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# Standardization of Parameters for the Mycelium Growth of *Hericium erinaceus* : An Edible Medicinal Mushroom

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**ABSTRACT**

The parameters such as temperature and pH have an imperative impact on the sporophore expansion of mushroom and may turn out to be as a limiting factor in cultivation. The present study was aimed at investigating the optimal growth parameters for the mycelia of the *Hericium erinaceus* (HE 203) strain under submerged conditions. The optimum temperature for mycelial growth was found to be 24°C, although growth occurred in the broader temperature range of 20 to 28°C. In case of pH, the range lied in between 5-8 but ideal pH for maximum mycelial development was at pH 6. Further under *ex-vivo* conditions, the optimized inoculum showed maximum yield on saw dust as compared to wheat straw.

**Key words :** *Hericium erinaceus*, growth parameters, saw dust

## INTRODUCTION

*Hericium erinaceus*, commonly known as the monkey head mushroom, lion's mane mushroom, or pom pom mushroom, has been utilized in traditional Chinese medicine for generations. This mushroom was initially farmed in Japan in the 1960's and is today one of the most significant commercially produced mushroom species in the world (Phan *et al.*, 2014; Yamanaka, 2017; Gong *et al.*, 2022).

*H. erinaceus* is known for its therapeutic capabilities as well as its nutritional and culinary value. This mushroom is high in protein, vitamin $B_{12}$ and minerals, Polysaccharides, lectin, hericirine, hericenone, erinacol and erinacine (Heleno *et al.*, 2015; Wolters *et al.*, 2015). Terpenoids, phenolics, steroids, pyranones, fatty acids and alkaloids are also among the major chemical elements of *H. erinaceus*. According to many researches; over 80 small molecular compounds have been extracted and characterized from *H. erinaceus* (Cohen e*t al.*, 2014; Cheng *et al.*, 2016; Tsai-Teng *et al.*, 2016; He *et al.*, 2017; Atila *et al.*, 2021). Pharmacological research revealed that *H. erinaceus* had a wide range of pharmacological effects including neuroprotection, gastric mucosa protection, antidepressant, anticancer antioxidant, antihyperlipidemic, antihyperglycemic and immune regulation (Lew *et al.*, 2020; Liu *et al.*, 2021; Wu and Huang, 2021).

In most nations throughout the world, mushroom growing is a lucrative agribusiness and multibillion-dollar industry. The mushroom business has grown quickly in the last two decades as new species of mushrooms have been introduced for commercial cultivation. Although production of *H. erinaceus* is popular in Far Eastern countries, interest in Hericium spp. has grown dramatically in other regions of the world in recent years, owing to its therapeutic benefits. In order to popularize the production of this fungus in other regions, it is necessary to screen local isolates from distinct environments and geographic origins.

The goal of this study was to learn more about how temperature and pH affect mycelia formation and growth, as well as overall yield in *H. erinaceus*. For mushroom cultivation, both wheat straw and saw dust substrates are

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in practices in large scale production but evaluation is necessary for high-yielding, high-quality commercial product formation.

## MATERIALS AND METHODS

The isolate HE 203 of *H. erinaceus* used in present study was collected from Regional Research Center, Murthal (Sonipat) of Maharana Pratap Horticultural University (MHU), Karnal (Haryana). The strain was transferred to potato dextrose agar (PDA) plates and stored at 4°C for future use.

A 5 mm disc of strain of *H. erinaceus* was inoculated by an actively developing pure culture in a 250 ml flask containing 150 ml liquid medium. To find the best temperature for mushroom mycelial development, these flasks were incubated at different temperatures 16, 20, 24, 28 and 32°C for 14 days with initial pH of medium at pH 6. Each treatment was replicated five times for calculating the average biomass production. To test the influence of pH, a 5 mm agar plug was taken and put as described above. Using 0.1 N, HCL and 0.1 N, NaOH solutions, different pH values (4, 5, 6, 7, 8 and 9) were adjusted, and the pH was maintained in potato dextrose broth using a Systronic manufacture pH 362 pH metre. The same technique was used to measure mycelial growth at optimal temperature.

Two distinct straw substrates, saw dust and wheat straw, were utilized to see how they affected fruit body formation and yield. Each substrate was soaked for 6-7 h in regular water, then the extra water was drained and the moisture content was adjusted at 65-70%. The correct amounts of wheat bran and $CaCO_3$ were combined into both. The 1.515 kg dry weight of prepared substrate substrates (1 kg straw + 500 g wheat bran + 15 g $CaCO_3$) was filled and plugged into polypropylene bags (12" x 18" – 150 gauges), which were then sterilized for 2 h at 121.6°C. After overnight cooling, sterilized bags were inoculated aseptically with spawning of *H. erinaceus* (HE 203) @ 10% dry weight basis of substrate. Five replicates were performed for both the substrates.

After the inoculation, bags were placed in a spawn running chamber with a temperature of 25 °C and a relative humidity of 80-85%. The bags were placed in mushroom culture rooms after the spawning stage was over at 24°C and the relative humidity was maintained to 85-90%. Harvesting was done before the fruit bodies became yellow and the tassels were ready (around 5-10 mm in length). Two flushes yielded the total mushroom crop.

