Indian Journal of Ecology (2022) 49(6): 2389-2394
DOI: https://doi.org/10.55362/IJE/2022/3838

Manuscript Number: 3838
NAAS Rating: 5.79

Evaluation of Different Grain Substrates for Spawn Production and Yield Performance of Blue Oyster Mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead] through Bio-Conversion of Agri/Industrial Wastes

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Abstract: Blue oyster mushroom is a novel species that is widely gaining popularity nowadays owing to its simple and low production technology with high biological efficiency. Since spawn serves as the planting materials in mushroom production and its quality is the most important factor for the successful cultivation of any edible mushroom. Hence, the study was undertaken to determine with the objective to find out the best grain spawn for getting early and high yield crop of *Hypsizygus ulmarius* on different substrates. Seven locally available grains *viz*; barley, bajra, paddy, wheat, oat, maize and sorghum were used for spawn production. Bajra grains were found as an excellent substrate for spawn preparation as it supports faster and enhanced growth (11 days) followed by sorghum, wheat and maize grain substrate. Linear growth (50.52 mm) and growth rate (0.39 mm/h) of the fungus was found maximum on bajra grains as compared to other grains. Maximum yield (640.00 g/0.5 Kg substrate) and biological efficiency (128.00 %) was also recorded in bajra grains spawn on wheat straw substrate closely followed by wheat grains spawn on the same substrate. The performance of *H. ulmarius* on wood chips, curry leaves and saw dust was found minimum when spawned with either of the grain spawn.

Keywords: Biological efficiency, Grain spawn, *Hypsizygus ulmarius*, Substrate, Yield

The global food and nutritional security of the growing population is a great challenge that looks for alternative crops as a source of food and nutrition. Mushrooms are among the favoured alternative crops. Mushrooms are fleshy, spore-bearing and multicellular fungi, enriched with quality proteins, vitamins, minerals and also possess therapeutic and medicinal properties (Aditya and Bhatia 2020, Aditya et al 2022c). Blue oyster mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead] is gaining popularity nowadays among small and marginal farmers because of its simplicity of growing on soyabean, sawdust, paddy straw, wheat straw and other agro-wastes (Singh et al 2018, Aditya et al 2022d). Being saprophytic it can easily be introduced in any part of the country. Its fast growth and high resistance against competitive micro-organisms are likely to make its cultivation more economical and less tedious. This mushroom has also very high biological efficiency compared to other oyster mushrooms which makes this fungus the mushroom of the future in the coming years (Aditya et al 2022a, Aditya et al 2022c).

Mushroom spawn is the mushroom mycelium growing on a given grain substrate which is used as a planting medium for growing mushrooms. Spawn production is a technologically advanced process that necessitates a high level of expertise, specific knowledge and attention on the part of those involved in it (Aditya et al 2022b). It is considered as the bedrock of the mushroom industry and the limiting factor to mushroom cultivation all over the world (Chinda and Chinda 2007). Spawn quality is the most important factor in the production of edible mushroom. In nature, mushroom use spores for generative multiplication and these are microscopic and very difficult to handle. As an alternative, spawn can be prepared using tissue cultures extracted from cap tissues. Grain spawn is in common use because of its ability to ramify the substrate faster and ease of planting. In India, not much research work has been done on the cultivation technology of blue oyster mushroom. It is in light of these facts, the aim of the current work was to evaluate the different grain substrates for spawn production and yield performance of blue oyster mushroom [*Hypsizygus ulmarius* (Bull.: Fr.) Redhead] on different growing substrates under the sub-tropical zone of Himachal Pradesh, India.

MATERIAL AND METHODS

Study area: 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 during the year 2019-2021. The cultivation experiments were carried out under natural climatic conditions in the bamboo hut constructed for mushroom cultivation. Neri is located at an altitude of 650 m in the valley of Himachal Pradesh in the northern sub-tropical zone of India at 31.67°N and 36.48°E with an annual temperature of 16.8°C, precipitation 1342.72 mm and relative humidity is around 69 per cent.

Procurement, maintenance and preservation of culture: The pure culture of blue oyster mushroom (*Hypsizygus ulmarius)* was procured from the Directorate of Mushroom Research, ICAR complex, Chambhaghat, Solan (H.P). The culture, thus obtained was maintained on a potato dextrose agar (PDA) medium (subcultured periodically at an interval of 30-45 days). Full-grown culture was stored at 2-4°C in the refrigerator until used further for the entire work.

