

Full Length Research Paper

Optimization of humidifying procedure in controlled environment for indoor cultivation of *Pleurotus pulmonarius*

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This study investigated mushroom cultivation in controlled indoor environment. For this, the environmental factors including humidity and temperature were controlled by humidifier and ventilation. Four ventilations were installed on tops to bring the air from inside to outside and four small ventilations were installed on side wall along with black filter to bring the air from outside to inside. Four humidifiers were placed in four different positions inside the cultivation house. A single unit of humidifier was applied along with two top ventilations and two side ventilation to optimize the humidifying duration for 80 to 90% humidity. In this process 15 min humidifying duration was optimized to retain 80 to 90% humidity. Then the four units of humidifier were applied for 15 min by one combined application and five individual applications to optimize the configuration to ensure the 80 to 90% humidity in the whole cultivation room with tolerable temperature. Finally, the individual application as 15 min interval period by following after every 15 min humidifying treatment from four different points had been optimized and applied for the cultivation of method. During cultivation $28.60 \pm 1.02^\circ\text{C}$ temperature, $87.96 \pm 2.26\%$ humidity, $0.035 \pm 0.005\%$ CO₂ and 34.53 ± 19.003 lux light intensity had recorded. The total 261.8 kg mushroom was harvested from the controlled cultivation system, a fairly good production of mushroom was harvested till 7 flushes within 3 months of cultivation. This method of humidifying optimization and configuration procedure may ensure the continuous mushroom production throughout the years.

Key words: Indoor, cultivation, model, mushroom, environment, optimization.

INTRODUCTION

Mushroom has been considered as human diet since 200 years for its taste, nutritional and medicinal properties in the world. The demand of mushroom is increasing by times but in Malaysia, the number of cultivators and production is decreasing (Haimid et al., 2013). This is due to the inconsistent environmental condition with high

temperature (32-35°C) and low humidity (60-70%). *Pleurotus* species can grow in ranges of 25 to 30°C temperature and 80 to 90% humidity (Chang and Miles 2004; Uddin et al., 2011).

Jang et al. (2003) investigated the morphological differences of *Pleurotus ostreatus* grown in the artificial

environmental conditions. They controlled temperature and humidity from 13°C to above 16°C and from 60% to above 80% to cultivate *P. ostreatus* by using air cooler, over pressure ventilation system and under pressure ventilation system. In the humidity in excess of 80% at 13 to 16°C temperature, the best morphology and yield of *P. ostreatus* was investigated. In other conditions, *P. ostreatus* generally showed the morphology closing to malformation. Another study of Yang et al. (2013) in china was investigated the morphology and yield of *Pleurotus eryngii* in indoor controlled environment. The temperature and humidity were controlled from 14 to 18°C and from 89 to 97% by using air conditioners, humidifiers and fans. The maximum yield was found in the growing room from 16 to 18°C and 93% or above respectively.

Pleurotus sajor-caju was cultivated indoor house with electronic coolinics Model CTG 250 Yamamota and found the highest yield from 20 to 25°C temperature verified by farmers in local area (Seung et al., 1984). Pink Oyster mushroom was successfully cultivated in the artificially mushroom cultivation chamber at 23 to 25°C (Subramanian et al., 2014). Thepa et al. (1999) found that the combination of evaporative cooling and continuous ventilation system provided maximum yield and a suitable environmental condition for growing *Lentinula edodes*. Veena and Pandey (2012) was cultivated *Tramete versicolor* in 25±2°C temperature and 80 to 85% humidity and kept open the door of the cultivation room three times in a day for 30 min to supply fresh air. Generally outdoor cultivation provides lower yields and longer production times compare with indoor cultivation (Barney, 2000). Recently a study reported that humidifying treatment had influenced the mushroom growth in different stages of production but no optimized condition had identified (Islam et al., 2016).

In the environmental profile of Malaysia, it must need to apply any artificial system to provide optimum growing environment for mushroom. Although, some researches had performed on controlling and maintaining environmental factors to find out optimum range of humidity and temperature which are expensive and not suitable for convention commercial cultivation. So, this study has investigated to develop a new method and way of controlling the indoor environmental condition for commercial cultivation which ensure the continuous mushroom production throughout the years in Malaysia and other similar ecological regions.

