



# Optimization of mycelial growth and cultivation of wild *Ganoderma sinense*

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## Abstract

*Ganoderma sinense*, a well-known medicinal macrofungus of Basidiomycetes, is widely used in traditional medicine for promoting health and longevity in East Asia. The fruiting bodies of *G. sinense* contain polysaccharides, ergosterol, and coumarin, which have antitumor, antioxidant, and anticypenia activities. Mushroom cultivation requires suitable conditions for the formation of fruiting bodies and yield. However, little is known about the optimal culture conditions for mycelial growth and cultivation of *G. sinense*. In this study, the successful cultivation of a *G. sinense* strain collected from the wild was reported. The optimal culture conditions were identified by examining one factor at a time. The results of this study revealed that the nutritional requirements for the optimal mycelial growth of *G. sinense* were fructose (15 g/l) as the carbon source and yeast extract (1 g/l) as the nitrogen source. The optimal pH and temperature for *G. sinense* were 7 and 25–30 °C, respectively. The mycelia grew fastest in treatment II (69% rice grains + 30% sawdust + 1% calcium carbonate). *G. sinense* produced fruiting bodies under all tested conditions and showed the highest biological efficiency (2.95%) in treatment B (96% sawdust, 1% wheat bran, 1% lime). In summary, under optimal culture conditions, *G. sinense* strain GA21 showed satisfactory yield and a high potential for commercial cultivation.

**Key words:** *Ganoderma sinense*, mycelial growth, spawn, lingzhi mushroom

## Introduction

The fungal genus *Ganoderma* has long been used as a traditional medicinal herb in East Asia (Luangharn et al., 2021). Because the morphologies of *Ganoderma* species are similar, their taxonomies remain unclear (Yalcin et al., 2020). Approximately 400 species have been described in this genus. *Ganoderma sinense*, commonly known as “Lingzhi,” is distributed in subtropical–tropical regions (Hapuarachchi, 2019a). Similar to other species of the genus *Ganoderma*, *G. sinense* is used as herbal medicine and health supplement. It has been reported to have antitumor (Han et al., 2021), anti-aging (Teseo et al., 2021), anti-inflammatory (Mei et al., 2019), and anticancer (Zheng et al., 2018) activities and nitric oxide inhibitory properties (Wang et al., 2020).

Polysaccharides are the major biological components of *G. sinense* (Y. Zhang et al., 2019).

One of the most significant challenges in the mushroom cultivation industry is identifying superior mushrooms with high yields and high stress tolerance. The cultivation of wild strains before their extinction is considered a realistic approach to overcome this challenge. Previous studies have reported the successful cultivation of several wild mushroom strains, such as *Lepista sordida* (Thongbai et al., 2017), *Trametes versicolor* (Nguyen et al., 2021), and *Pycnoporus sanguineus* (Dulay and Damaso, 2020). However, to the best of our knowledge, no study has evaluated the potential cultivation of wild *G. sinense* strains.

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Culture conditions are the primary factors affecting the mycelial growth and fruiting body formation in mushrooms (Nguyen et al., 2021). Among *Ganoderma* species, the majority of the studies focused on identifying the culture conditions for the mycelial growth of *Ganoderma lucidum* (Jayasinghe et al., 2008) and *Ganoderma applanatum* (Jo et al., 2009), but not for other species. Reportedly, the optimal culture conditions for the mycelial growth of *G. lucidum* are the yeast malt extract medium (YM) at pH 5 and 25–30°C (Jayasinghe et al., 2008), whereas *G. applanatum* grows well on potato dextrose agar (PDA), yeast mannitol agar, and mushroom complete medium at 25–30°C (Jo et al., 2009).

