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# A comparative study on substrate sterilization methods for cultivation of Pink Oyster mushroom [*Pleurotus eous* (Berk.) Sacc.]

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**Abstract**

The experiment for the cultivation of Pink Oyster Mushroom *Pleurotus eous* was carried out to determine the effect of different substrate sterilization methods on the growth and yield of mushrooms through three methods of substrate sterilization: Chemical method, Hot-water method and Autoclave or steam sterilization. Paddy straw was used as the basal substrate for growing *P. eous*. Results showed that the time taken for the appearance of pinhead was the shortest with duration of 2.6 days on the substrate treated through autoclave or steam sterilization, and also gave the highest average mushroom yield of 258.8 g. Also, this substrate treatment method showed the highest biological efficiency (BE) of 85.34 % among all the treatments. However, the time taken for complete spawn running in both hot-water and steam sterilized substrate was recorded similar taking 17 days respectively. Among all the three substrate sterilization methods, the chemical treatment gave lowest yield of 176.93 g followed by hot-water of 242.2 g. However, the highest numbers of fruit bodies (37) and stipe length and diameter of quality fruit body with value of 0.96 cm and 1 cm respectively was recorded from the substrate treated by chemical method.

**Key words :** Biological efficiency, Mushroom yield, *Pleurotus eous*, Substrate sterilization.

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***P****leurotus* spp., also known as Oyster mushrooms are a group of edible saprotrophic fungi that belongs to the order Agaricales under family Pleurotaceae[16]. Almost all the species of genus, the members of *Pleurotus* are commercially cultivated all around the world and presently, it ranks as second leading commercially cultivated edible mushrooms after button mushrooms, *Agaricus bisporus*[17]. *Pleurotus eous* commonly known as pink oyster mushroom is an edible summer mushroom species that usually grows in the room temperature (RT) range of about 22 to 30°C and relative humidity (RH) of 85 to 95% [20]. They can be grown on a wide range of lignocellulosic materials composed of lignin, celluloses and hemicelluloses. The mushroom mycelia feeds upon these substrates by the presence of oxidizing enzymes (ligninases, laccases, peroxidases, manganese) and hydrolyzing enzymes (tannases, cellulases, and xylanases)[11,16,18].

For successful oyster mushroom cultivation, the selection of good quality substrate is a crucial step that determines the growing medium to be in their best condition for efficient and rapid colonization of mushroom mycelia and early cropping[8,15]. However, competitors such as bacteria and fungi may restrict the mushroom growth in the substrate which adversely results in low yield and poor quality fruit body production[1]. Therefore, the substrates for growing these mushrooms need to be sterilized properly in order to eradicate harmful microorganisms and other undesirable competitor moulds fungi[3]. According to researchers[1,9] preparation of the growing substrate materials is considered as one of the most significant steps for a successful oyster mushroom cultivation in order to defend against other saprophytic fungi which participate with the mushroom mycelia throughout their cropping period that results in various diseases and consequently, produce less mushroom yields. Different sterilization methods viz., steam pasteurization, autoclave or steam sterilization, chemical treatment and hot-water immersion extensively adopted worldwide to eradicate competitive fungi and other harmful microorganisms[12]. Bano *et al*.,[4] recommended hot-water dip of the substrate (cereal straw) at 65±5ºC for atleast 60 minutes. Suggested[21] hot-water treatment at temperature 80ºC for duration of 30-60 minutes for enhanced mushroom growth.

Atila[3] compared four different sterilization methods, viz: boiling in hot-water at 60, 80 and 100°C, and chemical treatment at 1% formaldehyde with steam sterilization by autoclaving at 121°C for about 90 minutes. Result showed that different sterilization methods affects the mycelium colonization rate, average weight of fruit body, yield and biological efficiency (BE) and morphology of fruit body such as stipe length and the width and size of pileus. However, the effectiveness of these substrate sterilization methods which affects the mushroom yield and the morphology of fruit body produced are yet to be explored and established so far. Therefore, the present studies aims to find out the most suitable method of substrate sterilization, which will have a significant effect on the rate of substrate colonization, biological efficiency and yield of *P. eous* mushroom.

*Specimen collection and spawn preparation:*

The strain of *P. eous* (Berk.) Sacc. was collected from ICAR Directorate of Mushroom Research, Chambaghat, Himachal Pradesh and it was maintained on medium, Potato Dextrose Agar (PDA) and then incubated in B.O.D incubator at 25±2°C for 1-2 weeks. Whole wheat grain was used as master spawn. It was sterilized by autoclaving at 121°C for 2 h and then inoculated with 14 days old pure culture of *P.eous* (Berk.) Sacc. and then, placed in the incubator for 15 days[5].

*Preparation of substrate and cropping :*

Paddy straw was used as the basal substrate for growing *Pleurotus eous*. The substrate was sun dried properly and then cut into length of 2-4 cm. Three different methods of substrate sterilization: chemical method, hot-water method and steam sterilization was used preparation of substrate. We followed Vijay & Sohi's[23] method for chemical sterilization by dipping the presoaked chopped straw in the mixture of formaldehyde+carbendazim. Hot-water treatment was done as explained by Singh & Dwiwedi[21]. Steam sterilization was carried out by autoclaving the substrate at 121°C for 15 mins as recom-mended by Mondal *et al*.,[14]. Spawning was done @5% by layering method and five replicates were kept for each treatment (n=5). The inoculated bags were then incubated in dark room for 2-3 weeks for spawn running.