MATERIALS AND METHODS

Model and design of indoor cultivation room

The indoor cultivation room was measured at 5.8 Lx4.57 Wx2.74 H

m³ in size inside a concrete building located at Taman Pauh Indah, Arau, Perlis. The wall was made by bricks at one side and by gypsum board on the rest of the walls. The roof of the room was build up by also gypsum board under the main concrete roof. The room was installed with four rows of racks; each rack contained four iron bars distance by 15 cm from each other and 55 cm distance between the racks. Roof ventilation, light and side ventilation were installed inside the room in order to facilitate fresh air and maintain proper light intensity respectively. The roof ventilations was installed with top roof to bring air from inside to outside and the side ventilations was installed at bottom of the wall with black filter to bring air outside to inside.

The bags were arranged horizontally through the racks by using nylon rope and made it tight by cable tie and hanged vertically. Twelve bags were arranged in one column of rope by 3 bags in together in 4 parts. The columns of rope were arranged at 25 cm distance from each other in every rack so that the air and humidity can easily flow through the spaces between the bags, preventing the temperature of the bags from increasing. Mushroom mycelia emitted heat during incubation, so the bags can be easily overheated if they touch each other (Curvetto et al., 2004). The distance of the top end bag from the ceiling was 76 cm and the down end bag from the floor was 30 cm. The humidifiers (TAY-RING TL-3600) were placed between the two columns of bags where the distance of humidifier from the opening part of the bags (front view) was 25 to 30 cm; from the side view of bags was 20 to 25 cm and from the back view of bags was 10 to 15 cm. The Hygrothermometers (EXTECH 445703), Luxmeters (HI 97500) and CO₂ meter (Drager Pac 7000) were installed in different positions inside the cultivation room to monitor temperature, humidity, light intensity and CO₂ level during the experimental procedure. The design and indoor cultivation model is followed as in Figure 1.

Optimized humidifying duration

In this method, four humidifiers were placed in four different points as surrounding 2.9x2.29 (m²) inside the room where the distance among the humidifier positions was 2 to 2.5 (m) from each other's as shown in Figure 2. The duration of humidifying was optimized in order to maintain the humidity values ranging between 80 and 90% in the surrounding 2.9x2.29 (m²) by using single unit of humidifier and two units of continuous roof and side ventilation. Here, side ventilation was applied since the room was closed and two roof ventilation was running continuously which had a strong tends to bring air out, so it is important to use an air incoming ventilation along with air out ventilation which also helps to proper air circulation and reduce the indoor temperature. For this, the humidifier and ventilation was run to achieve maximum humidity from initial range to higher than 90%. After that, the humidifying process was stopped and ventilation was still running. Then, the humidity was monitored until the reading dropped to lower than 80%. The changes of humidity with time were measured at 5 min interval. The same procedure was used in all 4 replicates. The data of increasing humidity from all replicates were analysed and a graph was plotted using duration of humidifying (Min) on the X-axis while the percentages of humidity (%) value on the Y-axis to obtain the equation for by which optimized the duration of humidifying for maintaining 80 to 90% of humidity.

