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EVALUATION OF VARIOUS SPAWN GRAINS AND LOCALLY AVAILABLE SUBSTRATES FOR THE PRODUCTION OF *Pleurotus eous* (Berk) Sacc.

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ABSTRACT

Pleurotus eous **(pink oyster mushroom) is an important edible mushroom known for its high nutritional value and ability to grow on lignocellulosic agro-residues. However, successful cultivation largely depends on the selection of suitable spawn substrates. The present study was aimed to evaluate different grain substrates for spawn production, standardize spawn dose, and identify suitable locally available substrates for cultivation of *P. eous*. The experiment was conducted during 2020-2022 at the Mushroom Research Laboratory, College of Horticulture and Forestry, Neri, Hamirpur (India), using six grain substrates *viz*., wheat (*Triticum aestivum*), paddy (*Oryza sativa*), maize (*Zea mays*), kodo millet (*Paspalum scrobiculatum*), bajra (*Pennisetum glaucum*), and jowar (*Sorghum bicolor*), and several lignocellulosic substrates. Among the spawn grains, bajra recorded maximum linear mycelial growth (91.58 mm), highest growth rate (9.62 mm day^{-1}), and minimum spawn run period (9.33 days). Maize grain gave the highest mushroom yield (2134 g kg^{-1} dry substrate) with biological efficiency of 213.40%. Standardization of spawn dose revealed that 3% spawn rate resulted in shortest spawn run, earliest primordia formation, and highest yield (1850 g kg^{-1}). Among locally available substrates, paddy straw gave highest yield (2803 g kg^{-1} dry substrate) with biological efficiency of 280.3%). The study concludes that maize grain spawn with 3% spawn dose and paddy straw substrate provides an efficient and cost-effective cultivation strategy for *P. eous*.**

Keywords: Agro-residues, biological efficiency, grain substrate, mushroom cultivation, *Pleurotus eous,* spawn

INTRODUCTION

The pink oyster mushroom [*Pleurotus eous* (Berk.) Sacc.] is an economically important basidiomycete belonging to the family Pleurotaceae (Singh *et al*., 2020). It is regarded as a functional food owing to richness in proteins, vitamins, and minerals, and simultaneously has low fat and cholesterol contents (Kortei and Waife-K-Wagyer, 2015; Sharma *et al.*, 2017). *P. eous* has the ability to utilize lignocellulosic substrates and can be cultivated under a wide range of climatic conditions across the temperate to tropical regions (Kortei *et al*., 2018).

The spawn quality is a critical factor influencing the successful mushroom production (Alavi *et al*., 2006). Spawn production is a specialized process that requires a high level of expertise, precision, and careful management (Chinda and Chinda, 2007). For *P. eous*, cereal grains such as paddy, jowar, and bajra have been identified as suitable substrates for spawn production (Eswaran and Ramabadran, 2000; Thulasi *et al*., 2010; Kaur, 2018; Jatwa, 2019).

Spawn rate is another crucial factor in oyster mushroom (*Pleurotus* spp.) cultivation, as it significantly affects mycelial colonization, yield, and biological efficiency (Chang and Miles, 2004).

An optimal spawn rate ensures rapid and uniform colonization of substrate, reduces the contamination risks and enhances the overall crop performance (Mondal *et al.*, 2010; Royse, 2014). In contrast, lower spawn rates delay colonization, while excessively high rates raise production costs without any significant increase in yield (Chang and Miles, 2004). Therefore, evaluation of optimal spawn rate is essential for efficient and economical production.

