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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.374.382

Research Article
Successful Rescue of Wild *Trametes versicolor* Strains Using Sawdust and Rice Husk-based Substrate

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Abstract

Background and Objective: *Trametes versicolor* has not only been valued in medical use but also in environmental protection. One of the major challenges currently faced in the commercial cultivation of *T. versicolor* is finding superior strains that can produce high yields. In an attempt to search for high-yield potential *T. versicolor*, two wild strains, namely VNUA and BV, were isolated and evaluated for potential cultivation. **Materials and Methods:** Optimized culture conditions were set up by one-individual factor-at-a-time. Four different kinds of culture media, including Czapek, Raper, PGA and modified Potato Dextrose Agar (PDA), were investigated to ascertain the optimal media. The efficiency of sawdust and rice grain for mother spawn production was evaluated. Different combinations of sawdust and rice husk were tested to investigate the most favorable substrate mixtures. **Results:** The ideal medium and temperature for the favorable mycelial growth of *T. versicolor* were PGA and 30°C, respectively. The optimal spawning material for upscaling of the mycelium was Treatment D (20% rice grain, 79% sawdust and 1% calcium carbonate). The strains were successfully cultivated in a basal substrate combination of sawdust and rice husk supplemented with wheat bran. Investigated strains responded differently to different substrates cultivation. Of note, compared with strain BV, strain VNUA showed a significantly higher biological efficiency (7.3%). **Conclusion:** Wild *T. versicolor* strains were successfully fructified under artificial cultivation conditions. Strain VNUA can be considered as a potential strain for commercial cultivation. The use of sawdust for the spawn production of *T. versicolor* can reduce the cost of manufacturing.

Key words: *Trametes versicolor*, wild strain, mycelial growth, cultivation substrate, spawns, medicinal mushroom, sawdust

Citation: Nguyen, B.T.T., V.V. Le, H.T.T. Nguyen, L.T. Nguyen, A.D. Tran and N.X. Ngo, 2021. Successful rescue of wild *Trametes versicolor* strains using sawdust and rice husk-based substrate. Pak. J. Biol. Sci., 24: 374-382.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

T. versicolor (common name: Turkey Tail), a member of the family *Polyporaceae*, is one of the most popular medicinal macro fungus[1-3]. This macrofungus can produce various biologically active components with pharmacological properties[3]. As the main bioactive components, polysaccharopeptides isolated from *T. versicolor* can suppress breast cancer cell proliferation[4], induce cancer cell death[5,6] and stimulate IgM production in B-cells[7]. Along with *Pleurotus ostreatus*, *T. versicolor* appeared to be the model macro fungus that produces enzyme laccase applied in environmental protection[8]. The enzyme laccase secreted by *T. versicolor* has been used in the pretreatment of lignocellulosic biomass[9], bioremediation[10], in triclosan biodegradation[11], blue wastewater biodegradation[12] and dye decolorization[13].

Since the first successful artificial cultivation of *T. versicolor* was reported in Vietnam, the Vietnamese *T. versicolor* industry has been finding potential strains that can produce high yields. For promoting economic efficiency in *T. versicolor* cultivation, the search for wild strains before their extinction is a useful strategy. However, to our knowledge, no study to date has been carried out to determine the potential of wild *T. versicolor* strains for commercial cultivation.

Culture conditions and cultivated strains are two key factors to improve the crop performance of mushrooms. However, the optimized culture conditions for the mycelial growth and fructification of *T. versicolor* have been barely studied. At the time of writing, sawdust was found to be the only suitable basal substrate for the cultivation of *T. versicolor*[14]. It is worth noting that the substrate for mushroom cultivation is generally selected according to the locally available lignocellulosic waste. Therefore, further research is needed to explore varieties of substrates suitable for the cultivation of *T. versicolor* at an industrial scale in Vietnam.

Overall, this study aimed to rescue and evaluate the potential cultivation of Vietnamese wild *T. versicolor* strains through optimizing the cultural conditions. We highlighted the successful rescue of wild strains and their ability to produce fruiting bodies under artificial cultivation conditions.