As Lingzhi mushrooms are scarce in nature, they are artificially cultivated to meet the increasing demands of international markets. Due to the abundance of available agro-industrial waste and human resources, there are good prospects for growing *Ganoderma* in Vietnam. The Lingzhi mushroom cultivation industry improves sustainable rural development in Vietnam (Ngo et al., 2019). Various methods such as log cultivation, sawdust cultivation, and substituted cultivation have been developed to cultivate Lingzhi mushrooms (Zhou et al., 2012), with sawdust, corncobs, cottonseed husk, and bagasse being the primary substrates (Zhou, 2017). Little is known about the optimal conditions required for mycelial growth and fruiting body formation in *G. sinense*.

In this study, a wild *G. sinense* strain collected from Lang Son Province was isolated, and the culture conditions for its mycelial growth and fruiting body formation were optimized. This study may provide useful information about spawn production and cultivation of wild *G. sinense* strains.

## Material and methods

### Fungal strains

Strain GA21 was collected from Lang Son Province in northern Vietnam (GPS location: 21°39'57"N 107°07'45"E) – Figure 1. The voucher specimen (no. GA21) was deposited at the Mushroom Research and Development Center, Vietnam. Pure mycelial cultures were isolated from healthy fruiting bodies using a previously described tissue culture technique (Nguyen et al., 2021). The inner tissue (approximately 3 cm) of the fruiting body was inoculated in a PDA medium in a Petri dish at 25°C for 7 days in a dark room. The

mycelium was maintained on PDA slants at 4°C for further studies. To identify strain GA21, the internal transcribed spacer (ITS) was amplified and sequenced using the universal primer set ITS5 (5'GGA AGT AAA AGT CGT AAC AAG G3') and ITS4 (5'TCC TCC GCT TAT TGA TAT GC3') (White et al., 1990). The ITS sequence of strain GA21 was deposited in GenBank under the accession number OP106957. In addition, the ITS regions of *G. sinense* MFLU 19-2198, *G. sinense* 19-2197, *G. sinense* 19-2173, *Ganoderma nasalanense* LPDR 17060211, *Ganoderma orbiforme* URM83336, *Ganoderma ecuadorensis* Dai 17418, *Ganoderma fornicatum* BCRC35374, and *Ganoderma australe* G88 were retrieved from GenBank. Phylogenetic trees were constructed using maximum-likelihood algorithms (Felsenstein, 1981) in the Molecular Evolutionary Genetics Analysis (MEGA) X software (Kumar et al., 2018). The robustness of the phylogenetic trees was evaluated using a bootstrap procedure based on 1000 replications.

### Nutrition effects on mycelial growth

The optimal nutritional requirements for the growth of *G. sinense* were identified by examining one factor at a time. The basal medium was the potato–agar medium containing 200 g/l infused potato and 15 g/l agar powder (Hailong Co Ltd, Vietnam). Potato infusion was prepared as described previously (Nguyen et al., 2019). Carbon sources were tested at a concentration of 20 g/l, which included glucose, lactose, saccharose, starch, fructose, maltose, xylose, and dextrin (Merk, Germany). Based on the obtained results, fructose was selected as the optimal carbon source. To optimize the concentration of the carbon source, fructose was enriched into the basal medium at multiple concentrations (0, 10, 15, 20, 25, and 30 g/l). Similarly, *G. sinense* was inoculated in a basal medium supplemented with various nitrogen sources (ammonium chloride [NH<sub>4</sub>Cl], ammonium nitrate [NH<sub>4</sub>NO<sub>3</sub>], ammonium sulfate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>], peptone, yeast powder, and urea) (Merk, Germany). The yeast extract was evaluated at various concentrations (0, 1, 2, 3, 4, and 5 g/l) as the best nitrogen source for the mycelial growth of strain GA21.

### Effects of temperature and pH on mycelial growth

To optimize the culture conditions in terms of temperature, mycelium disks of *G. sinense* (approximately 1 cm diameter) were inoculated on PDA plates and in-

cubated at different temperatures (15, 20, 25, 30, and 35 °C). In addition, eight pH values (pH 3, 4, 5, 6, 7, 8, 9, and 10) were evaluated to identify the optimal pH for the favorable growth of *G. sinense*. The pH of the growth media was adjusted using 0.1 M NaOH or 0.1 M HCl.