Although several studies have investigated oyster mushroom cultivation, most have focused on either spawn grain types or cultivation substrates independently, without clearly establishing the relationship between spawn quality and crop performance (Girmay *et al.*, 2016). Further, limited information is available on the combined effects of spawn grain type, spawn rate, and locally available lingo-cellulosic substrates on the growth and yield of *P. eous* under subtropical conditions of India. India generates nearly 350 million tons of agro-forestry waste annually, much of which remains unutilized (Das and Mukherjee, 2007). The potential of agroforestry wastes for mushroom cultivation has not so far adequately been explored. Kaur *et al.* (2025) identified bajra as a superior grain for mycelial growth but did not assess its effect on yield or biological efficiency. Similarly, Patil and Baig (2025) reported variation in biological efficiency of *P. eous* on different agro-wastes without considering spawn type or spawn dose. Other studies on oyster mushrooms also lacked integrated evaluation across both spawn and cultivation phases (Netam *et al.*, 2024), and spawn dose standardization for *P. eous* remained unaddressed. To address these gaps, a systematic evaluation of suitable spawn grains along with the locally available substrates is necessary to optimize mushroom production and improve biological efficiency. The present study adopts an integrated approach by evaluating different cereal grains for spawn production, standardizing spawn rates, and validating the performance of selected spawn on various locally available lignocellulosic substrates. This comprehensive assessment may provide a practical and cost-effective strategy for the sustainable utilization of agro-residues and enhance cultivation of *P. eous*.

Pleurotus spp. are lignocellulolytic fungi capable of growing on a wide range of agroforestry wastes. These fungi, particularly white-rot fungi, degrade lignin and cellulose, although some species preferentially decompose lignin over cellulose (Amirta *et al*., 2016). These residues are often disposed of through open burning, leading to environmental pollution and serious health hazards. Mushroom cultivation offers an effective and sustainable alternative for bioconversion of these wastes into protein-rich food. Lignocellulosic materials such as paddy straw, coffee husk, and wheat straw have been reported as suitable substrates for the cultivation of *P. eous* (Ingale and Ramteke, 2010; Thulasi *et al*., 2010; Jatwa *et al*., 2016; Kaur, 2018). Therefore, it was considered essential to evaluate the locally available substrates to identify the most suitable options for both spawn production and the cultivation of *P. eous*.

MATERIALS AND METHODS

Evaluation of various grain substrates for spawn production of Pleurotus eous

The present study was conducted at the Mushroom Research Laboratory, College of Horticulture and Forestry, Neri, Hamirpur (H.P.), Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (India) during the years 2020-2022. Different grain substrates, namely wheat (*Triticum aestivum*), paddy (*Oryza sativa*), maize (*Zea mays*), kodo (*Paspalum scrobiculatum*), bajra (*Pennisetum glaucum*), and jowar (*Sorghum bicolor*), were evaluated for their suitability in spawn production of *P. eous* (Chang and Miles, 2004). The grains were thoroughly cleaned, soaked in water for 2 h, and subsequently boiled for 45 min. Excess water was drained off, and a mixture of lime and gypsum in a ratio of 1:3 (w/w) was added at 0.7 and 2.1%, respectively, on a wet weight basis. The treated grains were filled into 1000 mL bottles up to two-thirds of their capacity, plugged with non-absorbent cotton, and sterilized in an autoclave at 22 psi for 2 h 20 min. After cooling, the bottles were transferred to an inoculation chamber and exposed to ultraviolet light for at least 20 min for surface sterilization (Sharma and Suman, 2005). Each bottle was aseptically inoculated with four mycelial discs (5 mm dia) of the test fungus. The inoculated bottles were incubated at 25°C until complete mycelial colonization of the grains. The observations were recorded for linear growth (mm), time required for complete spawn run (days), and growth characteristics. Growth rate (mm day^{-1}) was calculated using the formula:

$$r_g = \frac{d_g t_2 - d_g t_1}{t_2 - t_1}$$

Wherein, r_g = growth rate, $d_g t_2$ = diametric growth after time t_2, $d_g t_1$ = diametric growth after time t_1; t_2-t_1 = change in time intervals

Evaluation of spawn doses for production of P. eous.

Different doses of standard check (wheat) were evaluated at different concentrations i.e. 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% to assess their effect on the yield and yield contributing parameters of *P. eous*. The data were recorded in terms of the time taken for complete mycelial colonization (days) in spawn bottles, time taken for spawn run (days), primordia initiation (days), the number of fruiting bodies, morphometric characteristics, and yield (g kg^{-1} dry substrate). The biological efficiency (BE%) was calculated by using the formula of Chang *et al*. (1981).

$$BE\ (\%) = \frac{FW_{(M)}}{DW_{(S)}} \times 100$$

Wherein BE = Biological efficiency, $FW_{(M)}$ = Fresh weight of Harvested mushroom; $DW_{(S)}$ = Dry weight of substrate.