MATERIALS AND METHODS

Study area: All the experiments were carried out during November, 2019-December, 2020 in the Mushroom Research and Development Center, Faculty of Biotechnology, Vietnam National University of Agriculture, Vietnam.

Fig. 1(a-b): Morphological features of (a) *T. versicolor* strain VNUA and (b) Strain BV

Strain isolation: *T. versicolor* strains VNUA and BV were collected from botanic gardens the Vietnam National University of Agriculture, Vietnam (GPS location: 21°0 16.56"N, 105° 56 19.32"E) and Ba Vi National Park (GPS location: 21° 4 41"N, 105° 21 30"E), respectively (Fig. 1a, b). Pure mycelial cultures were isolated from the collected fruiting body using tissue culture technique and sub-cultured onto Potato Glucose Agar (PGA) medium at 25°C. The culture was routinely maintained in PGA slants at 4°C in complete darkness[15].

Effect of temperature: For the effect of temperature, *T. versicolor* strains were inoculated on PGA medium at five different temperatures (15, 20, 25, 30 and 35°C). The mycelial density and growth diameter were recorded after 2, 4, 6 and 8 days of incubation.

Culture media: Three different commercial culture media including Czapek (30 g sucrose, 2 g $NaNO^{-3}$, 1 g KH_2PO_4, 0.5 g

$MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 0.5 g KCl), Raper (2 g yeast extract, 2 g peptone, 0.46 g KH_2PO_4, 1 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 20 g glucose), PGA (20 g glucose, 250 g potatoes) and one modified PDA medium (20 g glucose, 250 g potatoes, 2 g yeast extract and 2 g peptone) were used to optimize the mycelia growth performance of *T. versicolor*. The incubation of cultures was performed at 30°C under complete darkness conditions. The diameter growth was measured on days 2, 4 and 6. The main macroscopic mycelial characteristics such as density (poor, moderate and abundant), texture (flat, semi-cottony and cottony) and pigmentation were recorded.

Spawn substrate: Rice grain and sawdust were used as the main substrates for mother spawn production. The ingredients for the different culture media were as follows: Treatment A (99% rice grain, 1% calcium carbonate), Treatment B (79% rice grain, 20% sawdust, 1% calcium carbonate), Treatment C (47.5% rice grain, 47.5% sawdust, 1% calcium carbonate) and Treatment D (20% rice grain, 79% sawdust, 1% calcium carbonate). The substrates were dispensed into glass bottles with a cotton plug and autoclaved at 121°C for 90 min. Sawdust was mixed with a lime solution of 0.4% and pasteurized for 5-7 days. Rice grain was soaked in water for 12 hrs, boiled until the grain became swell and drained to prevent excessive moisture.

Substrate preparation and cultivation: For the cultivation of *T. versicolor*, sawdust and rice husk were used as basal substrates, whereas wheat bran was employed as a nitrogen-rich supplement. All the treatments with different combinations of basal substrates are as follows: Treatment I (94% sawdust+0% rice husk+5%wheat bran+1% calcium carbonate), Treatment II (84% sawdust+10% rice husk+5% wheat bran+1% calcium carbonate), Treatment III (74% sawdust+40% rice husk+5% wheat bran+1% calcium carbonate) and Treatment IV (64% sawdust+40% rice husk+5% wheat bran+1% calcium carbonate).

The moisture content of the growth substrate was adjusted to 65% (w/w). The formulated substrate (1 kg) was packed into an autoclavable polyethylene bag and sterilized at 121°C for 90 min. The spawned bags were incubated in a dark room at 25°C and 70±5% air relative humidity. Once the mycelium thoroughly colonized the substrate, the bags were transferred into a mushroom cultivation house for fructification at 25±2°C and 85±5% air relative humidity.

Spawn running time was determined as the days needed for the mycelium to colonize the substrate completely. Primordia formation time was determined as the days needed from inoculation to the formation of the primordia. Biological Efficiency (BE) (%) was calculated as follows: the weight of the fresh fruiting body (g) to the dry weight of the substrate (g) and expression as a percentage.

