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# Cotton waste as an optimal substrate for cultivation of the pink oyster mushroom *Pleurotus djamor*

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**ABSTRACT**

*Pleurotus djamor*, commonly known as the pink oyster mushroom, has garnered attention for its significant medicinal and nutritional value. Due to the limited scientific information available on the optimal cultivation conditions for this macro fungus, the objective of this study was to identify suitable temperature, media, and spawning material for upscaling mycelium, as well as an appropriate substrate for cultivating the *P. djamor* strain PN2. The mycelium of strain PN2 exhibited the most robust growth when cultured on YMA and Raper media at 25°C. The optimal spawning material for upscaling the mycelium of strain PN2 comprised 79% rice grain, 18% sawdust, 2% rice bran, and 1% $CaCO_3$. Additionally, cotton waste was identified as the optimal substrate for cultivating *P. djamor,* resulting in a remarkable biological efficiency of 48.56%. Collectively, our findings provide useful information for the industrialization of P. djamor cultivation.

## 1. INTRODUCTION

The genus *Pleurotus*, commonly known as the oyster mushroom, belongs to the family *Pleurotaceae* within the order *Agaricales* [1]. The commercial production of this genus has significantly increased over the past few decades [2]. In addition to their nutritional and medicinal value, *Pleurotus* species can also biosorb various environmental contaminants [3].

*P. djamor* is widely distributed across tropical and subtropical regions [4]. Like other *Pleurotus* species, this species possesses high medicinal and nutritional value. The protein and carbohydrate content of *P. djamor* ranges from 11.3% to 43.1% and 35.5% to 42.4%, respectively [5]. RNAse purified from *P. djamor* has shown the ability to inhibit the proliferation of breast cancer and hepatoma cells [6]. Among the *Pleurotus* species, *P. djamor* stands out for its capacity to accumulate significant quantities of heavy metals, particularly lead [7]. Therefore, *P. djamor* can be used to bioremediate heavy metal-contaminated soil in the environment [8]. Chromium, copper, iron, and zinc contents found in wild *P. djamor* were 0.38-0.95, 12.08-16.26, 22.92-32.07, and 12.02-19.58 mg/kg, respectively [9]. The cultivation of *P. djamor* on heavy metal-containing substrates may pose risks to consumers.

The cultivation of mushrooms is a cost-efficient method for transforming lignocellulosic waste materials [10]. *Pleurotus* mushroom species are decomposers with the capacity to effectively break down and absorb components of substrate materials [11]. Therefore, oyster mushrooms can be easily cultivated on substrates made from organic agricultural waste. *Pleurotus* species are among the most widely cultivated mushrooms [6]. In general, the substrate for mushroom cultivation comprises basal substrates such as straws, sugarcane bagasse, and cotton waste, along with supplements like rice bran, urea, and wheat bran [12]. *P. djamor* can be grown on corn cobs [13], paddy straw [14], wheat straw [15], combined dairy manure-food waste digestate [16], and coffee pulp [5]. *P. djamor* cultivated on cotton waste exhibited a higher yield than that on wheat straw and paddy straw [17]. According to Selvakumar, straw is a more suitable basal substrate for cultivating *P. djamor* than sugarcane bagasse, cotton waste, sawdust, and coir pith [18]. Quinoa stalk was identified as the best basal substrate for the cultivation of *P. djamor* [19]. Bean straw and safflower can be used as an alternate substrate to sawdust and wheat straw for *P. djamor* growth [20]. There is a relationship between the cultivation substrate and the concentration of bioactive molecules in the fruiting body of *P. djamor* [21]. Combinations of substrates can enhance the nutritional parameters of *P. djamor* [5]. For example, *P. djamor* cultivated on wheat straw and quinoa stalk exhibited a higher total antioxidant assay than wheat straw or quinoa stalk alone [22]. The spent substrate from *P. djamor* cultivation has been reported to control the reproduction of

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*Meloidogyne javanica* on lettuce [23]. *P. djamor* can grow in regions characterized by elevated temperatures, less rainfall, and humidity [24].

Although *P. djamor* possesses high medicinal and nutritional values, no studies have been conducted to optimize culture conditions for *P. djamor* cultivation in Vietnam. This study aimed to investigate the optimal temperature, media, and spawning material for upscaling mycelium and the basal substrate for cultivating *P. djamor*. The recommended cultural conditions are expected to contribute significantly to the development of the mushroom industry in Vietnam.

## 2. MATERIALS AND METHODS

### 2.1. Mushroom Strain

*P. djamor* strain PN2 was procured from the Research Institute of Edible and Medicinal Mushroom, Vietnam National University of Agriculture. The strain was preserved on potato glucose agar (PGA) slants at 10°C under complete darkness for further use. The optimal cultural conditions for the mycelial growth of pure culture and master spawn and the basal substrate for cultivation of strain PN2 were identified by one individual factor at a time [Figure 1].

### 2.2. Determination of Culture Conditions for the Mycelial Growth of *P. djamor*

The effect of temperature on the growth of strain PN2 was determined at 15°C, 20°C, 25°C, 30°C, and 35°C on PGA medium. From experiment #1, 25°C was identified as the optimal temperature for the mycelial growth of strain PN2. Therefore, to determine the optimal agar medium for mycelial growth, strain PN2 was incubated at 25°C in five different media [i.e. Potato Dextrose Agar (PDA), Potato Glucose Agar (PGA), Yease Mannitol Agar (YMA), Czapek, and Raper]. The composition of each medium is listed in Table 1.

