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Optimization of parameters for maximal mycelial growth of enoki mushroom (*Flammulina velutipes*)

Laxmi Devi[1], Sachin Gupta*[1], Moni Gupta[2], Stanzin Diskit[1], Meh-run-nisa[1], Nentia Chib[1], Ranbir Singh[1], Upma Dutta[3] and Julie D. Bandral[4]

[1]*Division of Plant Pathology, FoA, SKUAST-Jammu, J&K, 180009, India*
[2]*Division of Biochemistry, FBSc, SKUAST-Jammu, J&K, 180009, India*
[3]*Division of Microbiology, FBSc, SKUAST-Jammu, J&K, 180009, India*
[4]*Division of Post Harvest Management, FoH&F, SKUAST-Jammu, J&K, 180009, India*

ABSTRACT

The present study was conducted to evaluate the influence of culture media, pH and temperature on the growth dynamics of two strains of Flammulina velutipes (DMRX-166 and DMRO-253) for enhancing mycelium proliferation under in vitro conditions. Five different solid media were tested, and distinct differences in growth were observed between the strains and across the media. Among the media, Malt Extract Agar (MEA) supported the highest mycelial proliferation with average growth rate (5.45mm/day for DMRX-166 and 5.12 mm/day for DMRO-253), followed by Potato Dextrose Agar (PDA) and Wheat Straw Agar (WSDA). Irrespective of strain, among the four different temperature regimes (10°C, 15°C, 20°C and 25°C), the highest radial growth was recorded at 25 °C, while lower temperatures (10-15 °C) resulted in slower mycelial growth. Similarly, across pH levels (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0), optimal mycelial development occurred within the pH range of 7.0-8.0, with DMRX-166 showing maximal mycelial growth compared to DMRO-253. Maintaining consistent environmental conditions throughout the experiment was challenging. Nevertheless, the findings contribute valuable insights into the optimization of cultural conditions for efficient mycelial proliferation of F. velutipes, providing a scientific basis for large scale cultivation and strain improvement.

Keywords: *Flammulina velutipes, mushroom, mycelial growth, solid media, temperature, enoki.*

INTRODUCTION

Mushrooms are large, spore-bearing and macroscopic reproductive structures that are produced by ascomycetous and basidiomycetous fungi during their sexual life cycle. Various mushroom species have long been recognized for their dual significance as both food and medicine. Edible mushrooms are recommended by FAO as food to meet the protein requirements of developing countries, a large portion of which depends mainly on cereals [1]. Mushrooms are a rich source of bioactive compounds with notable medicinal potential, and their direct inclusion in the diet can promote health through the additive and synergistic effects of these bioactive constituents [2]. The mycelium, the vegetative network of fungal hyphae, is central to the cultivation of mushrooms serving as the primary structure for nutrient absorption and the precursor to fruit body formation. Beyond structural development, the mycelium is a source of bioactive compounds such as polysaccharides, phenolic compounds, terpenoids, and amino acids. These compounds exhibit various pharmacological activities, including antioxidant, immunomodulatory and anticancer effects [3]. Optimizing mycelial growth not only ensures robust fruit body production but can also enhance the accumulation of these bioactive molecules, thereby maximizing the therapeutic potential of mushrooms. The rate and proliferation of mycelial growth of different mushroom species are greatly influenced by the composition of the media [4,5]. Therefore, optimizing mycelial growth is pivotal for enhancing the yield, quality and production of bioactive compounds with medicinal properties. Environmental and nutritional factors, notably temperature, pH and culture medium composition, significantly influence mycelial development. *Flammulina velutipes* commonly known as Enoki mushroom is recognized as one of the earliest cultivated fungi, with records of its domestication dating back to 800 A.D. [6]. In East Asian countries, Enoki mushrooms are primarily grown for their culinary appeal and health-promoting properties. Globally, *F. velutipes* ranks fourth among edible mushrooms in terms of production and consumption [7]. It is well known for its mild crispy texture and rich nutritional profile as well as potential health benefits with a range of bioactive compounds. Despite its global distribution, the commercial popularity of *F. velutipes* is more prominent in Asia [8]. The wide range of bioactive compounds has been identified in *F. velutipes*, including polysaccharides, protein–glucan complexes, sterols, lectins and various enzymes such as peroxidases, laccases, cellulases, and proteases. These molecules exhibit diverse medicinal and pharmaceutical properties [9]. Both mycelial cultures and fruiting bodies of *F. velutipes* have been shown through *in vitro* studies to possess a wide spectrum of biological activities, such as immunomodulation through cytokine induction as well as antifungal, antibacterial, antiviral, antioxidant, antiprotozoal

