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Nutritional requirements for the enhanced mycelial growth and yield performance of *Trametes versicolor*

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ABSTRACT

As one of the most precious medicinal mushrooms, *Trametes versicolor* is widely used in the treatment and prevention of cancer. In an attempt to identify nutritional requirements, this study explores the influence of carbon source (C), nitrogen source (N), and cultivation substrate on the mycelial growth and yield of *T. versicolor*. The optimal C and N sources and their ratios for the mycelial growth of *T. versicolor* were fructose and yeast extract at a 3:1, respectively. *T. versicolor* cultivated on a substrate mixture of 62% sawdust + 30% rice husk + 3% wheat bran + 1% $CaCO_3$ exhibited the highest biological efficiency (12.58%). The findings in this study will provide important information regarding spawn production and *T. versicolor* cultivation at an industrial-scale.

1. INTRODUCTION

Trametes versicolor (synonym *Coriolus versicolor*), belonging to the family *Polyporaceae*, has been reported as one of the most popular medicinal mushrooms [1,2]. Several studies have reported that *T. versicolor* has the ability to produce a rich source of biologically active components such as tyrosol [3], friedelin, triterpenoids, alnusenone, α-D-glucan, and β-D-glucan [1]. Therefore, this mushroom has been widely consumed as a health supplement in the treatment and prevention of cancers and protective body functions [4]. Along with medicinal values, *T. versicolor* has been considered a useful white-rot fungus in environmental protection issues. The enzyme laccase that it produces, for instance, has been used for the biological pretreatment of lignocellulosic biomass [5], bioremediation [6], wastewater decontamination, pharmaceuticals, and polycyclic aromatic hydrocarbons degradation [7].

Two main factors affecting microbiological mycelia growth and fruiting body formation are nutrient and growth conditions [8]. However, at present, only a few studies have been carried out to optimize culture conditions for mycelial growth and fructification of *T. versicolor.* The optimal carbon and nitrogen sources and mineral salts were found to be dextrin, yeast extract and $MgSO_4.7H_2O$, respectively [1]. *T. versicolor* is able to grow in a wide range of pH values, between 4 and 9 [1]. The optimal temperature and humidity for fructification are 25 ± 2°C and 80–85%, respectively [9]. To date, only sawdust is known to be a suitable basal substrate for the cultivation of *T. versicolor* [9,10]. In general, the substrate used for mushroom cultivation is designed based on the local availability of agro-industrial wastes. Thus, for large-scale commercial cultivation in different regions, additional studies will be needed to identify various substrates for the development of *T. versicolor* cultivation technology. Taken together, the present study aims to ascertain the favorable culture conditions for promoting the vegetative growth and yield performance of *T. versicolor.*

2. MATERIAL AND METHODS

2.1. Mushroom Strains

T. versicolor strain VT1 was kindly provided by the Mushroom Research and Development Center, Vietnam. The culture was stored in sterilized potato dextrose agar slants at 4°C under complete darkness for maintenance [11].

2.2. Carbon Sources

To determine the carbon source most favored by *T. versicolor*, eight different sources, including fructose, glucose, maltose, lactose, saccharose, xylose, soluble starch, and dextrin, were screened. The individual carbon source was taken at 20 g/l and added into a basal medium (200 g infused potato, 15 g agar powder, and 20 g carbon

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source). The potato infusion was prepared according to Nguyen *et al*. [11]. Based on the obtained results, fructose with one-sixth of each case at specific concentrations (5 g/l, 10 g/l, 15 g/l, 20 g/l, 25 g/l, and 30 g/l, respectively) was supplemented into the basal medium to optimize the carbon concentration in culture media.

2.3. Nitrogen Sources

According to the study of carbon sources and concentration, *T. versicolor* was inoculated in a basal medium supplemented with 20 g/l fructose. To determine the nitrogen source and requirement for mycelial growth, three different inorganic nitrogen sources, including ammonium chloride (NH_4Cl), ammonium nitrate (NH_4NO_3), and ammonium sulfate $(NH_4)_2SO_4$, and four complex organic nitrogen sources, including peptone, yeast powder, casein, and urea, were tested with a concentration of 2 g/l.