Optimized humidifiers configuration

Then the four humidifiers were configured in two different ways of

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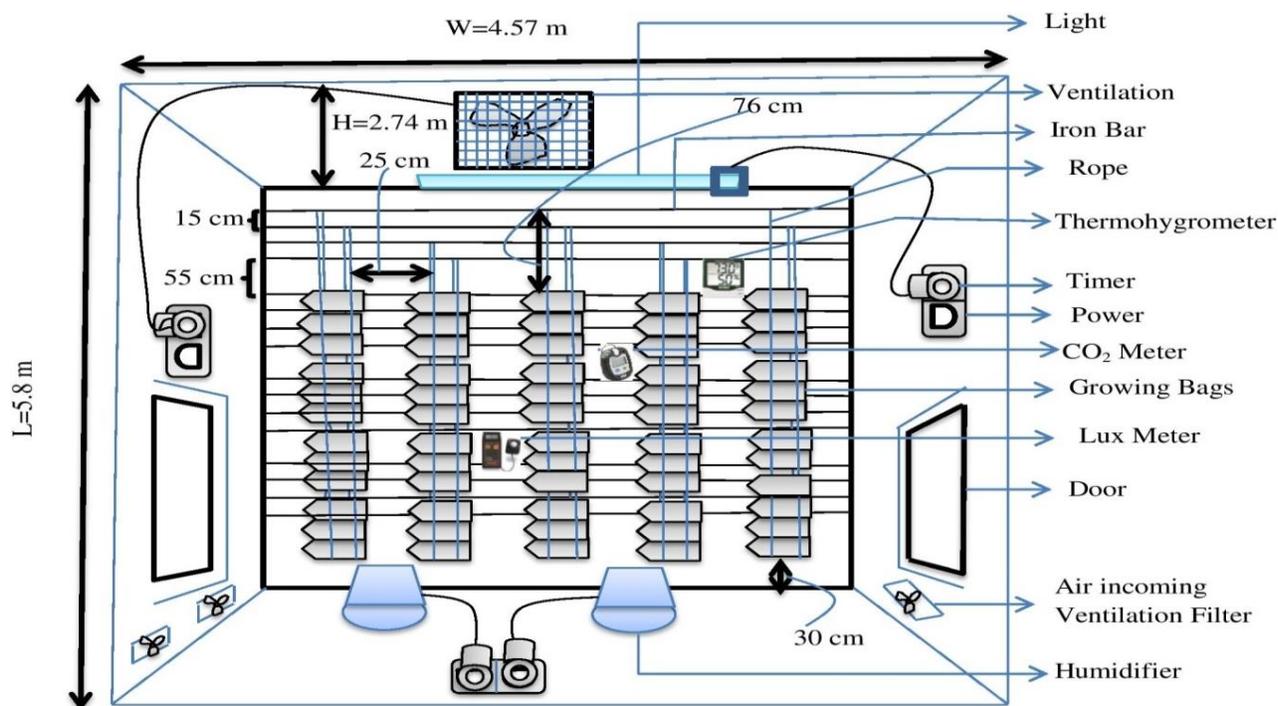


Figure 1. Floor plan and design of indoor mushroom cultivation house with bags.

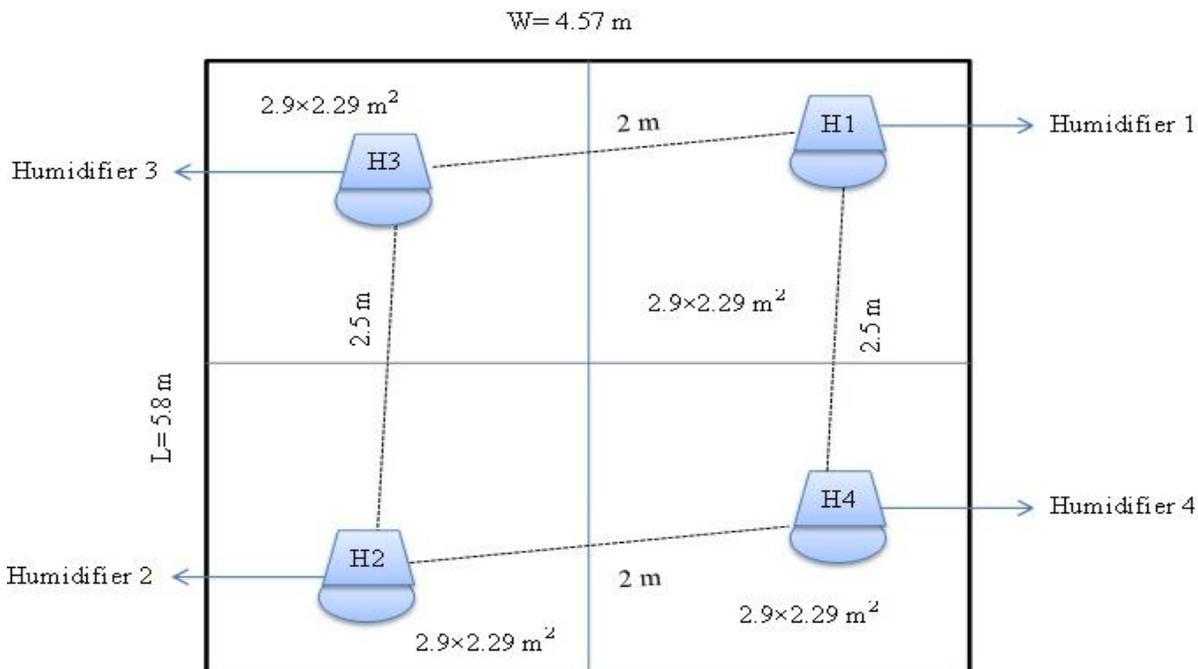


Figure 2. Configuration of humidifier position in different points inside the growing house.

application for 15 min optimized duration obtained from the result section (Optimize Humidifying Duration) in order to ensure 80 to 90% humidity. First one was "Combined Application" and another one was "Individual Application".