*Corresponding Author: ***Sachin Gupta***

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and mitogenic effects [10]. Enokipodins A, B, C, and D which are α-cuperene-derived sesquiterpenoids represent important secondary metabolites of *F. velutipes* and are produced as antimicrobial agents during the stationary phase of mycelial development in malt extract medium [11]. In addition to its therapeutic potential, successful cultivation depends heavily on its physiological requirements. Therefore, understanding the physiological requirements of *F. velutipes* is essential for optimizing the cultivation techniques to maximize the yield. Optimizing cultivation practices for *F. velutipes* requires careful evaluation of culture media, temperature and pH as these factors play a decisive role in enhancing mycelial growth and ultimately mushroom yield. Identifying ideal physiological requirements *viz*., media composition, temperature regime and pH levels can substantially improve mycelial colonization efficiency resulting in improved productivity and better quality fruit bodies of *F. velutipes*. Studies on mycelial growth parameters are essential for the cultivation of different species of mushrooms, as it also facilitate spawn production and disease resistance which is essential for successful cultivation. [12]. Therefore, the present study aimed to determine the influence of physiological factors *viz.*, culture media, pH and temperature on the growth dynamics of mycelial growth of two strains of enoki mushroom (*F. velutipes*).

MATERIAL AND METHODS

The experiment was carried out in the Mushroom Research Unit, Department of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology, Chatha, Jammu (India).

Source and Maintenance of Culture

Two strains of *F. velutipes* (DMRX-166 and DMRO-253) used in the present study were procured from the Directorate of Mushroom Research, Chambaghat, Solan. The cultures were subsequently sub-cultured on Potato Dextrose Agar (PDA) slants. These slants were maintained at ambient temperature 23±2°C for one week and then subsequently stored in a refrigerator at 4±2°C for further use.

Effect of different solid culture media on mycelial growth

In the present study, various solid media were assessed to determine the most suitable growth medium for the optimal development of the fungus. Five different solid agar-based media (Potato Dextrose Agar, Malt Extract Agar, Sawdust Decoction Agar, Wheat Straw Decoction Agar and Paddy Straw Decoction Agar) were evaluated and PDA was used a control.

Preparation of Media

Culture media used in the present study were prepared in accordance with the standard protocol, the individual components of each medium were dissolved separately in distilled water and 2% agar was incorporated for solidification [13]. In the case of WSDA, 200 g of wheat straw was boiled in 500 mL of distilled water for 45 minutes. The resulting decoction was filtered through muslin cloth and the extract was collected in a separate beaker. In a separate preparation, 20 g of agar-agar was dissolved in 500 mL of water by boiling followed by the addition of 20 g of dextrose. The wheat straw extract was then combined with the agar solution while continuously stirring with a glass rod. The final volume was adjusted to 1000 mL in a conical flask by adding additional distilled water [14].

A similar procedure was used for the preparation of sawdust decoction agar and paddy straw decoction agar. All prepared media were autoclaved at 15 psi for 20 minutes. Sterilized petri plates were used for pouring the prepared media. For each treatment, 20 mL of the respective medium was aseptically dispensed into each sterilized petri plate. The poured medium was then allowed to solidify before inoculation with the *F. velutipes*. After solidification, petri plates were inoculated at the center with a mycelial disc obtained from a pure culture of *F. velutipes*. The inoculated plates were then incubated at 23±2°C in a BOD incubator. Mycelial radial growth rate observations were recorded at 2 day intervals. Each treatment was replicated in triplicate.