Effect of different grain spawn substrates on production of P. eous

The experiment was conducted under natural bamboo hut conditions. Spawn prepared on different grain substrates from the previous experiment was evaluated for yield performance using wheat straw as the cultivation substrate in 3 kg capacity mushroom growing bags (1 kg dry substrate). The dried wheat straw was chopped into small pieces and soaked in water for 24 h to ensure complete wetting (Bhogal, 2024). Excess water was drained, and the substrate pasteurized using hot water treatment for 45 min. After cooling, the substrate was inoculated with spawn from different grain substrates @ 2%. The spawned substrate was filled into mushroom bags and incubated under natural conditions in a bamboo hut (Bhogal, 2024). Observations were recorded for time taken for spawn run (days), time to first flush (days), and yield (g kg^{-1} dry substrate). Biological efficiency (%) was calculated as described earlier (Chang *et al*., 1981).

Evaluation of different production substrates for P. eous cultivation

An experiment was conducted to evaluate locally available lignocellulosic substrates, including paddy straw, maize straw, pine needles (*Pinus roxburghii*), sawdust, and lantana leaves, along with wheat straw as the standard control. Substrate-preparation and spawning procedures were carried out as described previously. The observations were recorded for the time taken for spawn run (days), time taken for first flush (days), stipe length (mm), cap diameter (mm), and yield (g kg^{-1} dry substrate). Biological efficiency (%) was calculated using the standard formula (Chang *et al*., 1981).

Statistical analysis

The experiment was conducted using a completely randomized design with six treatments and 3-4 replications to ensure the reliability of the results (Panse and Sukhatme, 2000). Data obtained from the experiments were subjected to analysis of variance (ANOVA). The significance of treatment effects was tested at 5% level of probability ($p \leq 0.05$). All the statistical analyses, including ANOVA and related computations, were performed using the OPSTAT statistical software (Sheoran *et al*., 1998).

RESULTS AND DISCUSSION

Effect of different grain substrates on the growth parameters of Pleurotus eous

The evaluated grain substrates significantly influenced the growth parameters of *P. eous* (Table 1). Among the tested substrates, bajra exhibited maximum linear mycelial growth (91.58 mm), which was at par with jowar (90.42 mm), while wheat grain substrate showed minimum linear growth (76.17 mm) [Plate 1]. The growth rate was also highest in bajra (9.62 mm day^{-1}), followed by jowar (9.49 mm day^{-1}) and kodo (8.92 mm day^{-1}). The lowest growth rate (7.91 mm day^{-1}) was observed on wheat grain substrate (Fig. 1). The superior performance of bajra and jowar may be attributed to their favourable nutrient composition and better moisture-holding capacity, which support rapid mycelial proliferation.

Table 1: Evaluation of different grain substrate on the growth of *Pleurotus eous*

Grain substrates	Linear growth (mm) after 9 days incubation	Growth rate (mm day^{-1})	Time taken for spawn run (days)	Growth characteristics
Wheat	76.17	7.91	11.33	Thick strand white mycelium growth on all grains
Paddy	89.17	9.35	10.67	White mycelial growth on all grains but not compact
Maize	82.50	8.61	12.67	White fluffy mycelial growth on all the grains
Kodo	85.25	8.92	11.33	White mycelium is improperly distributed on all grains
Jowar	90.42	9.49	10.33	Dense white mycelial growth on all grains
Bajra	91.58	9.62	9.33	Thick, dense white mycelial growth on all grains
$CD_{0.05}$	1.50	0.86	1.04	-
$SE_{(d)}$	0.68	0.39	0.47	-