Statistical analysis: The growth performance was statistically analyzed using GraphPad Prism (version 8.0, GraphPad Software Inc., San Diego, CA). Significant differences among the group means were compared by a one-way ANOVA, followed by Tukey's multiple range tests ($p<0.05$) and indicated with letters.

RESULTS

Mycelial characteristics: The mycelium of strains VNUA and strain BV were isolated successfully using tissue culture technique on PGA medium at 25°C within one week. Characteristically, both the investigated strains' mycelial density and texture were found to be high and cottony, respectively. In terms of pigmentation, the mycelium of strain VNUA was white and similar to that of strain BV.

Effect of temperature on mycelial growth: The effect of incubation temperature on the growth of *T. versicolor* is shown in Fig. 2a, b and 3a. A significant influence of temperature on the mycelial growth of *T. versicolor* was found. The results demonstrated that the strains could grow at all tested temperature levels (Fig. 2a, b). Notably, after 6 days of incubation, strain VNUA and strain BV exhibited the highest growth mycelial diameter at 30°C with 41.95±0.6 and 42.67±0.5 mm, respectively. Very weak growth was observed when the strains were incubated at 35°C (Fig. 3a).

Mycelial growth on culture media: Strains VNUA and BV were capable of growing in a wide range of cultural media (Fig. 3b and 4). After six days of incubation, PGA and modified PGA were considered ideal media for strain VNUA while no significant difference was observed for strain BV in the mycelial diameter among the investigated media (Fig. 4a, b). Culture media affected mycelial pigmentation and density (Fig. 3b). Comparatively, the pigmentation was found to be whitish grey for Czapek medium but white for other media. Czapek medium exhibited moderate-density mycelium, whereas other media showed high-density mycelium. Therefore, the Czapek medium was likely not an efficient medium for the enhanced mycelial growth of *T. versicolor*.

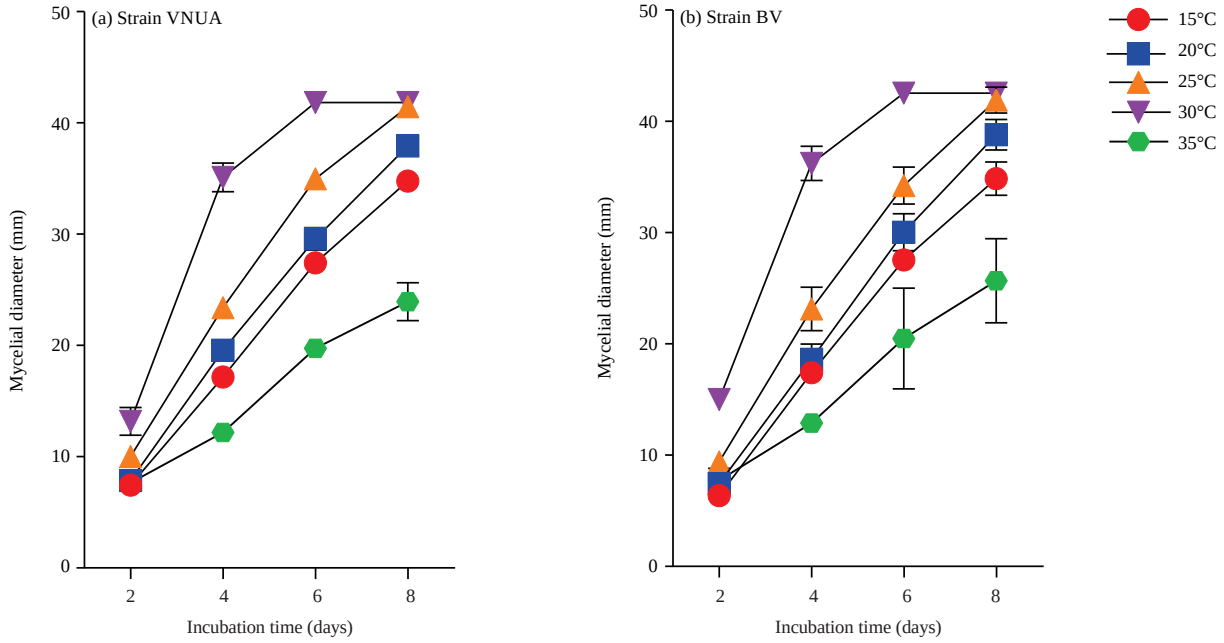

Fig. 2(a-b): Effect of temperature on mycelial growth of (a) *T. versicolor* strain VNUA and (b) Strain BV