Effect of different temperature regimes on mycelial growth

The influence of temperature on the mycelial growth of *F. velutipes* was investigated. The four different temperature regimes (10°C, 15°C, 20°C, 25°C) were used to standardize the optimal temperature. 20°C was used as the control. Mycelial discs were inoculated onto petri plates containing basal medium and incubated at four different temperatures in a BOD incubator and radial mycelial growth rate observations were recorded at 2 day intervals. Each treatment was replicated in triplicate.

Effect of different pH levels on mycelial growth

To examine an optimal pH for the mycelial growth of *F. velutipes*, the basal medium was adjusted with different pH levels. The pH of the medium was adjusted to a range of 5-8 using acids and bases before sterilization by using a calibrated digital pH meter. pH of 6.5 was used as the control. 20 ml of media was aseptically poured into sterilized petri plates and allowed to solidify. Once solidified, the plates were inoculated at the center with a mycelial disc of the culture and incubated at 23±2°C in a BOD incubator. The radial mycelial growth rate was measured at 2 day intervals. Each treatment was replicated in triplicate.

Statistical Analysis

The data recorded in each experiment were subjected to statistical analysis using a factorial completely randomized design.

RESULTS AND DISCUSSION

Effect of different solid culture media on mycelial growth of *F. velutipes*

Results presented in Tables 1 and 2 indicated that among the five different solid media evaluated, the tested media significantly influenced the mycelial growth rate of *F. velutipes* strains. Statistical analysis confirmed that the radial growth rate was maximum on malt extract agar followed by potato dextrose agar and all the treatments were significantly different from each other. However, the minimum average radial was observed on sawdust decoction agar for both strains. In the case of strain, DMRX-166, the highest average growth rate was 5.45mm/day recorded on MEA, followed by 5.28mm/day on PDA. The lowest average growth rate 3.96mm/day was recorded on SDA. For strain DMRO-253, the highest mycelial growth rate 5.12mm/day was recorded with MEA, followed by 4.86mm/day on PDA. In contrast, SDA proved to be the least effective media with lowest average growth rate of 3.50mm/day. The results obtained on solid media corroborate previous reports indicating enhanced mycelial growth of *F. velutipes* on malt extract agar [15].

Similar results were obtained with maximum mycelial growth of *F. velutipes* observed on malt extract agar medium [16]. Our results conform with earlier studies which reported that malt extract agar was the best culture media for the mycelial growth of *Ganoderma lucidum, Pleurotus ostreatus* and *Volvariella volvacea* [17, 18].

Effect of temperature on mycelial growth of *F. velutipes*

The results presented in Table 3 and Table 4 revealed the impact of different temperatures on the mycelial growth rate of strains (DMRX-166 and DMRO-253) of *F. velutipes*. Four different temperatures were evaluated for radial growth and the results showed that irrespective of the strains used, 25°C was found to be the most optimal temperature for maximum mycelial growth. In the present study, statistical analysis confirmed that the growth rate of *F. velutipes* was optimal at 25°C and significantly higher than other temperature regimes, followed by 20°C, whereas, the lowest radial growth was recorded at 10°C and 15°C. For strain DMRX-166, the maximum average growth rate 5.15mm/day was recorded at 25°C, followed by 4.40mm/day at 20°C. However, the minimum average growth rate 0.32mm/day was observed at 10°C. Similarly, for strain DMRO-253, 25°C was found to be the most optimal temperature with average growth rate of 4.85mm/day, followed by 20°C with an
average growth rate of 3.68mm/day. However, at 10°C lowest mycelial growth rate of 0.20mm/day was observed. Temperature plays a crucial role in regulating the mycelial development of mushrooms. The optimum temperature range is important not only for mycelial growth but also for the production of metabolic products and sporulation [19]. The above findings are in agreement with findings that reported 25°C to be the best temperature for mycelial growth of mushrooms [20]. The optimum temperature for mycelial growth of *F. velutipes* was found to be 25°C [21]. Baekjung is a variety of *F. velutipes*, which exhibits optimum mycelial growth at 25°C-30°C temperature [22].

