The Growth Morphology and Yield of Grey Oyster Mushrooms (*Pleurotus sajor-caju*) Subjected to Different Durations of Acoustic Sound Treatment

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Abstract. *Pleurotus sajor-caju* (grey oyster mushroom) is a typical edible fleshy fungi. It has fruiting bodies shape like an oyster that contain high nutrient such as carbohydrates, proteins, fats, minerals and multivitamins. Due to its high nutritional values, the demand of grey oyster mushrooms keeps increasing. In this study, the effect of different durations and intervals of acoustic sound treatment on growth morphology, yield enhancement and the quality of the mushroom were studied. Using sound of fire cracker at constant intensity of 75 dB, four different acoustic sound treatments had been applied on the mushroom bags which were 5 pops at 5-day interval, 15 pops at 5-day interval, 5 pops at 10-day interval and 15 pops at 10-day interval and mushroom bags without any sound treatment acted as control. The results showed that the controls had longer time for mycelium to fill-up the bags compared to sound treated bags. Besides, controls also showed lower yield in terms of number of fruiting bodies and biological efficiencies (%) compared to all acoustic sound treated bags. However, 15 pops at 10-day interval had the shortest time taken to complete all the growth stages (mycelium growth, emergence of pinhead and formation of fruiting bodies). For colour of the mushroom, there were significant differences (P<0.05) in L* values for 15 pops at 5-day interval and no significant different (P>0.05) was observed for a* (redness) and b* (yellowness) values. Controls had the highest firmness value compared to mushroom with sound treatment. In summary, 15 pops at 10-day interval was the best treatment duration and interval as it took the shortest time for different growth stages with the greatest yield of mushrooms.

1. Introduction

*Pleurotus* mushroom is a fleshy fungi that its fruiting bodies contains spore and comes under the family of Pleurotaceae [6]. It is commonly known as oyster mushroom as it fruiting bodies open up like a shape of an oyster during its morphogenesis process [7]. It provides various macro- and micronutrients for our health. Besides, it also accommodate a compelling levels of zinc, potassium, iron, calcium, phosphorus, Vitamin C, niacin, folic acid, low in calories, high in vegetable proteins, fibre and vitamins C, D and B-complex.[1]. The demand of mushroom market in Malaysia gradually increasing due to the increment of human population and impetus in mushroom uses whether in culinary and medicine. In order to fulfil the demand, various methods could be used to boost the growth and yield of the oyster mushroom. One of the method is sound treatment. Sound treatment has been verified could change the cell cycle based on different sound acoustics, sound intensities and sound frequencies [2]. Various acoustic sounds have been used as physical stimulant that can enhance the growth and yield of oyster mushrooms such as hard core music, soothing instrumental, thunder storm and quranic recital [4].

2. Methodology

2.1 Substrate Preparations
The sawdust will be mixed with rice bran, calcium carbonate and water in the ratio of 100:10:1 respectively. The substrate was manually mixed with water until the desired texture have been achieved. The wet substrate was then filled into polypropylene bags (29 x 46cm) about 850g in each bag. The bags were closed and tied up into cylindrical shape and closed with cotton-stuffed caps. The bags were then sterilized at 121\degree C for 30 minutes using autoclave or conventionally using steamer for 10 hrs. After sterilization the bags were cooled overnight and ready to be inoculated with Pleurotus sp. spawn. The inoculation process was carried out in a laminar flow.

2.2 Mushroom Cultivation
The inoculated bags were incubated (spawn running) at 28-30\degree C in the dark room (5% light) with RH of 75%-85% until mycelia had completely covered the bags. All the bags were labelled according to the treatment applied and arranged vertically on the racks in the mushroom cultivation chamber. This is because it can accelerate the process of mycelium colonization due to the pulling force of gravity. It took about 29 to 35 days for the mycelium to fill-up the bags. After completion of spawn running stage, the bags were then placed into a mushroom house where the environmental conditions were maintained at 28-30\degree C; 80 – 90% RH and about 30% light. Water was sprayed on the floor regularly to maintain the relative humidity up to the desired level in the form of fine mist with the help of a nozzle. The bags were opened for cropping (emergence of pinhead and fruiting body formation). Fruiting bodies that have achieved maturity level were then harvested by pulling from the substrate bags. The bag was then covered with the caps to close of the mushroom bags and left for 7 days for the next fruiting cycle in order to let the tissue of mycelium to accumulate the nutrients and to recover from injury.

