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# Oyster Mushrooms Spent Substrate in Arbuscular Mycorrhizal Inoculum Production

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Abstract

Mushrooms are the edible fruiting bodies of fungi, belonging to either phylum Basidiomycota or Ascomycota which is mainly cultivated for food. Spent mushroom substrate (SMS) is the substrate left after harvesting of mushrooms. The present study was undertaken to know the effect of oyster mushrooms spent substrate on Arbuscular mycorrhizae (AM) inoculum production. Two oyster mushroom species (*Pleurotus eous* and *Hypsizygus ulmarius*) were cultivated and among them *Hypsizygous ulmarius* showed significantly higher yield (577.94g) and bio-efficiency (113.73%). *Hypsizygous ulmarius* spent substrate shown higher nutrient content like N, P, K and narrow C: N ratio. Two oyster mushrooms (*Pleurotus eous* and *Hypsizygus ulmarius*) spent substrates were incorporated to pots with sterilized sand : soil (1:1) as substrate and another set with unsterilized sand : soil (1:1) as a substrate with AM fungi *Glomus mosseae* and *Glomus fasciculatum*. Sorghum was used as a host plant. Both the SMS were found to enhance plant growth and mycorrhizal associations like per cent root colonization and AM spores in the substrate. Among the two SMS, *Hypsizygus ulmarius* SMS significantly increased the plant height of sorghum, number of spores and per cent root colonization of *Glomus fasciculatum* compared to *Pleurotus eous* SMS. Increased mycorrhizal association characteristics were observed in sterilized substrate compared to in unsterilized substrate.

*Keywords:* Oyster mushroom, SMS, Mycorrhizl inoculum

Oyster mushroom is consumed for its taste, medicinal and its nutritional properties and has been recommended to patients with cholesterol-related ailments. Oyster mushrooms are efficient lignin degraders and can be grown well on various types of lignocellulosic materials. Spent mushroom substrate (SMS) is the substrate left after harvesting of mushroom fruiting bodies. SMS is useful as soil amendment for improving the physical and chemical properties of the soil providing nutrients for the plants. It improves the structure of the soil, reduces surface crusting and compaction, increases microbial activity and provide nutrients and intern promotes faster crop growth establishment, improved crop density and yield.

The word Mycorrhiza is derived from the two Greek words, *Mycos* and *Rhiza* which means fungus and roots, respectively. Arbuscular mycorrhizae are the fungal symbionts of plants, associated with more than 80 per cent of terrestrial plants. Mycorrhizal fungi are also known as endophytes and necrotrophs. AM fungi are obligate biotrophic fungi forming symbiotic relationship with roots of many plants. They confer benefits directly to the host plant's growth and yield through acquisition of P and other immobile micro and macro-nutrients like P, K, Ca, Cu and Zn and thus become a significant component in low input Agri system.

Broad use of AM fungi has been limited because none of the AM fungi have been cultivated *in vitro* and hence it is difficult to obtain large quantity of prime inoculum. AM fungi can be grown in presence of host plants. The growth and physiology of host plants have been postulated to influence the spore production of AM fungi.

With this background, the study was aimed to know the effect of oyster mushrooms spent substrate on Arbuscular mycorrhizal inoculum production.

## Methodology

The pure cultures of Oyster mushrooms (*Pleurotus eous* and *Hypsizygus ulmarius*) used were collected from Mushroom Laboratory, Department of Agricultural Microbiology, UAS, GKVK, Bengaluru.

*Spawn production and Cultivation of Oyster mushrooms*: Spawn for the cultivation of *Pleurotus eous* and *Hypsizygus ulmarius*was prepared by following the standard procedure (Krishnamoorthy and Muthuswamy, 1997). Cultivation of Oyster mushrooms was carried out by following the method of Yang *et al*. (2013). After bud initiation, water was sprayed on the buds at regular intervals to avoid drying of the buds. Fruiting bodies of mushroom were harvested before the basidiospores were shed.

The number of days taken for bud initiation was recorded. The fresh weight of the mushrooms was recorded at different flushes and the total yield of mushrooms per bag was calculated. Bags were maintained up to three harvests for about 45 days. Bioefficiency of the mushrooms was calculated using the formula:

$$\text{Bio efficiency (\%)} = \frac{\text{Fresh weight of mushrooms (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

*Preparation of mushrooms spent substrate:* The substrate gets degraded after the cultivation of mushroom. The degraded substrate was shade dried for 2 days. After shade drying, it was oven-dried at 45 ºC for three days in a hot air oven until the constant weight was gained. Then, the substrate was powdered using a mixer grinder and sieved using 2mm mesh size sieve. The finely powdered substrate was stored in polythene covers for use in further experiments.