Combined application

Combined application was followed by applying four units of humidifier in together for 15 min optimized duration with 2 units of

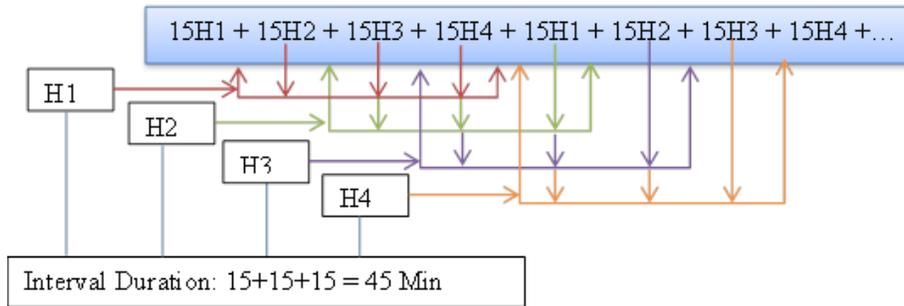


Figure 3. Humidifying configuration 2 for individual application.

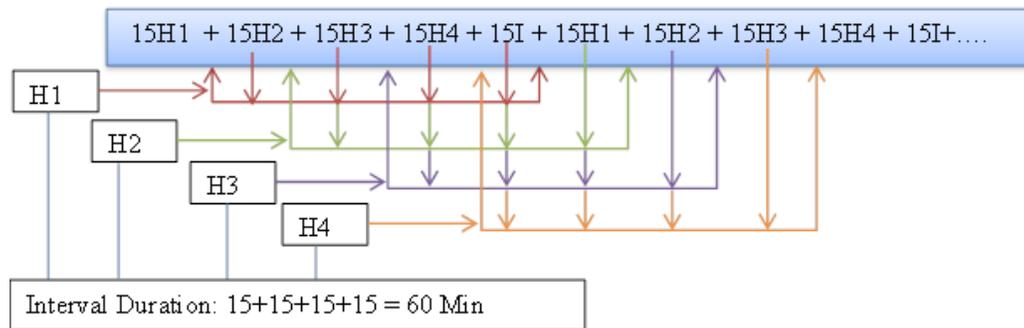


Figure 4. Humidifying configuration 2 for individual application.

continuous roof and side ventilation. All humidifiers were applied together for 15 min by 45 min interval from four different parts of the room. The procedure was continued for 24 h with 4 replicates. The changes of humidity and temperature with time were measured at 5 min interval. The data from all replicates were analysed and made a profile for combined application of humidifiers.

Individual application

The individual application was followed by applying humidifier one after another with or without interval along with 2 units of continuous roof and side ventilation. Then the four humidifiers were configured by the optimized humidifying duration to ensure 80 to 90% humidity in whole the growing room. Four humidifiers were fixed by 15 min optimized duration as following the five configurations to optimize the procedure and environmental condition that able to provide optimum humidity and corresponding tolerable temperature for mushroom cultivation.

Configuration 1

In this procedure, the four humidifiers were configured as 15 min running one after another without any interval among them and followed the process for 24 h. For this, every humidifier had to run after 45 min left as in Figure 3. Ventilations process was running continuously throughout the whole procedure. This procedure was controlled by fixing with electrical timer and the changes of humidity with time were measured at 5 min interval. The same procedure was used in all 4 replicates. The data from all replicates were analysed and made a profile for humidity with corresponding

temperature.

Configuration 2

In this procedure, the four humidifiers were configured as 15 min running one after another with one 15 min interval between humidifier 4 (H4) and humidifier 1 (H1) and followed the process for 24 h. For this, every humidifier had to run after 60 min left as in Figure 4. Ventilations process was running continuously throughout the whole procedure. This procedure was controlled by fixing with electrical timer and the changes of humidity with time were measured at 5 min interval. The same procedure was used in all 4 replicates. The data from all replicates were analysed and made a profile for humidity with corresponding temperature.

Configuration 3

In this procedure, the four humidifiers were configured as 15 min running one after another with two 15 min interval, one was between humidifier 2 (H2) and humidifier 3 (H3) and another one was between humidifier 4 (H4) and humidifier 1 (H1) and followed the process for 24 h. For this, every humidifier had to run after 75 min left as in Figure 5. Ventilations process was running continuously throughout the whole procedure. This procedure was controlled by fixing with electrical timer and the changes of humidity with time were measured at 5 min interval. The same procedure was used in all 4 replicates. The data from all replicates were analysed and made a profile for humidity with corresponding temperature.