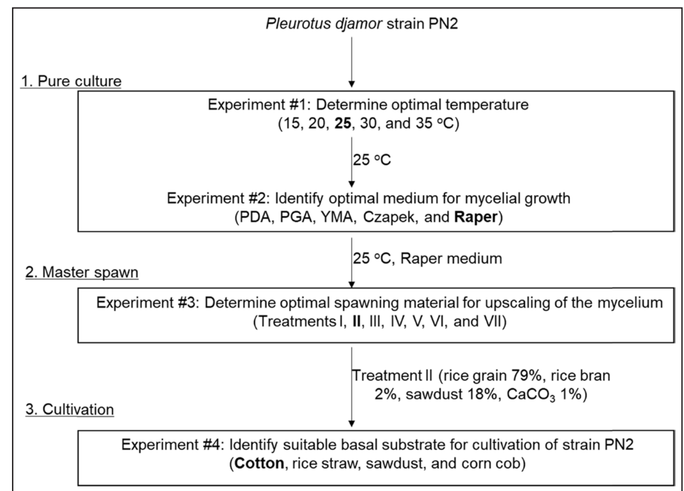


**Figure 1:** Flow chart showing the steps for optimizing culture conditions for cultivating strain PN2. Initially, strain PN2 was cultured on PDA medium at temperatures of 15°C, 20°C, 25°C, and 35°C to determine the ideal temperature for mycelial growth. The selected optimal temperature was then used to assess the impact of different media (PDA, PGA, YMA, Czapek, and Raper media) on the growth of pure culture mycelium. Strain PN2 cultivated on Raper medium at 25°C was transferred to spawning materials to scale up the mycelium. Following the identification of treatment II as the optimal spawning material for master spawn, the strain grown on this treatment was inoculated onto basal substrates. The optimized conditions are highlighted in bold.

**Table 1:** The composition of the culture medium was used to determine the optimal culture conditions for the mycelial growth of *P. djamor.*

| Composition (g/l) | PDA | PGA | YMA | Czapek | Raper |
|---|---|---|---|---|---|
| Potato | 200 | 200 | - | - | - |
| Dextrin | 20 | - | - | - | - |
| Glucose | - | 20 | - | - | 20 |
| Yeast extract | - | - | 2 | - | 2 |
| Pepton | - | - | - | - | 2 |
| Maltose | - | - | 20 | - | - |
| Sucrose | - | - | - | 30 | - |
| $NaNO_3$ | - | - | - | 2 | - |
| $KH_2PO_4$ | - | - | - | 1 | 0.46 |
| $MgSO_4.7H_2O$ | - | - | - | 0.5 | 0.5 |
| $FeSO_4.7H_2O$ | - | - | - | 0.01 | - |
| KCl | - | - | - | 0.5 | - |
| $K_2HPO_4$ | - | - | - | - | 1 |
| Agar | 17 | 17 | 17 | 17 | 17 |

### 2.3. Effect of Spawning Material on Upscaling of the Mycelium

From experiment #2, Raper medium was identified as the optimal agar medium for the mycelial growth of pure spawn. Subsequently, mycelia grown in Raper medium was inoculated on seven different spawning materials (Experiment #3). The composition of those materials in Experiment #3 is given in Table 2. Each treatment was conducted with six replicates.

In the preparation of the spawning materials, rice grains were first washed and soaked in water overnight and were subsequently boiled for 20-25 min until achieving a soft consistency. Rubberwood sawdust was mixed with a lime solution (0.4%) and fermented for 5-7 days. The

**Table 2:** The composition of spawning material was used to optimize media for mycelial growth of mother spawn.

| Composition (%) | Treatment I | Treatment II | Treatment III | Treatment IV | Treatment V | Treatment VI | Treatment VII |
|---|---|---|---|---|---|---|---|
| Rice grain | 99 | 79 | 59 | 39 | 19 | 0 | 0 |
| Rice bran | 0 | 2 | 4 | 6 | 8 | 10 | 0 |
| Sawdust | 0 | 18 | 36 | 54 | 72 | 89 | 99 |
| $CaCO_3$ | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

**Table 3:** Effect of temperature on the mycelial growth of strain PN2.

| Temperature (°C) | Mycelial diameter (mm) | |
|---|---|---|
| | 3 days | 6 days |
| 15 | 0.00 ± 0.00[d] | 17.08 ± 0.24[d] |
| 20 | 10.58 ± 0.24[c] | 29.75 ± 0.67[c] |
| 25 | 33.17 ± 0.77[a] | 83.25 ± 0.28[a] |
| 30 | 22.25 ± 1.17[b] | 69.75 ± 1.77[b] |
| 35 | 0.00 ± 0.00[d] | 0.00 ± 0.00[e] |
| Tukey's $HSD_{0.05}$ | 2.65 | 3.59 |

Values are mean ± standard error. Different letters represent significant differences ($P < 0.05$).

**Table 4:** Effect of media on the mycelial growth of strain PN2.

| Media | Mycelial diameter (mm) | |
|---|---|---|
| | 3 days | 6 days |
| PDA | 0.00 ± 0.00[d] | 0.00 ± 0.00[d] |
| PGA | 16.58 ± 0.47[b] | 39.17 ± 1.71[b] |
| YMA | 30.58 ± 1.60[a] | 78.17 ± 1.54[a] |
| Czapek | 12.08 ± 0.20[c] | 22.75 ± 0.40[c] |
| Raper | 33.75 ± 1.09[a] | 82.33 ± 0.62[a] |
| Tukey's $HSD_{0.05}$ | 3.71 | 4.48 |

Values are mean ± standard error. Different letters represent significant differences ($P < 0.05$).

moisture content of rubberwood sawdust was adjusted to 65% using tap water. Rice grains, rubberwood, rice bran, and $CaCO_3$ were mixed based on the composition of each spawning material listed in Table 2, filled into three-quarters of 24 mL round-bottom tubes, and sterilized for 60 min at 121°C. The spawning materials were cooled down to 25°C. Then, a (5 mm × 5 mm) piece of mycelia culture grown in Raper medium was inoculated into the spawning material and incubated for 20 days at 25 ± 1°C in a light-free environment.

## 2.4. Substrate Preparation for Cultivation of *P. djamor*

Cotton waste without seeds, rice straw, rubber wood sawdust, and corncob were selected to determine the optimal basal substrate for cultivating *P. djamor* (Experiment #4). Rice straws were chopped into 5-6 cm pieces. Basal substrates (cotton waste, rice straw, sawdust, and corncob) were soaked in a 0.4% lime solution until they reached a moisture content of 65%. Basal substrates were then composted separately in 1.5×1.5×1.5 m piles: rice straw, sawdust, and corncob for 5 days and cotton waste for 2 days. 1.2 kg of cultivation substrate (87% basal substrate, 12% wheat bran, and 1% $CaCO_3$) was mixed and placed in polythene bags and autoclaved for 90 min at 121°C.

