

Comparative evaluation of different spawn substrates on the growth and yield of oyster mushroom (*Pleurotus* spp.)

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ABSTRACT

Oyster mushroom is well known for its nutritional qualities and ease of cultivation. The spawn quality is important in obtaining the optimum yield in different mushrooms. In India, wheat grain is the most popular substrate for spawn preparation. Due to the high cost of wheat grain, alternative spawn substrates are one of the major concerns of the mushroom industry. In this study, we have investigated oyster mushrooms' growth and yield-related parameters using wheat straw spawn and liquid spawn with wheat grain spawn as control. Days for spawn run, pin head formation and days of first harvest were recorded in the wheat straw spawn as compared with control, i.e. wheat grain spawn in oyster mushroom species viz. *Pleurotus djamor* and *P. ostreatus* var. Florida. Notably, high yield was observed in the control compared to wheat straw spawn in both the oyster mushroom species. The average fruit body weight and diameter of pileus & stipe were higher in the wheat straw spawn as compared with control in *P. ostreatus* var. Florida. In *P. djamor* early spawn run and pin head formation were observed in control compared with liquid spawn. The yield was higher in control than in other spawn substrates used in the study.

Keywords: Oyster mushroom, wheat straw spawn, liquid spawn, yield

The genus *Pleurotus*, often known as the oyster mushroom, is a member of the family Pleurotaceae and class Basidiomycota. After shiitake, *Pleurotus* is the most commonly grown mushroom worldwide (Royse *et al.*, 2017, Barh *et al.*, 2019, 2020). Its resemblance to a white, shell-like fruiting body gave rise to the common name "oyster mushroom", and also referred as "Dhingri mushroom" in India. The fan or spatula-shaped fruit body can be found in various colours depending on the species, including white, pink, yellow, grey, and blue (Stanley and Odu, 2012). The oyster mushroom is an edible mushroom with excellent flavour and unique taste. This mushroom is very popular in India, especially in th country's North-East,

Chhattisgarh, and Bihar states (Singh *et al.*, 2021). Its popularity is due to its ability to grow in different kinds of agricultural waste (Sanchez, 2010). Beside this, *Pleurotus* species are enriched with various nutritional components viz. protein, minerals and vitamins, along with its therapeutic value for diabetics and cancer (Sivrikaya, 2002; Szabova, 2013). The growth of the oyster mushroom depends on the type and quality of the substrate, spawn and environmental conditions (Royse *et al.*, 2004; Jafarpour *et al.*, 2010). Oyster mushroom can be cultivated in different types of agricultural wastes, which are inexpensively available in the farmer's yard (Pokhrel, 2016).

Spawn quality and production are the key determinant factors for mushroom cultivation, which requires controlled aseptic environment (Sofi *et al.*, 2014). In general, sterilized grains are mostly used for spawn preparation due to their superior performance and biological features (Siddhant *et al.*, 2013). Wheat grain is used as their primary substrate for producing spawn for various edible mushroom species in India. There are many reports available in the literature on the evaluation of various grains types for the spawn preparation of diverse oyster mushroom species (Asghar *et al.*, 2007; Pal *et al.*, 2008; Stanley and Herbert, 2010 and Shukla and Jaitly, 2011). Liquid spawn preparation was reported in stirred submerged cultures (Abdullah *et al.*, 2013). Because of rapid mycelia colonization and simplicity in implementing automatic inoculation, liquid spawn has emerged as potential spawning material for commercial cultivation (Kawai *et al.*, 1996; Wang *et al.*, 2011; Ma *et al.*, 2016). In this study, we evaluated wheat straw and liquid spawn as spawning materials and wheat grain spawn as control. Further, the morphological and yield-related parameters of the two oyster mushroom species (*P.djamor* and *P.ostreatus* var. Florida) was also evaluated during the study.

MATERIALS AND METHODS

Germplasm

The cultures of two strains of oyster mushroom species *P.djamor* (DMRP-205) and *P.ostreatus* var. Florida (DMRP-136) were procured from the ICAR-Directorate of Mushroom Research (ICAR-DMR), Solan, Himachal Pradesh, India. The cultures were maintained and propagated on malt extract agar (MEA) medium autoclaved at 15psi for 25-30 minutes. A culture bit (3-5mm) of young mycelial colony from the culture tube was used to inoculate the MEA petri-plates and incubated at 25±2°C for growth.