*Chemical analysis of oyster mushrooms spent substrates:* Chemical analysis of oyster mushrooms spent substrate was carried out. N, P and K analysis were carried out by following the standard procedure as described by Piper (1966). C:N ratio and organic carbon by the following standard methods as described by Mikhailova *et al.* (2003).

*Collection of arbuscular mycorrhizal inoculum:* Sand and soil-based cultures of arbuscular mycorrhizal inoculum of *Glomus mosseae* and *Glomus fasciculatum* were collected from Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru. The inoculum was mass multiplied for larger quantity using sand and soil (1:1) as substrate and finger millet as host plant. Mass multiplied AM inoculum was observed for spore numbers/ g of substrate by followingWet-sieving and Decanting method (Gerdemann and Nicholson, 1963).

*Glass House evaluation*: Soil was collected from Zonal Agriculture Research Station, UAS, GKVK, Bengaluru. The soil was red sandy loam. Pot culture experiment was conducted to study the effect of mushroom (*Pleurotus eous* and *Hypsizygus ulmarius*) spent substrates on the AM (*Glomus mosseae* and *Glomus fasciculatum*) inoculum production using sand : soil (1:1) as substrate and Sorghum (*Sorghum bicolar*) as a host plant. Sand and soil were sterilized in an autoclave at 121 ºC under 15 psi pressure for 45 minutes. One kg of sterilized sand and 1 kg of sterilized soil were mixed and filled into the pots. Another set of pots were filled with unsterilized sand and soil in 1:1 ratio. Hundred gram of mushroom spent substrate and 100 g of mycorrhizal inoculum (1:1 ratio) were mixed with the following treatments and put in the pots. A layer of sand and soil mixture was put on the inoculum. Sorghum seeds were sown. Thirty ml of Hoagland's nutrient solution was added to each pot once in 15 days. The plants were maintained for about 45 days.

There were six treatments like, $T_1$- *Glomus mosseae*: $T_2$- *Glomus fasciculatum*: $T_3$- *Pleurotus eous* SMS + *Glomus mosseae*: $T_4$- *Pleurotus eous* SMS + *Glomus fasciculatum*: $T_5$- *Hypsizygus ulmarius* SMS + *Glomus mosseae*: $T_6$- *Hypsizygus ulmarius* SMS + *Glomus fasciculatum*

Data on Plant height (cm) / plant and Spore numbers (/g of substrate) were recorded. Per cent root colonization by AM fungi was assessed by the following method described by Philips and Hayman (1970). Root colonization was measured using the below formula:

$$\text{Root colonization (\%)} = \frac{\text{No. of roots infected}}{\text{Total No. of roots taken}} \times 100$$

The data obtained from the experiment were subjected to statistical analysis by using Completely Randomized

Design (CRD). Comparison between treatment means was made using Duncan's Multiple Range Test (DMRT) for drawing conclusions

## Results and Discussion

The number of days taken for first bud initiation and the number of buds / per bag was recorded. Earlier (14 days) bud initiation of oyster mushroom species *Pleurotus eous* was observed on paddy straw substrate followed by *Hypsizygus ulmarius* (21 days). Number of buds per bag was observed higher in *Pleurotus eous* (32.50) followed by *Hypsizygus ulmarius* (20.75). (Table 1). Higher total yield of mushrooms was recorded in *Hypsizygus ulmarius* (577.94 g/bag) with higher bio-efficiency (113.73%) compared to *Pleurotus eous* yield (422.92 g/bag) and bio-efficiency (92.16%). The results of the present study agreed with Mohapatra and Behera (2013) who reported higher bioefficiency of *Hypsizygus ulmarius* (102.83%) when compared to *Pleurotus eous* (84.41%) (Table 1).

Table 1

Days for bud initiation, number of buds, total yield and bioefficiency of oyster mushrooms grown on paddy straw

| Oyster mushrooms | Number of days for bud initiation | Number of buds/bag | Total yield (g) | Bioefficiency (%) |
|---|---|---|---|---|
| *Pleurotus eous* | 14 [b] | 32.50 [b] | 422.92 [b] | 92.16 [b] |
| *Hypsizygus ulmarius* | 21 [a] | 20.75 [a] | 577.94 [a] | 113.73 [a] |

**Note**: Values are mean of 13 replications
Means superscribed by the same letter in a column do not vary significantly

The chemical properties such as nitrogen (2.17%), phosphorus (0.74%) and potassium (2.52%) were recorded higher in *Hypsizygus ulmarius* spent substrate when compared to nitrogen (1.81%), phosphorus (0.63%) and potassium (2.14%) content of *Pleurotus eous*spent substrate. C:N ratio (20.65:1) and organic carbon (37.64%) were higher in *Pleurotus eous* spent substrate compared to C:N ratio (17.27:1) and organic carbon (33.47%) of *Hypsizygus ulmarius* spent substrate (Table 2). These results were in agree with Ahlawat *et al*. (2005) who reported that *Hypsizygus ulmarius* SMS contains higher nutrient content when compared to *Pleurotus eous* and *Pleurotus florida* SMS. Spent mushroom substrate contains narrow C:N ratio and higher amount of nutrients.