2.4. Carbon/Nitrogen (C/N) Ratios

Following the study of carbon and nitrogen sources, 20 g/l fructose as a carbon source and yeast extract with individual concentrations (1, 2, 3, 4, and 5 g/l), similar to the nitrogen source, were used to determine the most favorable C/N ratio.

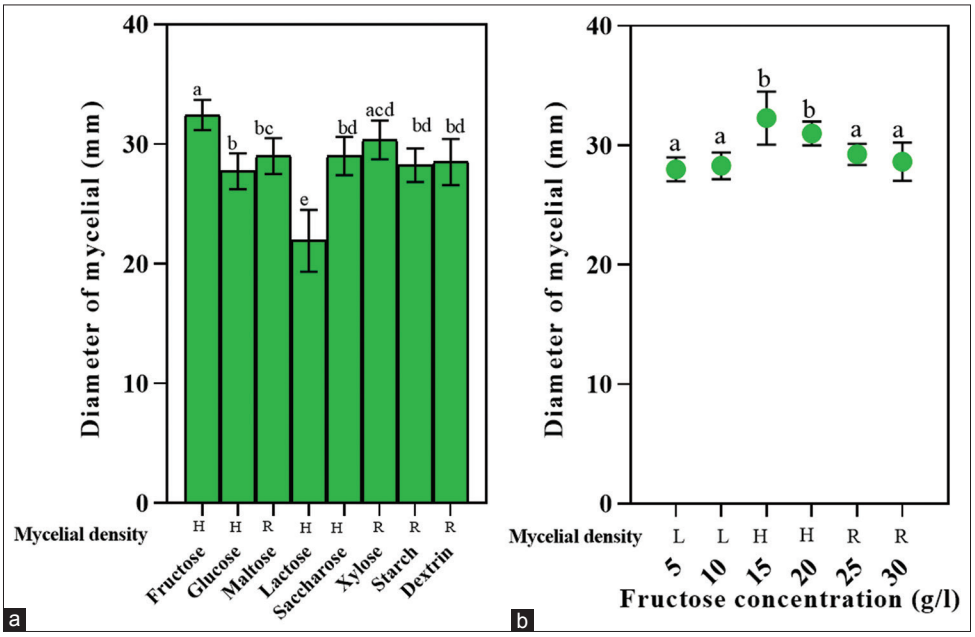

Figure 1: Effect of carbon sources (a) and fructose concentration (b) on mycelial growth of *Trametes versicolor*. Different letters denote statistically significant differences at $P < 0.05$. H: high, R: regular.