From experiment #3, treatment II (79% rice grain, 18% sawdust, 2% rice bran, and 1% $CaCO_3$) was found to be the ideal spawning material, mother spawn grown on this treatment was inoculated into cultivation substrate substrates, transferred to the spawn running room, and incubated in darkness for 20 days at 25°C. To induce the formation of fruiting bodies, *P. djamor* was transferred from the spawn running room to the cultivation room and incubated at 25°C with 85% relative air humidity. The air humidity was controlled using a humidity controller. Mature mushrooms were carefully handpicked without causing any damage to the substrate as soon as they began to show wrinkling.

## 2.5. Data Records and Statistical Analysis

The mycelial morphology of strain PN2 grown on agar medium was characterized by assessing the following parameters: mycelial growth diameter (mm), growth rate (mm/day), and density. The mycelial growth rate on agar media was calculated by dividing the diameter of the mycelial (mm) by the incubation time (day) [25]. For spawning material and cultivation substrate, the growth rate was estimated by dividing the mycelial height (mm) by the colonization time (days) [26]. The density was determined as high, regular, or low based on visual observation.

The yield was calculated based on the total fresh weight of the fruiting body (g) per bag. The spawn running period (in days) referred to the time it took for strain PN2 to fully colonize the substrate. The day of pinhead formation (days) was determined as the time required for primordia to appear after the substrate was fully colonized by the mycelium. To calculate the biological efficiency (BE, as a percentage), we used the ratio of the fresh weight of the fruiting bodies (in grams) to the dry weight of the substrate (in grams) [27]. The cultivation substrate was kept in an oven at 65°C overnight after autoclaving to determine the dry weight.

The data analysis was performed by utilizing GraphPad Prism (version 9.0, GraphPad Software Inc., San Diego, CA). Each experiment was carried out in triplicate. The collected data were subjected to a one-way analysis of variance (ANOVA), followed by Tukey's honestly

**Table 5:** Effect of spawn substrates on the mycelial diameter (A) and growth rate (B) of strain PN2.

| Treatment | Mycelial diameter (mm) | | | Growth rate (mm/day) | | |
|---|---|---|---|---|---|---|
| | 5 days | 10 days | 15 days | 5 days | 10 days | 15 days |
| I | 30.17 ± 0.82[a] | 69.33 ± 1.63[a] | 106.33 ± 1.26[a] | 6.03 ± 0.17[a] | 6.93 ± 0.16[a] | 7.09 ± 0.08[a] |
| II | 25.50 ± 2.31[ab] | 59.50 ± 1.23[ad] | 99.42 ± 1.96[ab] | 5.10 ± 0.46[ab] | 5.95 ± 0.12[ab] | 6.63 ± 0.13[ab] |
| III | 21.83 ± 1.85[ab] | 51.75 ± 2.52[cd] | 93.87 ± 2.50[b] | 4.67 ± 0.37[ab] | 5.18 ± 0.25[bc] | 6.26 ± 0.17[b] |
| IV | 25.50 ± 2.49[ab] | 56.00 ± 2.48[bd] | 95.17 ± 1.92[b] | 5.10 ± 0.50[ab] | 5.60 ± 0.25[b] | 6.34 ± 0.13[b] |
| V | 26.50 ± 2.71[ab] | 56.42 ± 3.02[bd] | 95.25 ± 2.53[b] | 5.30 ± 0.54[ab] | 5.64 ± 0.30[b] | 6.35 ± 0.17[b] |
| VI | 17.92 ± 2.76[b] | 45.08 ± 3.08[c] | 83.75 ± 2.73[c] | 3.58 ± 0.55[b] | 4.51 ± 0.31[c] | 5.58 ± 0.18[c] |
| VII | 29.75 ± 2.68[a] | 59.75 ± 2.53[abd] | 93.33 ± 2.44[bc] | 5.95 ± 0.54[a] | 5.98 ± 0.25[ab] | 6.22 ± 0.16[bc] |
| Tukey's $HSD_{0.05}$ | 10.27 | 10.78 | 9.91 | 2.05 | 1.08 | 0.66 |

Values are mean ± standard error. Different letters represent significant differences ($P < 0.05$).

**Table 6:** Effect of basal substrates on the mycelial growth (A), growth rate (B), complete spawn mycelium running on the substrate (C), and times required for primordia formation (D) of strain PN2.

| Basal substrates | Mycelial diameter (mm) | | | | Growth rate (mm/day) | | | | Complete spawn mycelium running on the substrate (days) | Times required for primordia formation (days) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 days | 10 days | 15 days | 25 days | 5 days | 10 days | 15 days | 25 days | | |
| Cotton waste | 26.4 ± 1.2[a] | 66.5 ± 1.8[b] | 100.2 ± 1.2[b] | 133.3 ± 0.8[a] | 5.29 ± 0.23[a] | 6.65 ± 0.18[b] | 6.68 ± 0.08[b] | 5.33 ± 0.03[a] | 25.88 ± 0.30[b] | 11.25 ± 0.25[b] |
| Rice straw | 26.8 ± 1.1[a] | 70.2 ± 1.0[ab] | 109.9 ± 1.1[a] | 133.0 ± 0.9[a] | 5.36 ± 0.22[a] | 7.02 ± 0.10[ab] | 7.33 ± 0.08[a] | 5.32 ± 0.04[a] | 26.22 ± 0.47[b] | 10.33 ± 0.29[b] |
| Sawdust | 26.6 ± 0.7[a] | 60.0 ± 0.6[c] | 89.4 ± 1.0[c] | 119.8 ± 1.2[c] | 5.31 ± 0.15[a] | 6.00 ± 0.06[c] | 5.96 ± 0.07[c] | 4.79 ± 0.05[c] | 27.33 ± 0.33[b] | 14.00 ± 0.29[a] |
| Corn cob | 26.0 ± 0.8[a] | 72.0 ± 1.5[a] | 103.1 ± 1.2[b] | 126.7 ± 1.0[b] | 5.20 ± 0.15[a] | 7.20 ± 0.15[a] | 6.87 ± 0.08[b] | 5.07 ± 0.04[b] | 29.11 ± 0.42[a] | 10.44 ± 0.29[b] |
| Tukey's $HSD_{0.05}$ | 0.36 | 0.50 | 0.43 | 0.37 | 0.73 | 0.50 | 0.29 | 0.15 | 1.50 | 1.09 |

Values are mean ± standard error. Different letters represent significant differences ($P < 0.05$).