Table 2

Chemical properties of oyster mushrooms spent substrate

| SMS | N content (%) | C: N ratio | P content (%) | K content (%) | Organic carbon (%) |
|---|---|---|---|---|---|
| *Pleurotus eous* | 1.81 [b] | 20.65: 1 [b] | 0.63 [b] | 2.14 [b] | 37.64 [b] |
| *Hypsizygus ulmarius* | 2.17 [a] | 17.27: 1 [a] | 0.74 [a] | 2.52 [a] | 33.47 [a] |

*Note*: Values are mean of 13 replications
Means superscribed by the same letter in a column do not vary significantly

At 30 DAS in sterilized substrate, significantly increased plant height (28.00 cm) was recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by in $T_5$ (*Hypsizygus ulmarius* SMS + *Glomus mosseae*) (25.25 cm). Minimum plant height was observed in $T_1$ (*Glomus mosseae*) (21.75 cm). At 45 DAS, significantly increased plant height (43.50 cm) was recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by in $T_5$ (*Hypsizygus ulmarius* SMS + *Glomus mosseae*) (39.00 cm). Minimum plant height was observed in $T_1$ (*Glomus mosseae*) (34.75 cm) (Table 3).

At 30 DAS un sterilized, significantly increased plant height (35.33 cm) was recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by in $T_5$ (*Hypsizygus ulmarius* SMS + *Glomus mosseae*) (32.33 cm). Minimum plant height was observed in $T_1$ (*Glomus mosseae*) (25.66 cm). At 45 DAS, significantly maximum plant height (48.33 cm) was recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by in $T_5$ (*Hypsizygus ulmarius* SMS + *Glomus mosseae*) (46.67 cm). Minimum plant height was observed in $T_1$ (*Glomus mosseae*) (38.00 cm) (Table 3).

The findings of this study are similar to that of Gbolagade *et al*. (2013) findings in which they reported

TABLE 3

Effect of oyster mushrooms spent substrate and AM fungi on plant height of sorghum in sterilized and unsterilized substrate

| Treatments | Plant height (cm) in sterilized substrate | | Plant height (cm) in unsterilized substrate | |
|---|---|---|---|---|
| | 30 DAS | 45 DAS | 30 DAS | 45 DAS |
| $T_1$- *Glomus mosseae* | 21.75 $^{d}$ | 34.75 $^{d}$ | 25.66 $^{d}$ | 38.00 $^{d}$ |
| $T_2$- *Glomus fasciculatum* | 21.50 $^{cd}$ | 33.75 $^{cd}$ | 25.67 $^{d}$ | 39.33 $^{d}$ |
| $T_3$- *Pleurotus eous* SMS + *Glomus mossae* | 20.00 $^{c}$ | 39.00 $^{bc}$ | 27.67 $^{cd}$ | 44.00 $^{c}$ |
| $T_4$- *Pleurotus eous* SMS + *Glomus fasciculatum* | 18.50 $^{c}$ | 37.00 $^{b}$ | 28.33 $^{c}$ | 45.00 $^{c}$ |
| $T_5$- *Hypsizygus ulmarius* SMS + *Glomus mossae* | 25.25 $^{b}$ | 39.00 $^{b}$ | 32.33 $^{b}$ | 46.67 $^{b}$ |
| $T_6$- *Hypsizygus ulmarius* SMS + *Glomus fasciculatum* | 28.00 $^{a}$ | 43.50 $^{a}$ | 35.33 $^{a}$ | 48.33 $^{a}$ |

**Note**: Values are mean of 4 replications
Means superscribedby the same letter in a column do not vary significantly DAS – Days after sowing

significantly higher plant height of *Abelmoschus esculentum* L. in AM fungi (*Glomus mosseae*) + SMC (*Pleurotus pulmonarius*) treatment than AM fungi alone.