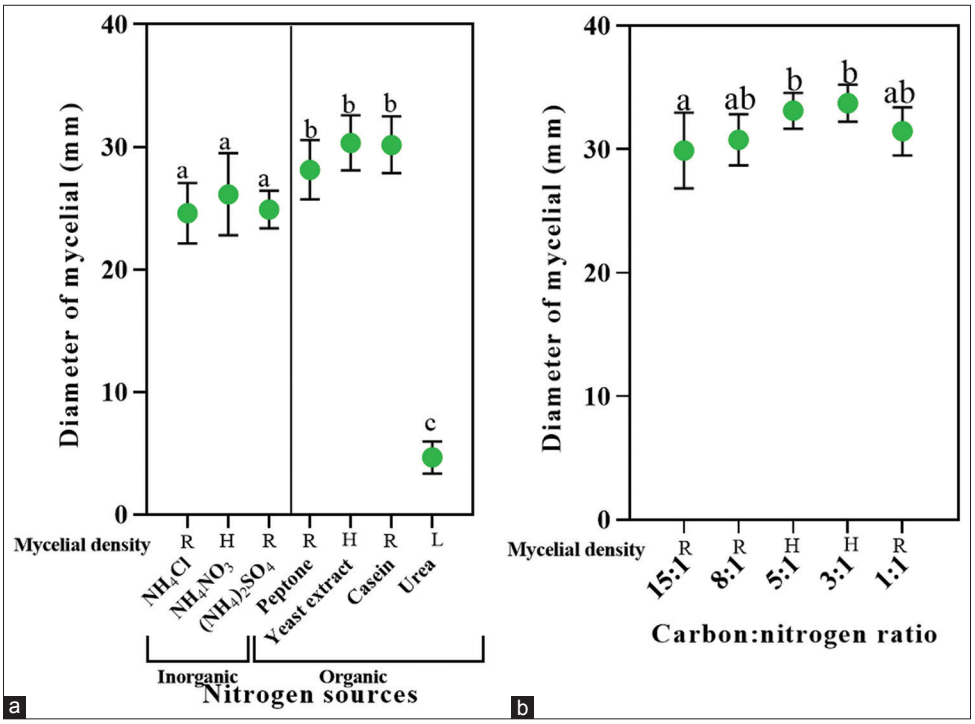

Figure 2: Effect of nitrogen sources (a) and Carbon : Nitrogen ratio (b) on mycelial growth of *Trametes versicolor*. Different letters denote statistically significant differences at $P < 0.05$. H: high, R: regular, L: low.

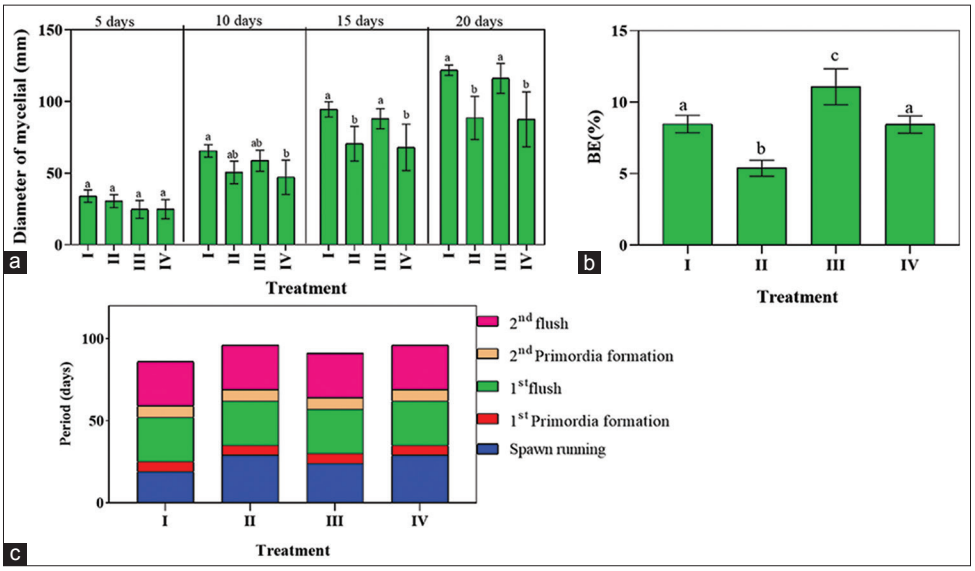

Figure 3: Effect of basal substrates on mycelial growth (a), biological efficiency (b), and growth period of *Trametes versicolor* (c). Different letters denote statistically significant differences at $P < 0.05$.

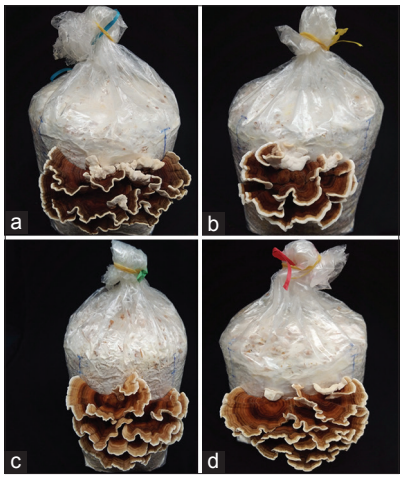

Figure 4: Effect of basal substrates on fruiting body morphology of *Trametes versicolor*. a: Treatment I (92% sawdust + 7% wheat bran + 1% $CaCO_3$), b: Treatment II (62% sawdust + 30% cotton waste + 7% wheat bran + 1% $CaCO_3$), c: Treatment III (62% sawdust + 30% rice husk + 7% wheat bran + 1% $CaCO_3$), d: Treatment IV (62% sawdust + 30% corn cob + 7% wheat bran + 1% $CaCO_3$).

