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Mycelia Growth of Pink Oyster (*Pleurotus Djmour*) Mushroom in Different Culture Media & Environmental Factors

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Abstract

The study was undertaken to determine the optimum culture media and different other factors viz: optimum culture media composition, pH and temperature for a well-known mushroom- pink oyster (Pleurotus djmour). Four different types of culture media as-Potato dextrose agar (PDA), malt extract agar (MEA), potato yeast dextrose agar (PYDA), and yeast extract agar (YEA) was prepared as different media for pure culture growth. Calculation was conducted on average mycelium growth & duration of complete mycelium growth. The highest mycelia growth rate (0.24 cm) was observed for potato dextrose agar media and the lowest mycelia growth (0.11 cm) was observed in yeast media. The highest mycelia growth rate (0.41 cm) was observed at 25^0C and the lowest mycelia growth (0.20cm) was observed at 30^0C temperature. Among the nutrient media compositions the fastest mycelium growth rate was observed in PDA media at ratio of 15:150 (Dextrose: Potato) and Minimum mycelia growth rate was observed in PDA media at ratio of 25:250 (Dextrose: Potato). The pH that gives the highest mycelia growth rate (0.45cm) was observed in the pH 6.5 & the lowest (0.11cm) was observed in the pH 4.5 which statistically similar with pH 9.0.

Keywords: Pink oyster, Mycelium growth, Potato dextrose agar (PDA), Malt extract agar (MEA), Potato yeast dextrose agar (PYDA), Yeast extract agar (YEA).

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1. Introduction

Mushroom describes a variety of gilled fungi, with or without stems, and the term is used even to describe both the fleshy fruiting bodies of some Ascomycota and the woody or leathery fruiting bodies of some Basidiomycota. *Pleurotus djamor*, commonly known as the pink oyster mushroom, is a species of fungus in the family Pleurotaceae. The potential of mushroom as fungal protein and as a source of medicinal compounds make the production of mycelium an attractive prospect. Mushroom mycelium is used for medicinal and therapeutic purposes; mycelial biomass powder can be used to formulate various types of health tablets and capsules [1]. The biological efficiency of mushroom depends on the development of mycelia in the first cultural stage. Healthy and active mycelial growth plays crucial roles in protecting themselves against several stress factors [2]. Mushrooms are the source of extra ordinary power and virility and are used in the preparation of many continental dishes and have medicinal properties like anti-cancerous, anti-cholesteral, anti-tumorous. Mushrooms are useful against diabetes, ulcer and lungs diseases [3]. Mushrooms are the good source of protein, vitamins and minerals [4]. Mushrooms contain about 85-95% water, 3% protein, 4% carbohydrates, 0.1% fats, 1% minerals and vitamins [5]. Mushrooms contain appreciable amount of potassium, phosphorous, copper and iron but low level of calcium [6]. Mushroom protein is intermediate between that of animals and vegetables [7]. Mushroom also contain appreciable amount of Niacin, pantothenic acid and biotin [8].

It is also important in biotechnological researches which are dealing with large scale production of mycelium (biomass) to obtain anti-cancer and antimicrobial substances, volatile compounds, biomass for food industry and enzymes production [9]. Mycelia growth is strongly influenced by *in vitro* conditions and they obtain their nutrients by absorbing soluble inorganic and organic materials from medium which assures the maximum and most vigorous germination [10]. It has also been reported that healthy and active mycelia growth in used medium play crucial role for protecting themselves against several stress factors [2]. Beside media the factors like temperature, pH and light affect to the mycelia growth.

Mycelium growth depends on several factors such as growing media, different media concentration, pH, temperature, nutrient element & some environmental factors. Mycelia stage of mushroom was significantly influenced by the carbohydrate source, nitrogen source, temperature, pH and light intensity. Fasola, et al. [11] reported that potato dextrose agar (PDA) is the best culture media for mycelia growth of *Volvariella speciosa*. Fasola, et al. [11] also reported that the maximum, optimum and minimum mycelia growth of *Volvariella speciosa* at the pH of 9.0, 6.0 and 3.0, respectively while no growth was observed at pH 2.0 and 10.0. Generally, the growth of this fungus reduced at very strong acidic and alkaline pH. Jonathan and Fasidi [12] observed very good mycelia growth of *Volvariella esculenta* at pH 6.0. This study was attempted to investigate the effects of different media, pH level, temperature, and different nutrient media composition on vegetative growth of pink oyster (*Pleurotus djamor*) mushroom.