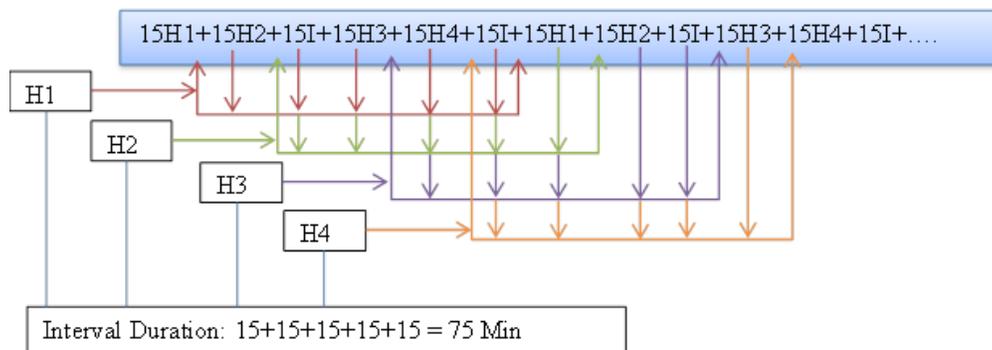


Figure 5. Humidifying configuration 3 for individual application.

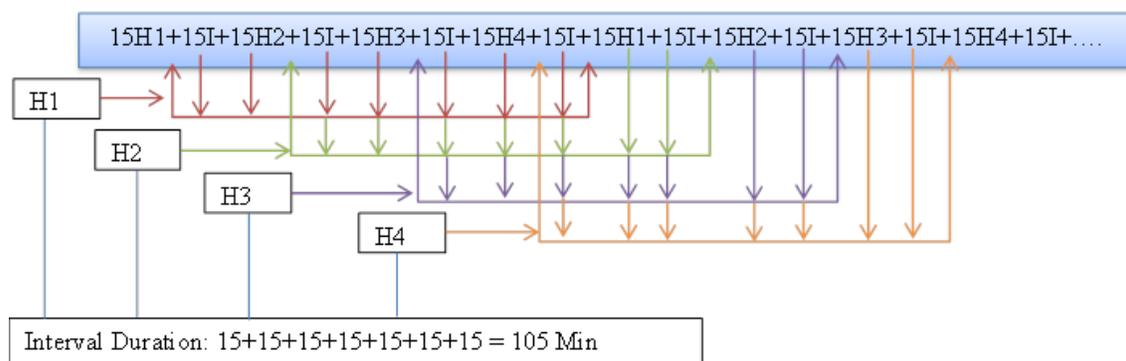


Figure 6. Humidifying configuration 4 for individual application.

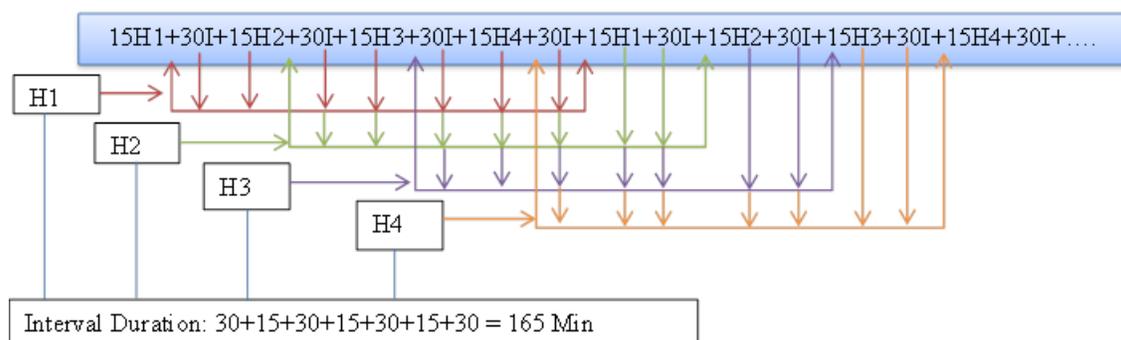


Figure 7. Humidifying configuration 5 for individual application.