## RESULTS AND DISCUSSION

An experiment was set up to determine the optimal temperature for *H. erinaceus* (HE 203) and biomass. The outcomes were collected (Fig. 1). Temperature had a substantial impact on the growth and biomass of strain HE 203, and there was a considerable variation in growth at the different temperatures. Considerably greater HE 203 mycelium growth was detected at 24°C (5.95), followed by 20°C (4.01), 28°C (4.23), and 16°C (1.93), all of which differed from each other, while significantly less (0.68) growth was observed at 32°C. The foregoing findings are consistent with Atila *et al.* (2021). *Hericium* isolates tested in the study exhibited good potential for cultivation at 25°C. Grace and Mudge (2015) found 25°C as the optimal temperature for *H. erinaceus* and *H. americanum* strains to develop and produce biomass.

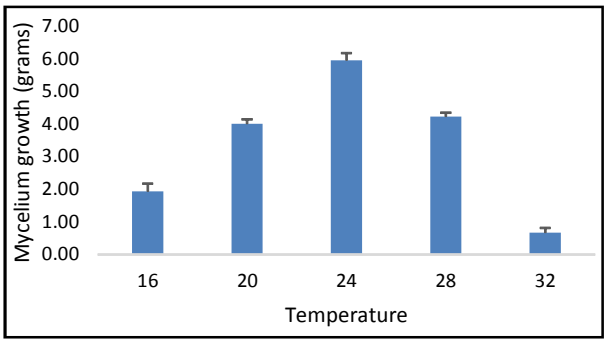


Fig. 1. Effect of temperature on the mycelial growth of *Hericium erinaceus*. The growth was measured after 14 days of incubation in PDB at pH 6.

A study was conducted to determine the best pH for *H. erinaceus* strain HE 203 to achieve optimal growth and biomass. The development of HE 203 at different pH values varied substantially (Fig. 2). Mycelia development of HE 203 was much higher (5.67 g) at pH 6.0, whereas it was significantly lower (1.06) at pH 5.0, followed by pH 9.0 (1.82), pH 8.0 (2.57), and pH 7.0. (4.46). At pH 4.0, no mycelia development was seen.

In this study, the effect of locally and inexpensively available agricultural residual

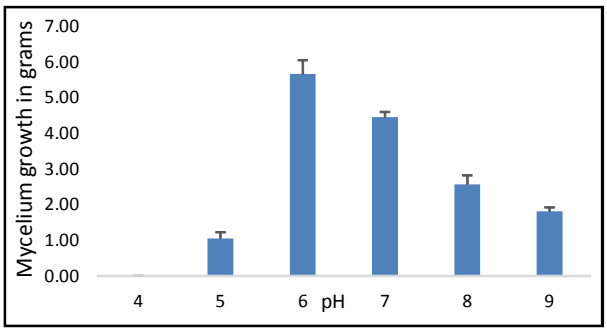


Fig. 2. Effect of pH on the mycelial growth of *Hericium erinaceus*. The growth was measured after 14 days of incubation in PDB at temperature 24°C.

substrates (wheat straw and saw dust) was investigated on the yield of *H. erinaceus*. It was observed that total fresh yield of *H. erinaceus* was significantly influenced by evaluated substrates. In case of saw dust, there was a significantly higher yield of *H. erinaceus* as compared to wheat straw. The net produced was 321.89 g per one kg bag of saw dust substrate and 237.755 g in one kg wheat straw bag (Fig. 3).The current findings were consistent with those of Atila *et al.* (2017), Atila (2019), Atila *et al.* (2021) and Turk *et al.* (2021). Atila (2019) evaluated substrates for *H. erinaceus* and production, and found that common oak sawdust straw produced the earliest spawn run and greater yield with the highest lignin degradation rate.

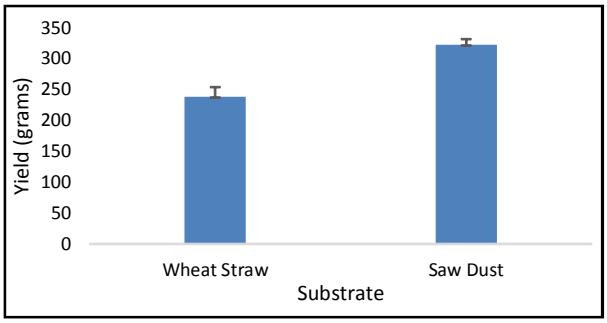


Fig. 3. Effect of substrate on the yield.

## CONCLUSION

The isolate belonging to the *Hericium erinaceus* species He 203 required optimum temperatures of 24°C and pH 6 for maximum mycelium growth. In the current investigation, the fruiting bodies of *H. erinaceus* were produced with greater ease and yield on saw dust rather than wheat straw substrate. This research will help to stimulate the cultivation of *H. erinaceus*, particularly in the Northern Himalayan area.

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