2. Materials and Methods

The experiment was conducted in the tissue culture laboratory of National Mushroom Development and Extension Centre (NAMDEC), sub centre (Strengthening Mushroom Development Project), Horticulture Center, Mehedibag, Sylhet, Bangladesh.

2.1. Preparation of Culture Media for Pure Culture

Potato dextrose agar (PDA), malt extract agar (MEA), potato yeast dextrose agar (PYDA), and yeast extract agar (YEA) was prepared as different media for pure culture growth according to standard methods. Media was autoclaved at a pressure of 15 pound square inch (psi) for twenty minutes at 121°c. Calculation was conducted on average mycelium growth & duration of complete mycelium growth.

2.2. Sterile Culture Technique

A healthy, fresh, clean mushroom fruit body was selected for culture. A section of mushroom was broken off. Pour the different media into sterile tubes. Using the index finger and thumb of the left hand, a piece of the mushroom was firmly grasped with the clean surface facing upward or sideways. With the right hand, the scalpel was grasped firmly by the handle. Starting with the tip of the scalpel, a very small triangular shape was cut from the clean inner tissue. The tissue was gently "teased" free, and placed it quickly just inside the lip of the tube. Then the bottom of the tube was taped for a few times on the heel of hand to position the mushroom tissue on the surface of the agar. The tube was then placed in a second storage can. These steps were repeated for the remaining tubes and mushroom materials. It was important to make several tubes from each mushroom specimen, since even with the most careful technique contamination can sometimes occur.

2.3. Preparation of Different Synthetic PDA Media

Different amount of potato & dextrose were used for PDA media. The PDA was prepared by 0g, 5g, 10g, 15g, 20g, 25g of dextrose & 0g, 50g, 100g, 150g, 200g, 250g potato mixed with20g agar at pH 6.5. The mixture was boiled in gas burner until the agar dissolved. The media was poured into petri plate at 20 ml/plates and sterilized and solidified. Then the plates were inoculated with testing mushroom species. Then plates were transferred in incubation room for mycelium running at 20-25° C temperature.

2.4. Study of the Different Temperature on Mycelial Growth of P. Djamor

The selected medium was employed to evaluate the suitable temperature for mycelium growth of *P. djamor*. Some composition was adjusted at 6.5 ph value before autoclave The inoculated plates of *P. djamor* on PDA (Potato Dextrose Agar) were placed at six different temperature values such as 10^0 C, 15^0 C, 20^0 C, 250 C 30^0Cand 35^0 C, with the pH value of each medium being 6.5.

2.5. Study of the Different Ph on Mycelial Growth of P. Djamor

The media were adjusted to different pH values at 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 with the addition of 1N HCl or 1N NaOH before pouring into Petri plates and autoclaving. The Petri plates were inoculated with the inocula of the mushroom. After autoclaving, the Petri plates were inoculated and transferred into incubation room for mycelial growth at 21^0 to 25^0 C temperatures. Average mycelia growth and duration of completion of mycelium running of each replication were calculated.

3. Results

3.1. Study of the Different Growth Media on Mycelial Growth of Pleurotus Djamor

3.1.1. Mycelium Growth per Day

Four different culture media such as potato dextrose agar (PDA), malt extract agar (MEA), yeast extract agar (YEA), malt extract yeast agar (MEYA) and potato yeast dextrose agar (PYDA) medium were used to investigate the mycelial growth of *Pleurotus djamor*.

The highest mycelial growth rate (0.24cm/day) was observed in the potato dextrose agar (PDA) media where lowest mycelial growth was found in YEA media (0.11cm/day) (Table 1). The density of mycelium on PDA was higher than other culture media.

Table-1. Mycelial growth rate per day by different media

Media	Mycelial growth rate (cm/day)
Potato dextrose agar	0.24 a
Malt extract agar medium	0.20 a
Potato yeast dextrose agar	0.16 ab
Yeast agar medium	0.11 b
C.V (%)	9.16

Note: Means followed by same letters are not significantly different from each other at 5% level

3.2. Total Time Required for Complete Mycelium Growth

In case of duration of complete mycelium running, the minimum time (5.25 days) was recorded in PDA medium and the maximum time (10.25 days) was found in YEA medium (Figure 1).