2.5. Basal Substrates

For the cultivation of *T. versicolor*, sawdust, cotton waste, and corncob were used as growing substrates. All treatments with different combinations of basal substrates used to cultivate *T. versicolor* are as follows: Treatment I (92% sawdust + 7% wheat bran + 1% $CaCO_3$), Treatment II (62% sawdust + 30 % cotton waste + 7% wheat bran + 1% $CaCO_3$), Treatment III (62% sawdust + 30 % rice husk + 7% wheat bran + 1% $CaCO_3$), and Treatment IV (62% sawdust + 30 % corn cob + 7% wheat bran + 1% $CaCO_3$).

2.6. Wheat Bran

T. versicolor was cultivated in a combination of sawdust and rice husk as a basal substrate enriched by wheat bran at different percentages (0%, 3%, 5%, 7%, and 9%).

2.7. Substrate Preparation and Cultivation

Sawdust, cotton waste, and corncob were soaked in a lime solution (4 kg of lime per 1000 L of water), fermented for 7 days, and then allowed to sit an extra 1–2 days until the water content of the substrates reached 65% moisture level. The resulting substrate was filled with 1 kg of the substrate in a polyethylene bag and autoclaved at 121°C for 90 min. The inoculated bags were incubated at 25°C in the spawn-running room under dark conditions and then transferred into the cropping room after complete colonization of the substrate. For fruiting body formation, the temperature and relative humidity in the cropping room were set to 25 ± 2°C and 85 ± 5%, respectively.

2.8. Data Collection and Statistical Analysis

The diameter growth, mycelial density (high, regular, and low), texture (cottony, floccose), and colonial diameter were recorded after 4 days of incubation. The period of spawn running (days) is defined as the time required for the mycelium to colonize the substrate completely. The period of primordia formation (days) is defined as the day of inoculation to the formation of the primordia. Biological efficiency (BE) (%) was calculated as follows: The ratio of the weight of the fresh fruiting body (g) per the dry weight of the substrate (g) and expression as a percentage.

The raw data were statistically analyzed using GraphPad Prism (version 8.0, GraphPad Software Inc., San Diego, CA). Significant differences among the group means were determined by a one-way ANOVA, followed by Tukey's multiple range tests ($P < 0.05$) and indicated with letters.

3. RESULTS AND DISCUSSION

3.1. Effect of Carbon Sources on Mycelial Growth of *T. versicolor*

It was observed that strain VT1 was able to use the eight carbon sources [Figure 1a]. The mycelium color and texture of the *T. versicolor* grown in all media were found to be white and cottony. However, comparatively, among the eight evaluated carbon sources in this study, fructose and xylose showed the highest growth rates ($P < 0.05$). By