The biomass accumulation and crude extract yield of *F. velutipes* were found to be increased markedly when the incubation temperature was increased to 25°C [23]. All these earlier findings are in close agreement with the results of the present investigation.

Effect of pH on mycelial growth of *F. velutipes*

The results presented in Table 5 and Table 6 revealed the effect of pH on mycelial growth of strains (DMRX-166 and DMRO-253) of *F. velutipes*. Seven different pH *viz.*, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 were evaluated for the radial growth and the result showed that irrespective of the strains used, pH 7.5 was found to be the most optimal pH for the growth of *F. velutipes*. Statistical analysis confirmed that the average radial growth rate at pH 7.5 was maximum, while the minimum average radial growth was recorded at pH 5.0. For strain DMRX-166, the highest average growth rate 6.69mm/day, was observed at pH 7.5, followed by pH 7.0 with a growth rate of 6.40mm/day. However, the lowest growth rate of 1.80mm/day was recorded at pH 5.0. Similarly, in case of strain DMRO-253, a maximum average growth rate 6.50mm/day was recorded at pH 7.5, which is followed by 6.22mm/day with pH 7.0, while the minimum growth rate of 1.60mm/day was recorded at pH 5.0. In the present study, pH 7.0-8 exhibited the maximum mycelial growth of *F. velutipes*. The literature on the influence of pH for *F. velutipes* is limited, but similar observations have also been reported for other mushrooms. The previous studies reported that *Cordyceps militaris* (L.) achieved maximum growth on solid media at pH 7.5 [24]. Likewise, through statistical and evolutionary optimization of medium composition, it was demonstrated that pH was a key factor affecting exopolysaccharides production in *Ganoderma lucidum*, with the highest yield predicted at pH 7.5 [25]. Similar results were recorded, wherein 7.0-8.0 was recorded as optimal pH for mycelial growth of *Pleurotus djamor* strains [26].

Table 1: Effect of different solid culture media on mycelial growth (mm) of Flammulina velutipes strain (DMRX-166)

Media	Days of incubation								
	2	4	6	8	10	12	14	16	Avg. growth rate
PDA	4.00	4.65	5.13	5.76	6.22	5.90	5.50	5.10	5.28
MEA	4.30	4.72	5.46	5.85	6.32	6.05	5.70	5.25	5.45
SDA	2.52	3.05	3.80	4.54	5.05	4.64	4.20	3.95	3.96
WSDA	2.94	3.50	4.30	4.86	5.43	5.25	4.75	4.35	4.42
PSDA	2.70	3.25	4.15	4.68	5.20	4.80	4.52	4.20	4.18
	CD (0.05)				SE (d)				
Media	0.020				0.010				
Days	0.025				0.013				
Media x Days	0.057				0.029				

Table 2: Effect of different solid culture media on mycelial growth (mm) of Flammulina velutipes strain (DMRO-253)

Media	Days of incubation								
	2	4	6	8	10	12	14	16	Avg. growth rate
PDA	3.50	4.11	4.85	5.20	5.75	5.55	5.11	4.80	**4.85**
MEA	4.01	4.42	4.96	5.55	6.00	5.84	5.32	4.90	**5.12**
SDA	2.00	2.88	3.76	3.98	4.14	4.00	3.75	3.55	**3.50**
WSDA	2.41	2.92	3.88	4.22	4.95	5.10	4.40	3.95	**3.97**
PSDA	2.33	2.90	3.80	4.00	4.47	4.68	4.05	3.77	**3.75**
	CD (0.05)				SE (d)				
Media	0.013				0.007				
Days	0.017				0.009				
Media x Days	0.038				0.019				

Table 3: Effect of different temperature regimes on mycelial growth (mm) of Flammulina velutipes strain (DMRX-166)