Spawn preparation

Spawn was prepared using wheat straw and liquid culture along with wheat grain as control. The wheat

grain (control) spawn was prepared as described by Sharma and Kumar (2011). For wheat straw spawn, straw (3-5 cm) was soaked in water for 6-8 hrs and excess water was removed. The straw was added with wheat bran (20%) and CaCO₃ (1%). Further, it was filled in the polypropylene (PP) bags (40 x 20 cm size) @ 1 kg/bag with a polypropylene ring (height 2 cm, dia 4 cm) placed around the neck of the bag and sealed with non-absorbent cotton. These PP bags were autoclaved at 22psi pressure for 1.5 h and cooled down to room temperature. Further, master wheat grain spawn (14±2 g) was inoculated in these PP bags followed by incubation (24±2°C). Oyster mushroom species, *P. djamor* and *P. ostreatus* var. Florida, were used to prepare the wheat straw based spawn while liquid spawn was prepared for *P. djamor* only using malt extract medium. For preparation of liquid spawn, 25 g malt extract powder (Hi-Media, India) was dissolved in 1 litre distilled water and 50ml broth was dispensed in 100 ml Erlenmeyer flasks with two glass beads. The flasks were sterilized in an autoclave at 15psi for 15-20 min. 2-3 mm mycelial bit from actively growing culture was inoculated to each flask and incubated at 24±2°C in an incubator and monitored for colonization.

Cultivation trial

Wheat straw was used as a substrate for the cultivation trial of oyster mushroom species. Steam pasteurization methodology for the preparation of the substrate was adopted from Upadhyay (2011). The substrate was then thoroughly spawned with wheat straw and wheat grain spawn @3% on wet weight basis and 5 kg substrate was filled per bag. Each bag was perforated with 12–14 holes. The inoculated bags were incubated at 24±2°C for complete mycelium colonization and fruiting in the cropping room of ICAR-DMR, Solan. After the complete spawn run, humidity was maintained at 80±5%. The data was recorded in terms of days to complete spawn run, pinhead formation, first harvest, pileus diameter (cm), stipe length/diameter (cm), and yield (BE%).

P. djamor (DMRP-205) was used for cultivation trial using liquid spawn. Wheat straw was soaked overnight in water followed by removal of excess water. Further, this substrate with optimum moisture content (60-65%) was mixed with wheat bran (20%) and CaCO₃ (1%) followed by filling (750g) in polypropylene (PP) bags and sealed with polypropylene rings and non-absorbent cotton. The PP bags were autoclaved at 22psi for 1.5 h and inoculated with liquid spawn (3 ml/bag) along with control. The bags were shifted in the cropping room of ICAR-DMR, Solan and temperature was maintained (24±2°C) for the spawn run and fruiting. The different morphological and yield parameters viz. complete spawn run, pinhead formation and yield was estimated in the liquid spawn and control bags.

Statistical analysis

The experiment was conducted in two factorial randomized block design with five replications. The data was analyzed using OPSTAT for ANOVA (Sheoran *et al.*, 1998). Principal component analysis was performed to find the relation of spawn with the *Pleurotus* species and the traits observed in the study using XLSTAT (Vidal *et al.*, 2020). The second trial was conducted for *P.djamor* with grain based spawn and liquid spawn in randomized block design with five replication and data was recorded for complete spawn run, days of pinhead formation, days of 1st harvest and biological efficiency (%).

RESULTS AND DISCUSSION

Impact of wheat straw spawns on the growth and yield of oyster mushroom species

In general, grain spawn is used for mushroom cultivation. Being staple food, the cost of the grain is high, which impacts the cost of cultivation and benefit cost ratio in the mushroom farming. During the study, to reduce the cost of cultivation, wheat straw was

used as spawning material and its effect on the growth and yield of the oyster mushroom species {*P. djamor* (DMRP-205) and *P. ostreatus* var. Florida (DMRP-136)} was studied against grain spawn as control. Similar time periods were observed for spawn preparation in both wheat grain and straw spawn. Both the species *P. djamor* (Fig. 1) and *P. ostreatus* var. florida (Fig. 2) were cultivated using both the spawns following the standard package of practice.