#### **Grain spawn preparation**

To upscale the mycelium of strain GA21, rice grains and rubber wood sawdust were selected as spawn substrates. Rice grains and sawdust (Thanh Cao KINOKO I.E Co., Ltd) were prepared according to previously reported protocols (Nguyen et al., 2019). In brief, rice grains were washed and soaked in water overnight. The grains were boiled for 20–25 min until they became soft. Rubber wood sawdust was mixed with a lime solution (0.4%) and fermented for 5–7 days. Then, the moisture level of the substrates was adjusted to 65% using tap water. The following compositions of the culture media were used: treatment I (99% rice grains + 1% calcium carbonate), treatment II (69% rice grains + 30% sawdust + 1% calcium carbonate), and treatment III (39% rice grains + 60% sawdust + 1% calcium carbonate). The mixtures were filled into 24-ml round-bottom tubes to their three-fourth capacity and sterilized at 121 °C for 60 min. As soon as the culture media reached room temperature, the sterilized tubes were inoculated with a piece of mycelia culture (5 mm × 5 mm) and incubated for 20 days at 25 ± 1 °C under dark conditions.

#### **Substrate preparation and cultivation**

In the cultivation of *G. sinense*, rubber wood sawdust was selected as the primary substrate medium. Treatments with various combinations of sawdust and wheat bran (Thanh Cao KINOKO I.E Co., Ltd) were used to cultivate *G. sinense*: treatment A (99% sawdust, 0% wheat bran, 1% calcium carbonate), treatment B (98% sawdust, 1% wheat bran, 1% calcium carbonate), and treatment C (96% sawdust, 3% wheat bran, 1% calcium carbonate).

Polythene bags were filled with 1.2 kg of the substrates and autoclaved at 121 °C for 90 min. After cooling, the substrates were inoculated with the master spawn in 5% of substrate wet weight. The inoculated bags were moved to the spawn running room and incubated at 25 °C with an air relative humidity of 65% under dark conditions until complete colonization of the substrate (20 days). The bags were incubated at 25 °C

and 85% air relative humidity in the cultivation room to induce fruiting body formation.

#### **Data collection**

The morphology of *G. sinense* mycelium was characterized based on the growth rate (mm/day), density [high, 3+; regular, 2+; and low, 1+], texture (cottony and floccose), and radial growth distribution (uniform and uneven) of mycelium. The mycelial growth rate (mm/day) was calculated after 8 days of incubation.

The growth performance of *G. sinense* was determined based on the following cultivation parameters: spawn running period, pinhead formation, and fruiting body development. The spawn running period (days) was the period required for *G. sinense* to achieve complete colonization of the substrate. The day of pinhead formation (days) was considered the day of inoculation to the primordia. The biological efficiency (BE, in %) was calculated based on the ratio of the dry fruiting body weight (g) to the dry weight of the substrate (g). To calculate dry weight, the fruiting bodies of *G. sinense* were dried at 60 °C for 24 h.

#### **Statistical methods**

Data were analyzed using GraphPad Prism (version 9.0, GraphPad Software Inc., San Diego, CA). All experiments were conducted in triplicate. The obtained data were subjected to one-way Analysis of variance (ANOVA), followed by Tukey's multiple-range tests ( $P < 0.05$ ), which were represented by letters or asterisks.

