Time taken for spawn run (days)*	Time taken for first flush (days)*	No. of sporocarps in first flush*	Stipe length (cm)*	Cap diameter (cm)*	Yield (g/0.6 kg dry substrate)*	Biological efficiency (%)
Wheat straw	14.66	20.66	37.33	5.66	7.90	744.00	124.00
Maize straw	16.33	23.33	28.33	5.77	10.13	679.50	113.20
Pine needles	19.33	30.33	20.66	6.46	6.76	576.00	96.00
Saw dust	19.33	30.00	22.33	4.26	4.83	517.62	86.27
Wood chips	22.33	34.00	16.66	3.36	5.10	436.79	72.80
Lantana leaves	19.33	26.33	20.33	4.06	6.53	566.80	94.47
Curry leaves	20.66	28.00	18.00	3.90	5.93	462.45	77.07
$CD_{p\geq 0.05}$	1.22	1.39	5.42	0.86	1.30	12.73	
$SE_{(d)}$	0.39	0.45	1.77	0.28	0.42	5.88	

*Values indicates mean of three replicates.

Table 2. Effect of substrate supplementation on spawn run period of *Hypsizygus ulmarius.*

Supplements	Average time (days) taken for spawn run at different supplement doses (%)*						Overall mean
	5.0	6.0	7.0	8.0	9.0	10.0	
Cotton seed hull	14.33	13.33	11.33	10.66	9.00	8.33	11.17
Gram flour	15.66	15.00	12.33	11.00	9.66	8.66	12.05
Rice bran	15.00	14.00	13.00	12.00	10.00	9.00	12.16
Wheat bran	15.33	14.33	13.33	12.33	10.33	9.67	12.56
Maize bran	15.67	15.00	14.00	12.66	11.00	10.00	13.06
Mustard cake	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Wheat straw (Control)	15.68	15.68	15.68	15.68	15.68	15.68	15.68
Overall mean	13.09	12.48	11.38	10.61	9.38	8.76	
	$CD_{p\geq 0.05}$	$SE_{(d)}$					
Supplements	0.53	0.27					
Doses	0.49	0.25					
Interaction	1.29	0.65					

*Values indicates mean of three replicates.

Results revealed that average minimum time (8.33 days) taken for spawn run was recorded in wheat straw supplemented with cotton seed hull, which did not differ significantly with the same substrate supplemented with gram flour (8.66 days) at the rate of 10.0 per cent. The average maximum time taken for spawn run was recorded in wheat straw without any amendments (15.68 days) which did not differ significantly with the amendments of maize bran (15.67 days), gram flour (15.66 days) and wheat bran (15.33 days) at the rate of 5.0 per cent (Table 2).

Data recorded on the production of number of fruit bodies of *H. ulmarius* have been presented in Table 3 clearly indicate that irrespective of the different supplement doses used, the average maximum number (56.83) of basidioma were harvested from wheat straw when supplemented with cotton seed hull which was followed significantly by the same substrate supplemented with gram flour (45.44) and rice bran (44.27). However, the average minimum number (32.33) of basidioma was recorded in wheat straw alone (without any amendments) which was significantly followed by same substrate when supplemented with maize bran (41.28) and wheat bran (42.33). The latter two treatments were statistically at par with each other. Irrespective of the different supplements used, significantly mean maximum number (43.66) of basidioma were harvested when the standard substrate was amended at the rate of 10.00 per cent which was followed at 9.00 (41.76) and 8.00 (38.76) per cent while, mean minimum number (32.38) of basidioma were harvested when the supplement was added at the rate of 5.00 per cent followed at 6.00 (32.68) per cent. However, both treatments did not differ significantly from each other. Table 3 further revealed that average maximum number of basidioma (70.64) was recorded in wheat straw supplemented with cotton seed hull at the rate of 10.0 per cent, which was highly significant with the same substrate supplemented with the same supplement (63.65) at the rate of 9.0 per cent. However, average minimum number (32.33) of basidioma was observed in wheat straw where no supplement was added significantly followed by the same substrate supplemented with maize bran (34.68) at the rate of 5.0 per cent. However, minimum number (32.33) basidioma were harvested from standard substrate without supplementation which was statistically at par with those harvested from standard substrate supplemented with maize bran at the rate of 5.00 (34.68) and 6.00 (35.00) per cent rate of supplementation.

Table 3. Effect of substrate supplementation on basidioma production of *Hypsizygus ulmarius*.

