IJIRR

International Journal of Information Research and Review
Vol. 03, Issue, 11, pp. 3125-3128, November, 2016

Research Article

# OPTIMIZATION OF PHYSICAL PARAMETERS FOR THE GROWTH OF A WHITE ROT FUNGUS- *TRAMETES VERSICOLOR*

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**ARTICLE INFO**

*Article History:*

Received 18th August, 2016
Received in revised form
22nd September, 2016
Accepted 14th October, 2016
Published online November, 30th 2016

*Keywords:*

*Trametes versicolor*,
White rot Fungus,
Brown's-II Medium.

**ABSTRACT**

The effect of 12 different basal media, different temperatures (16°C, 20°C, 24°C and 28°C), pH of 3-8 anddays of incubation (for 30 days) on mycelial growth and sporulation of medicinally important white rot fungus *Trametes versicolor* (L.) Lloyd has been investigated. The pure culture was obtained by single spore isolation and maintained on Malt Extract Agar medium at ±4°C for physiological studies. Basal medium composition, temperature, pH and days of incubation have a significant effect on mycelial growth, however, no spore formation have been reported in any of these experiments. Mycelial growth was found to be optimum on Brown's-II medium. The optimum temperature and H-ion concentration for mycelial growth were observed to be 24°C and pH 6.0 respectively. The fungus gave maximum growth after 21 days beyond which it declined.



## INTRODUCTION

Many edible and non-edible mushrooms have long been used worldwide, especially in the Orient, for medicinal purposes. Important pharmaceutical products with proven medical applications have been derived from many fungi like *Ganoderma lucidum*, *Hericium erinaceus*, *Lentinus edodes*, *Schizophyllum commune*, *Tremella fusiformis*, *Trametes versicolor* and *Grifola frondosa* (Cun *et al*., 1994; Mizuno, 1995, 1999; Gao *et al*., 1996; Kim *et al*., 1999; Siaand Candlish, 1999; Wasser, 2002). At present there are at least 270 species of mushroom that are known to have various therapeutic properties (Ying *et al*., 1987). Medicinal mushrooms have become even more widely used as traditional medicinal ingredients for the treatment of various diseases and related health problems largely due to the increased ability to produce the mushrooms by artificial methods. As a result of large numbers of scientific studies on medicinal mushrooms especially in Japan, China and Korea, over the past three decades, many of the traditional uses have been confirmed and new applications developed (Wasserand Weis, 1999; Rai *et al*., 2005). *Coriolus versicolor* is a mushroom used in traditional Asian herbal remedies. The substances extracted from the mushroom, polysaccharide K (PSK) and polysaccharide-peptide (PSP), are being studied as possible complementary cancer treatments (Collins and Ng, 1997; Chu *et al*., 2002; Fisher and Yang, 2002; Cui and Chisti, 2003). The action of purified laccase from the basidial fungi *Trametes versicolor* on various dyes and industrial wastes was studied (Lee *et al*.,1999; Couto *et al*., 2002; Selvam *et al*., 2002; Xavier *et al*., 2007). The influence of glucose concentration, addition of a vegetable oil surfactant emulsion, nature of the surfactant and the presence of manganese and copper on the growth, pH and production of laccase, total peroxidase and manganese-dependent peroxidase activities were evaluated (Mikiashvili *et al*., 2005; Minussi *et al*., 2007; Iqbal *et al*., 2011). The studies reveals that much work have been done on the enzymatic activities of *Trametes* (Paice *et al*., 1993;Tanaka *et al*., 1999; Novotný *et al*., 2004; Gamelas *et al*., 2005; Wu *et al*., 2005; Xavier *et al*., 2007; Stoilova *et al*., 2010; Iqbal *et al*., 2011) and a very little work have been done on the physiological studies of this fungi (Carroad & Wilke, 1977; Park *et al*., 1989;Liao, 1990; Xavier *et al*., 2007; Jo *et al*., 2010).

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## MATERIALS AND METHODS

**Material:** The specimen of *Trametes versicolor* was collected from Palampur, (Himachal Pradesh, India). The pure cultures were isolated and maintained on Malt Extract Agar (Malt extract- 20g, Agar- 20g and distilled water up to 1000 ml). The part of tissue was removed from the fruiting body, cultured on MEA (malt extract agar) and incubated at 24°C in an incubator.

The species was further studied for physiological investigations.