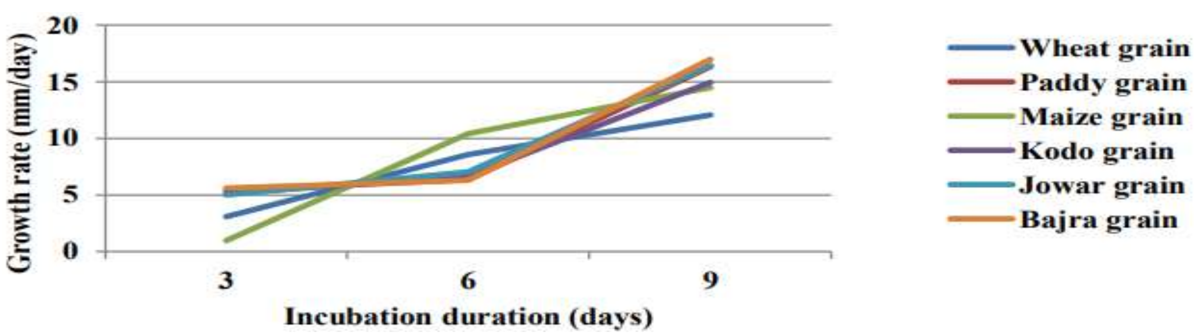

Fig. 1: Growth rate of *Pleurotus eous* on different grain substartes

Regarding the time taken for spawn run, the study revealed that maize grains required maximum time (12.67 days) for complete colonization, whereas bajra took minimum time (9.33 days) for completion of spawn run, indicating faster substrate utilization. Variations in spawn run duration among grain substrates could be attributed to the differences in grain size, nutrient availability, and surface characteristics affecting the mycelial spread. The qualitative observations of mycelial

Plate 1: Effect of different grain substrates on the linear growth of *Pleurotus eous*

growth revealed dense and thick mycelial development on bajra, while jowar supported dense, white, and uniform growth. Wheat grains showed thick, strandy mycelial growth, whereas paddy grains showed comparatively loose and less compact growth. In kodo millet, mycelial growth was irregular and unevenly distributed (Fig. 1). These variations further highlight the suitability of bajra and jowar as superior substrates for spawn production.

Evaluation of spawn dose/rate on the yield of P. eous

The results demonstrated that spawn dose/rate significantly affected growth, yield, and yield-related parameters of *P. eous* (Table 2). The minimum time required for the completion of spawn run was 10.66 days recorded at the highest spawn dose (3.0%), which was at par with other higher doses (Fig. 2). Similarly, the earliest primordia initiation (12.33 days) was observed at 3.0% spawn rate, whereas the lowest dose (0.5%) resulted in delayed development.

Table 2: Evaluation of different spawn doses/rates on the yield and yield attributes of *P. eous*

Treat-ments (%)	Time taken for spawn run (days)	Time taken for primordia formation (days)	No. of fruiting bodies	Cap diameter (mm)	Stipe length (mm)	Yield (g kg^{-1} dry substrate)	Biological efficiency (%)
0.5	13.67	18.99	13.55	90.23	11.29	923.33	92.33
1.0	13.66	17.33	15.33	93.82	11.33	1013.33	101.33
1.5	13.33	15.55	22.33	97.83	14.88	1450.00	145.00
2.0	12.00	13.77	24.33	97.32	13.00	1500.00	150.00
2.5	11.33	12.77	25.33	95.02	12.66	1583.33	158.33
3.0	10.66	12.33	26.77	94.98	11.83	1850.00	185.00
$CD_{0.05}$	4.74	0.69	0.90	4.28	2.15	93.17	-
SE(d)	2.15	0.31	0.41	1.94	0.97	42.42	-