Results obtained in the cultivation trial of *P. djamor* using the wheat straw spawn indicated earlier spawn run in wheat grain spawn (8 days) while wheat straw spawn took 10 days for spawn run. Similarly, pin head formation was also earlier in control (13 days) than wheat straw spawn (15 days). Moreover, a higher average fruit body weight (9.14g), longer stipe length (1.78cm) was recorded in wheat straw spawn than the control (average fruit body weight: 7.86 g and stipe length: 1.68cm). With respect to pileus diameter (6.42 cm) and days to first harvest (20 days), both the treatments were at par. Notably, high yield (BE %) was observed in control (67.20%) than wheat straw spawn (56.07%) (Fig 3).

Also in *P. ostreatus* var. Florida cultivation trial, significantly earlier spawn run (12 days), pin head formation (20 days), first harvest (24 days) was observed in control (wheat grain spawn) than wheat straw spawn (spawn run - 19 days; pinhead formation- 28 days, first harvest- 31 days). However, higher average fruit body weight (40 g), pileus diameter (11.56 cm) and stipe diameter (2.52cm) was recorded in wheat straw spawn than control (average fruit body weight: 12.38g, pileus diameter: 8.32cm, stipe diameter: 2.18cm). On the other hand, a significantly higher stipe length was observed in the control (8.04cm) compared to wheat straw spawn (1.42cm). The yield was significantly higher in the control (72.77%) than wheat straw spawn (63.38%) (Fig 3).

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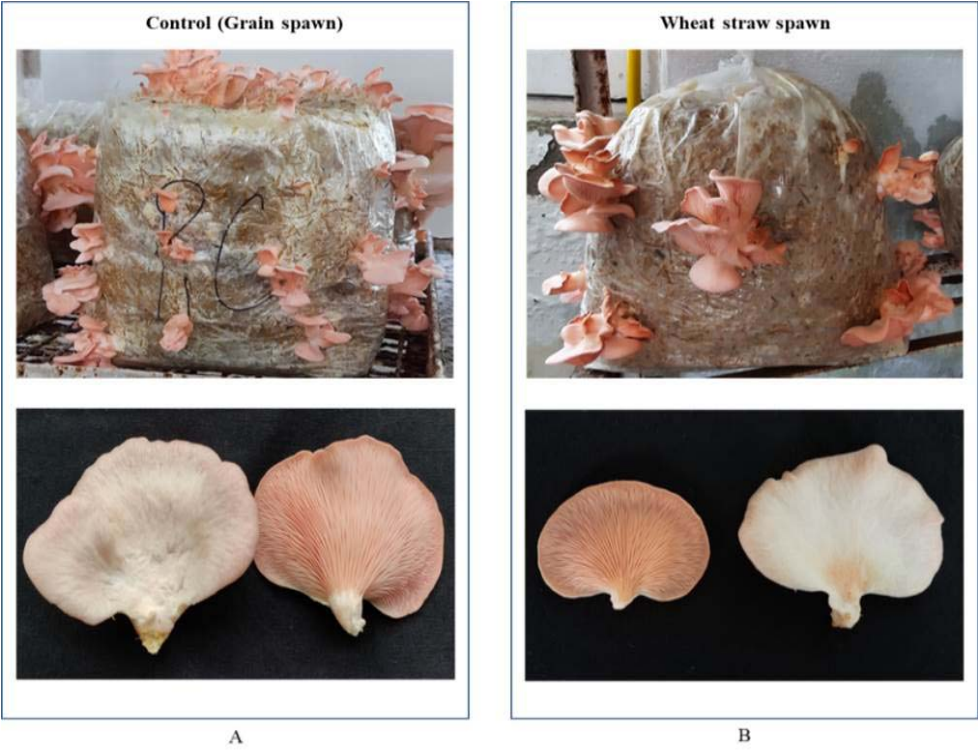


Fig 1. Oyster mushroom crop (*P. djamor* strain DMRP-205). A: Control - wheat grain spawn and B: Wheat straw spawn