In sterilized substrate, significantly higher per cent root colonization (85.25 %) was recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by $T_5$ (*Hypsizygus ulmarius* SMS + *Glomus mosseae*) (80.50%). Lowest per cent root colonization (61.00%) was recorded in $T_1$ (*Glomus mosseae*) (Table 4). In unsterilized substrate, significantly higher Per cent root colonization (67.50%) was recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by in $T_5$ (*Hypsizygus ulmarius* SMS + *Glomus mosseae*) (62.00 %). Lowest Per cent root colonization (46.25 %) was observed in $T_1$ (*Glomus mosseae*) (Table 4). The results of the study are similar to that of Tallapragada *et al*. (2011) in which they observed that addition of *Pleurotus florida* SMS increased root colonization of *Glomus intraradices* compared to *Glomus intraradices* alone.

In sterilized substrate, significantly more number of spores (43.75/g) were recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by in $T_5$ (*Hypsizygus ulmarius* SMS + *Glomus*

TABLE 4

Effect of oyster mushrooms spent substrate on per cent root colonization by AM fungi in sorghum roots in sterilized and unsterilized substrate

| Treatments | Root colonization (%) in sterilized substrate | Root colonization (%) in unsterilized substrate |
|---|---|---|
| $T_1$- *Glomus mosseae* | 61.00 $^{e}$ | 46.25 $^{c}$ |
| $T_2$- *Glomus fasciculatum* | 62.75 $^{e}$ | 48.50 $^{bc}$ |
| $T_3$- *Pleurotus eous* SMS + *Glomus mossae* | 74.25 $^{cd}$ | 55.25 $^{bc}$ |
| $T_4$- *Pleurotus eous* SMS + *Glomus fasciculatum* | 78.00 $^{bc}$ | 58.50 $^{b}$ |
| $T_5$- *Hypsizygus ulmarius* SMS + *Glomus mossae* | 80.50 $^{b}$ | 62.00 $^{b}$ |
| $T_6$- *Hypsizygus ulmarius* SMS + *Glomus fasciculatum* | 85.25 $^{a}$ | 67.50 $^{a}$ |

**Note**: Values are mean of 4 replications
Means superscribedby the same letter in a column do not vary significantly
DAS – Days after sowing

*mosseae*) (39.50/g). Lowest numbers of spores (28.00/g) were recorded in $T_1$ (*Glomus mosseae*) (Table 5). In unsterilized substrate, significantly higher number of spores (34.75/g) were recorded in $T_6$ (*Hypsizygus ulmarius* SMS + *Glomus fasciculatum*) followed by in $T_5$ (*Hypsizygus ulmarius* SMS + *Glomusmosseae*) (30.00/g). Lowest number of spores (22.00/g) was recorded in $T_1$ (*Glomus mosseae*)

TABLE 5

Effect of oyster mushrooms spent substrate on spore numbers of AM fungi in sterilized and unsterilized substrate

| Treatments | Spore numbers / g of sterilized substrate | Spore numbers / g of unsterilized substrate |
|---|---|---|
| $T_1$- *Glomus mosseae* | 28.00 $^{e}$ | 22.00 $^{d}$ |
| $T_2$- *Glomus fasciculatum* | 29.25 $^{e}$ | 23.25 $^{d}$ |
| $T_3$- *Pleurotus eous* SMS + *Glomus mossae* | 33.75 $^{d}$ | 26.75 $^{c}$ |
| $T_4$- *Pleurotus eous* SMS + *Glomus fasciculatum* | 36.00 $^{c}$ | 28.50 $^{bc}$ |
| $T_5$- *Hypsizygus ulmarius* SMS + *Glomus mossae* | 39.50 $^{b}$ | 30.00 $^{b}$ |
| $T_6$- *Hypsizygus ulmarius* SMS + *Glomus fasciculatum* | 43.75 $^{a}$ | 34.75 $^{a}$ |

**Note**: Values are mean of 4 replications
Means superscribedby the same letter in a column do not vary significantly
DAS – Days after sowing

(Table 5). The results of the study are similar to that of Tallapragada *et al*. (2011) in which they observed that addition of *Pleurotus florida* SMS increased spore numbers of *Glomus intraradices* compared to *Glomus intraradices* alone.

From the above experiments, it could be concluded that, both the oyster mushrooms spent substrates enhances the mycorrhizal symbiosis characteristics like spore numbers and per cent root colonization. Among two SMS, *Hypsizygus ulmarius* SMS increases the mycorrhizal symbiosis characteristics of *Glomus fasciculatum* compared to *Pleurotus eous* SMS. Sterilized substrate enhances the mycorrhizal symbiosis characteristics compared to unsterilized substrate.

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