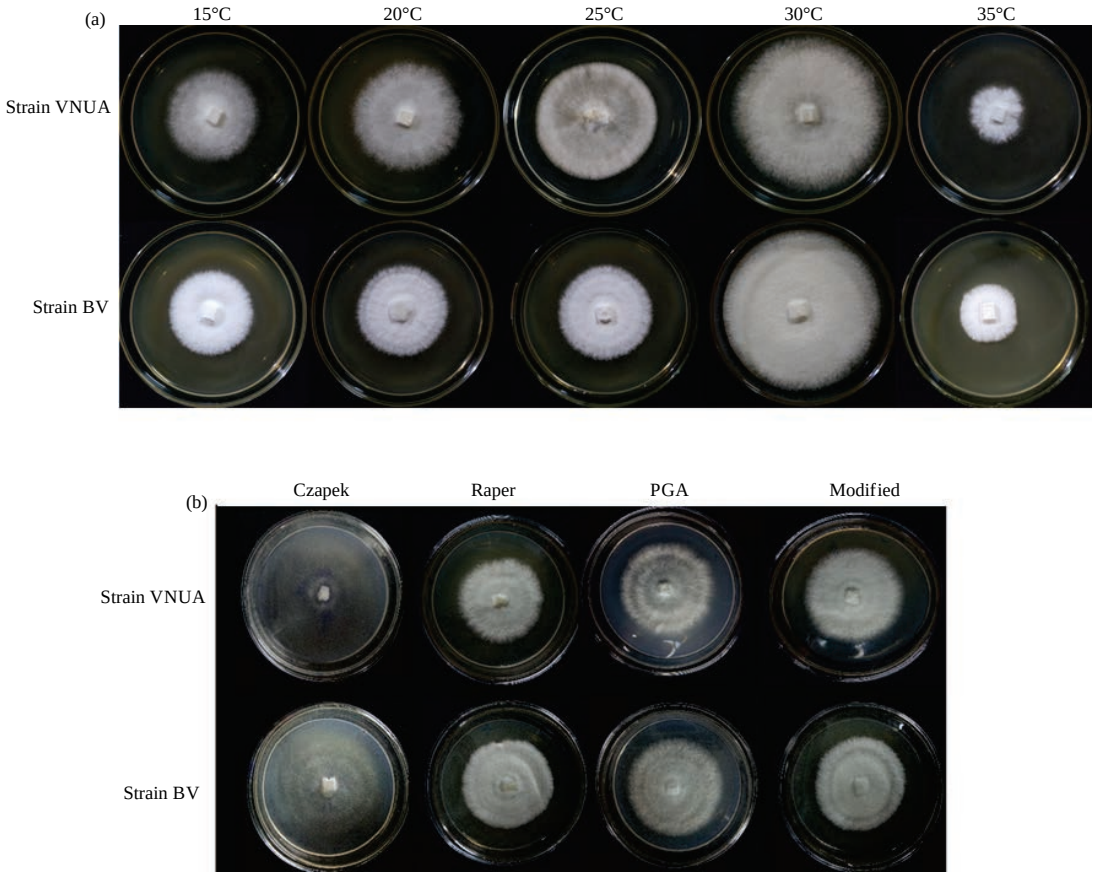

Fig. 3(a-b): Mycelial growth of *Trametes versicolor* strains on (a) Different temperature and (b) Culture media