**Table 7:** Effect of substrates on the number of fruiting bodies per bunch, cap diameter, yield, and biological efficiency of strain PN2.

| Basal substrates | Number of fruiting bodies/bunch | Cap diameter (cm) | Yield (g/bag) | BE (%) |
|---|---|---|---|---|
| Cotton waste | 8.81 ± 0.53[a] | 5.17 ± 0.12[a] | 582.71 ± 21.48[a] | 48.56 ± 5.37[a] |
| Rice straw | 7.91 ± 0.64[ab] | 4.60 ± 0.15[b] | 462.04 ± 13.39[b] | 38.51 ± 3.35[b] |
| Sawdust | 9.12 ± 0.75[a] | 4.62 ± 0.14[b] | 447.380 ± 17.44[bc] | 37.28 ± 4.40[bc] |
| Corn cob | 6.40 ± 0.35[b] | 3.5 ± 0.16[c] | 398.88 ± 7.87[c] | 33.24 ± 1.97[c] |
| Tukey's $HSD_{0.05}$ | 2.07 | 0.53 | 60.78 | 5.07 |

Values are mean ± standard error. Different letters represent significant differences ($P < 0.05$).

significant difference test ($P < 0.05$), with the results indicated using letters to show the significance of the differences between treatments.

## 3. RESULTS

### 3.1. Effect of Temperature on Mycelial Growth

The mycelial growth of strain PN2 was influenced by incubation temperature [Table 3]. Strain PN2 can grow within the temperature range of 15°C-30°C but not at 35°C. After 6 days of incubation, the mycelial diameter of the strain grown on PGA medium was highest at 25°C (83.25 mm), followed by 30°C (69.67 mm) and 20°C (29.75 mm). The mycelial density of strain PN2 was significantly higher at 25°C and 30°C, while it was lower at 15°C. Therefore, 25°C was considered the optimal temperature for the mycelial growth of strain PN2.

### 3.2. Effect of Media on Mycelial Growth

After 3 days of incubation at 25°C, no mycelial growth of strain PN2 was observed on the PDA medium [Table 4]. Strain PN2, when grown on Raper medium, exhibited a mycelial diameter of 82.33 mm on day 6, which was higher than that on other media. The strain displayed high-density mycelial growth when cultured on YMA and Raper media. Collectively, the Raper medium was determined to be the optimal medium for the growth of strain PN2.

### 3.3. Mycelial Growth on Spawn Substrates

Strain PN2 displayed growth on all spawn substrate formulas. After 15 days of incubation, the strain exhibited the highest mycelial diameter and high mycelial density in Treatment I (106.33 mm) and Treatment II (99.42 mm) [Table 5]. In contrast, the strain showed lower mycelial diameter in Treatment VI (83.75 mm) compared to all other treatments ($P < 0.05$), except Treatment VII. The lowest growth rate was observed when strain PN2 was grown on Treatment VI. Considering mycelial density, growth rate, and the cost-effectiveness of sawdust compared to rice grain, Treatment II (79% rice grain, 18% sawdust, 2% rice bran, 1% $CaCO_3$) was the optimal spawn substrate for producing mother spawn for the cultivation of strain PN2.

### 3.4. Cultivation Characteristics

Mycelial growth of strain PN2 was observed in all cultivation substrates [Table 6]. The time required for complete mycelial growth significantly varied ($P < 0.05$) among the treatments. After 25 days of incubation, strain PN2 displayed a higher mycelial diameter on cotton waste (13.33 cm) and rice straw (13.3 cm) compared to sawdust (11.98 cm) and corn cob (12.67 cm) ($P < 0.05$). The growth rate of strain PN2 was not significantly different across substrates on day 5. However, rice straw resulted in the highest growth rate of strain PN2 (7.3 mm/day) on day 15. The strain cultivated on corn cobs required 29.11 days after spawn inoculation to complete spawn mycelium running, which was longer than on cotton waste, rice straw, and sawdust. In contrast, strain PN2 exhibited the fastest mycelial extension when grown on cotton waste, requiring a relatively short time (26.22 days) for complete mycelium to run on this substrate. The minimum time required for pinhead initiation of strain PN2, when cultivated on cotton waste, rice straw, sawdust, and corn cob, was 11.25, 10.33, 14.0, and 10.44 days, respectively. In terms of mycelial density, strain PN2 exhibited a higher density when grown on cotton waste compared to other substrates [Figure 2].

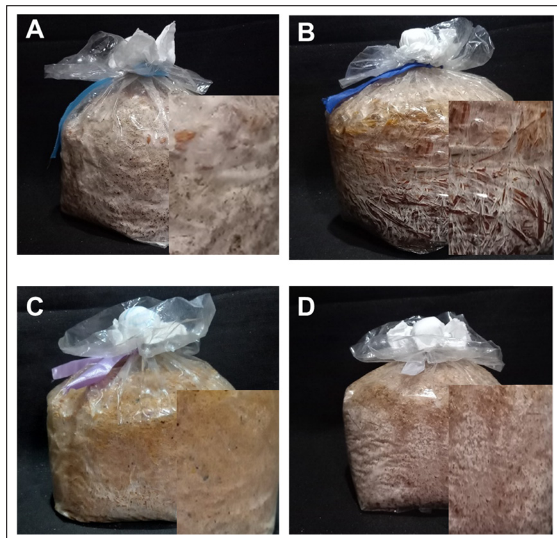


**Figure 2:** Density of mycelium of strain PN2 cultivated on cotton **(A)**, rice straw **(B)**, sawdust **(C)**, and corn cob **(D)**.