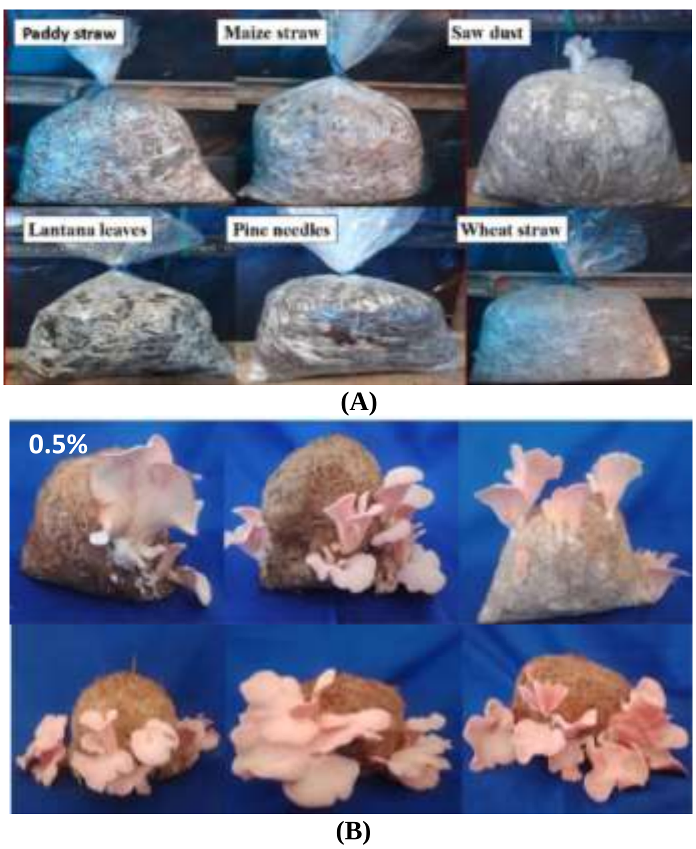

Plate 2: Effect of A) various production substrates on spawn run, and B) spawn dose on yield of *P. eous*

The number of fruiting bodies increased with increase in spawn dose, with highest number of fruiting bodies (26.77) observed at 3.0%, followed by 2.5%. The lowest number of fruiting bodies (13.55) was observed at 0.5%. Morphometric parameters showed a slightly different trend, with maximum cap diameter (97.83 mm) and stipe length (14.88 mm) observed at 1.5% spawn dose, suggesting that moderate spawn rates favour better individual fruit body development. The highest yield (1850 g kg^{-1} dry substrate) and biological efficiency (185.0%) were recorded at 3.0% spawn dose, followed by 2.5 and 2.0%; while the lowest values were observed at 0.5% spawn rate (Plate 2). The enhanced performance at higher spawn doses may be attributed to faster colonization, reduced contamination risk, and efficient substrate utilization. These findings are in agreement with Sarita *et al*. (2021), Chowdhury *et al*. (2024), and Bhogal (2024), who also

Table 3: Effect of different grain spawn on growth and yield parameters of *P. eous*

Grain substrates	Time taken for spawn run (days)	Time taken for first flush (days)	Yield (g kg^{-1} dry substance)	Biological efficiency (%)
Wheat	12.67	19.00	2120.67	212.07
Paddy	13.00	19.67	2020.67	202.07
Maize	14.00	21.67	2134.00	213.40
Kodo	14.67	25.67	2014.00	201.40
Jowar	13.67	23.33	2057.33	205.73
Bajra	15.67	24.67	2017.33	201.73
$CD_{p\geq0.05}$	1.34	1.20	54.48	-
$SE_{(d)}$	0.61	0.54	24.73	-

reported increased yield and biological efficiency with higher spawn rates. Therefore, a spawn dose of 3.0% was found optimal for efficient cultivation of *P. eous*.

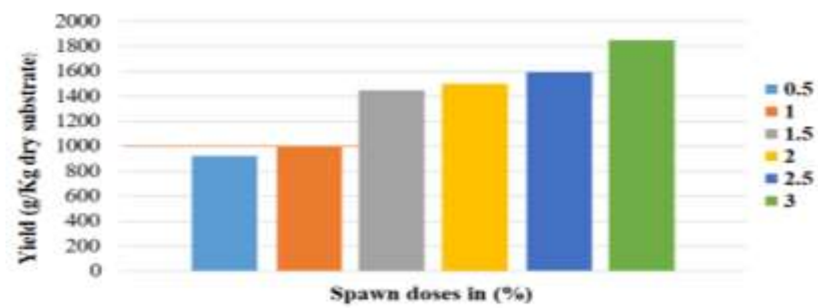

Fig. 2: Effect of different spawn doses on yield parameter of *P. eous*

Effect of different grain spawn on production parameters of P. eous

All the spawn grain substrates were evaluated on wheat straw for different production parameters. The results revealed maximum and minimum time taken for spawn run was 15.67 and 12.67 days, respectively, recorded on bajra grain and wheat grain substrates, respectively (Table 3). Further, maximum time for the 1^{st} flush was recorded on kodo grains (25.67 days) and minimum on wheat grain substrate (19.0 days). Despite faster colonization with wheat spawn, maize grain spawn produced the highest yield (2134.0 g kg^{-1} dry substrate) and biological efficiency (213.4%), whereas whereas the lowest yield (2014.0 g kg^{-1}) and biological efficiency (201.4%) were recorded with kodo spawn (Plate 3; Table 3). The superior yield performance of maize grain spawn may be attributed to its higher carbohydrate content and better nutrient availability, which support enhanced fruiting. These findings are in agreement with Mbogoh *et al*. (2011) and Sahu *et al*. (2014), who also reported maximum yield and biological efficiency with maize grain spawn in *Pleurotus* spp. However, the results differ from those of Kumbhar (2012), who reported higher yields with ragi, maize, bajra, and jowar.