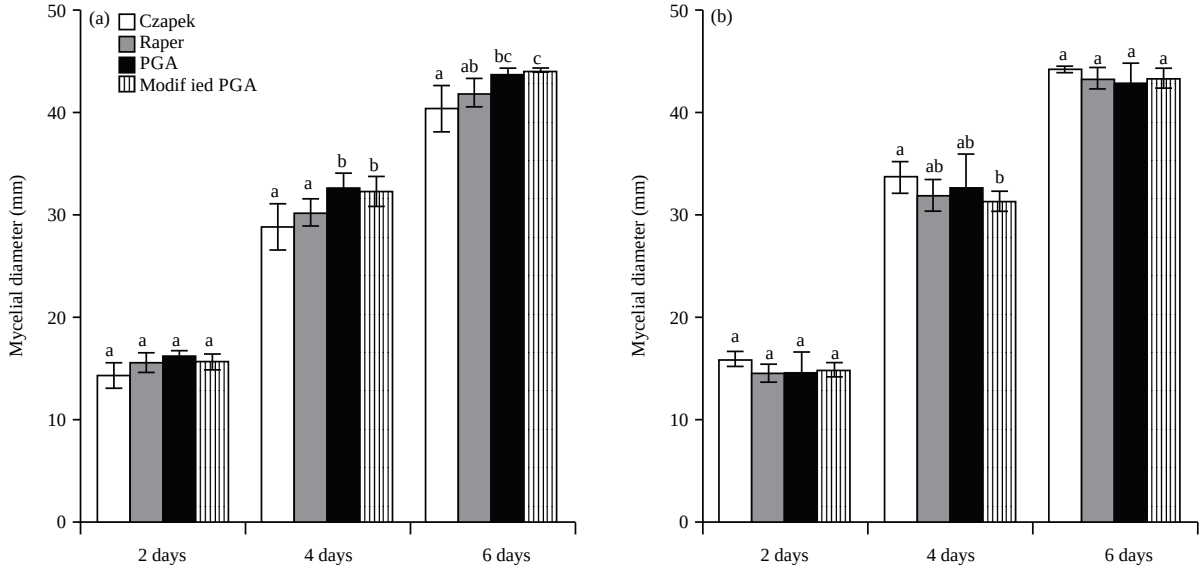

Fig. 4(a-b): Effect of different culture media on mycelial growth of (a) *T. versicolor* strain VNUA and (b) Strain BV
Different letters denote statistically significant differences at p<0.05

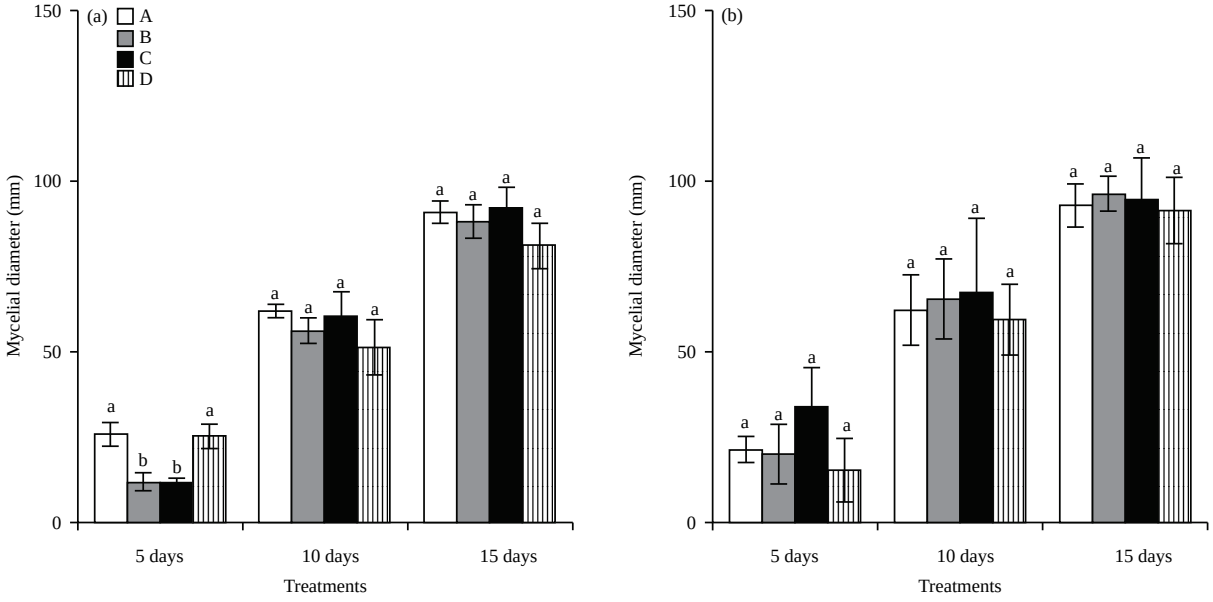

Fig. 5(a-b): Effect of different spawning material on mycelial growth of (a) *T. versicolor* strain VNUA and (b) Strain BV
Different letters denote statistically significant differences at p<0.05