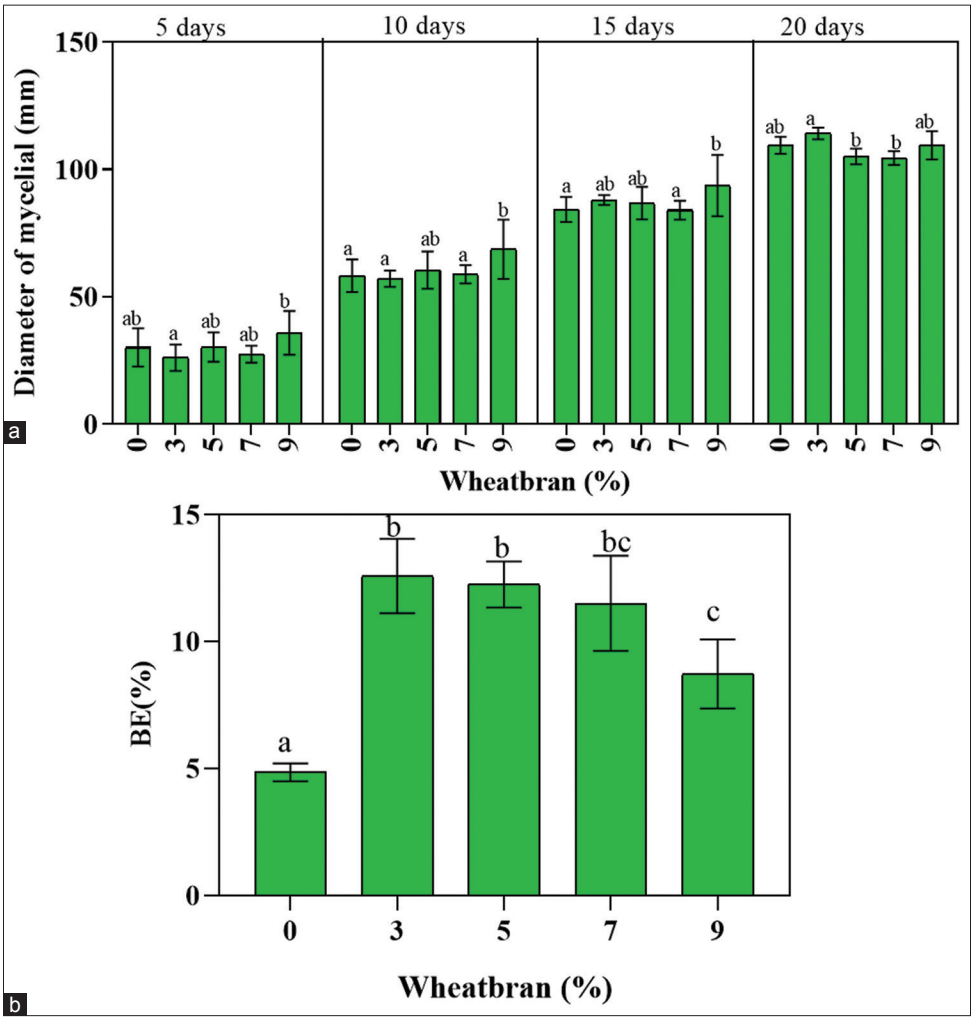

Figure 5: Effect of wheat bran concentration on mycelial growth (a), biological efficiency (b) of *Trametes versicolor*. Different letters denote statistically significant differences at $P < 0.05$.

contrast, lactose was not observed as an efficient carbon source for enhanced mycelial growth. In addition, the mycelial density was found to be high for fructose, glucose, and saccharose but regular for maltose, lactose, xylose, starch, and dextrin. Therefore, fructose was selected as the most suitable carbon source for further experiments.

Fructose at various concentrations (5 g/l, 10 g/l, 15 g/l, 20 g/l, 25 g/l, and 30 g/l) was tested for its effects on growth performance. As shown in Figure 1b, 15 g/l, 20 g/l, and 25 g/l fructose treatments exhibited high mycelial density, whereas the 5 g/l and 10 g/l fructose treatments showed a thin mycelial density. The highest diameter of mycelia was found in the media containing 15 g/l of fructose (32.29 ± 2.22 mm), while the lowest was detected in the 5 g/l (28.00 ± 1.0 mm). Based on the colonial diameter and mycelial phenotype, the best growth performance of *T. versicolor* was obtained in media containing 15–20 g/l fructose. Thus, fructose with 15 g/l was selected as the carbon concentration for further experiments.

Due to providing essential nutrients, the growth medium is considered to be the most important factor for mushroom production [12]. As structural and storage compounds in the cell, carbon sources play a critical role in mycelial growth [13]. Therefore, among the nutritional composition of the medium, the carbon source was selected as the first ingredient for optimization in the present study. In general, mushrooms can use a variety of compounds such as monosaccharides, polysaccharides, organic acids, amino acids, alcohols, and natural products as a carbon source [14]. Furthermore, the influence of carbon source on growth rate strongly depends on species, growth condition as well as the medium [15]. Jo *et al*. [1] proved that the efficient carbon sources for mycelial growth of *T. versicolor* were dextrin, fructose, and mannose. In our study, fructose with economic feasibility was the most beneficial carbon source for the radial growth of the mycelium and should be used as an ingredient for *T. versicolor* spawn production at the industrial-scale.