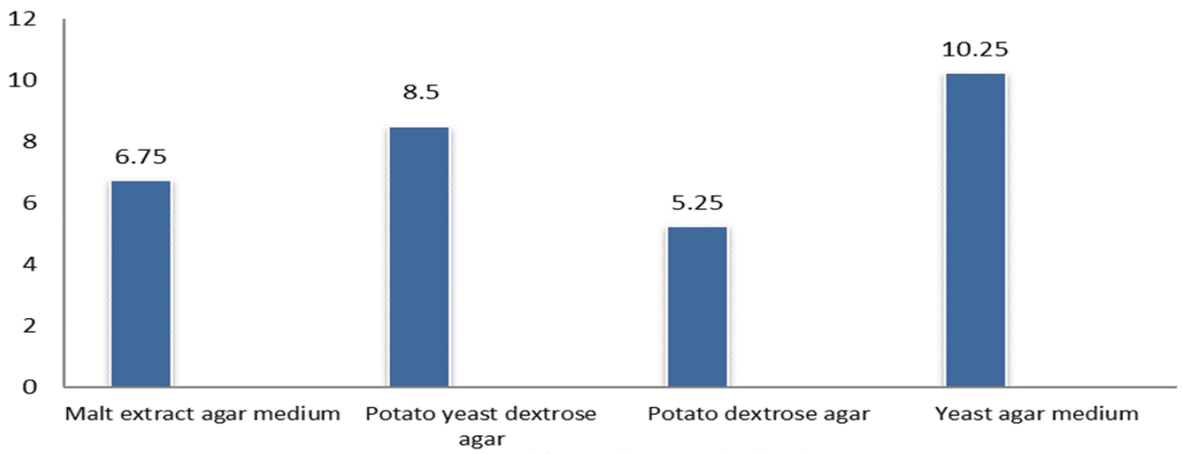

Figure-1. Days required for mycelium growth in line diagram

The diagram describes that PDA media takes the least amount of time to complete mycelium growth and YEM takes the highest amount of time.

3.3. Study of the Different Ph on Mycelial Growth of Pleurotus Djamor

To determine the suitable pH on PDA media with the pH range of 4.5 to 9.0 were used for mycelial growth of *Pleurotus djamor*. The best mycelia growth (0.45 cm/day) was observed in slightly acidic medium (pH 6.5) which was statistically different from other pH values (Table 2).

Table-2. Mycelial Growth Rate (cm/day) at different pH level

pH	Mycelial growth rate (cm/day)
4.5	0.11 c
5.0	0.13 c
5.5	0.15 c
6.0	0.31 b
6.5	0.45 a
7.0	0.31 b
7.5	0.30 b
8.0	0.26 b
8.5	0.15 c
9.0	0.12 c
C.V%	4.93

Note: Means followed by same letters are not significantly different from each other at 5% level

By interpreting the results we find that means are not significantly different from each other for pH of 4.5, 5.0, 5.5, 8.5, 9.0 and 6.0, 7.0, 7.5, 8.0 are not significantly different but 6.5 is significantly different mean than others.

3.4. Time Required for Complete Mycelium Growth Depending on Ph

In case of duration of complete mycelium running, the minimum time (5.0 days) was recorded at pH 6.0 and 6.5 which was statically similar to pH 6.0 and pH 7.0 and the maximum time (9.25 days) was found at pH 4.5 (Figure 2).