**Procedure**

The effects of basal media, temperature, pH, days of incubation, carbon and nitrogen source on growth and reproduction of *T. versicolor* were observed in still cultures grown in 100 ml Erlenmeyer flask containing 25 ml of the basal medium (autoclaved at 15 psi pressure for 15-20 mins). Each flask was inoculated with an agar plug of 10 mm diameter cut from the margin of a 4-days old culture with a mycelial inoculum equivalent to 2.5 mg dry weight. Three replicates were kept for each parameter.

**Determination of mycelial dry weight and final pH**

At the end of each experiment, the mycelia were harvested through pre-weighed Whatman filter paper No. 1 and dried at 45°C in a hot air oven and their dry weights were measured using an electronic balance (Sartorius Analytical BL 210S). The final pH of the culture filtrate of the individual replicate was checked over Digital pH Meter 813.

**Basal Media**

The fungus was grown in twelve different media viz. Raulin's, Richard's, Dox's, Coon's, Brown's I, Brown's II, Glucose-peptone, Glucose-nitrate, Czapek's I, Czapek's II, Asthana& Hawker's and Elliot's medium (Prasher and Chauhan, 2015). The initial pH of all the different media was not changed and checked before and after autoclaving at 15 lbs psi steam pressure for 15 minutes. It was incubated at 24°Cfor 15 days.

**Temperature**

In the experiment on the effect of temperature, the fungus was incubated at 16, 20, 24, 28 and 32°C, in basal medium (Brown's-II medium) with a pH of 5 (selected arbitrarily) for 15 days of incubation.

**H-ion concentrations**

In the experiment on the effect of pH, the pH levels of the medium were adjusted to 3.0-9.0 with a difference of unit pH. The pH of each aliquot was adjusted to a separate unit value aseptically with sterile 1N-HCl and 1N-KOH and checked over Digital pH Meter 813. The flasks were incubated for 15 days at 24°C.

**Days of Incubation**

A period of 30 days was selected to observe the optimum days of incubation for growth and sporulation of the fungus. The basal medium was adjusted to optimum pH of 6.0 with sterilized solution of 1N KOH after sterilization of 15 lbs psi steam pressure for 15 minutesand incubated for 30 days at optimum temperature i.e. 24°C and at pH-6.0 (found optimum).

**Statistical analyses**

All the experiments were performed in triplicates. The means of three replicate values for all data in the experiments obtained were tested in a one way ANOVA at P=0.05 using PASW Statistics 18 software and Tukey's test was used to evaluate differences between treatments.

## RESULTS AND DISCUSSION

**Effect of basal media**

The study has revealedthat *T. versicolor* gives maximum growth with Brown's-II and Glucose-peptone media (Fig. 1) followed by Czapek's II, Glucose-nitrate, Richard's and Czapek's Imedia. The least growth was obtained with Coon's, Dox's, Brown's-I, Asthana& Hawker's, Elliot's media and Raulin's medium. Brown's-II medium was selected for further experimental studies. The final H-ion concentration of the basal medium did not change significantly with the growth of the fungus. It didnot sporulate with any of the basal medium.

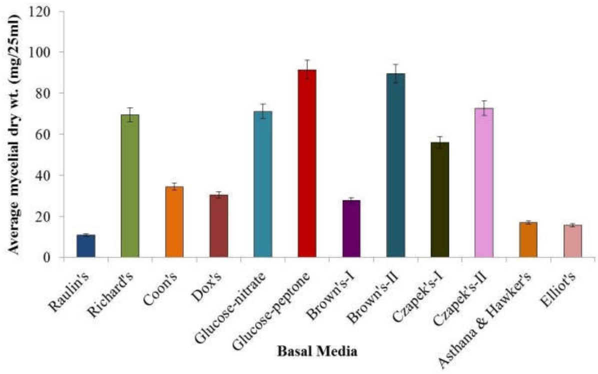


**Fig.1** Growth (average mycelial dry wt.-mg/25ml) of *Trametes versicolor* with different basal media at 24°C after 15 days of incubation (taken tentatively).

**Effect of temperature:** The optimum temperature of 24°C is found to be optimum for mycelial growth of *T. versicolor* after 15 days of incubation (Fig. 2).