3.2. Effect of Nitrogen Sources

With regard to nitrogen sources, the highest mycelial growth performance of *T. versicolor* was achieved with peptone (28.14 ± 2.41 mm), yeast extract (30.33 ± 2.25 mm), and casein (30.167 ± 2.32 mm) [Figure 2a]. By contrast, very weak growth (4.667 ± 1.32 mm) was observed in urea treatment. Among the seven nitrogen sources, yeast extract showed the highest mycelial density, whereas the rest of the nitrogen sources exhibited moderately thin or thin mycelial density. Based on the results of mycelial characteristics and growth rates, the yeast extract was considered as the optimal nitrogen source for luxuriant mycelial growth of *T. versicolor*.

Nitrogen source is an essential nutrient that the growth culture medium must provide for the synthesis of all nitrogen-containing compounds and chitin cell wall components [14]. Nitrogen sources used for the growth of mycelium are inorganic (salts of nitrate, salts of the ammonium ion, etc.), and organic nitrogen [14]. Therefore, in this study, both organic and inorganic nitrogen sources were utilized to determine their effects on mycelium growth. According to the obtained results, organic and inorganic nitrogen sources exhibited a difference in supporting the growth of mycelium. Relative to organic nitrogen sources, except for urea, all investigated inorganic sources did not benefit the mycelial growth, which is consistent with the observations of Jo *et al*. [1]. Most of the mushrooms exhibited a preference for using complex organic nitrogen sources. For instance, peptone and beef extracts were considered to be useful nitrogen sources for the enhanced mycelial growth of *Cordyceps sinensis* [16]. Similar to *C. sinensis*, the mycelial growth of *Volvariella esculenta* [17] was promoted considerably in the medium containing organic nitrogen sources as compared to inorganic nitrogen. Inorganic nitrogen does not enhance the growth of mycelium [18], probably due to inorganic nitrogen sources being unable to be used for the biosynthesis of essential amino acids [19].

3.3. Effect of Carbon/Nitrogen Ratio

Based on the results obtained from the carbon and nitrogen sources, fructose and yeast extracts were utilized to optimize the C/N ratio. The C/N ratios 5:1 and 3:1 showed the fastest mycelial extension growth rate [Figure 2b], whereas other ratios exhibited a lower rate. In addition, in terms of mycelial density, compared with the other C/N ratios, the C/N ratio of 3:1 exhibited a thicker mycelial density and can be considered an optimal C/N ratio.

An ideal balance between the carbon and nitrogen sources plays a key role in the growth of mycelium. The mycelium could stop growing under nitrogen deficiency conditions. By contrast, due to its metabolism by-products, a very high concentration of nitrogen can result in inhibiting the growth of mycelium [20]. The optimal C/N ratio suitable for the mycelial growth may vary according to species. For instance, the suitable ratios for the enhanced mycelial growth of *Cystoderma amianthinum* [21], *Macrolepiota procera* [22], *Oudemansiella radicata* [23], *Paecilomyces fumosoroseus* [24], and *Ganoderma applanatum* [25] were found to be 30:1, 10:1, 20:1, 40:1, and 2:10, respectively. Our result indicated that a C/N ratio of 3:1 was optimal for mycelial growth of *T. versicolor,* which is in high agreement with studied by Jo *et al*. [1].

3.4. Effect of Basal Substrates

For primordia formation and development, different species require different basal substrates [26]. To ascertain the most suitable treatment for the cultivation of *T versicolor,* four treatments with different combinations of basal substrates were used. The duration of the growth cycle, fruiting body morphological characteristics, and BE (%) are shown in Figure 3a and c. The obtained results reveal that basal substrates significantly affected ($P < 0.05$) the mycelium run rate. *T. versicolor* showed the ability to grow on all tested treatments and completed the period of mycelial colonization between 19 days (Treatment I) and 29 days (Treatment II and IV). Of the substrate treatments, Treatment I exhibited the fastest mycelia extension rate. Primordia formation was observed on day 25 (Treatment I) and day 35 (Treatment II and IV), after inoculation. Morphologically, no significant differences in fruiting bodies were observed in all treatments used for cultivation [Figure 4]. All treatments showed two flushes during the cultivation period. Among the investigated basal substrates, Treatment III (sawdust and rice husk) showed the best yield performance with a BE value of 11.07%, followed by Treatment I (8.45%) and Treatment IV (8.43%) [Figure 3b]. *T. versicolor* was capable of completing the growth cycle within 86–96 days.