Spawn preparation: Seven different cereal grains i.e., wheat, paddy, sorghum, bajra, oat, maize and barley were evaluated to see their effect on spawn production of *H. ulmarius.* Healthy grains were cleaned, washed and rotten grains or those floating on the surface of the water were removed. As much as 10 Kg of each grain substrates were boiled in 15 liters of water and care was taken not to overcook the grains, so that they may not rupture. Excess water was drained off by keeping on a wire mesh for 1-2 h. The substrates were mixed with 2.0 per cent calcium sulfate and 0.5 per cent calcium carbonate on a wet-weight basis to obtain the desired pH and to avoid the stickiness of the grain substrates. Thereafter, grains were filled in graduated glass bottles (1000 ml capacity) plugged with non-absorbent cotton and sterilized at 22 psi for 2 h. After cooling, the bottles were shaken vigorously to restore the transparency of the glass and were inoculated with uniform-sized mycelial bits (5.00 mm) of *H. ulmarius* under aseptic conditions. These bottles were then incubated at 25±1° C in the BOD incubator, till the grains were fully impregnated with the mycelium of inoculated fungus culture (11-19 days).

Substrate preparation: Seven different agro and industrial wastes *viz*., wheat straw, maize straw, pine needles (*Pinus roxburghii*), wood chips, saw dust, lantana leaves (*Lantana camara*) and curry leaves (*Murraya koenigii*) were collected from the locality of College of Horticulture and Forestry Neri, Hamirpur, Himachal Pradesh and used as cultivation substrates. At first, all the growing substrates were chopped into small pieces (2-4 cm long) and thereafter soaked separately in water to get completely wet and then treated with a solution of formalin (0.5 %) and carbendazim (0.075 %). After about 18 hours, the substrates were taken out and the excess water in the substrate was drained off by placing the substrate on a clean wire mesh. After that, all of these chemically treated substrates were thoroughly spawned using a 5.0 percent spawn dose of each grain spawn. These spawned substrates were then filled in the mushroom growing bags of polythene having 2.0 Kg capacity (0.5 Kg dry substrate) and later shifted to the growing mushroom hut. Standard mushroom growing packages and practices were followed to raise the crop.

Observations recorded: Data were recorded in terms of time taken for spawn run (days), growth characteristics and linear growth (mm) at 24 h intervals until the mycelium ramified completely on the grains. The growth rate (mm/h) was further calculated as per the following formula:

$r_g = dgt_2 - dgt_1 / t_2 - t_1$

Where, dgt_2 = Diametric growth (mm) at time t_2; dgt_1 = Diametric growth (mm) at time t_1.

Yield of mushroom: This is the quantity/weight of mushroom produced per bag of substrate per harvest time.

Biological efficiency: The matured mushroom was harvested, which differed depending on the substrate type. The curl margin of the cap denoted mature mushrooms, which were harvested by twisting to uproot from the base. The yield was expressed in biological efficiency (%) and calculated (Chang et al 1981). The biological efficiency was calculated thus:

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

Statistical analysis: The experiments were conducted in a completely randomized block design with four replications and analyzed by using the statistical package of the program OPSTAT.

RESULTS AND DISCUSSION

Effect of different grain substrates for spawn production of *Hypsizygus ulmarius*: There was a significant difference in spawn development of *H. ulmarius* on different grain substrates, except paddy and oat grain substrates which took 17 and 17.5 days respectively for complete colonization of the grains by the test fungus (Table 1). Latter both treatments were statistically at par with each other. Among the different tested grain substrates, a significant minimum period for the spawn run of tested fungus was recorded in bajra grains (11 days) followed by sorghum (13 days) and wheat (15 days) grains. However, barley grain substrates took a significantly maximum time (19 days) for spawn development of *H. ulmarius.* The growth characteristics of *H. ulmarius* spawn varied considerably with respect to different grain substrates. A white mycelial growth was recorded on all types of grains under study but, it was more conspicuous in bajra, sorghum, wheat and maize grains spawn. The grains were tightly held with each other after a complete spawn run. The mycelia growth was faster, thick, strandy and dense white on bajra grains while, observed to be dense to thick in the case of sorghum, wheat and maize grains. However, comparatively slower, thin and fluffy growth was noticed and grains were also loosely held in oat, paddy and barley grains. A perusal of the data depicts that irrespective of the durations of incubation, significantly maximum (50.52 mm) linear growth was recorded in bajra grains followed by sorghum, wheat and maize grain substrates (Table 2). However, significantly minimum (17.97 mm) linear growth of the test fungus was in barley followed by oat and paddy grains. Keeping aside the spawn substrates, the linear growth rate of the fungus increased in all the grain substrates with the passage of time and it attained maximum growth in each grain substrates after 11 days of incubation. The linear growth was significantly higher in bajra grains (107.13 mm) followed by sorghum grains after 11 days of incubation while, minimum linear growth was observed in barley grains (1.27 mm) after 1 day of incubation. An intermediate level of linear growth was recorded in the rest of the grain substrates after different durations of incubation.

