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Performance of Vegetative Growth and Artificial Fruit Body Formation of *Hypsizygus marmoreus* in Bangladesh

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Hypsizygus marmoreus is an edible and medicinal mushroom belongs to Basidiomycetes. The optimal culture conditions for the vegetative growth of brown and white strains of *H. marmoreus* were investigated to obtain suitable farming condition. Temperature suitable and unsuitable for vegetative growth was obtained at 20 and 35°C, respectively. The both strains of *H. marmoreus* love medium temperature and the mycelial growth was well developed at 20–25°C. This mushroom prefer pH ranging 6–8 for mycelial growth. The highest and lowest mycelial growth was found at pH 7 and 5 of both strains, respectively. Among 5 different carbon sources, sucrose was the best, whereas, maltose and lactose were the worst. The most suitable nitrogen sources were CaNO₃ (in brown strain 65.6 mm and in white strain 56.3 mm) and the most unsuitable was NH₄H₂PO₄ (in brown strain 44.02 mm and in white strain 40.2 mm). Five different culture media were used to screen the optimal mycelial growth of *H. marmoreus*. PDA and YM (yeast–malt extract) were the most suitable and Czapek dox and Hennerberg were the most unfavorable for vegetative growth, whereas, glucose peptone was moderately suitable. The highest and lowest vegetative growth was observed in media prepared with mango (*Mangifera indica*) and koroi (*Albizia procera*) sawdust, respectively. However, for both strains, 3 weeks were required to complete mycelium running in mango sawdust, whereas, 4 weeks were spent to get 100% mycelium running in mahagoni (*Swietenia mahagoni*) and koroi sawdust. The primordia and fruiting bodies of *H. marmoreus* were formed in the mango sawdust medium sooner. The fruiting bodies of brown strain were formed earlier than white strain after occurrence of primordia. In case of limiting ventilation, the stem development was found to be good but no cap was seen to be formed. Ventilation influenced the growth and development of fruit body of *H. marmoreus*. Therefore, it could be said that physiochemical requirement for the vegetative growth is strictly considerable and results focused in this paper could be followed by farmers to scale up farming of *H. marmoreus* in Bangladesh.

Key words: Mushroom farming, Fruit body, Influencing factors, Substrates, Vegetative growth

INTRODUCTION

Hypsizygus marmoreus is one of the popular edible mushrooms cultivated world widely in autumn and winter with light brown or white umbrella–shaped small cap and known as shimeji or beech mushroom. This mushroom grows in a bunch and each cluster may include 15–50 individual plant showing attractive morphological approach. Shimeji should always be cooked since it is not a good mushroom to serve raw due to its somewhat bitter taste, but the bitterness disappears completely upon cooking. They are firm and crunchy in texture and they have a delicate mild, sweet flavor (Waites *et al.*, 2001). *H. marmoreus* is also called *Hypsizygus tessulatus* belonging to Tricholomataceae, Agaricales, Hymenomycetidae, Basidiomycetes. Its taste is fresher than that of *Pleurotus ostreatus*, context is thicker than that of *Pholiota nameko*, character is more pliable than that of *Lentinula edodes*, and it even has unique fragrance of crab, so its mouth feel is good. Apart from its popular/exotic culinary status, *H. marmoreus* has exceptional medicinal

application including antitumour role. Matsuzawa (1998) reported that adding fruit bodies of beech mushroom to the diet of tumor–bearing mice resulted in a potent anti-tumor effect. Studies reported that *H. marmoreus* has an antioxidant effect (Matsuzawa *et al.*, 1998) and an antiatherosclerotic effect that lowers total serum cholesterol (Mori *et al.*, 2008). This mushroom contains complete set of 8 necessary amino acids and several kinds of polysaccharide. The extracts drawn by hot water or organic solvent from the fruit body has potential role to clear away the free radical of human body, which suggests that the mushroom has the effects of preventing and curing cancer, raising immunity, defending senescence and prolongs life. Moreover, polysaccharide, S–(1–3)–D–glucan isolated from *H. marmoreus*, showed a very high antitumor activity (Akavia *et al.*, 2009). Due to multipurpose uses; production and consumption is ever increasing of this medicinal mushrooms in the world. A lot of studies focused on its nutrient substances, physiological characters, and healthcare functions.