## **Results and discussion**

#### **Mycelial morphology of strain GA21**

The naturally occurring pileus was kidney shaped, and the color of strain GA21 was dark brown (Fig. 1A). The pileus size of strain GA21 was 7.9 cm in length, 10.8 cm in width, and up to 2.5 cm in thickness at the base (Fig. 1A). This strain grows well on the PDA medium. The mycelial morphology of strain GA21 was cottony with white pigmentation and uniform radial growth (Fig. 1B). Strain GA21 showed the highest ITS sequence similarity of 99.82% with *G. sinense* MFLU 19-2198, *G. sinense* MFLU 19-2197, *G. sinense* MFLU 19-2173, and *G. sinense* GACP171012239. In the phylogenetic tree, strain GA21 formed a robust cluster with *G. sinense* MFLU 19-2198, *G. sinense* MFLU 19-2197, and

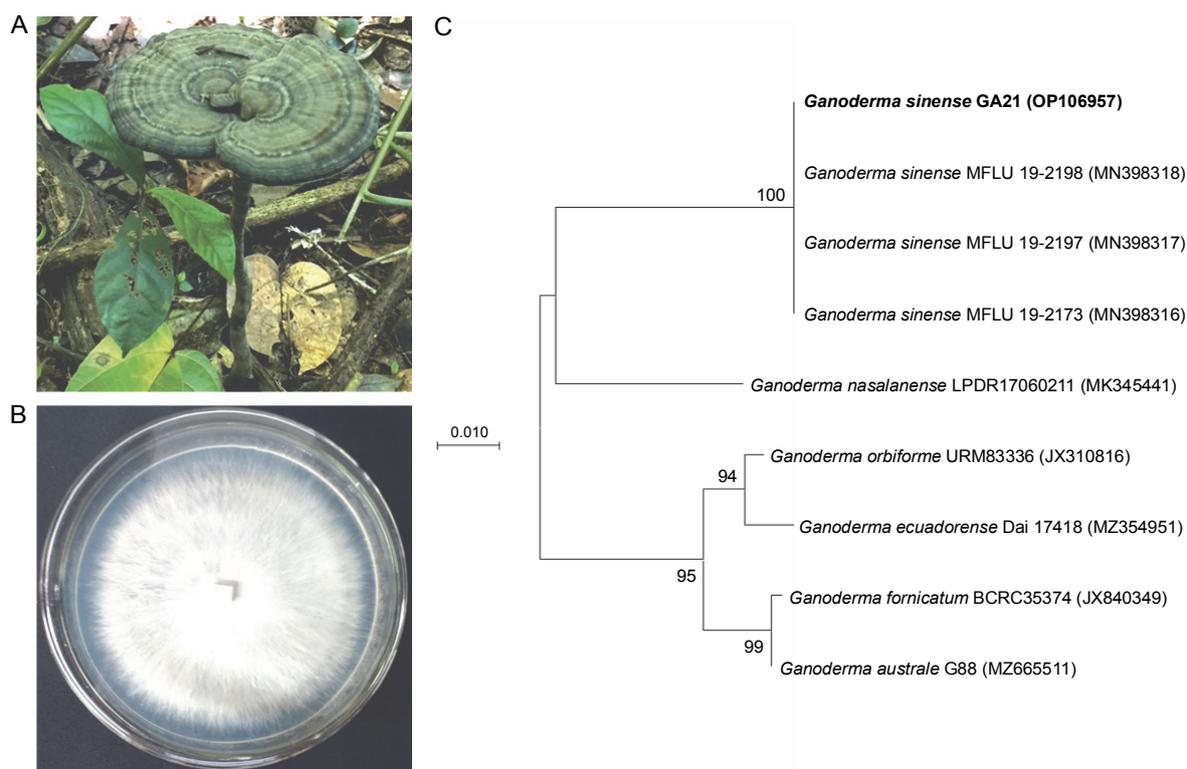


Fig. 1. Morphological features of *Ganoderma sinense* strain GA21: mature basidiomes (A), mycelium grown on potato dextrose agar medium (B); maximum-likelihood phylogenetic tree based on internal transcribed spacer sequences showing the relationship of strain GA21 to other members of the genus *Ganoderma*

*G. sinense* MFLU 19-2173, with a high bootstrap support value of 100% (Fig. 1C).