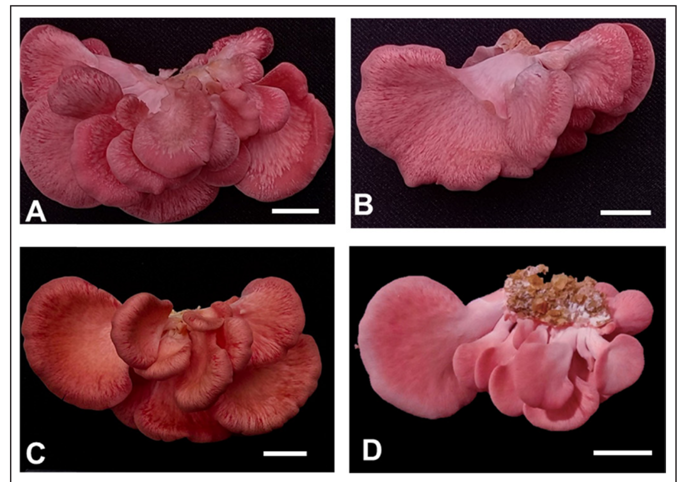


**Figure 3:** Fruiting body of strain PN2 cultivated cotton waste **(A)**, rice straw **(B)**, sawdust **(C)**, and corn cob **(D)**. Bar scale, 2 cm.

Different cultivation substrates exhibited significant effects on the number of fruiting bodies per bunch, cap diameter, yield, and BE of strain PN2 [Table 7]. Among the tested cultivation substrates, corn cob showed the lowest number of fruiting bodies per bunch (6.39 fruiting bodies/bunch) and the smallest cap diameter (3.55 cm) [Figure 3]. In contrast, cotton waste yielded the largest cap diameter (5.17 cm) and the highest yield (582.711 g/bag). Strain PN2 achieved the highest biological efficiency (BE) when cultivated on cotton waste (48.56%), followed by rice straw (38.51%) and sawdust (37.28%). Based on the mycelial growth rate, the time required for complete spawn mycelium colonization of the substrates, primordia formation, and BE, cotton waste was identified as the most suitable substrate for cultivating strain PN2.

## 4. DISCUSSION

Determining the optimal culture conditions for mushroom mycelial growth is a crucial step in the cultivation of mushrooms. Generally, the ideal temperature for mycelial growth varies depending on the mushroom species. For instance, *Tuber koreanum*, *Pleurotus ostreatus*, and *Lepista sordida* exhibited their highest mycelial growth rates at 25°C [28], 28°C [29], and 20-25°C [30], respectively. Based on growth diameter and mycelial density, 25°C is considered the optimal temperature for the mycelial growth of strain PN2 [Table 3]. This temperature falls within the optimal range (ranging from 23°C to 30°C) for the mycelia of five wild *P. djamor* strains [31].

Since the medium is the primary source of essential nutrients for mushroom growth, it plays a pivotal role in mushroom production [30]. The optimal medium for mushroom growth appears to be genus- and species-specific. For example, PDA, oatmeal yeast agar, and malt yeast peptone agar media were highly effective in promoting the mycelial growth of *Lepista sordida* [30], *Hericium erinaceus* [32], and *Coprinus comatus* [33], respectively. PDA and YDA were the most suitable media for the mycelial growth of the oyster mushroom *Pleurotus ostreatus* [34]. In contrast, four media (PDA, YDA, sweet potato dextrose agar, and malt extract agar) did not significantly differ in supporting mycelium growth for the oyster mushroom *Pleurotus cystidiosus* [34]. Considering mycelial growth rate and density, Raper medium (glucose 20 g/l, yeast extract 2 g/L, pepton 2 g/L, $KH_2PO_4$ 0.46 g/L, $MgSO_4.7H_2O$ 0.5 g/L, $K_2HPO_4$ 1 g/L, agar 17 g/L) was identified as the optimal medium for the growth of *P. djamor* PN2.

Spawn materials significantly impact the time required for spawn colonization and the mushroom yield [35]. Four types of spawn materials are used in mushroom cultivation: sawdust spawn, grain spawn, liquid spawn, and stick spawn [35]. Although grain spawn is the most commonly used, it causes increased production costs. Given that rice grain is more expensive than sawdust, using sawdust as a substrate for upscaling the mycelium is more economically efficient [36, 37]. Moreover, the nutrient content of rice grain is higher than that of sawdust, leading to a higher risk of contamination. Rice grain comprises protein (5%-12%), starch (50%-90%), vitamins (thiamine, riboflavin, and niacin), minerals (Ca, Mg, and P), and trace elements (Fe, Cu, Zn, and Mn) [38]. The main components of the sawdust are cellulose (47.82%) and lignin (33.29%) [39]. Therefore, in this study, we aimed to optimize spawn substrates for upscaling pink oyster mushroom mycelium by investigating various ratios of rice grain to sawdust. The ratio of grain to sawdust significantly affected the mycelial growth rate of strain PN2 [Table 5]. While no significant difference in mycelial diameter and growth rate was observed between treatment I (99% rice grain and 1% $CaCO_3$) and treatment II (79% rice grain, 18% sawdust, 2% rice bran, and 1% $CaCO_3$), treatment II was identified as a suitable spawn substrate for scaling the mycelial growth of strain PN2 due to its cost-effectiveness compared to treatment I. When mycelium is inoculated into a new medium, it requires time to adapt and begin colonizing the surrounding environment, resulting in a slow growth rate in this phase. As the mycelium grows and utilizes nutrients from the medium, the availability of these nutrients diminishes over time, causing a gradually declining growth rate [Tables 5 and 6]. This could explain why the mycelial growth rate of strain PN2 varied depending on the duration of incubation, which is consistent with findings from previous studies on *Pycnoporus sanguineus* [40], *Auricularia* sp. [41], and *Fomitopsis betulina* [42] [Tables 5 and 6].

