Mycopath (2015) 13(1): 25-28 **Research Article**

Effect of different culture medium and pH on the mycelial growth of shiitake mushroom

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Abstract

Different culturing medium viz. malt extract agar, potato dextrose agar, waksman agar, saboured agar and corn meal agar were investigated for the effective mycelial running of Shiitake mushroom [*Lentinula edode* (Berk) Pegler]. Maximum mycelial growth after 3, 6 and 9 days was observed on potato dextrose agar. Minimum mycelial growth after 3, 6 and 9 days was observed on waksman agar. Data regarding pH 5, 6, 7, 8 and 9 of growth medium was also recorded after 3, 6 and 9 days. Maximum mycelial growth was observed at neutral pH to slightly acidic (6). Maximum growth of 8 cm after nine days was recorded on potato dextrose agar medium followed by malt extract (7.58 cm), corn meal agar (5.35 cm), saboured agar (4.2 cm) and waksman agar (3.75 cm). This study concludes that potato dextrose agar and 6 pH are the best for the effective mycelial growth of Shiitake mushroom.

Keywords: Growth medium, mycelial growth, pH, Shiitake mushroom.

Introduction

Mushrooms are liked all over the world due to their delicate taste, flavor and health giving properties. They contain unsaturated fatty acids, good quality protein, vitamins and minerals. These are low in carbohydrates, fat, salts and rich source of dietary fiber. Moreover, these are considered a perfect diet for the patients of obesity, hypertension and diabetes because nucleic acid content in mushrooms are very low (Anonymous, 2003). Shiitake is a ligolytic and aerobic mushroom belonging to basidiomycete (Andrade *et al.,* 2008). It produces an edible mushroom that has been cultivated for centuries in China, Korea, Japan, Singapore, Thailand and other Asian countries (Ciesla, 2002). The word 'shiitake' was originally derived from Japanese words: *shii* which means oak and *take* means mushroom, reflecting the importance of oak wood as the natural host of the fungus (Davis, 1993; Royse, 2001). Over the last three decades, the commercial production of this mushroom has increased from an estimated 350,000 tons in 1965 to about 7.5 million tons in 2000 (Royse, 2001). During a period from 1940`s until 1986 Japan was the leading producer of shiitake worldwide. But in 1987 China overtook Japan by producing a large number of shiitake mushroom (178,800 tons). Shiitake mushroom has been reported to boost the immune system, lower cholesterol, function as an anticoagulant and may have use in treatment of some cancers (Puri, 2011; Pire *et al.,* 2001). It requires specific environment for successful growth and development. The optimum temperature for mycelial growth of Shiitake mushroom is variable ranging from 24-28 °C (Tokimoto and Komatsu, 1982) or 15-24 °C (Sabota 2007). The availability of good quality spawn is the limiting factor for mushroom cultivation in many developing countries. Spawn is prepared on different media depend upon the mushroom type and it is further multiplied on grains. Grains are preferred because they are very nutritious for fungi (Oei and Nieuwenhuijzen, 2005). Present work was done to find the best growth medium and pH for the effective culture establishment of Shiitake mushroom.

Material and Methods

Strain used in the experiment

In this study a promising strain of *L. edodes* from Taiwan was used. The strain was multiplied on malt extract agar medium (malt extract 20 g, agar 20 g, dextrose 20 g, peptone 4 g, water 1 L) for mother culture and incubated at 25±2 °C.

Preparation of culture medium

Five culture medium were tested. The composition of different media used during experiment were: malt extract agar medium

(MEA) [malt extract (20 g), dextrose (20 g), agar (20 g), peptone (4 g) and 1 liter water]; potato dextrose agar medium (PDA) [potato starch (20 g), dextrose (20 g), agar (20 g) and 1 liter water]; Saboured agar medium (SA) [dextrose (40 g), agar (20 g), peptone (10 g) and 1 liter water]; Waksman agar medium (WA) [agar (20 g), glucose (10 g), peptone (5 g), potassium dihydrogen phosphate (1 g) and 1 liter water]; corn meal agar medium (CMA) [corn meal (20 g), peptone (20 g), dextrose (20 g), agar (20 g) and 1 liter water]. The medium were sterilized in autoclave at 304 kPa and 121 °C for 30 minutes. Penciline was added in sterilized medium @ 1 g L^{-1}, when the medium was cooled up to about 45 °C. Then inoculation was done. Radial growth was measured till the mycelial growth completely covered the agar medium in the Petri plates and each treatment was replicated five times.

Different pH of the medium

In this part of study, different pH viz. 5, 6, 7, 8 and 9 of MEA medium were tested to evaluate the best pH for the mycelial growth of Shiitake mushroom with four replications of each treatment. Rest of the procedure was same as mentioned above.

Data recording and analysis

Radial growth of the fungus was measured in cm after 3, 6 and 9 days. Data were analyzed statistically by using ANOVA and means were separated with least significant difference (LSD) test at 5% level of significance.