To successfully cultivate mushroom, the three factors of spawns, substrate, and conducive environment must be considered [27]. The substrate provides essential nutrients for mycelium growth and fruiting body development stages and plays a critical factor in determining the success in mushroom cultivation [27]. Based on the available agro-industrial waste in Vietnam, we used sawdust, rice husk, cotton waste, and corn cob to optimize substrate for the cultivation of *T. versicolor.* The mycelial growth of *T. versicolor* was recorded in all treatments. During the colonization stage, to minimize the risk of fungal and bacterial contamination, the spawn running period should be reduced. Compared to other treatments, Treatment I showed the highest mycelium growth rate and, therefore, completed the colonization stage earlier. *T. versicolor* was able to form primordia and adapt to cultivation conditions for all four treatments. To the best of our knowledge, this is the first report showing that rice husk, cotton waste, and corn cob can be used as the basal substrates for the cultivation of *T. versicolor*. As one of the key factors in mushroom cultivation, BE is the main purpose of this set of experiments. Although the highest mycelial growth was observed in Treatment I, the BE value of this substrate (8.45%) was lower than Treatment III (11.07%). Thus, it would seem that the growth rate of mycelium does not have a very strong correlation with BE, which is in agreement with the findings of past studies by Liang *et al*. [28]. A combination of diverse substrates could enhance the yield performance of mushrooms because of a variation in the capability of such substrates to provide nutritional and environmental requirements and the difference in cellulose, hemicellulose, and lignin contents [29]. Compared to sawdust alone (Treatment I), Treatment III (62% sawdust + 30% rice husk + 7% wheat bran + 1% $CaCO_3$) exhibited higher BE and thus, should be used as the optimal substrate mixture to cultivate *T. versicolor.*

3.5. Effect of Wheat Bran

The highest mycelial extension of *T. versicolor* was found in 9% wheat bran in the first 15 days of spawn run [Figure 5a]. Further, no significant differences were observed in all treatments. The time required for the mycelium to colonize the substrate completely and for primordia formation across all the treatments was found to be 22 days and 6 days, respectively. As expected, the addition of wheat bran of up to 7% to the substrates showed an improvement in the yield as well as BE in all treatments, whereas the 9% wheat bran-supplemented basal substrate did not enhance the yield performance of *T. versicolor* [Figure 5b]. Substrate mixtures without wheat bran exhibited the lowest BE (4.85%). Substrates supplemented with 3–7% wheat bran could be considered as the most favorable substrate mixtures for fruiting body formation of *T. versicolor* with satisfactory yield (12.58–11.51%).

In general, basal substrates are known to be poor in nutrients. Thus, supplements are used as co-substrates to improve the nitrogen content for cellular protein and enzyme synthesis [30]. For the cultivation of mushroom, wheat straw was used to provide a reservoir of cellulose, hemicellulose, lignin, and nitrogen, which was utilized during the period of mycelial colonization and primordia formation [31]. To reduce the growth period, improve productivity, and enhance economic efficiency, wheat bran, which has high vitamin content, was used to supplement the cultivation substrate of *T. versicolor*. However, high supplementation of the substrate can inhibit the

growth of mushroom [32], increase contamination rate [31], and reduce the yield [33]. In addition, due to faster metabolic activities induced by extra nitrogen, supplementation has been reported to increase substrate temperatures [33].

4. CONCLUSION

This study presented the influence of nutrients on mycelial growth and yield performance of *T. versicolor*. Mycelial growth was found to be enhanced by using fructose as the carbon source and yeast extract as the nitrogen source. Along with sawdust, other agro-industrial wastes such as rice husk, cotton waste, and corn cob can be used basal substrates for the cultivation of *T. versicolor*. The highest yield of *T. versicolor* was obtained when cultivated on a substrate mixture of 62% sawdust + 30% rice husk + 3% wheat bran + 1% $CaCO_3$.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

6. CONFLICTS OF INTERSET

The authors declare that they do not have any conflicts of interest.

7. FINANCIAL SUPPORT

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