Configuration 4

In this procedure, the four humidifiers were configured as 15 min running one after another with 15 min interval after each 15 min humidifying procedure. For this, every humidifier had to run after 105 min left as in Figure 6. Ventilations process was running continuously throughout the whole procedure. This procedure was controlled by fixing with electrical timer and followed 24 h with 4 replicates. The changes of humidity and temperature were measured at 5 min interval. The data from all replicates were analysed and made a profile for humidity with corresponding

temperature.

Configuration 5

In this procedure, the four humidifiers were configured as 15 min running one after another with 30 min interval after each 15 min humidifying. For this, every humidifier had to run after 165 min left as in Figure 7. Ventilations process was running continuously throughout the whole procedure. This procedure was controlled by fixing with electrical timer and followed 24 h with 4 replicates. The



Figure 8. Side view of indoor mushroom cultivation in optimized condition.

changes of humidity and temperature were measured at 5 min interval. The data from all replicates were analysed and made a profile for humidity with corresponding temperature.

Cultivation in optimized procedure

After optimized the configuration procedure, 1200 complete colonized bags were transferred into the growing room and arranged as shown in Figure 1. The mushroom growing bags were prepared by mixing saw dust, rice barn and hydrated lime in sterilization process. In this treatment, the indoor environment was artificially maintained by humidifying and continuous ventilation process. The humidifier and ventilation was operated at duration suggested from optimized results obtained from optimization process mentioned in result section (Optimized Humidifier Configuration). The four units of humidifier were controlled by fixing with timer at 15 min running one after another with 15 min interval after each 15 min humidifying process. A light was applied from the centre of the roof only for 6 h from 12 to 6 am. Light also tends to increase indoor temperature due to heat. So, light was applied from midnight to early morning since that period considered as low temperature (Wafi et al., 2011) compared than other periods. Temperature, humidity, light intensity and CO₂ level of the room were recorded at every 1 h interval within 24 h and continued to record until production of fruit body throughout the cultivation period. Data for environmental factor and mushroom yield was recorded. Figure 8 showed the side view of indoor cultivation system.

Data collection and analysis

Environment factors

Environmental factors including temperature, humidity, light

intensity and CO₂ level were regularly monitored throughout all the investigation system. The maximum and minimum of indoor temperature and humidity were regularly recorded on daily basis during the cultivation.

Total yield

The total yield was determined by the sum of total mushroom harvested from all flushes throughout the cultivation period and calculated by the following Equation 1 (Shen and Royse, 2001):

$$\text{Total Yield (Kg)} = \sum \text{Weight in all flushes} \quad (1)$$

Statistical analysis

Descriptive statistics was applied to determine the maximum, minimum, mean, median and standard deviation (SD) executed using SPSS 17.0.

RESULTS AND DISCUSSION

Optimize humidifying duration

The data from the humidifying process were plotted using duration of humidifying (Min) on the X-axis while the percentages of humidity (%) value on the Y-axis (Figure 9).

For increasing humidity, a graph equation was obtained as Equation 2 with the correlation coefficient, R² of 0.9919 which is acceptable as the value closed to Equation.

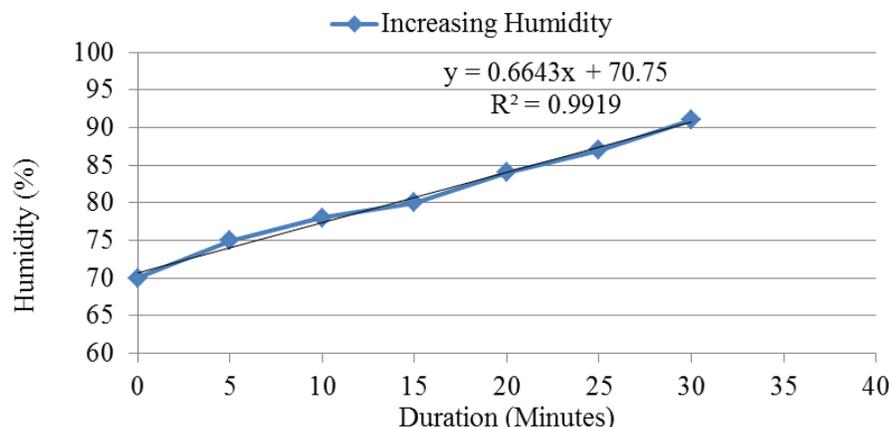


Figure 9. Duration optimization for increasing humidity in indoor controlled environment. Values are mean of 4 replicates.

Table 1. Descriptive of humidity and temperature in different ways of humidifier configuration.