Plate 3: Effect of different grain substrates on yield of *P. eous*

Effect of different substrates on the production parameters of P. eous

Significant variations were observed in production parameters across the different lignocellulosic substrates (Table 4). The shortest time of 8.33 days for spawn run was recorded on paddy straw, which was at par with maize straw (8.67 days), whereas lantana leaves took longest time of 15.0 days. Similarly, the earliest 1st flush took 18.33 days which was observed on wheat straw, while maximum delay (34.33 days) occurred on lantana leaves, indicating poor suitability of latter substrate for *P. eous* cultivation. Paddy straw exhibited the highest yield of 2803.0 g kg^{-1} dry substrate and maximum biological efficiency of 280.3%, whereas lowest yield (1084.67 g kg^{-1}) and biological efficiency (108.47%) were observed on wheat straw (Fig. 4). In terms of morphological parameters, maximum cap diameter (98.33 mm) was recorded on wheat straw, while maximum stipe length (15.47 mm) was observed on paddy straw. The minimum cap diameter and stipe length were recorded on maize straw and sawdust, respectively. The superior performance of paddy straw may be attributed to its favourable lignocellulosic composition, better aeration, and moisture retention capacity, which appear to have promoted efficient mycelial growth and fruiting. These findings are consistent with Jatwa *et al*. (2016), Kaur *et al*. (2018), Sharma *et al*. (2013), and Dubey *et al*. (2019), who also identified paddy straw as the most suitable substrate for *Pleurotus* cultivation.

Table 4: Effect of different agro-waste substrates on the yield and yield attributes of *P. eous*

Substrates	Time taken for spawn run (days)	Time taken for 1st flush (days)	Yield (g kg^{-1} dry substrates)	Biological efficiency (%)	Cap diameter (mm)	Stipe length (mm)
Paddy straw	8.33	24.67	2803.00	280.30	64.67	15.47
Maize straw	8.67	21.33	2684.33	268.43	48.33	9.47
Sawdust	12.67	34.00	1084.67	108.47	68.00	9.27
Pine needles	14.33	*	*	*	*	*
Lantana leaves	15.00	34.33	1132.33	113.23	72.00	14.73
Wheat straw	9.00	18.33	2126.67	212.67	98.33	14.03
$CD_{0.05}$	0.89	1.69	57.25		19.59	-
$SE_{(d)}$	0.39	0.77	25.99		8.89	

*Not included in the analysis

Plate 4: Effect of various production substrates on the yield of *P. eous*

Conclusion: The present study demonstrated that both spawn substrate and cultivation parameters significantly influence the growth, yield, and biological efficiency of *P. eous*. Among the grain substrates, bajra and jowar were found most suitable for spawn production due to their superior mycelial growth and faster colonization. However, maize grain spawn resulted in the highest yield and biological efficiency during cultivation. An increase in spawn dose enhanced mycelial colonization and yield, with 3.0% identified as the optimal rate for efficient production. Among the cultivation substrates, paddy straw proved to be the most effective, yielding the highest biological efficiency and overall productivity. Overall, the study highlights that the selection of appropriate spawn grain, optimal spawn dose, and suitable lignocellulosic substrate are critical factors for maximizing yield and ensuring sustainable and cost-effective cultivation of *P. eous*.

Ethical statement: The authors declare that the present study does not involve any experiments on humans or animals. All experimental procedures were conducted following standard laboratory and institutional guidelines. There are no ethical issues associated with this research.

Author's contributions: Conceptualization and experimental design were carried out by the first author. Laboratory experimentation, data collection and analysis were performed by the first and second authors. Manuscript drafting and revision were done by all authors. All authors have read and approved the final manuscript.

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