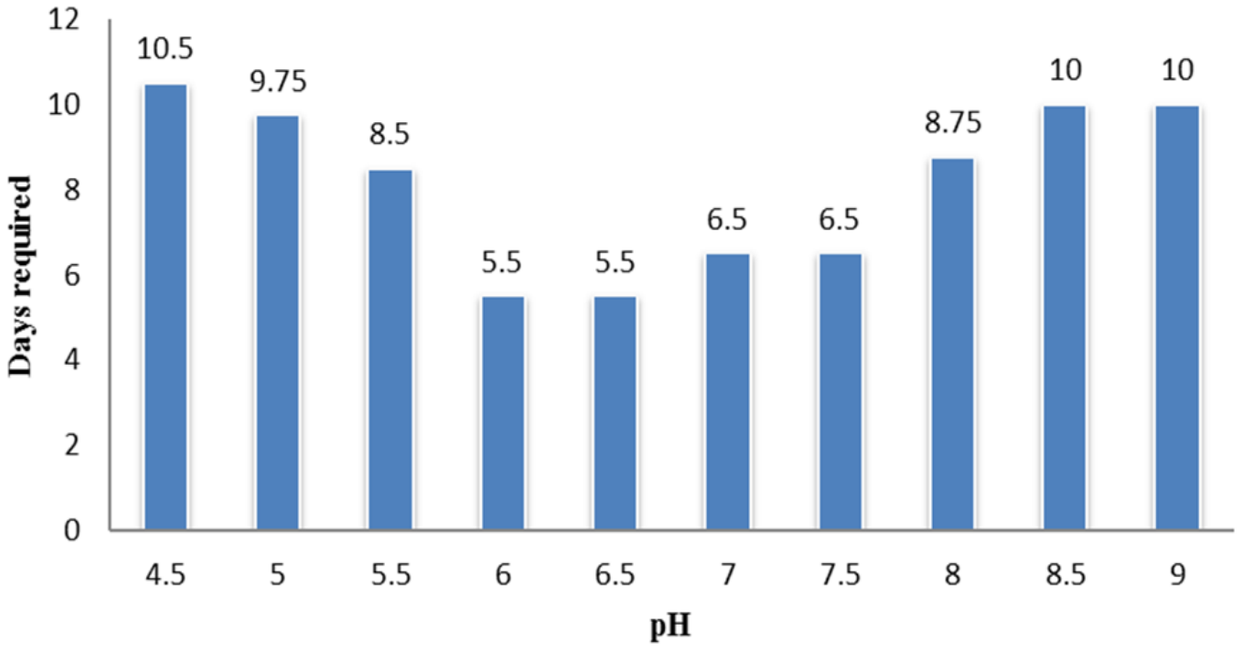

Figure-2. Days required for mycelium growth in line diagram at different pH.

Figure-3. Mycelium growth at pH 6.5, gives the highest mycelium growth rate

3.5. Study of the Different Temperature on Mycelial Growth of Pleurotus Djamor

The best mycelial growth (.41 cm/days) was obtained at 25^0 C which was significantly higher on compare to all the treatment. The lowest mycelial growth (0.20 cm/day) was recorded in 30^0 C.
10^0 & 35^0 has no mycelium growth at all (Table 3).

Table-3. Mycelial growth rate at different temperature

Temperature (^{0}C)	Mycelial growth rate (cm/day)
10	---
15	0.28 b
20	0.26 bc
25	0.41 a
30	0.20 c
35	---
C.V%	4.01

Note: Means followed by same letters are not significantly different from each other at 5% level

3.6. Time Required for Complete Mycelium Growth Depending on Tempereture

In case of duration of complete mycelium running, the minimum time (5.25 days) was recorded at 20^0C, which was statistically similar to 15^0C. In case of 35^0 C temperature, the duration of complete mycelium running was more than 7.75 days.

Table-4. Days required for complete mycelial growth at different temperature

Temperature (^{0}C)	Days required for complete mycelial growth
10	---
15	6.50 a
20	5.20 a
25	7.00 a
30	7.75 a
35	---
C.V%	9.24

Note: Means followed by same letters are not significantly different from each other at 5% level

3.7. Mycelial Growth Rate for Various PDA Media Composition (Cm/Day)

The result of mycelia growth rate in different ratio of potato & dextrose used in the culture media as well as variety is shown below. Highest mycelial growth rate (.33cm/day) was observed from the treatment T4 when *Pleurotus djmour* was inoculated in PDA at ratio of dextrose: potato (g/liter) (15/150) (Table 5).

Table-5. Mycelial growth rate (cm/day) for various PDA media composition

Treatment	Mycelial growth rate (cm/day)
T_1	---
T_2	0.28 ab
T_3	0.30 ab
T_4	0.33 a
T_5	0.31 ab
T_6	0.26 a
C.V%	9.57

Note: Means followed by same letters are not significantly different from each other at 5% level
[Here T1= Dextrose (g/liter): Potato(g/liter) (0:0), T2= Dextrose (g/liter): Potato(g/liter) (5:50), T1= Dextrose (g/liter): Potato(g/liter) (10:100), T1= Dextrose (g/liter): Potato(g/liter) (15:150), T1= Dextrose (g/liter): Potato(g/liter) (20:200), T1= Dextrose (g/liter): Potato(g/liter) (25:250)]

3.8. Duration of Mycelium Completion for Various PDA Media Composition (Days)

Duration of mycelia completion in different ratio of potato and dextrose used in culture media is shown in table below. Maximum days (14.00 days) required for the completion of mycelium running was observed from T6 when *P.djmour* was inoculated in PDA at ratio of dextrose (g/liter) (25:250) because excess nutrient delay the mycelial growth. Minimum days required for completion of mycelim running was observed from the treatment T4 when *P. djmour* was inoculated in PDA at ratio of dextrose (g/liter): Potato (g/liter) (15/150).