*Harvesting and data collection :*

Harvesting was done as soon as the majority of the pileus of the fruit bodies attained their highest size but before the cap margin rolls upwards without destroying the structure of mushroom beds as explained by[10]. Data of different growth parameters such as duration of spawn running, pinhead formation, maturation time, number of fruit bodies per replicate bag, average weight of the fruitbody (g), caps diameter (cm), stipe length and diameter (cm), total average mushroom yield (g) and the biological efficiency (%) were recorded. BE of *Pleurotus eous* was calculated in Eq. 1.

$$\text{BE (\%)} = \frac{\text{Total mushroom yield (g)}}{\text{Dry weight of the substrate (g)}} \quad (1)$$

*Statistical analysis :*

Data obtained in each replication was subjected to ANOVA. The results were given as mean ± standard deviation (SD) of 5 replicates (n=5). Comparison of mean values was done through least significant difference, LSD at $P \leq 0.05$ using SPSS Version 18.0.

*Spawn running, pinhead formation and cropping period :*

Chemical sterilization took the longest time for spawn running (17.6 days) in comparison to hot water and steam sterilization which required equal amount of time (17 days) for complete mycelial run. This result is in agreement with the findings of Ali *et al.*,[1] & Atila[3] who reported that oyster mushrooms treated with chemical took longer duration for complete mycelial colonization. The time taken for pinhead formation was found in the range of 2.6–3.2 days with the shortest duration observed on autoclaved or steam sterilized treatments (2.6 days) which is similar with the results of Shrestha *et al*.,[19]. Result shown by

Kortie *et al.*,[13] also indicates that the minimum time taken for pinhead formation in *P. eous* mushroom was only 2 days. Maturation of fruit bodies was observed in 3–3.2 days. The autoclave or steam sterilization method took the shortest duration for maturation of fruit-bodies which is corroborated with the results of[19] who evaluated the effect of three different methods of substrate sterilization: chemical sterilization, hot-water and autoclave or steam sterilization on the growth and yield of oyster mushroom grown on paddy straw. Found[24] that it took 2-7 days for the fruit body of *P. eous* mushroom to mature. Also, the flush duration took the minimum time of 13 days by autoclave or steam sterilization method and the longest duration of 18.2 days on chemical method.

Table-1. Duration of complete mycelial colonization, appearance of pinheads and cropping period

| Days Taken | | | | |
|---|---|---|---|---|
| Substrate treatment method | Complete mycelial (Mt) colonization | Pinhead for-mation (Pt) | Maturation | Flush duration |
| | RT=15-21°C;RH=66%- 89% | RT=15-20°C; | RH=62-91% | |
| Chemical | 17.6±1.34 | 3.2±0.83 | 3.2±0.83 | 18.2±3.83 |
| Hot-water | 17±0 | 3.2±0.44 | 3±0.70 | 14.6±3.97 |
| Autoclave | 17±0 | 2.6± 0.54 | 3±0.70 | 13±3.74 |

The results are the mean ± SD of 5 replicates (n=5).

*Effect of different substrate sterilization methods on some yield attributes of pink oyster mushroom, Pleurotus eous :*

Yield attributes such as pileus diameter (cm), stipe length, and diameter of fruit body and effective fruit body of Pink Oyster Mushroom, *P. eous* are shown in Table-2. There is no significant difference ($P \geq 0.05$) in the pileus diameter of the fruiting bodies produced from different substrates sterilization methods with values ranging from 5.6 to 5.8 cm. *P. eous* harvested from both hot-water and steam sterilization (autoclaving) method had the highest pileus diameter. The pileus diameter of an effective fruit body lies in the range of 7.64-9.43 cm, which was supported by the result of Kortei *et al.*,[13] who cultivated *P. eous* on eight different agricultural wastes and obtained pileus diameter of fruit body in the ranges of 6.2-9.5 cm. Mondal *et al.*,[14] obtained value of 9.3 to 9.6 cm for the diameters of pileus of 0.53-1.48 cm in oyster mushrooms as described by[7] who cultivated different varieties produced from three different substrates sterilization methods (chemical sterilization, hot-water and autoclave or steam sterilization). Fruiting-body with the highest stipe diameter (0.76 cm) was observed in chemical sterilization while the lowest diameter (0.62 cm) was observed in autoclave or steam sterilization. The chemical sterilization produced the highest stipe diameter of effective fruit body with value of 1.0 cm whereas lowest was observed in autoclave or steam sterilization (0.78cm). The obtained value is at par with the reported values of *Pleurotus* spp. on paddy straw. Fruiting bodies with the highest stipe