Mycelial growth on spawn substrates: The different cultural media did not significantly affect the mycelial growth of *T. versicolor* (Fig.5a, b). Strain VNUA and BV showed the ability to grow well and showed high-density mycelium with white color. Of the substrate treatments, Treatment D (20% rice grain, 79% sawdust, 1% calcium carbonate) was proposed as the substrate for spawn production of *T. versicolor*.

Cultivation characteristics: Five treatments with different combinations of basal substrates were used to evaluate the most suitable substrate mixtures to cultivate strains VNUA and BV. The spawn running, biological yield and fruiting body morphology of the investigated strains are shown in Fig. 6, 7 and 8, respectively. Interestingly, *T. versicolor* strains responded differently to the substrates' cultivation. About

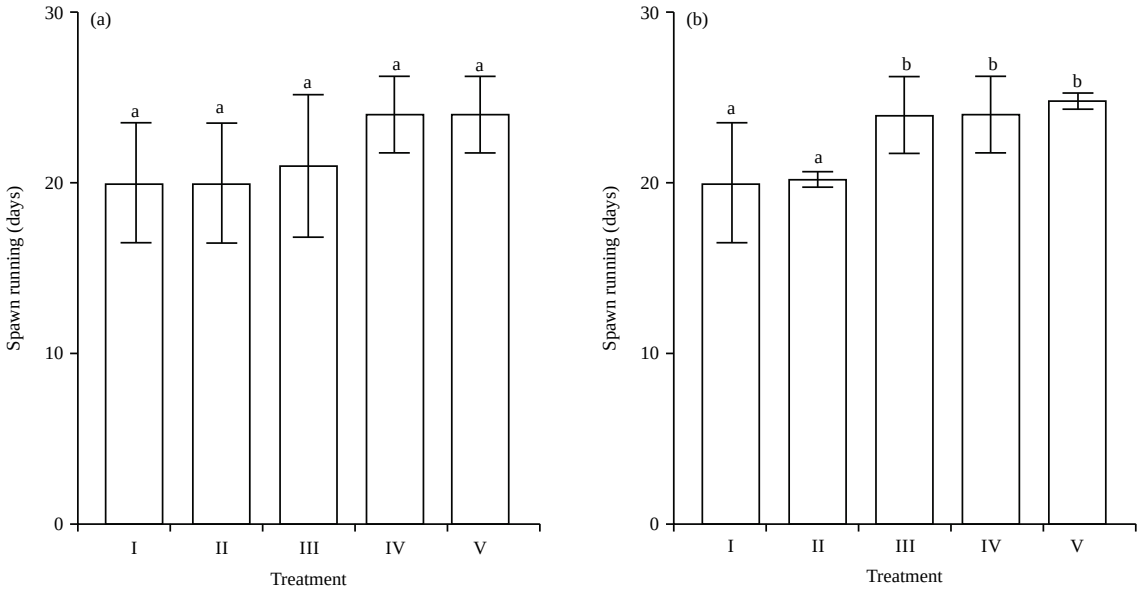

Fig. 6(a-b): Spawn running of (a) *T. versicolor* strain VNUA and (b) Strain BV cultivated on different treatments
Different letters denote statistically significant differences at p<0.05