Substrate composition is an essential factor affecting mushroom yield and nutritional value [43, 44]. Since substrates rich in lignocellulosic materials, such as straw, sawdust, and cotton waste, are cost-effective, renewable, and plentiful, they are the preferred choices for mushroom cultivation [45, 46]. In Vietnam, cotton, sawdust, rice straw, and corncob

are abundant agro-residues and are selected as the primary materials for mushroom cultivation. Therefore, we used these waste materials to optimize the cultivation of strain PN2. Nitrogen is an essential element required by all fungi for synthesizing nitrogen-containing compounds, such as pyrimidines, purines, proteins, and chitin, which forms the cell wall and consists of a (1–4)-linked unit of N-acetylglucosamine [44]. The substrate material used alone for cultivating mushrooms may lack sufficient nitrogen and other essential nutrients to ensure optimal growth [47]. For example, Mandeel et al. reported that *P. ostreatus* cultivated on sawdust exhibited a low yield due to the low nitrogen content of sawdust, which is insufficient to support mycelial growth [48]. Supplementing the cultivation substrate with wheat bran can enhance the yield and biological efficiency of oyster mushrooms [49, 50]. Accordingly, in this study, we added wheat bran to the cultivation substrate to provide a nitrogen source for the growth of pink oyster mushrooms.

The time required for complete mycelium colonization of spawn media depended on cultivation substrates [Table 6], which is consistent with a previous study conducted by Iqbal [51]. The minimum time for *Pleurotus* sp. to fully colonize substrates was 37 and 40 days for rice straw and sugarcane bagasse, respectively [51]. These differences in mycelium running time may be related to the different compositions of polysaccharide content in each type of substrate [10]. Additionally, the mycelial growth rate of oyster mushrooms is influenced by substrate particle size [52]. Smaller substrate particles (typically cut to 5-6 cm) provide a larger surface area for microorganisms to thrive. However, the use of very small particles can lead to substrate compaction, which may hinder proper aeration and reduce the oxygen available for microorganisms [52]. The variation in pinhead formation depends on various factors, such as temperature and substrate [53]. For *Pleurotus ostreatus* cultivated on waste paper, the typical pinhead initiation period falls within 9 to 14 days [53]. Substrates containing high-quality lignin and cellulose also take longer to initiate pinning than substrates with lower lignin and cellulose contents [54]. Since sawdust has higher-quality lignin and cellulose contents, the minimum time required for pinhead initiation of strain PN2 cultivated on this substrate is longer than that of other substrates [Table 6].

Few studies have optimized the substrate for cultivating *P. djamor*. This macro fungus can form fruiting bodies when cultivated on corn cobs [13], paddy straw [14], wheat straw [15], combined dairy manure-food waste digestate [16], and coffee pulp [5]. Previous studies identified quinoa stalk and bean straw as suitable substrates for the cultivation of *P. djamor* [19, 20]. Additionally, wheat straw has been found to be more effective than paddy straw and chickpea straw for cultivating *P. djamor* [15]. The highest biological efficiency for *P. djamor* was achieved using a combination of rice straw, cocopeat, and rice bran in a 7:3:1 ratio [55]. Selvakumar reported that straw is a more suitable basal substrate for cultivating *P. djamor* compared to sugarcane bagasse, cotton waste, sawdust, and coir pith [18]. The contrasting findings across studies could stem from variations in strains and culture conditions, such as temperature and humidity. Substrate selection for mushroom cultivation typically depends on locally available lignocellulosic waste. Because quinoa, wheat straw, chickpea straw, and cocopeat are not widely available in Vietnam, we could not use these substrates to optimize the cultivation of *P. djamor*. Instead, we selected cotton waste, rice straw, sawdust, and corncob, which are abundant in Vietnam. Our findings revealed that cotton waste is an ideal substrate for the cultivation of *P. djamor*, which is consistent with a study conducted by Ashraf [17]. Since each substrate contains different amounts of cellulose, hemicellulose, and lignin, the use of substrate mixtures may enhance the biological efficiency of mushrooms compared with using individual substrates [56]. Accordingly, further studies are required to analyze the composition of substrate materials and optimize the combination of cotton with other substrates to enhance the yield of *P. djamor*. Among the various wild pink oyster mushroom strains examined by Kalaw et al., strain TLPD3 displayed a biological efficiency of 25.07% [31], which was lower than that of strain PN2 in this study [Table 7]. Therefore, strain PN2 is a promising candidate for commercial cultivation on an industrial scale. Further research is needed to optimize culture conditions, such as temperature and humidity, to enhance biological efficiency for commercial-scale production.

## 5. CONCLUSIONS

The present study demonstrated that *P. djamor* PN2 exhibited the most robust mycelial growth when cultivated on YMA and Raper media at 25°C. The optimal spawning material for scaling up the mycelial growth of strain PN2 was determined to be treatment II (79% rice grain, 18% sawdust, 2% rice bran, and 1% $CaCO_3$). Our study emphasizes that the use of cotton waste as a substrate for cultivating *P. djamor* results in a high yield, making it a promising option for *P. djamor* cultivation. This study offers valuable insights into the large-scale cultivation of *P. djamor*.

## 6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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## 8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

## 10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

## 11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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## REFERENCES