2.3 Physical Stimulant Treatments
Acoustic sounds is the physical stimulant treatment that was carried out based on completely randomised design with two main factors which are treatment duration and treatment interval. Acoustic sound treatment was applied on the mushroom bag during spawn running stage and before fruiting body formation. The sound of fire cracker at 75 decibels was imposed on the mushroom bags for durations of 5 and 15 pops at 5- to 10-day intervals.

2.4 Growth Performance Analyses
The growth performance of the Pleurotus sajor-caju can be evaluate by determination of mycelium growth rate for each bag. The growth rate of mycelium for different durations and intervals of acoustic sound treatment and control were determined. Growth rate of mycelium treated with acoustic sounds were measured from the neck of the bag until the bottom of the bag when the mycelium has fill-up the bag by using a ruler in terms of unit of cm per day at every five day interval during spawn-running process.

Meanwhile, growth performance of the mushroom was determined by noting the numbers of days taken to reach the various growth stages of the grey oyster mushroom which were mycelium fill-up the bag, pinhead emergence and fruiting bodies formation. Each treatment was represented by 5 mushroom bags.

2.5 Mushroom Yield
Once the fruiting bodies have reached maturity, the mushroom can be harvested from the substrate bags. The yields of mushroom fruiting bodies were for each treatment were determined in terms number of fruiting bodies, fresh mushroom weight and percentage of biological efficiency, all the samples from the first harvesting until the last harvesting cycle were collected. All fruiting bodies harvested. Percentage biological efficiency (BE) was calculated as total fresh weight of harvested mushroom (g)/dry weight of substrate x 100.

2.6 Physico-chemical Analyses
Physico-chemical analyses of the mature fruiting bodies of grey oyster mushroom were established based on pileus diameter (largest and smallest), colour, texture (firmness) and percentage of moisture content.
2.6.1 Pileus Diameter. The largest and smallest sizes of the pileus of the mushrooms that had been harvested from different sound treatments and control were chosen. The mushroom’s pileus was measured side to side using a ruler as the diameter measurement.

2.6.2 Colour Determination. Colour Measurement was determined on the mushroom pileus using Minolta Chroma Meter (Model CR200 Trimulus Colour Analyser, Minolta Camera Co. Ltd., Japan.) using CIELAB colour parameters, L* (lightness), a* (greenness (-) to redness (+)), and b* (blueness (-) to yellowness (+)) values. A standard white tile was used to calibrate the equipment. The colour measurements was carried out immediately after harvesting.

2.6.3 Texture (firmness) Determination. Texture of fresh mushroom was determined by using texture analyser TA.XTplus (Stable Macro System) using P/2 (2 mm stainless steel probe). The selected pileus was placed on the stage of the texture analyser. Three different points were taken from the same surface of the harvested grey oyster mushroom to analyse the firmness of the pileus. In the penetration test, a 2 mm diameter cylinder probe was used to penetrate the harvested grey oyster mushroom pileus from top to 5mm depth. The firmness of the mushroom was determined by the maximum positive force for each test.

2.6.4 Moisture Content. Moisture analyser AND MX-50 was used to determine the moisture content of the mushroom pileus and stem. Freshly harvested grey oyster mushroom was weighed and placed on the pan at 105℃ for about 30-45 minutes to obtain the percentage moisture content of the samples.

2.7 Experimental Design and Statistical Analysis
The experiment was designed based on Completely Randomized Design (CRD) with 1 control and 4 different durations and intervals of acoustic sound treatment. Each treatment was represented by 5 replicates. All the data obtained were analysed using statistical package SAS version 16.0 software. One-way Analysis of variances (ANOVA) was used to analyse the differences among the treatments. The significant means (P<0.05) was tested through Tukey's Studentized Range Test.

3. Results and Discussion

3.1 Mycelium Growth Rate
Mycelium growth rate of grey oyster mushroom’s inflicted to constant sound intensity of 75dB, 5 or 15 pops of fire cracker explosion at 5- or 10-day interval are showed in Table 1. It showed that all the mushroom bags treated with acoustic sound treatment had significantly higher mycelium growth rate (P<0.05) compared to control. The fastest rate of mycelium growth (0.791 cm/day) to fully colonized the bags was from the treatment of 15 fire cracker pops at 10-day interval. However, control bags showed the slowest of mycelium growth rate (0.698 cm/day). This showed that acoustic sound treatment had significant effect on the growth of mycelium of grey oyster mushrooms.