Table-6. Duration of mycelium completion for various PDA media composition

Treatment	Days required to mycelium complete (days)
T1	---
T2	13.75 a
T3	13.00 ab
T4	11.50 b
T5	12.25 ab
T6	14.00 a
C.V%	9.83

Note: Means followed by same letters are not significantly different from each other at 5% level

4. Discussion

The growth of mycelium depends on different factors such as media, pH, temperature, nutrient element and some environmental factors [13]. Medium is important because it supplies necessary nutrient for the growth of mushroom mycelium. Culture media permit acceleration of mycelial growth; ensure quality and year round production [14]. The highest mycelial growth rate (0.24cm/day) was observed in the potato dextrose agar media which was similar to Khan, et al. [15] who reported that malt extract potato extract & wheat extract were the best media for cultivation of *Auricularia politrica.*

Fasola, et al. [11] reported that pH 6.0 is best for mycelial growth of *V. speciosa.* Ibekwe, et al. [16] reported that the optimum mycelia growth of *Pleurotus ostreatus* was recorded at pH 6.5 which supports our proposition.

Likewise, Chandra and Purkayastha [17] and Jonathan and Fasidi [12] obtained very good growths of *Agaricus campestris* and *V. esculenta* at pH 6.0. This result is not similar with the findings of Chang and Miles [10] who reported 7.5 as the best pH level for mycelial growth of *V. volvacea.* The lowest mycelial growth (.11 cm/day) was recorded at pH 4.5.

Mushroom mycelium is significantly influenced by various environmental factors such as light, temperature, pH, carbon sources etc. pH has great effects on nutrition and morphological development of mushrooms. Generally the growth of fungi is reduced at very strong acidic and alkaline pH. Different agar media such as Potato Dextrose Agar, Malt Extract agar, Lamberts Agar, Compost Extract Agar are mostly used for the growth of mycelium [18].

The effect of mycelial growth rate in four different culture media were shown in the results. Significantly the highest mycelium growth (0.24cm/day) of *pleurotus djamor* was observed in Potato Dextrose Agar media which is statistically similar (0.20cm/day) to Malt Extract Yeast Agar media and the lowest mycelium growth rate (0.11 cm/day) was found in Yeast Extract medium. This result is similar to Kalm and Kalyoncu [9]. The highest mycelium growth of *Pleurotus geesternaus* was observed in Potato Dextrose Agar media (0.41 cm/day) and this medium has been demonstrated to be highly supportive of mycelial growth of mushrooms by several authors such as Jeffers and Martin [19]. The lowest mycelium growth rate (0.29 cm/day) was found in Yeast Extract Agar media.

The days required to the completion of mycelium running in different media is shown in the results. The lowest time (5.25 days) required for the completion of mycelium running of *Pleurotus djamor* was observed in Potato Dextrose. Agar which was statically similar to Malt extract agar medium (6.75days) and highest time (10.25 days) was observed in Yeast Extract Agar media. Kalm and Kalyoncu [9] reported that MEA and PDA are more suitable media for mycelial growth of *Morchella spp.*

For *Pleurotus djamor* the temperature range of 10-35^{0}C was considered to find out the most suitable one. The highest mycelial growth (0.41 cm/day) was found in 25^{0}C in the case of *Pleurotus djamor* .This result is supported by Sung, et al. [20]; they stated that the favorable temperature for mycelial growth was 30^{0}C for *Macrolepiota procea* and *Pleurotus ostreatus*. This result is similar to the report of Shim, et al. [21]; they stated that the mycelial growth of *P. fumosoroseus* was favorable at the temperature of 20 to 25^{0}C. For *pleurotus djamor*, 10^{0}C & 35^{0}C was found to be not suitable for mycelial growth.

The test was conducted also to determine the best ratio in PDA media for mycelia growth for *Pleurotus djmour*. Highest mycelial growth rate (.33cm/day) was observed from the treatment T4 when pleurotus djmour was inoculated in PDA at ratio of dextrose: potato (g/liter) (15/150) which was statistically similar to T5. Similar findings were reported by Khandaker, et al. [22], who reported that PDA media gave highest (0.33cm/day) mycelial growth of *Grifola frondosa*.

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