Additionally, growth rate of *H. ulmarius* on different grain substrates was also calculated on 24 h intervals up to 264 h (11 days). The maximum mean growth rate was observed in bajra grains (0.39 mm/h) followed by sorghum, wheat, maize and oat and paddy grain substrates, irrespective of the time of incubation (Table 3). The growth rate in the case of oat and paddy was statistically at par with each other and the minimum growth rate was in barley grain substrate (0.15 mm/h), irrespective of the different days of incubation. Irrespective of the spawn substrates, the growth rate of the test fungus increased significantly in all the test grain substrates with the passage of time being significantly minimum (0.09 mm/h) between 0-24 h and maximum (0.36 mm/h) between 240-264 h of incubation. The maximum growth rate (0.57 mm/h) was in bajra grains between 240-264 h of incubation significantly followed by same grain substrate (0.54 mm/h) between 216-240 h of incubation. The minimum growth rate was in barley grains (0.05 mm/h) between 0-24 h of incubation. An intermediate range of growth rate was recorded in the rest of the grain substrates after different durations of incubation.

Effect of different grain substrates on yield performance of *Hypsizygus ulmarius*: After screening of different grain

Table 1. Effect of different grain substrates on spawn production and growth characteristics of *Hypsizygus ulmarius*

Grain substrate	Time taken for spawn run (days)	Growth characteristics
Bajra	11.00	Thick, strandy and dense white mycelial growth on all grains.
Sorghum	13.00	Dense white mycelial growth on all grains but intact with each other.
Wheat	15.00	Thick, strandy white mycelial growth on all grains.
Maize	16.00	Dense, fluffy white mycelial growth on all grains.
Oat	17.00	Thin, fluffy white mycelial normal growth on all grains.
Paddy	17.50	White mycelial growth on all over the grains but not tightly held.
Barley	19.00	White mycelial normal growth on all grains.
CD (p=0.05)	0.51	

Table 2. Effect of different grain substrates on linear growth of *Hypsizygus ulmarius*

Grain substrate	Average linear growth (mm) after different incubation duration (days)											Overall mean
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	
Bajra	5.93	12.47	19.50	27.77	36.90	46.33	57.23	68.70	80.30	93.47	107.13	50.52
Sorghum	3.38	6.53	12.67	19.23	26.20	33.33	42.67	52.07	62.37	72.60	83.33	37.67
Wheat	3.23	6.18	9.70	15.60	22.30	29.67	37.23	44.83	52.90	61.57	69.83	32.09
Maize	2.37	4.70	8.40	12.73	18.20	24.70	31.27	37.97	45.03	52.40	59.57	27.03
Oat	2.30	5.00	7.80	11.53	16.57	22.20	28.00	34.23	41.03	48.40	55.63	24.79
Paddy	2.43	4.68	8.03	11.83	16.93	23.00	29.00	35.67	42.40	49.70	57.03	25.51
Barley	1.27	2.57	4.83	7.83	11.70	15.83	20.00	24.96	30.47	35.90	42.30	17.97
Overall mean	2.99	6.01	10.13	15.22	21.26	27.87	35.05	42.63	50.64	59.15	67.83	
CD (p=0.05)	Grain substrate 0.12		Duration 0.15		Interaction 0.39							

substrates under *in vitro* conditions, cultivation trials were conducted to assess the yield performance of *H. ulmarius* on different substrates viz., wheat straw, maize straw, pine needles (*Pinus roxburghii*), wood chips, saw dust, lantana leaves (*Lantana camara*) and curry leaves (*Murraya koenigii*) using commercial spawn prepared on different grain substrates. The yield was recorded and biological efficiency was further calculated and have been presented in Table 4. The results evinced that irrespective of the different substrates used, bajra grains spawn produced maximum average yield (468.28 g/0.5 Kg substrate) followed by wheat grains spawn which was statistically at par. Moreover, it was significantly followed by sorghum grains spawn and maize grains spawn (Table 4). Minimum yield was recorded in barley grains spawn (431.76 g/0.5 Kg substrate) which was statistically at par with oat grains spawn and paddy grains spawn. Irrespective of the different grains spawn used, wheat straw substrate produced maximum average yield (614.52 g/0.5 Kg substrate) significantly followed by maize straw substrate. However, minimum yield (337.95 g/0.5 Kg substrate) was recorded in wood chips substrate significantly followed by curry leaves and saw dust. Interaction of the table clearly depicts that maximum yield (640.00 g/0.5 Kg substrate) was recorded in wheat straw substrate spawned with bajra grains spawn which was statistically at par with the same substrate spawned with wheat grains spawn. Minimum yield spawned with barley grains spawn was recorded in wood chips substrate (321.00 g/0.5 Kg substrate) followed by the same substrate spawned with, paddy, oat and maize grains spawn respectively, which was also statistically at par