Table 1: The mycelium growth rate (cm/day) of grey oyster mushroom subjected to different conditions of acoustic sound treatments.

| Acoustic sound treatments  | Mycelium growth rate (cm/day) |
|----------------------------|--------------------------------|
| Control                    | 0.698±0.0359<sup>a</sup>       |
| 5 pops at 5-day interval   | 0.761±0.036<sup>a</sup>        |
| 15 pops at 5-day interval  | 0.766±0.0385<sup>a</sup>       |
| 5 pops at 10-day interval  | 0.791±0.0148<sup>a</sup>       |
| 15 pops at 10-day interval | 0.761±0.0328<sup>a</sup>       |

Note: Values are means of 5 replicates. Means (n=5) ± standard deviation.
a-b : Values bearing the different superscript within the same column are significantly different at 5% level (P<0.05).
3.2 The Number of Days for Mycelium to Fill-up the Bag, Pinhead Emergence and Fruiting Body Formation

There were significant differences (P<0.05) in the number of days taken for mycelium to fill-up the mushroom bags among all of the different acoustic sound treatment (Figure 1). It can be seen that the acoustic sound treated bags had shorter time for mycelium to fill-up the substrate bags compared to control. Acoustic sound treatment with 75dB, 15 pops at 10-day interval took the shortest time for mycelium to fill-up the substrate bags which was 29.6 days followed by 15 pops at 5-day interval (30.6 days), 5 pops at 5-day interval and 5 pops at 10-day interval (30.8 days).

![Figure 1: The number of days for mycelium to fill-up the bag, pinhead emergence and fruiting body formation of grey oyster mushroom (Pleurotus sajor-caju) subjected to different conditions of acoustic sound treatments. Vertical bars represent standard error.](image)

A study done by [3], had reported that sound wave was able to significantly generate the protective enzymes and endogenous hormones under the suitable intensity, frequency and durations of the acoustic sound treatment.

There were significantly (P<0.05) longer time needed for pinhead emergence for the treatment of 5 pops at 5-day interval compared to other acoustic sound treatments and even control. The shortest time taken was 1.2 days which was shown by the treatment of 15 pops at 5-day interval and 15 pops at 10-day interval. High sound intensity at 75dB with 5 day interval has the shortest time taken for mycelium to fill-up the bag with 23.6 days and the control took the longest time with 40.6 days [5].

The number of days for fruiting bodies formation was counted from the pinhead emergence until the matured mushrooms had been harvested. Treatment of 5 pops at 5-day interval took the shortest time for the fruiting bodies to form which was 1.4 days. However, 15 pops at 5-day interval took the longest compared to all sound treated bags and control which was 2.4 days.

In overall, acoustic sound treatment with durations of 15 pops at 10-day interval took the shortest time for mycelium growth rate and the shortest time taken to complete all the different stages. In this study, the enzymes activity of mushrooms actively stimulated by acoustic sound treatment under suitable durations and intervals thus actively promoted the growth of mushrooms.

3.3 Yield

For the number of fruiting bodies, there was no significant different (P>0.05) among all the different conditions of acoustic sound treatments (Table 2) even though all the acoustic sound treated bags had higher number of fruiting bodies as compared to control.
Table 2: The yield of *Pleurotus sajor-caju* subjected to different conditions of acoustic sound treatments.

| Acoustic sound treatments | No. of fruiting bodies | Total weight of fruiting bodies (g) | Biological efficiency (%) |
|---------------------------|------------------------|-------------------------------------|---------------------------|
| Control                   | 35.000±24.238*a        | 333.000±159.515*a                   | 47.334±23.951*a           |
| 5 pops at 5-day interval  | 51.000±29.875*a        | 456.000±64.265*a                    | 64.762±9.455*a            |
| 15 pops at 5-day interval | 78.000±48.683*a        | 329.000±241.076*a                   | 68.666±13.696*a           |
| 5 pops at 10-day interval | 46.000±6.519*a         | 428.000±32.519*a                    | 62.093±8.707*a            |
| 15 pops at 10-day interval| 65.000±23.184*a        | 538.000±159.044*a                   | 79.563±23.184*a           |

Note: Values are means of 5 replicates. Means (n=5) ± standard deviation.

a: Values bearing the different superscript within the same column are significantly different at 5% level (P<0.05).