Ways of humidifier configuration	Humidity			Temperature		
	Mean±Std. Dev.	Min.	Max.	Mean ±Std. Dev.	Mini.	Max.
Combined application	87.6±3.7	80.00	94.00	31.1±1.1	29.00	32.50
Configuration 1	93.2±3.7	87.00	99.00	28.1±1.1	26.50	30.50
Configuration 2	92.5±3.3	84.00	97.00	28.3±1.0	27.00	30.60
Configuration 3	90.4±4.8	83.00	97.00	28.4±.98	27.50	30.60
Configuration 4	87.1±3.5	80.00	92.00	28.6±1.02	27.50	30.80
Configuration 5	74.2±8.8	62.00	90.00	30.2±.87	28.50	31.40

*Values are mean of 4 replicates.

$$y = 0.6643x + 70.75$$

2

Where y is the value of humidity (%) and x is the duration of humidifying (min).

From the Equation 2, the humidifying duration for 90% humidity was calculated 28.98 min and for 80% humidity was calculated 13.92 min. For increasing from 80 to 90% humidity the duration of humidifying was 15.06 min. So, for this System, the humidifying procedure for increasing humidity had optimized as 15 min for surrounding 2.9x2.29 (m²) by using single unit of humidifier and two units of continuous roof and side ventilation.

Optimized humidifier configuration

In the configuration of combined application the maximum humidity 94% was found where the mean was 87.6±3.7% and the maximum temperature 32.5°C was found where the mean was 31.1±1.1°C (Table 1). In this configuration, although the maximum humidity was found 94% but the mean was within the optimum range for mushroom cultivation. On the other hand, the maximum and mean temperature was found higher than the

optimum range. Sarker et al. (2008) reported that *Pleurotus* species able to grow till 30°C. Here although the four units of humidifier were run in together for 15 min but the temperature was higher than optimum since the interval period was 45 min. When the humidifiers were running the temperature was start to decrease but its needed to stop after 15 min to maintain optimum humidity, after that 45 min needed to wait for next humidifier application and the temperature was increased since 45 min was long interval period. Moreover a lot of vapour was produced within the 15 min application by four units of humidifier in together and after stopping, all the vapours condensed on the floor within very few min because the concentration of vapour inside the room was high which was mixed to each other and converted into small water which is heavier than vapour and condense on floor very quickly. So, this study not recommended this combined application of humidifier for ensuring optimum environmental condition during indoor cultivation.

The mean value of humidity 93.2±3.7, 92.5±3.3 and 90.4±4.8% had found in configuration 1, 2 and 3 respectively where the maximum humidity were 99 and 97%. The mean temperature 28.1±1.1, 28.3±1.0 and

Table 2. Overview of environmental factors (temperature, humidity, CO₂ and light) during the optimized cultivation of system.

Environmental factors	Mean	Std. Dev.	Std. Error	Minimum	Maximum
Temperature (°C)	28.60	1.02	0.038	26.00	30.80
Humidity (%)	87.96	2.26	0.085	80.00	92.00
Carbon dioxide (%)	0.035	0.005	0.0005	0.03	0.04
Light (lux)	34.53	19.003	1.09	5.00	65.00

28.3±1.0°C were found in the configuration of 1, 2 and 3 respectively where the maximum was 30.5°C and 30.6°C (Table 1). In these three configurations, the mean temperature was within the optimum range for mushroom cultivation but the maximum and mean humidity was too high rather than optimum range. Kong (2004) reported that *P. ostreatus*, *P. florida*, *P. sajor-caju* reach their optimum growth at 25 to 30°C temperature. Yang et al. (2013) reported that, during the fruiting period, the suitable air relative humidity can be as high as 85 to 90%.

In these three configurations (1, 2 and 3), the humidity were found too high since there were without or very short interval period within the humidifier application. A single unit of humidifier was run one after another for 15 min without any interval period (Configuration 1), only 15 min interval between Humidifier 4 and Humidifier 1 (Configuration 2) and 15 min interval Humidifier 2 and Humidifier 3 and 2nd 15 min interval between Humidifier 4 and Humidifier 1 (Configuration 3). Among the three configurations, the highest mean value of humidity was found in Configuration 1 and the lowest was found in Configuration 3. So, the mean value of humidity was started to decrease with the increasing interval frequency. Deacon (2013) reported that excessive water or humidity surrounding the environment can cause diseases, contamination and poor quality of mushrooms. So, this study had also rejected these three procedures of humidifier configuration to ensure optimum environmental condition during indoor cultivation of *Pleurotus pulmonarius*.