1. Raman J, Jang K-Y, Oh Y-L, Oh M, Im J-H, et al. Cultivation and nutritional value of prominent *Pleurotus* spp.: An overview. Mycobiology. 2021;49:1-14.
2. Sánchez C. Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Appl Microbiol Biotechnol. 2010;85:1321-37.
3. Kapahi M, Sachdeva S. Mycoremediation potential of *Pleurotus* species for heavy metals: a review. Bioresour Bioprocess. 2017;4:32.
4. Salmones D, Mata G. Laccase production by *Pleurotus djamor* in agar media and during cultivation on wheat straw. Rev Mex Mic. 2015;42:17-23.
5. Vega A, De León JA, Miranda S, Reyes SM. Agro-industrial waste improves the nutritional and antioxidant profile of *Pleurotus djamor*. Clean Waste Syst. 2022;2:100018.
6. Corrêa RCG, Brugnari T, Bracht A, Peralta RM, Ferreira ICFR. Biotechnological, nutritional and therapeutic uses of *Pleurotus* spp. (Oyster mushroom) related with its chemical composition: A review on the past decade findings. Trends Food Sci Technol. 2016;50:103-17.
7. Mleczek M, Budka A, Siwulski M, Mleczek P, Budzyńska S, *et al.* A comparison of toxic and essential elements in edible wild and cultivated mushroom species. Eur Food Res Technol. 2021;247:1249-62.
8. Dulay RMR, Bernardo AP, Cruz AGD, Kalaw SP, Reyes RG. Effects and myco-accumulation of lead (pb) in five *Pleurotus* mushrooms. Int J Biol Pharm Allied Sci. 2015;4:1664-77.
9. Širić I, Kumar P, Adelodun B, Abou Fayssal S, Bachheti RK, et al. Risk assessment of heavy metals occurrence in two wild edible oyster mushrooms (*Pleurotus* spp.) collected from Rajaji National Park. J Fungi. 2022;8:1007.
10. De A, Mridha D, Roychowdhury T, Bandyopadhyay B, Panja AS. Substrate level optimization for better yield of oyster mushroom (*Pleurotus ostreatus*) production, using different ratio of rice straw and sugarcane bagasse. World J Microbiol Biotechnol. 2023;39:270.
11. F. Mohamed M, Nassef DMT, A. Waly E, M. Kotb A. Production of oyster mushroom (*Pleurotus* spp.) intercropped with field grown Faba Bean (Vicia faba L.). Asian J Crop Sci. 2013;6:27-37.
12. Naraian R, Singh MP, Ram S. Supplementation of basal substrate to boost up substrate strength and oyster mushroom yield: An overview of substrates and supplements. Int J Curr Microbiol Appl Sci. 2016;5:543-53.
13. García-Hernández PN, Baas-López JM, Toledano-Thompson T, Valdez-Ojeda R, Pacheco-Catalán D. Revalorization of *Pleurotus djamor* fungus culture: fungus-derived carbons for supercapacitor application. Sustainability. 2021;13:10765.
14. Jegadeesh R, Lakshmanan H, Kab-Yeul J, Sabar-Atnam V, Raaman N. Cultivation of Pink Oyster mushroom *Pleurotus djamor* var. roseus on various agro-residues by low cost technique. J Mycopathol Res. 2018;56:213-20.
15. Satpal S, Gopal S, Kumar R. Effect of different substrates on the growth and yield of Oyster mushroom (*Pleurotus djamor*). Int J Agric Sci. 2017;9:3721-3.
16. Jasinska A, Prasad R, Lisiecka J, Roszak M, Stoknes K, et al. Combined dairy manure-food waste digestate as a medium for *Pleurotus djamor*—mineral composition in substrate and bioaccumulation of elements in fruiting bodies. Horticulturae. 2022;8:934.
17. Ashraf J, Ali MA, Ahmad W, Ayyub CM, Shafi J. Effect of different substrate supplements on oyster mushroom (*Pleurotus* spp.) production. Food Sci Technol. 2013;1:44-51.
18. Selvakumar P, Rajasekar S, Babu AG, Periasamy K, Raaman N, et al. Improving biological efficiency of *Pleurotus* strain through protoplast fusion between *P. ostreatus* var. *florida* and *P. djamor* var. *roseus*. Food Sci Biotechnol. 2015;24:1741-8.
19. İnci Ş, Kirbağ S, Akyüz M. Valorization of local agro-residues for the cultivation of *Pleurotus djamor* (Rumph. Ex Fr.) Boedijn and their effects on nutritional value. *Biomass Convers Biorefinery*. Epub ahead of print 22 March 2024.
20. Atila F. Evaluation of suitability of various agro-wastes for productivity of *Pleurotus djamor*, *Pleurotus citrinopileatus* and *Pleurotus eryngii* mushrooms. J Exp Agric Int. 2017;17:1-11.
21. Cruz-Moreno BA, Pérez AAF, García-Trejo JF, Pérez-García SA, Gutiérrez-Antonio C. Identification of secondary metabolites of interest in *Pleurotus djamor* using agave tequilana bagasse. Molecules. 2023;28:557.
22. Şule I, Akyüz M, Kirbag S. Antimicrobial, antioxidant, cytotoxicity and DNA protective properties of the pink oyster mushroom, *Pleurotus djamor* (Agaricomycetes). Int J Med Mushrooms. 2023;25:55-6.
23. Lopes AD, De Melo Santana Gomes S, Schwengber RP, Carpi MCG, Dias-Arieira CR. Control of *Meloidogyne javanica* with *Pleurotus djamor* spent mushroom substrate. Chem Biol Technol Agric. 2023;10:13.
24. Akhil GL, Susha ST, Radhika NS, Sajeesh PK, Binitha NK. Performance of different oyster mushrooms in five agro-ecological zones of Kerala. J Tropic Agri. 2023;61:207-17.
25. Yang W, Guo F, Wan Z. Yield and size of oyster mushroom grown on rice/wheat straw basal substrate supplemented with cotton seed hull. Saudi J Biol Sci. 2013;20:333-8.
26. Pham V, Pham N, Nguyen H, Nguyen T, Nguyen T, et al. The relationship between mycelial growth and fruit body's yield of oyster mushrooms (*Pleurotus* spp.) collected from southern Vietnam. Int J Agric Technol. 2023;19:203-14.
27. Dissasa G. Cultivation of different oyster mushroom (*Pleurotus* species) on coffee waste and determination of their relative biological efficiency and pectinase enzyme production, Ethiopia. Int J Microbiol. 2022;2022:1-10.
28. Gwon J-H, Park H, Eom A-H. Effect of temperature, ph, and media on the mycelial growth of *Tuber koreanum*. Mycobiology. 2022;50:238-43.
29. Hoa HT, Wang C-L. The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology. 2015;43:14-23.
30. Alim Y, Sidhoum W, Dib S. First record of the edible mushroom *Lepista sordida* in Western Algerian Forest: nutritional value and physicochemical parameters of mycelial culture. J Fungi. 2023;9:858.
31. Kalaw SP, Dulay RMR, Damaso Jr EJ, Ramos JC, Rosario MAG del, et al. Nutritional and physical requirements for mycelial growth and fruiting body production of six strains of *Pleurotus* djamor from Luzon Island, Philippines. Asian J Agric Biol. Epub ahead of print February 2022:3.
32. Gonkhom D, Luangharn T, Hyde KD, Stadler M, Thongklang N. Optimal conditions for mycelial growth of medicinal mushrooms belonging to the genus *Hericium*. Mycol Prog. 2022;21:82.
33. Jang M-J, Lee Y-H, Liu J-J, Ju Y-C. Optimal Conditions for the Mycelial Growth of *Coprinus comatus* Strains. Mycobiology. 2009;37:103-8.
34. Hoa HT, Wang C-L. The Effects of Temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). Mycobiology. 2015;43:14-23.
35. Zhang W-R, Liu S-R, Kuang Y-B, Zheng S-Z. Development of a novel spawn (Block Spawn) of an edible mushroom, *Pleurotus ostreatus*, in liquid culture and its cultivation evaluation. Mycobiology. 2019;47:97-104.