In general, it is recognized that growing mycelia in a defined medium is a rapid and alternative method to obtain fungal biomass for further use (Yang *et al.*, 2009). Moreover, it is also well known that the mycelial cultivation of mushrooms is enhanced by different environmental and nutritional factors as well as propagation of mycelia is an earlier and essential step as spawning to culti-

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vate fruiting bodies of mushrooms. Comprehending this consequence, a proportional study has been conducted on the vegetative growth and artificial fruit body formation of *H. marmoreus* using different physiochemical factors to assess the optimal farming condition.

MATERIALS AND METHODS

Mushroom sample

The mycelial cultures of 2 strains (Brown–buna shimaji and White–bunapi shimaji) of *H. marmoreus* were collected from Kyushu University Mushroom Bank (KUMB), Japan and used in this experiment. To facilitate the study, strains of *H. marmoreus* were transferred to potato dextrose agar (PDA) plates and incubated at 23°C in the dark condition until they showed a full growth and then preserve at 8°C for further use. Unless otherwise stated, the tests were performed at least 3 times.

Effects of temperature and pH

The experiment was conducted to screen the optimum temperature and pH for the mycelial growth of the mushroom. Five different temperatures (15, 20, 25, 30 and 35°C) were used. A 5 mm diameter agar plug of an inoculum removed from 10 day old cultures grown on PDA and placed in the centre of each plate filled with 20ml of PDA. The medium was adjusted to pH 6 and incubated for 10 days at 15, 20, 25, 30 and 35°C separately. Radial growth of mycelia on each Petri dish was measured at 3 directions and average value was calculated out of those 3 measurements. To calculate final mean value of mycelial growth of each strain, 3 replications were used. For pH effect, a 5 mm diameter agar plug of an inoculum was removed and placed similarly. The medium was adjusted to pH 5, 6, 7, 8 and 9 with the addition of 1 N NaOH or HCl, and incubated for 10 days at 23°C. The measurement of mycelial growth was performed following same technique as optimum temperature tests.

Effects of carbon and nitrogen sources

To screen carbon and nitrogen sources favorable for the vegetative growth of selected mushroom strains, the tests were performed on potato agar (PA) medium following the modified technique used by Sung *et al.*, 1993. Each of 5 carbon sources (fructose, glucose, lactose, maltose and sucrose) were supplemented at the concentration of 2 g/100 ml with PA medium separately. In case of each nitrogen source, $\text{NH}_4\text{H}_2\text{PO}_4$, CaNO_3 , KNO_3 , NaNO_3 and urea were supplemented separately at the concentration of 0.02 M with 20 g of glucose to 100ml of PA medium. In both cases, the medium was adjusted to pH 6 before autoclaving. To measure the colony diameter on the media, all plates were incubated for 10 days at 23°C. Radial growth of mycelia was measured following same method which described earlier.

Effects of culture media

Five different culture media (Czapek dox, Hennerberg, Glucose peptone, PDA and YM) were pre-

pared to investigate the mycelial growth of the strains. The media were adjusted to pH 6 before autoclave. All culture media were inoculated with an inoculum similarly. After 10 days of incubation at 23°C, measurement of mycelial growth was performed with same manner used before.

Effect of sawdust and additives on mycelium running

To determine suitable additives for the mycelium running, sawdusts of *Mangifera indica* (mango), *Albizia procera* (koroi) and *Swietenia mahagoni* (mahagoni) were used with additives. The substrate was prepared supplimented (v/v) with 80% sawdust, 10% rice bran, 10% wheat bran and 1% calcium carbonate (CaCO_3) for mycelium running of *H. marmoreus*. After adding 60–70% water, round glass columns (2 cm × 22 cm) were filled with individual sawdust mixture separately and autoclaved for 90 minutes at 121°C. After 10–12 hours cooling, all glass columns were inoculated with mother culture of *H. marmoreus* and incubated in a mycelium running room for 4 weeks at 23°C. Thereafter, mycelial growth in glass columns was measured to screen out suitable substrate to propagate *H. marmoreus* and mycelial running rate (MRR) was noted.