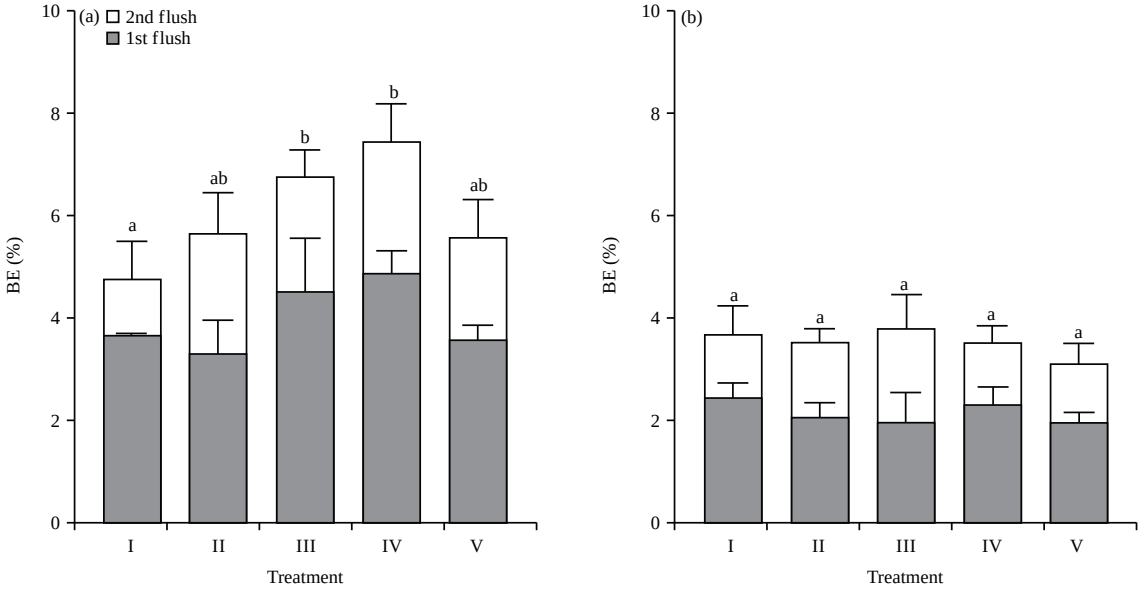

Fig. 7(a-b): Biological yield of (a) *T. versicolor* strain VNUA and (b) Strain BV cultivated on different treatments
Different letters denote statistically significant differences at p<0.05

spawning, for instance, there was no significant effect of the different treatments on the mycelia growth rate for strain VNUA (Fig. 6a). In contrast, the spawn running of strain BV was faster in treatments I (20) and II (20.2 days) than in treatments III (24 days), IV (24 days) and V (24.8 days) (Fig. 6b). Strain VNUA and strain BV were able to initiate and develop primordia for all the treatments (Fig. 7a, b). Among the investigated basal substrates, treatments III and IV showed the best yield performance with a BE value of 7.3 and 6.7% for strain VNUA, followed by treatments II (5.5%) and V (5.5%) (Fig. 7a). Although the highest mycelial growth was found in treatments I and II for strain BV, no significant difference in BE was recorded among all treatments (Fig. 7b). The BE of strain BV was between 3.1-3.5%. Compared

Fig. 8(a-b): Fruiting body morphology of (a) *T. versicolor* strain VNUA and (b) Strain BV

to strain BV, strain VNUA produced a higher total yield and was considered a suitable candidate for commercial cultivation. No differences in fruiting body morphology were observed regardless of the investigated substrate cultivation (Fig. 8a, b).

DISCUSSION

In this study, two investigated wild *T. versicolor* strains show high adaptation under artificial culture conditions. The optimal medium and temperature for the best mycelial growth were PGA and 30°C, respectively. Temperature plays a vital role in determining successful mycelial growth, primordial formation and yield[15]. The optimal growth temperature for mycelial growth varies according to species. For example, *Macrolepiota proceara*, *Ganoderma tropicum*, *Lentinus strigosus* and *Tricholoma* spp. exhibited efficient mycelial growth at 25[16], 25-28[17], 32[18] and 20-25°C[19], respectively. *T. versicolor* was able to grow and produce enzyme laccase at temperatures ranging from 5-35°C[20] but grew poorly above 30°C and below 20°C[1]. In agreement with the previous study conducted by Jo *et al.*[1], in this study, the optimal temperature for the mycelium growth of *T. versicolor* was observed at 30°C, followed by 25°C (Fig. 2 and 3a).