36. Nguyen LT, Le VV, Nguyen BTT, Nguyen HTT, Tran AD, et al. Optimization of mycelial growth and cultivation of wild *Ganoderma sinense*. BioTechnologia. 2023;104:65-74.
37. Nguyen BTT, Le VV, Trang Thi H, Thi Nguyen L, Dong Tran A, *et al.* Successful rescue of wild *Trametes versicolor* strains using sawdust and rice husk-based Substrate. Pak J Biol Sci. 2020;24:374-82.
38. Razzaq A, Ali A, Safdar LB, Zafar MM, Rui Y, et al. Salt stress induces physiochemical alterations in rice grain composition and quality. J Food Sci. 2020;85:14-20.
39. Boadu KB, Nsiah-Asante R, Antwi RT, Obirikorang KA, Anokye R, et al. Influence of the chemical content of sawdust on the levels of important macronutrients and ash composition in Pearl oyster mushroom (*Pleurotus ostreatus*). PLOS ONE. 2023;18:e0287532.
40. Wilson CM, Rich Milton RD, Mary Jhane GV, Renato GR. Mycelial biomass and biological activities of Philippine mushroom *Pycnoporus sanguineus* in time-course submerged culture. J Appl Biol Biotechnol. Epub ahead of print 14 September 2020;8(05):88-93.
41. Devi MB, Singh SM, Singh NI. Biomass production in *Auricularia* spp.(Jew s ear) collected from Manipur, India. Int J Curr Microbiol App Sci. 2015;4(6):985-9.
42. Krupodorova TA, Barshteyn VYu, Kizitska TO, Pokas EV. Effect of cultivation conditions on mycelial growth and antibacterial activity of *Lentinula edodes* and *Fomitopsis betulina*. Czech Mycol. 2019;71:167-86.
43. Elkanah FA, Oke MA, Adebayo EA. Substrate composition effect on the nutritional quality of *Pleurotus ostreatus* (MK751847) fruiting body. Heliyon. 2022;8:e11841.
44. Hoa HT, Wang C-L, Wang C-H. The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus* ). Mycobiology. 2015;43:423-34.
45. Akcay C, Ceylan F, Arslan R. Production of oyster mushroom (*Pleurotus ostreatus*) from some waste lignocellulosic materials and FTIR characterization of structural changes. Sci Rep. 2023;13:12897.
46. Devi KB, Malakar R, Kumar A, Sarma N, Jha DK. Ecofriendly utilization of lignocellulosic wastes: mushroom cultivation and value addition. In: Kuddus M, Ramteke P, eds, Value-Addition in Agri-food Industry Waste Through Enzyme Technology. Elsevier; 2023:237–254.
47. Mohamed MF, Refaei EFS, Abdalla MMA, Abdelgalil SH. Fruiting bodies yield of oyster mushroom (*Pleurotus* columbinus) as affected by different portions of compost in the substrate. Int J Recycl Org Waste Agric. 2016;5:281-8.
48. Girmay Z, Gorems W, Birhanu G, Zewdie S. Growth and yield performance of *Pleurotus ostreatus* (Jacq. Fr.) Kumm (oyster mushroom) on different substrates. AMB Express. 2016;6:87.
49. Lucas De Jesus G, José Lavoranti O, Schafer G, Dias De Oliveira G, Scheffer De Andrade Silva R, et al. Nutrient uptake in supplemented substrate by oyster mushroom. World J Microbiol Biotechnol. 2023;39:175.
50. Vieira FR, De Andrade MCN. Optimization of substrate preparation for oyster mushroom (*Pleurotus ostreatus*) cultivation by studying different raw materials and substrate preparation conditions (composting: phases I and II). World J Microbiol Biotechnol. 2016;32:190.
51. Iqbal B, Khan H, Khan I, Shah B, Naeem A, et al. Substrates evaluation for the quality, production and growth of oyster mushroom (*Pleurotus* florida Cetto). J Entomol Zool Stud. 2016;4:98-108.
52. Bellettini MB, Fiorda FA, Maieves HA, Teixeira GL, Ávila S, et al. Factors affecting mushroom *Pleurotus* spp. Saudi J Biol Sci. 2019;26:633-46.
53. Tesfay T, Godifey T, Mesfin R, Kalayu G. Evaluation of waste paper for cultivation of oyster mushroom (*Pleurotus ostreatus*) with some added supplementary materials. AMB Express. 2020;10:15.
54. Oei P. Mushroom Cultivation: Appropriate Technology for Mushroom Growers. Backhuys; 2003. https://books.google.co.kr/books?id=1yk5AAAACAAJ.
55. Zurbano L, Bellere A, Savilla L. Mycelial growth, fruiting body production and proximate composition of *Pleurotus djamor* on different substrate. *CLSU Int J Sci Technol*;2. Epub ahead of print 31 March 2017; 2( 1) : 7-13.
56. Owaid M, Abed A, Nassar B. Recycling cardboard wastes to produce blue oyster mushroom *Pleurotus ostreatus* in Iraq. Emir J Food Agric. 2015;27:537.

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