Supplements	Average number of basidioma produced at different supplement doses (%)*						Overall mean
	5.0	6.0	7.0	8.0	9.0	10.0	
Cotton seed hull	48.33	48.92	51.66	58.67	63.65	70.64	56.83
Gram flour	38.00	39.00	44.33	48.33	50.33	52.66	45.44
Rice bran	37.66	38.00	42.33	47.00	49.67	51.00	44.27
Wheat bran	35.67	36.33	40.68	43.00	48.33	50.00	42.33
Maize bran	34.68	35.00	39.00	42.00	48.00	49.00	41.28
Wheat straw alone (Control)	32.33	32.33	32.33	32.33	32.33	32.33	32.33
Overall mean	32.38	32.68	35.76	38.76	41.76	43.66	
	$CD_{p\geq 0.05}$	$SE_{(d)}$					
Supplements	1.23	0.62					
Doses	1.14	0.57					
Interaction	3.02	1.52					

*Values indicates mean of three replicates.

Results presented in Table 4 indicate that irrespective of the different supplement doses used, the average maximum yield (3088.67 g/2 Kg substrate) was recorded in standard substrate (wheat straw) supplemented with cotton seed hull significantly followed by same substrate when supplemented with gram flour (3015.56 g/2 Kg substrate) and rice bran (2926.00 g/2 Kg substrate). However, average minimum yield (2470.00 g/2 Kg substrate) was observed in wheat straw alone significantly followed by the same substrate when supplemented with maize bran

(2817.39 g/2 Kg substrate) and wheat bran (2877.78 g/2 Kg substrate). Irrespective of the different supplements used the average maximum yield (3146.77 g/2 Kg substrate) was recorded in substrate supplemented at the rate of 10.0 per cent which was significantly followed by the substrate supplemented at the rate of 9.0 per cent (3033.66 g/2 Kg substrate), 8.0 per cent (2867.88 g/2 Kg substrate), and 7.0 per cent (2801.66 g/2 Kg substrate). However, average minimum yield (2625.50 g/2 Kg substrate) was produced in the substrate supplemented at the rate of 5.0 per cent significantly followed by the substrate supplemented at the rate of 6.0 per cent (2749.88 g/2 Kg substrate).

Table 4 revealed that maximum (3537.33 g/2 Kg substrate) yield was recorded on wheat straw supplemented with cotton seed hull at the rate of 10.00 per cent which was highly significant with the same substrate supplemented with gram flour (3378.67 g/2 Kg substrate) and rice bran (3240.00 g/2 Kg substrate) at the same rate which was statistically at par with the yield recorded in wheat straw amended with cotton seed hull at the rate of 9.0 per cent (3258.67 g/2 Kg substrate). However, average minimum yield was observed in wheat straw (2470.00 g/2 Kg substrate) when no supplements were added in the substrate. Biological efficiency was recorded to be maximum (176.87 %) in wheat straw supplemented with cotton seed hull at the rate of 10 per cent followed by the same substrate supplemented with gram flour (168.93 %) and rice bran (162.00 %) at the same rate. Minimum biological efficiency (123.50 %) was observed in wheat straw where no supplement was added followed by the same amendment with maize bran (128.95 %) and wheat bran (130.47 %) at the rate of 5.0 per cent.

Table 4. Impact of substrate supplementation on yield of *Hypsizygus ulmarius*.

Supplements	Average yield (g/2 Kg dry substrate) at different supplement doses (%)*						Overall mean
	5.0	6.0	7.0	8.0	9.0	10.0	
Cotton seed hull	2,777.33	2,914.67	2,981.33	3,062.66	3,258.67	3,537.33	3,088.67
	(138.87)	(145.73)	(149.07)	(153.13)	(162.93)	(176.87)	
Gram flour	2,688.00	2,881.33	2,941.33	3,005.33	3,198.67	3,378.67	3,015.56
	(134.40)	(144.07)	(147.07)	(150.27)	(159.93)	(168.93)	
Rice bran	2,629.33	2,778.67	2,876.00	2,946.67	3,085.33	3,240.00	2,926.00
	(131.47)	(138.93)	(143.80)	(147.33)	(154.27)	(162.00)	
Wheat bran	2,609.33	2,760.00	2,792.00	2,890.68	3,050.66	3,164.00	2,877.78
	(130.47)	(138.00)	(139.60)	(144.53)	(152.53)	(158.20)	
Maize bran	2,579.00	2,694.67	2,749.33	2,832.00	2,958.68	3,090.67	2,817.39
	(128.95)	(134.73)	(137.47)	(141.60)	(147.93)	(154.53)	
Wheat straw alone	2,470.00	2,470.00	2,470.00	2,470.00	2,470.00	2,470.00	2,470.00
(Control)	(123.50)	(123.50)	(123.50)	(123.50)	(123.50)	(123.50)	
Overall mean	2,625.50	2,749.88	2,801.66	2,867.88	3,003.66	3,146.77	
	$CD_{p\geq0.05}$	$SE_{(d)}$					
Supplements	11.63	5.82					
Doses	11.63	4.82					
Interaction	28.49	14.26					