Conditions for primordial formation

Similar method used for mycelium running was followed to testify the formation of premordia and fruiting body of *H. marmoreus* but polypropylene bottles (12 cm × 30 cm) were used in stead of glass columns. All bottles were inoculated with mother culture of *H. marmoreus* and incubated in a mycelium running room for 35 days at 23°C. While substrates of all bottles were colonized with mycelia and turned into white color, excrete mycelial buff matter suggested its physiological maturation. Thereafter, bottles were opened and scratched mycelia from the neck and bottles were soaked with tap water for 3 hours and then incubate for 10–15 days at 17°C under alternative 12 hours light (350 lux) and dark conditions with relative humidity of 95%. After incubation, brown/white–milky colored primordia have been started to form on the top surface of the media.

Effect of ventilation

This experient was conducted in plastic boxes (18 inch × 15 inch × 12 inch) by filling the bottom of the boxes with tap water. Fourteen bottles of 35 days old spawn which were ready to form fruit body kept in the each plastic box. Then, three boxes were covered with transference polyethylene and similar set of boxes maintained to cover and open at 6 hours interval. In both cases, incubation was maintained at 19°C for 10–15 days. After incubation, the condition of cultures and fruit bodies in all boxes was obseved and recorded.

RESULTS AND DISCUSSION

Effect of temperature

The environmental requirements for the artificial veg-

Table 1. Effect of temperature and pH on mycelial growth of *H. marmoreus*

°C	Mycelial growth (mm)		Mycelial growth (mm)		pH
	Brown strain	White strain	White strain	Brown strain	
15	27.8	22.0	18.5	22.8	5
20	75.6	71.8	26.2	32.8	6
25	69.9	66.6	30.3	34.5	7
30	38.1	35.9	29.4	35.4	8
35	19.6	18.3	25.1	30.2	9
Mean	46.2	42.9	25.9	31.1	Mean

Mycelial growth was calculated (n=3) after 10 days of incubation and effect of pH on mycelial growth was recorded at 23°C.

etative growth of 2 strains (brown and white) of *H. marmoreus*, an edible and delicious medicinal mushroom were studied. To screen the optimum temperature, 5 different temperatures (15, 20, 25, 30 and 35°C) were studied (Table 1). The optimum temperature for the filamentous growth and density of studied mushroom was obtained at 20–25°C. The lowest mycelial growth and density were recorded at 35°C. The best mycelial growth of both white and brown strains of *H. marmoreus* was found at 20°C. The both strains of *H. marmoreus* love medium–low temperature for adequate vegetative proliferation. Lee *et al.* (1999) and Shim *et al.* (2003) reported that the mycelial growth of *Paecilomyces fumosoroseus* had been expedited gradually in proportion to the rise of temperature and the most suitable was at 25°C. Even though the mycelial growth of *P. fumosoroseus* was favorable at the range of 20–25°C and had been expedited in proportion to the rise of temperature, the mycelial growth appeared to be suppressed at the temperature higher than 30°C. Shim *et al.* (2005) and Jo *et al.* (2006) stated that the favorable mycelial growth of *Macrolepiota procera* and *Phellinus* spp. was at 30°C. Hur *et al.* (2008) observed the temperature effect on the mycelial growth of *P. linteus* and found that the temperature of 30°C was the best which does not comply with our result. Jayasinghe *et al.* (2008) studied the temperature effect on the mycelial growth of 8 strains belonging to *Ganoderma lucidum* and found suitable growth at 25°C with extension range of 20–30°C. So, it could be said that the mushroom used in this experiment was different from the mushroom used by Shim *et al.*, Jo *et al.* and Hur. Therefore, their results are not corresponded with that of our findings.