In Configuration 4, although the maximum 92% humidity was found but the mean value was 87.1±3.5% which was within the optimum range of mushroom cultivation. On the other hand, the maximum 30.80°C temperature was found with mean value 28.6±1.02°C (Table 1) which was also within the optimum range (Kong, 2004; Sarker et al., 2008). Here the mean humidity and temperature were within the optimum range since 15 min interval period was followed after every 15 min humidifying treatment.

In Configuration 5, the mean 74.2±8.8% humidity was found which was lower than optimum ranges. Jang et al. (2003) showed that, 80% or more relative humidity is suitable for the formation of highest cap size and individual weight of the *P. ostreatus* and the cap size and stipe thickness started to decrease at lower than 80% and decreased severely at 60% or less relative humidity.

The mean 30.2±.87°C temperature was found in configuration 5 which was higher than the optimum ranges (Table 1). In this configuration the low mean humidity and high mean temperature were found since there was 30 min interval period was followed after every 15 min humidifying treatment.

Among the configurations, configuration 4 was able to provide optimum environmental condition with optimum ranges of humidity and temperature. So, humidifier configuration 4 was followed in the experimental indoor controlled mushroom cultivation.

Environmental conditions during indoor cultivation

The maximum 30.8°C and minimum 26°C temperature had found throughout the cultivation period where the mean temperature was 28.60±1.02°C which was within the tolerable ranges of mushroom fruiting temperature. The maximum 92% and minimum 80% temperature had found throughout the cultivation period where the mean humidity was 87.96±2.26 which was within the optimum ranges of mushroom fruiting requirements. Another two environmental factors including CO₂ and light intensity had also found within the optimum ranges as 0.035±.005% and 34.53±19.003 lux respectively (Table 2).

Total yield

A total of 261.8 kg mushroom was harvested from the controlled cultivation System, a fairly good production of mushroom was harvested till 7 flushes within 3 months, Bano and Rajarathnam (1982) reported that increasing temperature and lower humidity had shortened spawn run and longer fruiting period which is also similar to the observation of Chang and Miles (2004). The highest 59.2 kg mushroom had harvested from the first flash and the production started to decrease after first flash. The lowest 17.3 kg mushroom had collected from the 7th harvesting flash (Figure 10). Although the production was decreasing by increasing flushes but the total production had found maximum similar to other studies (Jiskani et al., 1999; Dundar et al., 2008; Sharma et al., 2013).

Based on the result of current study, it could be concluded that humidifying and ventilation system

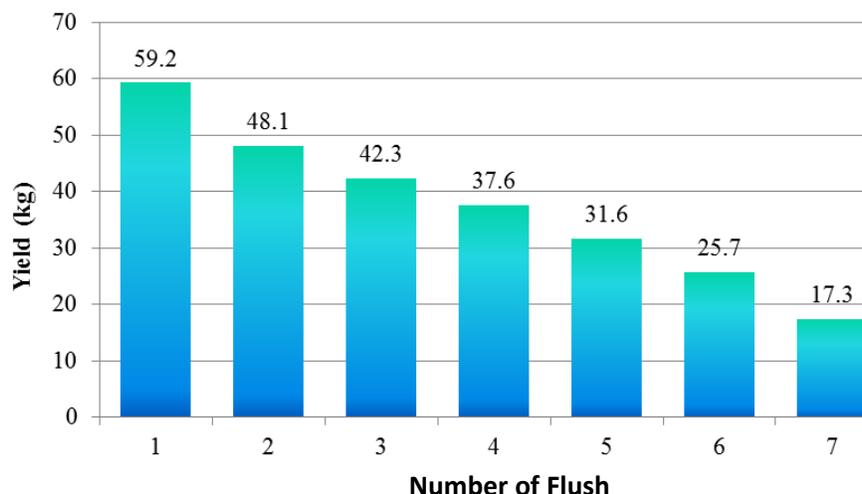


Figure 10. Total yield performance in the optimized cultivation of system.

influenced the indoor mushroom cultivation. It is very well known that low temperature and high humidity favoured the mushroom cultivation by many researches. This study showed the way of controlling indoor environment for commercial mushroom cultivation. The optimized procedure for humidifying able to maintain optimum humidity with tolerable temperature which may ensure the continuous production of mushroom all over the years by independent of weather.

Conflict of Interests

The authors have not declared any conflict of interests.

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