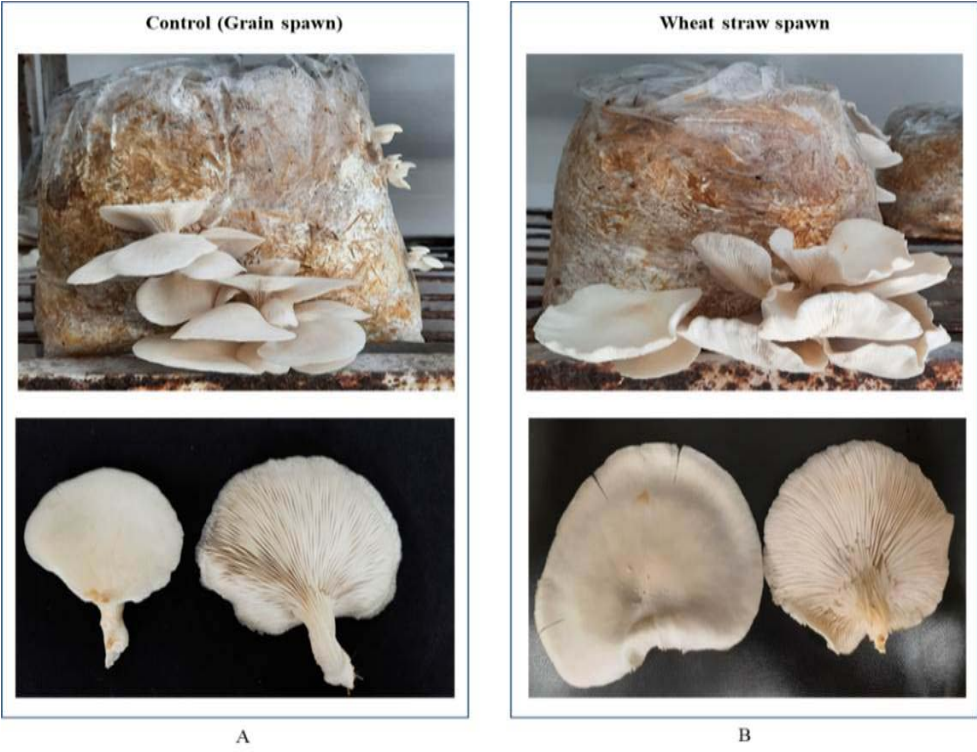


Fig 2. Oyster mushroom crop (*P. osteratus* var. Florida strain DMRP-136). A: Control wheat grain spawn; B: Wheat straw spawn

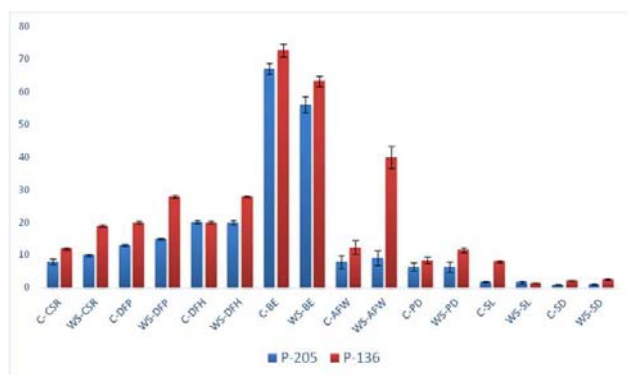


Fig 3. Morphological and yield-related parameters of the oyster mushroom. P-205: DMRP-205 (*P. djamor*); P-136: DMRP-136 (*P. ostreatus* var. Florida); C- control; CSR: control- complete spawn run (days); WS: wheat straw spawn; DPP: days to pinhead formation; DPH: days of first harvest; BE: biological efficiency; AFW: average fruit body weight; PD: pileus diameter; SL: stipe length; SD: stipe diameter

Recently, Njeru *et al.*, (2022) evaluated different alternative spawn substrates, viz. wheat straw, bean straw, maize cobs and sawdust to cultivate the indigenous oyster mushroom species collected from Kenya. They have reported bean straw as the best spawning material based on the shortest colonization period compared to others. No significant differences in days of mycelium colonization were observed for wheat grain and wheat straw spawn. While during this study, earlier spawn run in both the species was observed in control (wheat grain) spawn. In an earlier study, Nithyatharani and Kavitha (2018) analyzed the effect of the sorghum, pearl millet and wheat grain spawn on the growth of *P. ostreatus*. They observed higher yield using sorghum spawn compared with other substrates. Similarly, Zhang *et al.* (2019) analyzed the block saw dust spawn in *P. ostreatus* and observed similar cropping time and yield. Liu *et al.* (2018) reported a high yield in corn-stalk spawn compared to stick spawn in *P. ostreatus*. Our study observed a higher yield in wheat grain spawn (control) than wheat straw spawn.