Table-2. Effect of sterilization methods on some yield attributes of *Pleurotus eous* mushroom

| Substrate treatment | Pileus diameter of fruitbody (cm) | Pileus diameter of effective fruitbody (cm) | Stipe diameter of fruitbody (cm) | Stipe diameter of effective fruitbody (cm) | Stipe length of fruitbody (cm) | Stipe length of effective fruitbody (cm) |
|---|---|---|---|---|---|---|
| Chemical | 5.62±1.17[a] | 7.64±1.78[a] | 0.76±0.55[a] | 1±0.35[a] | 0.76±0.33[a] | 0.96±0.08[a] |
| Hot-water | 5.8±0.85[a] | 7.84±0.85[a] | 0.68±0.31[a] | 0.78±0.22[a] | 0.62±0.21[a] | 0.58±0.13[b] |
| Autoclave | 5.8±0.57[a] | 9.43±1.54[a] | 0.62±0.34[a] | 0.84± 0.23[a] | 0.54±0.28[a] | 0.66±0.23[b] |

The results are the mean ± SD of 5 replicates (n=5); means with similar letters within same column are not significantly different at P≥0.05.

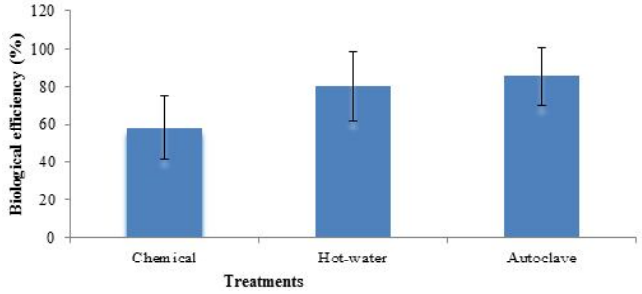


Figure 1. Bar plot indicating the effect of different sterilization methods on the biological efficiency of *P. eous*. The black bars in the bar plots represent the standard deviation of the mean.

length were observed in chemical sterilization (0.76 cm) while the lowest stipe length was observed in steam sterilization (0.54 cm). The stipe length of the effective fruit body shows significantly differences among the different substrate sterilization methods as given in Table 2 and was found in the ranges of 0.58-0.96 cm being highest observed in chemical sterilization. Arathy & Das[2] reported stipe length of fruit body of *P. eous* in the ranged from 0.87 to 1.57 cm cultivated on different agricultural wastes.

*Growth and yield performance of pink oyster mushroom (Pleurotus eous)* :

The effect of different substrate sterilization methods were evaluated and shows the influence on growth, yield, and biological efficiency (BE) of pink oyster mushrooms (Table-3 and Figure 1). The average number of fruit body per bag ranges from 23.2 - 37, in which the highest number of fruit bodies were obtained on substrate sterilized by chemical sterilization. The studies revealed by some authors[13,24] showed 29- 51 and 36 - 60 number of fruit bodies of oyster mushroom per bag respectively which is quite greater in numbers as compared to our study. The type of substrate sterilization methods had significantly influence (P≤0.05) the average weight of fruit body and was found in the range from 9.2 to 14.9 g. Autoclave or steam sterilization produced the highest weight of fruit

Table-3. Growth and yield performance of pink oyster mushroom ( *Pleurotus eous)* in three harvesting period

| Substrate treatment | No.of fruitbodies per bag in first flush | Average weight of fruitbody (g) in first flush | First flush | Second flush | third flush | Total yield per bag (g) |
|---|---|---|---|---|---|---|
| Chemical | 37±15.81[a] | 9.2±3.64[a] | 62.93±37.85[a] | 89.4±20.40[a] | 30.4±17.2[a] | 176.9±51.06[a] |
| Hot-water | 23.2±13.82[a] | 9.46±2.12[a] | 74.4±33.32[a] | 119.6±37.82[a] | 48.2±21.87[a] | 242.2±56.04[a] |
| Autoclave | 27.6±10.89[a] | 14.93±3.88[c] | 102.8±57.84[a] | 96.8±37.565[a] | 59.2±33.03[a] | 258.8±47.17[a] |

The results are the mean ± SD of 5 replicates (n=5); means with similar letters within same column are not significantly different at P≥0.05.

body (14.9 g). In the both first and third flush, autoclave or steam sterilization gave the highest average yield per bag of 102.8 g and 59.2 g respectively. However in the second flush, hot-water treatment of substrate obtained the highest average yield (119.6 g) which is followed by chemical sterilization (89.4 g) and autoclave or steam sterilization (96.8 g). The highest total yield of mushrooms was obtained on autoclave or steam sterilization and consequently it gave the highest biological efficiency (85.34%) among all the sterilization methods. Similar results were reported by various authors[6,19].

This experiment showed that different methods of substrates sterilization have variably influenced on the mushroom growth, morphology of the fruiting bodies, mushroom yield and biological efficiency of *Pleurotus eous* mushroom, but, the autoclave or steam sterilization method exhibited the best method with a shorter flushes duration, superior mushroom yields and higher biological efficiency among other methods tried. Thus, it is concluded from this study, that autoclave or steam sterilization method can be adopted as one of the best effective method of substrate sterilization for higher yields and production of oyster mushrooms.

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