Results and Discussion

Selection of culture medium on radial growth of Shiitake mushroom

The results of the *in-vitro* experiment conducted for the evaluation of five culture media viz. malt extract agar, potato dextrose agar, Waksman agar, Saboured agar and corn meal agar on the radial growth of Taiwan strain of shiitake mushroom are given in the Table 1. Composition of all medium used in this experiment based on Atri and Lata (2013). The fungus growth on different media revealed significant differences. Among the various media maximum fungus growth (8 cm) was recorded in potato dextrose agar after nine days of inoculation. While lowest after 9 days (3.75 cm) was recorded in Waksman agar. On the other hand malt extract agar showed (7.58 cm) growth after 9 days very closed to potato dextrose agar. The mycelial growth on medium was also significantly affected by different growth periods. The highest fungus growth was observed after 9 days that was (8 cm) as against the lowest (0.85 cm) after 3 days of inoculation.

As far as the interaction between days after inoculation is concerned, the highest growth of fungus was observed after 9 days with each media as against the lowest after 3 days with each media. Among the interactions means, the highest growth of fungus was noted in potato dextrose agar and malt extract agar after 9 days. The lowest fungus growth was observed in Saboured agar (0.85 cm) after 3 days and (3.75 cm) after 9 days in Waksman agar. The two Media malt extract agar and potato dextrose agar gave almost similar results. Khan *et al.* (2013) also conducted study on growth of *A. brunescens* and similar results were recorded. They reported optimum linear fungus growth on malt extract agar medium. The growth and sporulation of fungi depends on genetic, nutritional and environmental factors. Culture media is one of the important factor**s** for the growth and reproduction of fungus. It is evident from the present investigation that the Shiitake mushroom was successfully grown on five cultured media. Among them potato dextrose agar medium gave the best response in term of mycelial growth but statistically it was at per with malt extract agar media. Mycelium growth was found 0.87 cm in diameter on an average daily basis in potato dextrose agar and malt extract agar. Maximum growth found in malt extract agar and saboured agar. The results are comparable to that of Gbolagade *et al*. (2006) findings, who observed that potato dextrose agar and yellow corn agar are best for the culture establishment of shiitake mushroom. Atri *et al*. (2007) also reported maximum radial growth of *Lentinus squarrosulus* mycelium in yeast extract agar. Atri and Lata (2013) also conducted experiments by using eleven different liquid media to evaluate the best media for the vegetative growth of *Lentinus cladopus* and proved malt extract agar media as a best media. The findings of this experiment support the findings of our result.

Effect of pH on the radial growth of Shiitake mushroom

Fungus growth on media having different pH showed significant difference in radial growth of Shiitake mushroom. Among the various pH, neutral to slightly acidic media showed maximum radial growth. Maximum growth (8 cm) recorded after nine days of inoculation in media whose pH was adjusted to 6. Minimum growth of (5.75 cm)

was recorded after nine days in 9 pH media. In neutral pH (7.8 cm) of growth was recorded (Table 2). As composition of media played an important role in the mycelial growth of mushroom**,** similarly pH of the media also stimulated the growth. Almost all mushrooms perform and grow best at neutral pH (Khan *et al*., 2013). Because mycelium of mushroom obtain most of the nutrients at a specific level of pH from the media (Sarker *et al*., 2007). The pH of the media was adjusted by hydrochloric acid and sodium hydroxide solution. From the present study it is clear that Shiitake mushroom grew best on neutral to slightly acidic pH. Results showed best mycelial growth of shiitake mushroom on 6 pH but statistically it was at par with 7 pH. Results showed statistical comparison of different treatments after different days in which 6 pH gave maximum mycelial growth after 3, 6 and 9 days (Table 2). Our results also matched with the findings of Khan *et al.* (2013) that neutral pH have positive effect on mycelial growth and production of oyster mushroom.

Conclusion

Use of potato dextrose agar as a medium appeared to be the most suitable due to improved mycelial growth and useful for large scale culture establishment of Shiitake mushroom. While neutral to slightly acidic pH of medium is recommended for the best mycelial growth. The findings made in present study are useful for large scale culture establishment and spawn production of Shiitake mushroom.

Table 1: Effect of different culture media on the radial growth of Shiitake mushroom.

Medium	Fungal colony diameter (cm)		
	3 days	6 days	9 days
Malt extract agar	1.60 b	3.80 b	7.58 a
Potato dextrose agar	2.45 a	4.75 a	8.00 a
Waksman agar	1.05 c	1.55 e	3.75 c
Saboured agar	0.85 c	2.10 d	4.20 c
Corn meal agar	1.05 c	3.02 c	5.35 b

In a column, means with the same letters do not significantly differ by the least significant difference (LSD) test at $P \leq 0.05$ with a completely randomized design.

Table 2: Effect of different pH of media on the radial growth of Shiitake mushroom.

pH	Fungal colony diameter (cm)		
	3 days	6 days	9 days
5	2.56 bc	5.50 b	6.80 b
6	3.47 a	6.53 a	8.01 a
7	2.72 b	5.41 b	7.80 a
8	2.10 cd	4.00 c	6.01 c
9	2.01 d	4.01 c	5.75 d

In a column, means with the same letters do not significantly differ by the least significant difference (LSD) test at $P \leq 0.05$ with a completely randomized design.

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