*G. sinense* has high phenotypic plasticity (Hapuarachchi, 2019b), with its morphology varying depending on climate, nutrition, and geographical environment (Hapuarachchi, 2019b). The color of *G. sinense* fruiting bodies was yellowish brown, brownish orange, gray, brownish black, and dark brown (Hapuarachchi, 2019b). The morphological characteristics of strain GA21 were similar to those of *G. sinense*. However, to further confirm the taxonomic position of strain GA21, the ITS region was amplified and sequenced using the universal primer set ITS5 and ITS4. ITS is an informative DNA region used for the classification of fungi (Schoch et al., 2012). The ITS sequence similarity and the phylogenetic tree confirmed that strain GA21 belongs to *G. sinense*. To the best of our knowledge, this is the first report of the occurrence of *G. sinense* in Vietnam.

#### **Effect of carbon sources on mycelial growth**

The culture medium is a vital intrinsic factor since it provides essential nutrients for mycelium growth (Ta

et al., 2021). The nutrition requirements for the mycelium growth of Lingzhi mushrooms primarily include carbon and nitrogen resources (Zhou et al., 2012). Carbon sources, which act as structural and storage compounds in the cell, should be optimized first among the constituents of nutritional compositions (Nguyen et al., 2021). *G. lucidum* can use a wide range of carbon sources, including dextrin, galactose, mannose, maltose, sucrose, and xylose (Jayasinghe et al., 2008). Of the eight carbon sources tested in this study, *G. sinense* grew well on the medium supplemented with fructose ( $4.82 \pm 0.05$  mm/day), saccharose ( $4.71 \pm 0.41$  mm/day), and maltose ( $4.40 \pm 0.14$  mm/day) (Fig. 2A). In contrast, low growth was observed in the medium enriched with lactose, with a mycelial growth rate of  $2.60 \pm 0.20$  mm/day (Fig. 2A). In addition, fructose treatment showed a higher mycelial density (3+) than the other carbon sources (Fig. 2B). Therefore, fructose was considered the best carbon source for mycelial growth.

To evaluate the effects of the concentration of the carbon source on the growth rate of *G. sinense*, fructose was supplied into the medium at different concentra-