Table 3. Effect of different grain substrates on growth rate of *Hypsizygus ulmarius*

Grain substrate	Average growth rate (mm/h) between duration of incubation (h)											Overall mean
	0-24	24-48	48-72	72-96	96-120	120-144	144-168	168-192	192-216	216-240	240-264	
Bajra	0.16	0.27	0.29	0.34	0.38	0.39	0.46	0.46	0.48	0.54	0.57	0.39
Sorghum	0.07	0.13	0.25	0.27	0.29	0.29	0.38	0.39	0.42	0.42	0.44	0.30
Wheat	0.07	0.12	0.14	0.24	0.27	0.30	0.31	0.31	0.33	0.35	0.35	0.25
Maize	0.09	0.10	0.15	0.17	0.22	0.27	0.27	0.27	0.29	0.30	0.29	0.22
Oat	0.11	0.11	0.11	0.15	0.20	0.23	0.24	0.25	0.28	0.30	0.30	0.21
Paddy	0.10	0.10	0.13	0.15	0.21	0.25	0.25	0.27	0.28	0.30	0.30	0.21
Barley	0.05	0.05	0.09	0.12	0.16	0.17	0.17	0.18	0.21	0.22	0.26	0.15
Overall mean	0.09	0.13	0.17	0.21	0.25	0.27	0.29	0.31	0.33	0.35	0.36	
CD (p=0.05)	Grain Substrate 0.01		Duration 0.01		Interaction 0.02							

Table 4. Yield of *Hypsizygus ulmarius* as influenced by different grain spawn on different substrates

Grain spawn	Average yield (g/0.5 Kg substrate) in different substrates							
	Wheat straw	Maize straw	Pine needles	Saw dust	Wood chips	Lantana leaves	Curry leaves	Overall mean
Bajra	640.00 (128.00)	595.66 (119.13)	470.66 (94.13)	410.00 (82.00)	349.33 (69.87)	459.00 (91.80)	380.66 (76.13)	468.28
Sorghum	609.00 (121.80)	568.33 (113.67)	437.00 (87.40)	400.00 (80.00)	350.33 (70.07)	424.33 (84.87)	361.66 (72.33)	455.33
Wheat	626.66 (125.33)	587.33 (117.47)	465.00 (93.00)	420.66 (84.13)	347.33 (69.47)	457.66 (91.53)	368.33 (73.66)	466.23
Maize	616.66 (123.33)	587.00 (117.40)	420.33 (84.07)	407.00 (81.40)	340.00 (68.00)	412.33 (82.47)	353.00 (70.60)	448.04
Oat	613.33 (122.67)	531.33 (106.27)	419.33 (83.87)	396.00 (79.20)	334.33 (66.87)	421.66 (84.33)	350.00 (70.00)	438.00
Paddy	602.66 (120.53)	574.00 (114.80)	434.00 (86.80)	394.00 (78.80)	323.33 (64.67)	408.66 (81.73)	346.66 (69.33)	440.47
Barley	593.33 (118.67)	572.66 (114.53)	409.66 (81.93)	389.67 (77.93)	321.00 (64.20)	398.66 (79.73)	337.33 (67.47)	431.76
Overall mean	614.52	573.76	436.57	402.47	337.95	426.04	356.81	
CD (p=0.05)	Grain Spawn 9.86		Substrate 9.86		Interaction 26.09			

Figure given in parentheses represents biological efficiency (percentage) with each other. Biological efficiency was recorded to be maximum (128.00 %) in wheat straw substrate spawned with bajra grains spawn (Plate 1) followed by wheat straw substrate spawned with wheat grains spawn whereas minimum biological efficiency (64.20%) was achieved in wood chips spawned with barley grains spawn. Spawn, the vegetative seed material plays a vital role in mushroom cultivation and the substrate on which the spawn is prepared also affects the mushroom production to a significant extent. Grain spawn is of common use because of its ability to ramify the substrate faster and ease of planting (Bahl 1988). Several authors have tried a variety of grain substrates and agricultural wastes for preparation of spawn based on the easy availability, accessibility and lower cost for different edible mushrooms (Reddy et al 2020, Aditya et al 2022a). At present grains like wheat, maize, barley, oat, sorghum,