The results were statistically analysed and ANOVA showed the significance variations of all traits for spawn, species, and its interaction except pileus and stipe diameter. On the pileus and stipe

diameter, species showed a significant effect, while spawn and its interaction didn't significantly affect the characters. The biological efficiency also showed significant variations for species and spawn type but their interaction was insignificant. The oyster mushroom's morphological parameters and yield majorly depend upon quality of spawn/substrate, environmental conditions and strain type (Royse, 2003; Banik and Nandi, 2004). However, an optimum climatic condition varied for the different *Pleurotus* spp. and is the key factor for achieving augmented yield. The results of the principal component analysis indicated a total variation of PC1 and PC2, in which PC1 contributed 96.78% while PC2 contributed around 3.22% variation. The eigen value for PCA 1 and PC2 was 1.936 and 0.064, respectively (Fig 4).

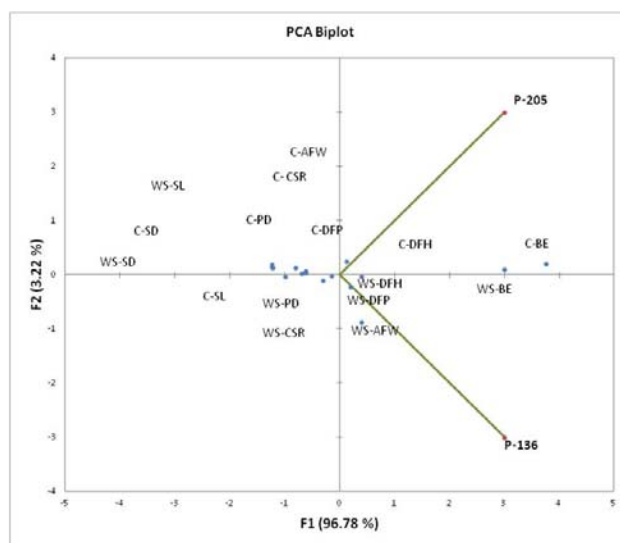


Fig. 4. PCA biplot analysis of the different morphological and yield-related parameters with two species *P. djamor* (P-205) and *P. ostreatus* var florida (P-136)

Impact of the liquid spawn on the growth and yield of oyster mushroom

During this study, liquid spawn technology was evaluated to explore its impact on the oyster mushroom's growth, yield parameters (*P. djamor*) and control (wheat grain spawn). The results indicated earlier spawn run (10 days) and pinhead formation (21days) in control than liquid spawn (spawn run 15

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days and pin head formation 23 days). In contrast days of first harvest were observed to be similar in both the treatments (27 days). Higher yield was recorded in control (68.7%) than liquid spawn (54.0%) during this study (Fig. 5).

In comparison to grain spawn, liquid spawn technology offers homogeneity, more aseptic environment and faster fruit body production (Friel and

McLoughlin, 2000). For instance, as compared with wheat grain, better colonization was reported in *P. pulmonarius* inoculated with liquid spawn generated through bioreactor based submerged fermentation (Abdullah *et al.*, 2013). However, more research is still required for the applicability of the liquid spawn in the oyster mushroom cultivation at the commercial level.