*Values indicates mean of three replicates. Figures given in parenthesis represents biological efficiency (%).

Pleurotus species grew well in varied type of substrates including agricultural wastes. Cellulose and lignin in the substrate were important components deciding the yield of sporocarp. A good substrate should be rich in nutrients, have good aeration and water holding capacity and have high lignocellulosic content (Sharma *et al.* 2013, Dhakal *et al.* 2020). Wheat straw and maize straw proved to be the best substrate and provided highest yield and biological efficiency. Results obtained were supported by the findings of Mondal *et al.* (2010) and Aditya *et al.* (2022b) who also reported wheat straw to be superior substrate over other types of agro-wastes in colonization and production rates of oyster mushroom. Time taken for spawn run ranged between 14-23 days which was in accordance with the findings of Jongman *et al.* (2013) and Aditya *et al.* (2022d) who also reported a spawn run period ranging between 12-21 days on maize substrate spawned with wheat, bajra, sorghum and barley grain spawn. The present results are also in conformity with the findings of *Sethi et al.* (2012) and Sharma (2016) who have reported wheat straw as most suitable substrate and took minimum time for spawn run of *H. ulmarius.*

During the present study, cap diameter and stipe length ranged between (4.83-10.13 cm) and (3.36-6.46 cm), respectively. Maximum stipe length, number of basidioma and cap diameter were recorded on wheat straw substrate. These results are in agreement with the results of Singh (2019) who reported cap diameter ranged from 6.00-8.73 cm and stipe length ranged from 7.50-11.17 cm in different substrates while, Shendge *et al.* (2019) recorded cap diameter ranged from 5.80-8.16 cm and stipe length ranged from 5.53-8.66 cm grown on soyabean straw for cultivating *H. ulmarius*. The findings of Neupane *et al.* (2018) further support the present results, who reported average cap diameter from 7.14-9.37 cm and stipe length from 4.60-6.76 cm in case of *P. florida* grown on banana leaves, rice straw, wheat straw and mixture of rice and wheat straw.

Various additives to the different substrates have been reported to give encouraging results. Singh and Parsad (2012) also reported maximum yield on wheat straw supplemented with soyabean flour at the rate of 5.0 per cent in case of *P. florida* and *P. sajor caju*. As far as the yield of *H. ulmarius* was concerned it was recorded maximum in wheat straw supplemented with cotton seed hull followed by gram flour and rice bran. The present findings are in agreement with the results reported by Yang *et al.* (2013) and Kumar *et al.* (2020) who have also reported cotton seed hull supplement to be superior over other types of supplements. However, Alananbeh *et al.* (2014) and Sharma (2016) in their studies reported gram powder supplement gave highest yield and biological efficiency by the addition of respective supplements in the substrates.

With the increase in rate of supplement dose, yield of *H. ulmarius* also increased significantly. Moreover in case of mustard oil seed cake supplementation in wheat straw, inhibitory effect of supplement was observed and even at the rate of 5.0 per cent supplement dose it completely inhibited the mycelial growth of the fungus. Similar results of poor yield and inhibition by supplementation with mustard oil seed cake was also reported by Krupodorova and Barshteyn (2015) and Sanjel *et al.* (2021) who have also substantiated the results of the present findings.

Food security for the world's ever-increasing population is a serious concern in the twenty-first century. *Hypsizygus ulmarius* may be proved as the future food for the future generations because of high biological efficiency, possessing quality parameters along with therapeutic values. The study has demonstrated that blue oyster mushroom can be successfully grown on locally available agro-industrial wastes. Further addition of supplement has great influence in the performance of *H. ulmarius* on the different substrates. Wheat straw substrate supplemented with cotton seed hull @10.0 per cent dose significantly gave highest mushroom yield and biological efficiency.

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