Temperature	Days of incubation						
	2	4	6	8	10	12	Avg. growth rate
10°C	0.15	0.31	0.32	0.36	0.37	0.42	**0.32**
15°C	0.84	0.95	1.00	1.11	1.28	1.40	**1.09**
20°C	3.75	4.03	4.30	4.57	4.82	4.96	**4.40**
25°C	4.21	4.66	5.00	5.24	5.79	6.04	**5.15**
	CD (0.05)			**SE (d)**			
Temperature	0.022			0.011			
Days	0.027			0.013			
Temperature x Days	0.054			0.027			

Table 4: Effect of different temperature regimes on mycelial growth (mm) of Flammulina velutipes strain (DMRO-253)

Temperature	Days of incubation						
	2	4	6	8	10	12	Avg. growth rate
10°C	0.00	0.08	0.19	0.29	0.33	0.36	**0.20**
15°C	0.40	0.55	0.68	0.80	0.96	1.05	**0.74**
20°C	2.90	3.25	3.69	3.92	4.10	4.22	**3.68**
25°C	4.00	4.41	4.78	5.05	5.32	5.54	**4.85**
	CD (0.05)			**SE (d)**			
Temperature	0.017			0.008			
Days	0.020			0.010			
Temperature x Days	0.041			0.020			

Table 5: Effect of different pH levels on mycelial growth (mm) of Flammulina velutipes strain (DMRX-166)

pH	Days of incubation						
	2	4	6	8	10	12	Avg. growth rate
5.0	1.42	1.60	1.80	1.92	2.00	2.10	**1.81**
5.5	1.70	1.91	2.10	2.33	2.42	2.50	**2.16**
6.0	5.02	5.22	5.47	5.70	5.82	5.95	**5.53**
6.5	5.28	5.49	5.84	5.99	6.18	6.33	**5.85**
7.0	5.77	5.99	6.21	6.50	6.88	7.05	**6.40**
7.5	6.11	6.41	6.66	6.80	6.96	7.20	**6.69**
8.0	5.58	5.85	6.10	6.30	6.58	6.79	**6.20**
	CD (0.05)			**SE (d)**			
pH	0.015			0.007			
Days	0.013			0.007			
pH x Days	0.036			0.018			

Table 6: Effect of different pH levels on mycelial growth (mm) of Flammulina velutipes strain (DMRO-253)

pH	Days of incubation						
	2	4	6	8	10	12	Avg. growth rate
5.0	1.16	1.29	1.55	1.72	1.89	2.00	**1.60**
5.5	1.67	1.80	1.92	2.02	2.24	2.41	**2.01**
6.0	4.80	5.01	5.21	5.39	5.50	5.59	**5.25**
6.5	5.00	5.22	5.41	5.64	5.90	6.13	**5.55**
7.0	5.82	5.92	6.15	6.25	6.48	6.70	**6.22**
7.5	6.05	6.24	6.48	6.60	6.77	6.86	**6.50**
8.0	5.45	5.75	5.92	6.15	6.31	6.00	**5.93**
	CD (0.05)			**SE (d)**			
pH	0.013			0.006			
Days	0.012			0.004			
pH x Days	0.032			0.016			

CONCLUSION

In this study, attempts were made to evaluate the effect of different solid agar media, pH, and temperature on the mycelial growth of two strains of *Flammulina velutipes*. Our results concluded that the best mycelial growth can be obtained on malt extract agar medium, for both the strains, and they grew well within a pH range of 7.5-8.0 (optimum 7.5), and optimal temperature for their growth was 25°C, which gave vigorous growth of mycelium and these conditions appeared to be the optimum while maintaining the culture and its sub-culture. These findings will aid in the selection of suitable culture media, pH, and temperature parameters for preserving genetic materials and cultivation of this mushroom at a large scale.

FUTURE SCOPE OF THE STUDY

Subsequent research could explore the influence of additional factors such as nutrient composition, carbon and nitrogen sources, humidity, and light exposure to enhance mycelial yield and fruiting body production. Further molecular level investigations on strain variability and stress tolerance could also contribute to the genetic improvement and commercial scalability of *F. velutipes* cultivation.

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