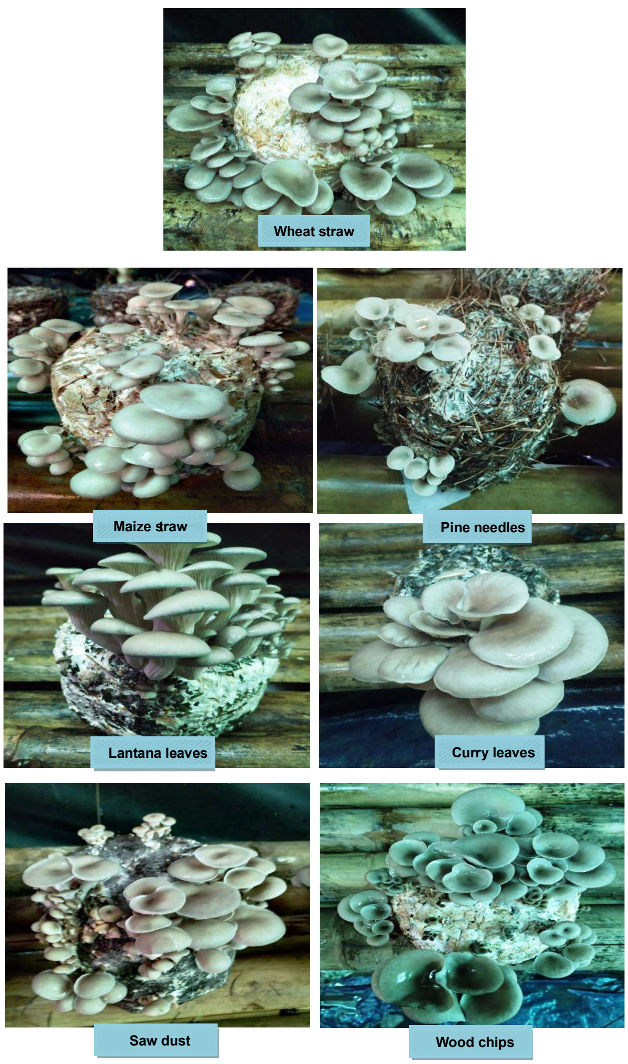

Plate 1. Mature sporocarps of *Hypsizygus ulmarius* on different substrates using bajra grains spawn

paddy and bajra are commonly used for the commercial spawn production and cultivation of a variety of edible mushrooms (Aditya 2021).

Spawn making is a rather complex task and not feasible for the common mushroom grower. It has been a primary concern in mushroom cultivation which is achieved by developing mushroom mycelia on a supporting medium under controlled environmental conditions. In all cases, the supporting material is sterilized grains which is preferred due to inherent biochemical properties and practical performance over others. The results of our present study are in accordance with the findings of Chauhan & Pant (1988) and Rathod et al (2002) who have also reported bajra and sorghum grains to be superior than other grains for spawn production of different *Pleurotus* spp. In the earlier reports, several other workers have also tested different grains for spawn development of *H. ulmarius* and found early spawn development in maize grains, sorghum grains and paddy grains (Sumi & Geetha 2017, Shendge 2018, Aditya 2021) as an ideal substrate for spawn production of blue oyster mushroom. Also Baghel (2017), further supports our results, as according to him bajra grains spawn took (11.2 days) followed by maize (13.4 days) and wheat grains spawn (14.5 days) for complete mycelia spread of *H. ulmarius* while, paddy grains took maximum time (17.60 days) for spawn run. These findings further support our results. In the present study, the highest yield and biological efficiency was recorded from the bajra grains spawn closely followed by the wheat grains spawn. The results of the present study are in accordance with the findings of Baghel (2017), Sumi & Geetha (2017) and Shendge (2018) who have also reported bajra, wheat and sorghum grains spawn exhibited higher yield and biological efficiency of *H. ulmarius.*

CONCLUSION

The mycelial growth of blue oyster mushroom (*H. ulmarius*) was faster on all the seven grain substrates used for spawn production. Bajra grains were the best substrate for spawn production as it supports much faster and enhanced growth of the fungus. The highest yield and biological efficiency of the mushroom was also recorded from the bajra grains spawn closely followed by wheat grains spawn on wheat straw substrate. The wood chips, saw dust and curry leaves substrates exhibited minimum yield on either of the grain spawn tested.

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Received 19 July, 2022; Accepted 12 November, 2022