The acoustic sound treatment of 15 pops at 5-day interval had significantly (P<0.05) the highest number of fruiting bodies which was 35 followed by 15 pops at 10-day interval (65), 5 pops at 5-day (51), and 5 pops at 10-day interval (46) for the total of 5 harvesting cycles. It is obvious that control had the lowest number of fruiting bodies which was only 35. It is reported that application of acoustic sound treatment at 111dB, had promoted the growth of paddy rice seeds. This is because the sound wave has the ability to change the cell cycle of the paddy rice cells thus increase the rate of reproduction [3]. However, 15 pops at 5-day interval had the lowest total weight of fruiting bodies (329 g) and 15 pops at 10-day interval had the highest total weight of fruiting bodies (538 g). Control obtained a total weight of fruiting bodies of 333 g, 5 pops at 5-day interval had 456 g, and 5 pops at 10-day interval had 428 g.

There was no significant different (P>0.05) among all the treatments. However, Control obtained the lowest biological efficiency as compared to acoustic sound treated bags (Table 2). From the results, it can be seen that control (47.3%) tend to show lower biological efficiency than all the sound treated bags (ranged from 62.093 % to 79.563 %). This showed that all acoustic sound treated bags had a greater yield potentials of mushrooms converted from the substrate.

3.4 Physico-chemical Properties

The pileus size of *Pleurotus sajo-caju* was determined by pileus diameter. There was no significant different (P>0.05) in the largest pileus diameter of harvested mushrooms among all the different durations of acoustic sound treatment (Table 2).

Table 3: The pileus diameter (cm) of *Pleurotus sajor-caju* subjected to different conditions of acoustic sound treatments.

| Acoustic sound treatments | Largest Pileus diameter (cm) | Smallest Pileus diameter (cm) |
|---------------------------|-------------------------------|-------------------------------|
| Control                   | 8.600±0.6325*a                | 2.700±1.446*a                 |
| 5 pops at 5-day interval  | 11.580±1.300*a                | 1.740±0.699*a                 |
| 15 pops at 5-day interval | 10.960±1.658*a                | 3.320±1542*a                  |
| 5 pops at 10-day interval | 11.760±3.713*a                | 2.740±1.757*a                 |
| 15 pops at 10-day interval| 10.500±1.149*a                | 1.900±0.418*a                 |

Note: Values are means of 5 replicates. Means (n=5) ± standard deviation.

a: Values bearing the different superscript within the same column are significantly different at 5% level (P<0.05).

However, all acoustic sound treated bags produced larger pileus diameter than control (Table 3). The largest pileus diameter for all acoustic sound treated bags were ranged from 10.5 cm to 11.76 cm. However, control had the largest pileus diameter of 8.6 cm only acoustic sound treatment of 5 pops at
10-day interval had the largest pileus diameter of 11.760 cm followed by 5 pops at 5-day interval (11.580 cm), 15 pops at 5-day interval (10.960 cm) and 15 pops at 10 day interval (10.50 cm). This showed that acoustic sound treatment had significant effect on the largest pileus diameter of the mushrooms.

For the smallest pileus diameter of the mushrooms, there was no significant different (P>0.05) observed among all different durations of acoustic sound treatment (Table 2). The range of the small pileus is between 3.320 cm to 1.740 cm. The smallest is pileus 1.740 cm (5 pops at 5-day interval).

The physico-chemical characteristics of grey oyster mushroom in terms of colours were analysed. The colour will be divided into 3 parts, which are L* value, a* value and b* value. L* represents the colour’s lightness, which L* 100 was pure white and L* 0 was matte black. a* values was in ranged of -60 (greenness) to +60(redness) and b* values was in ranged of -60 (blueness) to +60 (yellowness).