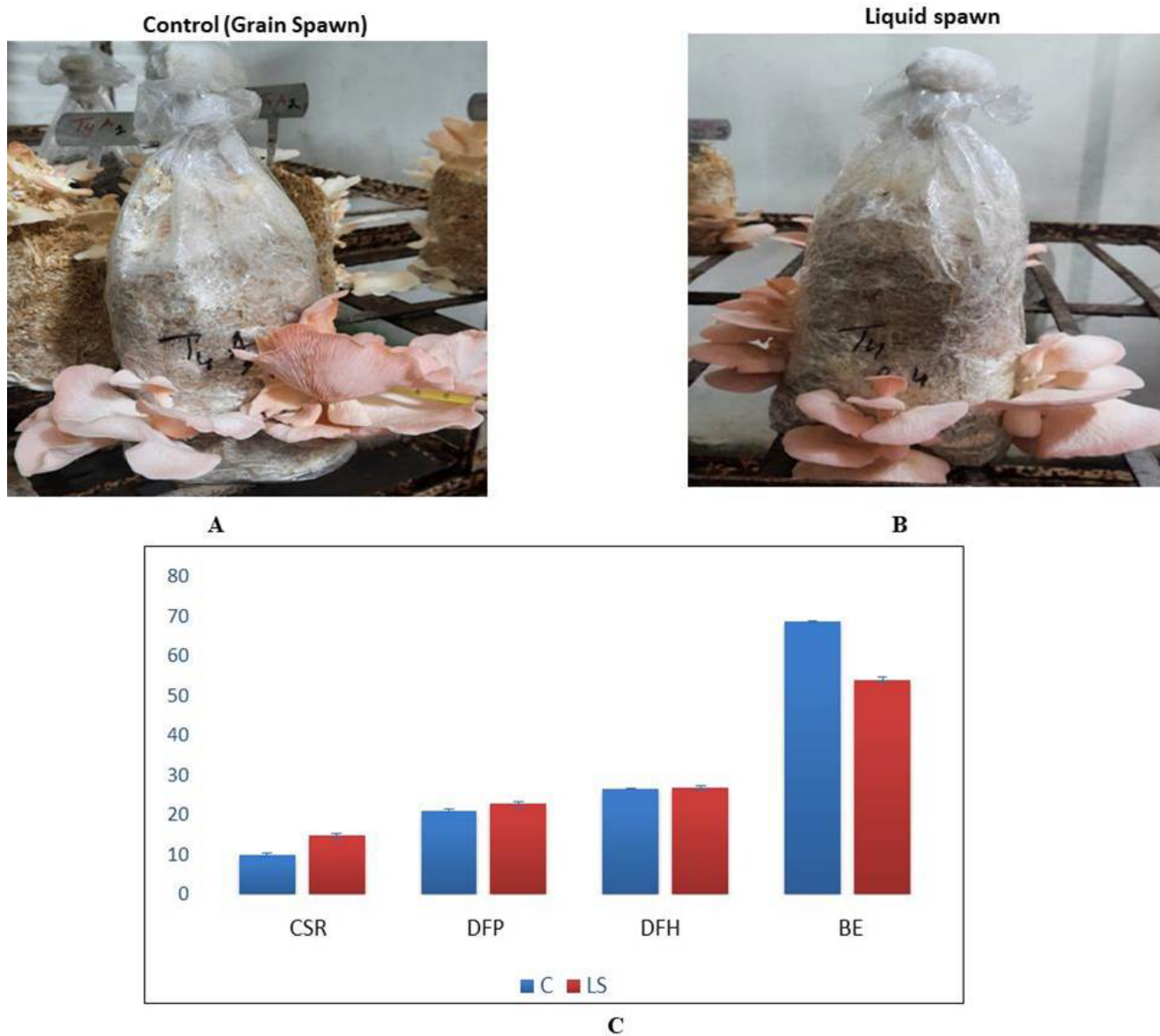


Fig. 5. Effect of liquid spawn on growth and yield of the oyster mushroom (*P. djamor*). A – crop using control spawn, B- crop using liquid spawn, C – Morphological parameters and yield (CSR: complete spawn run (days); DFP: days of pin head formation; DFH: days of first harvest; BE: biological efficiency; C: control; LS: liquid spawn)

CONCLUSIONS

The present study provides insight into utilizing various agricultural wastes as spawn substrates in cultivating oyster mushrooms. More research is needed to utilize alternative substrates in spawn production technology. Further, large-scale cultivation trial, feasibility and economics need to be explored in spawn production utilizing the different spawn substrates to promote mushroom cultivation and increase the income of the mushroom growers.

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