Effect of pH

Hydrogen ion concentration of the culture medium has a profound influence of overall metabolic activities of fungi. Highly acidic or alkalic media usually inhibit the growth of fungi and the minimum, optimum and maximum pH values as well as the pH range (5–9) for fungal mycelia was very considerable. Considering this, a research was conducted to screen out the suitable pH value for mycelial growth of *H. marmoreus*. This study found that the different strains may have an extensive

pH range for their spontaneous mycelial growth (Table 1). The highest mycelial growth of brown and white strains was found 35.4 mm and 30.3 mm at pH 7 and 8, respectively. For both strains, the lowest mycelial growth was found at pH 5. It was found that *H. marmoreus* prefer alkalic to acidic condition of media. Shim *et al.* (2005) revealed that pH 7 is the most suitable for the optimal growth of *M. procera*. Choi *et al.* (1999) and Chi *et al.* (1996) reported that mycelial growth of *Phellinus japonica* and *P. linteus* was optimal at pH 7 and 6–7, respectively. Shim *et al.* (2003) exposed that optimal pH was 8 for *Paecilomyces sinclairii*. Intiaj *et al.* (2008a) observed the pH requirement for the vegetative growth of 10 strains of *Schizophyllum commune* and reported that pH 5 was the best. After that decreased mycelial growth was found when pH value is gradually increased up to pH 9. This result suggested that mushrooms may have a broad pH range for their optimal mycelial growth in nature. The results of this study is completely similar to Shim *et al.* (2005), Shim *et al.* (2003), Choi *et al.* (1999) and Chi *et al.* (1996).

Effect of carbon sources

Carbon is the most required nutrient for mushroom. Carbon usually comes from organic compounds such as suger, organic acid, alcohols, starch, cellulose, hemi-celluloses and lignine. Five different carbon sources (glucose, fructose, lactose, maltose and sucrose) were used to find out the optimal farming condition and sucrose was the best carbon source for mycelial proliferation (Table 2). Fructose showed average mycelia growth of *H. marmoreus*. The lowest growth of vegetative development was obtained in maltose for both strains (brown strain 33.56 mm and white strain 28.90 mm). This result is completely similar to Shim *et al.* (1997) but partially similar to Shim *et al.* (2005). Shim *et al.* (1997) reported that *G. umbellata* was favorable to maximum carbon sources except salicin, cellobiose and lactose. Shim *et al.* (2005) proved that sucrose, maltose, dextrin and mannose were effective where lactose was highly negative.

Effect of nitrogen sources

To find out the effects of nitrogen sources for mycelial growth, 5 nitrogen sources were used in this study.

Table 2. Effect of carbon and nitrogen sources on mycelial growth of *H. marmoreus*

Carbon sources	Mycelial growth (mm)		Mycelial growth (mm)		Nitrogen sources
	Brown strain	White strain	White strain	Brown strain	
Glucose	37.60	32.09	48.30	50.90	Urea
Fructose	39.60	32.20	56.30	65.60	Calcium nitrate
Lactose	35.01	33.60	48.01	50.00	Sodium nitrate
Maltose	33.56	28.90	50.30	55.05	Potassium nitrate
Sucrose	40.70	36.06	40.20	44.02	Amm. phosphate
Mean	37.29	32.57	48.622	53.114	Mean

Mycelial growth was calculated (n=3) after 10 days of incubation at 23°C

Table 3. Effect of media on mycelial growth of *H. marmoreus*

Media	Mycelial growth (mm)	
	Brown strain	White strain
Czapek dox (Cza)	34.20	33.10
Glucose peptone (GP)	38.11	36.80
Hennerberg (Hen)	36.20	34.80
Potato dextrose agar (PDA)	53.50	50.30
Yeast-malt extract (YM)	43.90	41.30
Mean	41.18	39.26

Mycelial growth was calculated (n=3) after 10 days of incubation at 23°C.

The most suitable nitrogen sources were calcium nitrate (brown strain 65.6 mm and white strain 56.3 mm), potassium nitrate (brown strain 55.05 mm and white strain 50.3 mm) and average suitable was sodium nitrate (brown strain 50.00 mm and white strain 48.01 mm) and Urea (brown strain 50.90 mm and white strain 48.3 mm). The most unsuitable was ammonium phosphate (brown strain 44.02 mm and white strain 40.2 mm). CaNO_3 , potassium nitrate showed moderately thin or thin mycelial density (Table 2). Most of the nitrogen sources showed compact mycelial density. Shim *et al.* (2005) clarified that glycine was the most favorable and histidine, arginine and ammonium oxalate were the most unfavorable for the mycelial growth of *M. procera* on the culture media. Lee *et al.*, (2005) showed that soytone, malt extract, yeast extract and bacto-peptone were the most favorable but NaNO_3 and urea were the most unfavorable for the mycelial growth of *Ramaria botrytis*.