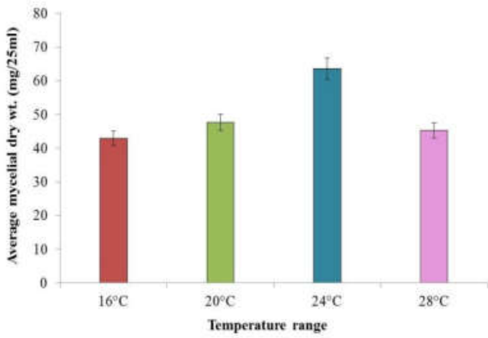


**Fig.2** Growth (average mycelial dry wt.-mg/25ml) of *Trametes versicolor* on the basal medium with selected temperatures after 15 days of incubation.

These results confirm the previous findings where *T. versicolor* showed optimum temperature range of 25-30°C for optimum growth (Park *et al*., 1989; Liao, 1990; Jo *et al*., 2010) on solid culture media. However in this study, it is observed that it also showed growth below 25°C (i.e. at 16°C). The optimal temperature can be different or same for species from the same family e.g. the optimal temperature was 24ºC for *G. fuligo* (Prasher and Chauhan 2013) and for *T. versicolor* it was 28ºC as reported earlier (Carroad and Wilke, 1977; Xavier *et al*., 2007) in different culture media. It did not sporulate *in vitro* at any temperature range.

**Effect of pH:** The maximum mycelial growth of this fungus is found with pH 6.0 (Fig. 3).

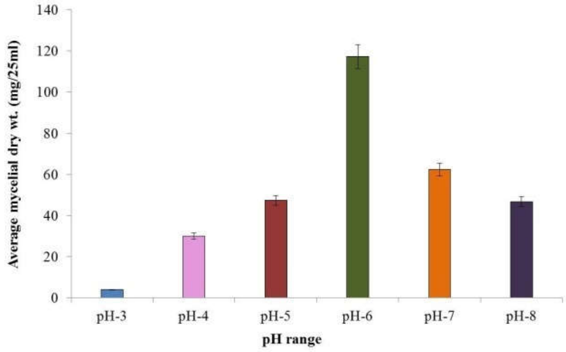


**Fig.3** Growth (average mycelial dry wt. -mg/25 ml.) of *Trametes versicolor* on the basal medium (Brown's-II medium) with selected H-ion concentrations after 15 days of incubation at optimum temperature (24°C).

It showed moderate growth with pH 5.0, 7.0 and 8.0 and poor mycelial growth with pH 4.0. However, highly acidic pH (i.e. pH 3) is found to be inhibitory for its growth.The results are in accordance with the previous findings where *T. versicolor* showed optimum pHrange of 5.0-5.8 (Park *et al*., 1989; Jo *et al*., 2010). It did not sporulate *in vitro* at any pH range.

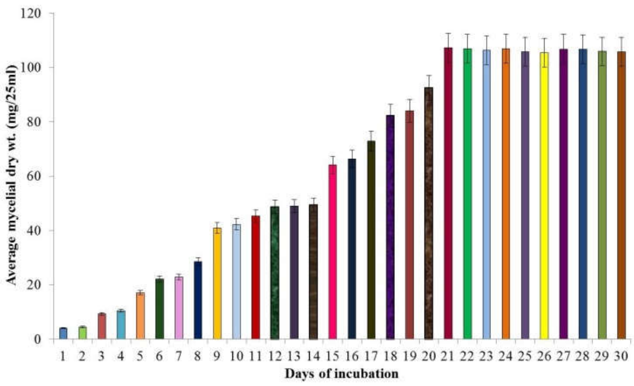


**Fig.4** Growth (average mycelial dry wt. -mg/25 ml.) of *Trametes versicolor* with different days of incubation at optimum temperature (24°C) and pH (6.0).

**Effect of days of incubation:** It has attained maximum growth in terms of average mycelial dry weight (mg) after 21 days of incubation (Fig. 4). The growth declined or remained the same after optimum days of incubation. The mean final pH of the culture filtrate did not changed to significant level up to 7 days thereafter it decreased and remained the same up to 30 days of incubation.

## Conclusion

The data can be utilized to increase the production of extracellular compounds such as polysaccharide K (PSK) and polysaccharide-peptide (PSP) which are known to be anti-cancerous compounds, Versicolor polysaccharide (VPS) a dietary supplement, laccase enzyme and other ligninolytic enzymes produced by this fungus. An understanding of the effect of physical factors on mycelial growth is a pre-requisite experimentation.

## Acknowledgement

The author acknowledges the Department of Science and Technology, Govt. of India, for the financial support in the form of fellowship under PURSE grant during the course of the studyand also thankful to the Chairperson, Department of Botany, Panjab University, Chandigarh, for providing the facilities used during part of the experiments in this study.

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