As one of the most critical factors, culture media provide essential nutrients such as carbon source, nitrogen source and minerals for mushroom growth. The optimal medium for mycelial growth of *Macrolepiota dolichaula* and *Cordyceps militaris* was MEA [21] and SDAY [22], respectively. To ascertain the optimal medium for the mycelial growth of *T. versicolor* strains, four different media were used to assess the mycelial growth performance of *T. versicolor*. Due to ease of use and economic feasibility, PGA was the most recommendable medium for strain VNUA and strain BV growth.

Spawn plays a fundamental role in mushroom cultivation[23,24]. The spawn running time and mushroom yield is related to the spawn type[25]. There are four spawn types, including sawdust spawn, grain spawn, liquid spawn and stick spawn, that are used to cultivate mushrooms[26]. As a nutritious carrier of mycelia, grain spawn served as an inoculant in the cultivation of mushrooms. To our knowledge, this is the first study to optimize spawn grain substrates for *T. versicolor* cultivation. It is worth noting that the use of grain for spawn production may increase the cost of manufacturing and lead

to a high contamination rate[27]. Therefore, we used a combination of sawdust and rice grain with a different ratio mixture as spawn substrate to reduce the production cost. Practical and economic spawn substrates to produce the mother spawn for *T. versicolor* was identified as Treatment D (20% rice grain, 79% sawdust, 1% calcium carbonate).

In addition to spawns and conducive environments, substrates need to be considered to cultivate mushrooms successfully. Cultivation substrate significantly contributes to the yield and medicinal value of mushrooms[27]. The mixture of Agro-wastes may improve yield due to such combinations' capability to differentiate lignocellulosic sources[28,29]. The selected local Agro-industrial wastes can reduce production costs and be a useful solution for waste management[30]. As one of the largest Rice-producing countries, Vietnam has plenty of rice husks. Accordingly, we used sawdust and rice husk as primary substrates for the cultivation of *T. versicolor.* The presence of rice husk in substrates exhibited an improvement in the mycelial growth rate for strain BV and enhanced the biological yield for strain VNUA. Generally, basal substrates are known to be deficient in nutrients. Thus, the addition of enrichment materials such as corn flour, wheat bran and rice bran is to increase the nitrogen level for cellular protein and enzyme synthesis[29]. In this study, for the enhancement of yield, wheat bran was used to provide a nitrogen reservoir, which was utilized during the mycelial colonization and primordia formation[31]. All the treatments used for the cultivation of *T. versicolor* strains showed two flushes during the cultivation period. In the second flush, the yield harvested from both strains is lower than the first flush. This may be due to the less nutrient accessibility in the substrate for the second flush[32]. Notably, strain VNUA exhibited satisfactory yield performance (7.3%) and could be considered a potential candidate for large-scale cultivation. *T. versicolor* has several medicinal properties that protect body function toward diseases. For determining the medicinal and nutritional value of strain VNUA, further studies are needed to assess the contents of tyrosol, friedelin, alnusenone, α-D-glucan and β-D-glucan. Disease outbreaks caused by bacteria, viruses and mold bring huge yield loss and enormous economic damage to the mushroom industry. The ability of strain VNUA to resist these diseases should be evaluated in further studies.

CONCLUSION

In this study, two wild *T. versicolor* strains were successfully rescued. PGA and 30°C were identified as the optimal medium and temperature, respectively, for the favorable mycelial growth of the investigated strains. A mixture of 20% rice grain, 79% sawdust and 1% calcium carbonate was proposed as suitable and economic substrates for the spawn production of *T. versicolor* at an industrial-scale. Compared with strain BV, strain VNUA exhibited higher biological growth efficiency (7.35%) and should be used for commercial cultivation.

SIGNIFICANCE STATEMENT

We successfully rescued two *T. versicolor* wild strains and proposed practice and economical protocol from spawn production to the cultivation of *T. versicolor*. This study will provide useful information to help the researcher isolate and cultivate wild *T. versicolor* strains. This study also contributes to the development of *T. versicolor* cultivation at the industrial scale and improves sustainable rural development.

ACKNOWLEDGMENT

This study was supported by a grant of Ha Noi Department of Science and Technology (01C-06/01-2019-3).

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