Screening of favorable culture media

Five different culture media were used to screen the optimal vegetative growth of *H. marmoreus*. According to mycelial growth and density, PDA (brown strain 53.5 mm and white strain 50.3 mm) and YM (brown strain 43.9 mm and white strain 41.3 mm) were the most suitable. Glucose peptone (GP) was medium suitable (brown strain 38.11 mm and white strain 36.8 mm). Czapek dox (brown strain 34.2 mm and white strain 33.1 mm) and Hennerberg (brown strain 36.2 mm and white strain

34.8 mm) were the most unfavorable for vegetative growth of mushroom (Table 3). Besides of slow growth, mycelial density was also somewhat thin to thin on Czapek dox. This result is corresponded with that of Imtiaz *et al.* (2008b) where filamentous growth of *Hericium erinaceus* was the highest observed on PDA, YM, Hamada and GP media. Whereas, the lowest mycelial growth was counted on Czapek dox, Hoppkins, Lilly and Hennerberg. Shim *et al.* (2005) also reported similar mycelial growth of *P. sinclairii* and *P. fumosoroseus* found suitable on PDA, YM, Mushroom complete and Hamada.

Effect of sawdusts on vegetative growth

The experiment was conducted to study the effect of different sawdusts on the vegetative growth and yield of studied mushroom. The mycelial growth of *H. marmoreus* was observed for 4 weeks. The highest and lowest vegetative growth was observed in mango and koroï sawdust media, respectively. However, for both strains, 3 weeks were required to complete mycelium running in mango sawdust, whereas, 4 weeks were spent to get 100% mycelium running in mahagoni and koroï. The most slowest filamentous growth was recorded in koroï sawdust (Table 4).

The highest number of primordia per bottle filled with mango sawdust was counted 34 for brown and 28 for white strains. Whereas, the lowest number of primordia per bottle filled with koroï sawdust was noted 30 and 23 for brown and white strain, respectively. The sawdust of mahagoni showed moderate performance of primordia formation for both strains of the mushroom. The substrate of mango was promoted to produce fruiting bodies of 29 (brown) and 24 (white) strains. The highest biomass weight of brown (88 gm) and white (73 gm) strain was obtained from mango sawdust and the lowest fresh weight of brown (80 gm) and white (61 gm) strain was recorded from koroï sawdust. The ongoing publicity of mushroom as a high source of protein with low cholesterol content which over ride meats and other fatty foods may soon get extra priority due to the use of sawdust as the best cheapest substrate for growing mushroom. It could be said that mango and mahagoni sawdusts are cellulose riched substrates suitable to grow *H. mar-*

Table 4. Effect of carbon and nitrogen sources on mycelial growth of *H. marmoreus*

Strain	Substrate	Mycelial growth (%) / week			
		1 st week	2 nd week	3 rd week	4 th week
Brown	Mango (<i>Mangifera indica</i>)	70.3	94.5	100	
	Mahagoni (<i>Swietenia mahagoni</i>)	35.2	67.5	97.0	100
	Koroi (<i>Albizia procera</i>)	34.0	53.3	76.3	
White	Mango (<i>Mangifera indica</i>)	65.4	90.4	100	
	Mahagoni (<i>Swietenia mahagoni</i>)	37.3	55.5	92.0	100
	Koroi (<i>Albizia procera</i>)	31.0	49.3	70.7	

Incubation for mycelial growth was conducted (n=3) at 23°C

moreus, whereas, koroi sawdust is lignin abundant that is complex to degrade and unsuitable for studied mushroom. Shim *et al.* (2006b) found that fruiting bodies of *Armillaria mellea* were produced abundantly in the oak sawdust medium mixed with 10% rice bran but contamination rate seemed to be increased in proportion to the higher contents of rice bran. Semerdzieva *et al.* (1988) also reported that fruiting bodies of *Oudemansiella radicata* were formed very well in the oak sawdust medium mixed with 10% rice bran. Therefore, rice/wheat bran might contain some ingredients to facilitate the good mycelial growth of mushrooms and our result corresponds with many evidences.