Table 4: The physico-chemical properties (colours) of Pleurotus sajor-caju subjected to different durations of acoustic sound treatment.

| Acoustic sound treatments       | Color | L* value  | a* value  | b* value  |
|--------------------------------|-------|-----------|-----------|-----------|
| Control                        |       | 57.232±4.056a | 5.538±0.566a | 14.093±0.735a |
| 5 pops at 5-day interval       |       | 59.863±1.697bc | 5.818±0.799a | 17.273±3.768a |
| 15 pops at 5-day interval      |       | 64.152±2.440a | 5.311±0.701a | 15.821±2.000ab |
| 5 pops at 10-day interval      |       | 57.944±1.575c | 4.850±1.023ab | 14.970±0.836ab |
| 15 pops at 10-day interval     |       | 62.546±2.672ab | 5.301±0.514a | 15.179±0.890ab |

Note: Values are means of 5 replicates. Means (n=5)±standard deviation.

Based on Table 3, in terms of L* values there were significant differences (P<0.05) among all different acoustic sound treatments. All acoustic sound treated grey oyster mushrooms had higher L* values compared to the control. The lightest pileus colour indicated by the highest L* values was 15 pops at 5-day interval which was 64.152, followed by 15 pops at 10-day interval (62.546), 5 pops at 5-day interval (59.863), 5 pops at 10-day interval (57.944) and control (57.232).

In terms of a* values, there was no significant different (P>0.05) and were in range of 4.850 to 5.818 which was in the region of red colour.

There were significant differences (P<0.05) in terms of b* values among all different acoustic sound treatments. All acoustic sound treated mushrooms had higher b* values indicating yellow colour compared to control. The highest b* values was from 5 pops at 5-day interval (17.273) and the lowest was from control (14.093).

Table 5: The physico-chemical properties (firmness,) of Pleurotus sajor-caju subjected to different durations of acoustic sound treatment.

| Acoustic sound treatments       | Firmness (gf) | Pileus’s Moisture Content (%) | Stem’s Moisture Content (%) |
|--------------------------------|---------------|-------------------------------|----------------------------|
| Control                        | 77.520±7.931a | 88.216±4.545a                 | 82.560±9.556a              |
| 5 pops at 5-day interval       | 55.555±11.165b | 89.880±0.966a                | 79.940±5.703a              |
| 15 pops at 5-day interval      | 70.088±11.832ab | 87.112±4.890a               | 72.800±7.476a             |
| 5 pops at 10-day interval      | 70.089±11.155ab | 72.300±30.969b              | 78.640±5.892ab            |
| 15 pops at 10-day interval     | 61.978±12.466b | 88.466±2.823a                | 75.720±5.042a             |

Note: Values are means of 5 replicates. Means (n=5)±standard deviation.

a-b : Values bearing different superscript within the same column are significantly different at 5% level (P<0.05).
Based on Table 4, the physico-chemical properties in terms of firmness of grey oyster mushrooms, there were significant differences (P<0.05) among all the treatments. The highest firmness value was from control (77.520 gF), followed by 5 pops at 10-day interval (70.089 gF), 15 pops at 5-day interval (70.088 gF), 15 pops at 10-day interval (61.978 gF) and 5 pops at 5-day interval (55.555 gF). There was no significant different (P>0.05) in terms of moisture content for both pileus and stem of the grey oyster mushroom among all different treatment (Table 4). This shows that the acoustic sound treatment does not have any effect towards the moisture content for all the harvested grey oyster mushrooms.

4. Summary
From this study, the effects of different acoustic sound treatment on the growth performance, yield enhancement and quality of grey oyster mushroom (Pleurotus sajor-caju) were investigated. Acoustic sound treated bags had faster time for mycelium growth rate compared to control. 15 pops at 10-day interval took the shortest time for mycelium to fully grown compared to all acoustic sound treatment bags and control (30 days). Besides that, time taken for all acoustic sound treatment has faster time taken to complete the stages of growth (spawn-running process, pinhead emergence and fruiting bodies formation). There were sign of improvement on yield of mushroom especially on number of fruiting bodies. There were significant differences (P<0.05) for L*values and firmness of the grey oyster mushroom harvested from acoustic sound treated bags compared to control. However, there was no significant different (P>0.05) on a* values, b* values and moisture content of pileus and stem of the grey oyster mushroom. In conclusion, acoustic sound treatment with 15 pops at 10 day interval was the best treatment duration especially for the higher growth rate of mycelium and shorter time taken to complete all growth stages.

5. References

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