Conditions for fruiting body formation

After formation of the primordia, the bottles were transferred to cultivation room at 19°C under 12 hours of illumination (350 lux) and darkness with relative humidity of 95%. The fruiting bodies of *H. marmoreus* were

formed in the mango sawdust medium sooner. The fruiting bodies of brown strain were formed earlier than white strain after occurrence of primordia. In case of limiting ventilation, the stem development was found to be good but no cap was seen to be formed (Fig. 1). Intiaj *et al.* (2008b) reported that 10% rice bran was better supplement with sawdust to produce fruiting bodies of *Hericiium erinaceus*. Shim *et al.* (2006a) and Semerdzieva *et al.* (1988) also reported that fruiting bodies of *O. radicata* were formed very well in the oak sawdust medium mixed with 10% rice bran. Rew *et al.* (2004) showed that the mycelial growth of *Phellinus baumii* was best on oak sawdust mixed with 20% of rice bran (v/v). Shim *et al.* (2006a) reported that mycelial growth and density of *O. radicata* were also good on oak sawdust mixed with 10% rice bran that might contain some ingredients to facilitate the good mycelial growth of *O. mucida* and the other mushrooms.

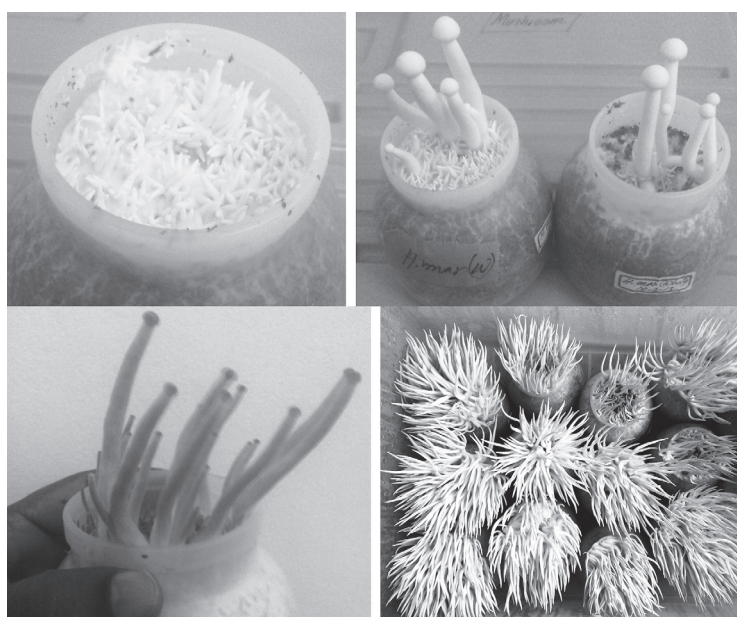


Fig. 1. Different stages of sporocarp of *H. marmoreus*. Premordia/pins formation of white strain (top-left), complete fruit body of white strain (top-right), complete fruit body of brown strain (bottom-left), capless fruit body of brown strain (bottom-right). young fruit body (middle) and mature fruit body (right).

CONCLUSION

This study was conducted for offering the best filamentous growth condition of brown and white strains of *H. marmoreus*. The requirement for the vegetative growth of 2 strains was slight different and vegetative growth of brown strain was faster than white strain in every case. Nowadays, we are devoted to improve the yield of biomass and quality of *H. marmoreus* through a new innovative experimental method by changing physicochemical factors, substrates and additives of the growing media. Ultimately, we might be able to produce commercially feasible basidiocarps of *H. marmoreus* using local agro-wastes and sawmill residues. After all, this is the first report associated with fruiting body production of *H. marmoreus* in Bangladesh.

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