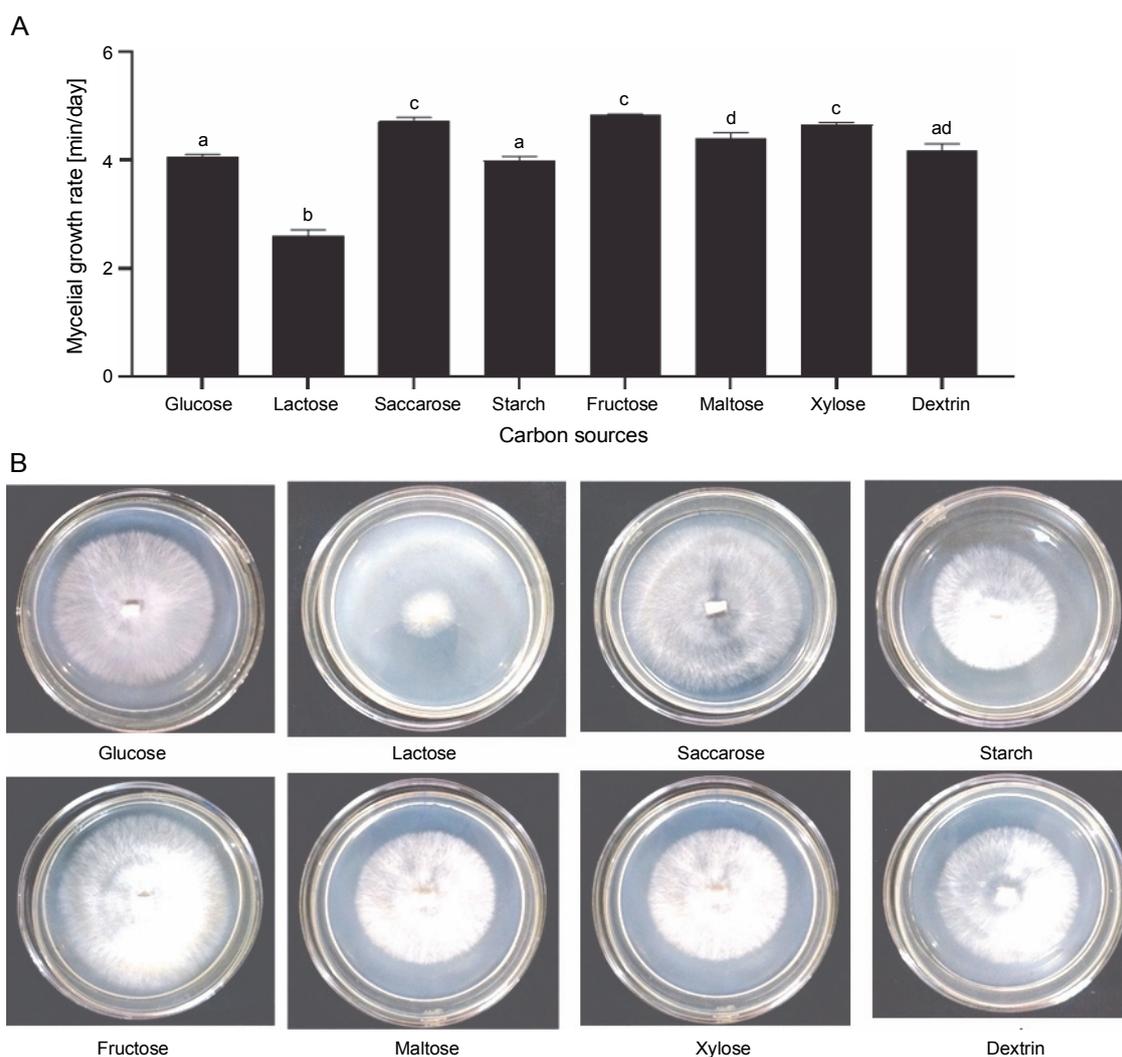


Fig. 2. Effect of carbon sources on the mycelial growth rate (A) and mycelial morphology of *Ganoderma sinense* on day 8 (B); different letters denote statistically significant differences at  $P < 0.05$

tions (10, 15, 20, 25, and 30 g/l), along with a medium devoid of fructose. The carbon content had a significant effect on the mycelial growth of *G. sinense*. The lowest mycelial diameter (5.45–5.60 mm/day) was observed in the medium containing 0–10 g/l of fructose (Fig. 3A). The highest mycelial growth was observed with 15 g/l ( $6.45 \pm 0.14$  mm/day), 20 g/l ( $6.82 \pm 0.10$  mm/day), and 30 g/l ( $6.75 \pm 0.10$  mm/day) fructose treatments. Thus, fructose with a carbon content of 15 g/l was selected for further experiments.

#### Effect of nitrogen sources on mycelial growth

Mushrooms require nitrogen for the synthesis of nitrogen-containing compounds and chitin in the cell wall (Miles and Chang, 1997). They can use both inorganic

(salts of nitrate, ammonium ions, etc.) and organic nitrogen sources (peptone, yeast extract, etc.) (Miles and Chang, 1997). Importantly, under nitrogen-deficient conditions, mycelium might stop growing. Alternatively, a high nitrogen content may inhibit mycelium growth due to the formation of metabolic byproducts (Junior Letti et al., 2018). The most suitable nitrogen sources for the mycelial growth of *G. lucidum* are ammonium acetate, glycine, arginine, and calcium nitrate (Jayasinghe et al., 2008). In the present study, three organic nitrogen sources (peptone, yeast extract, and urea) and three inorganic nitrogen sources [ $\text{NH}_4\text{Cl}$ ,  $\text{NH}_4\text{NO}_3$ , and  $(\text{NH}_4)_2\text{SO}_4$ ] at a concentration of 5 g/l were used to optimize the culture conditions of *G. sinense*. Results showed that *G. sinense* grow on the media supplementen-

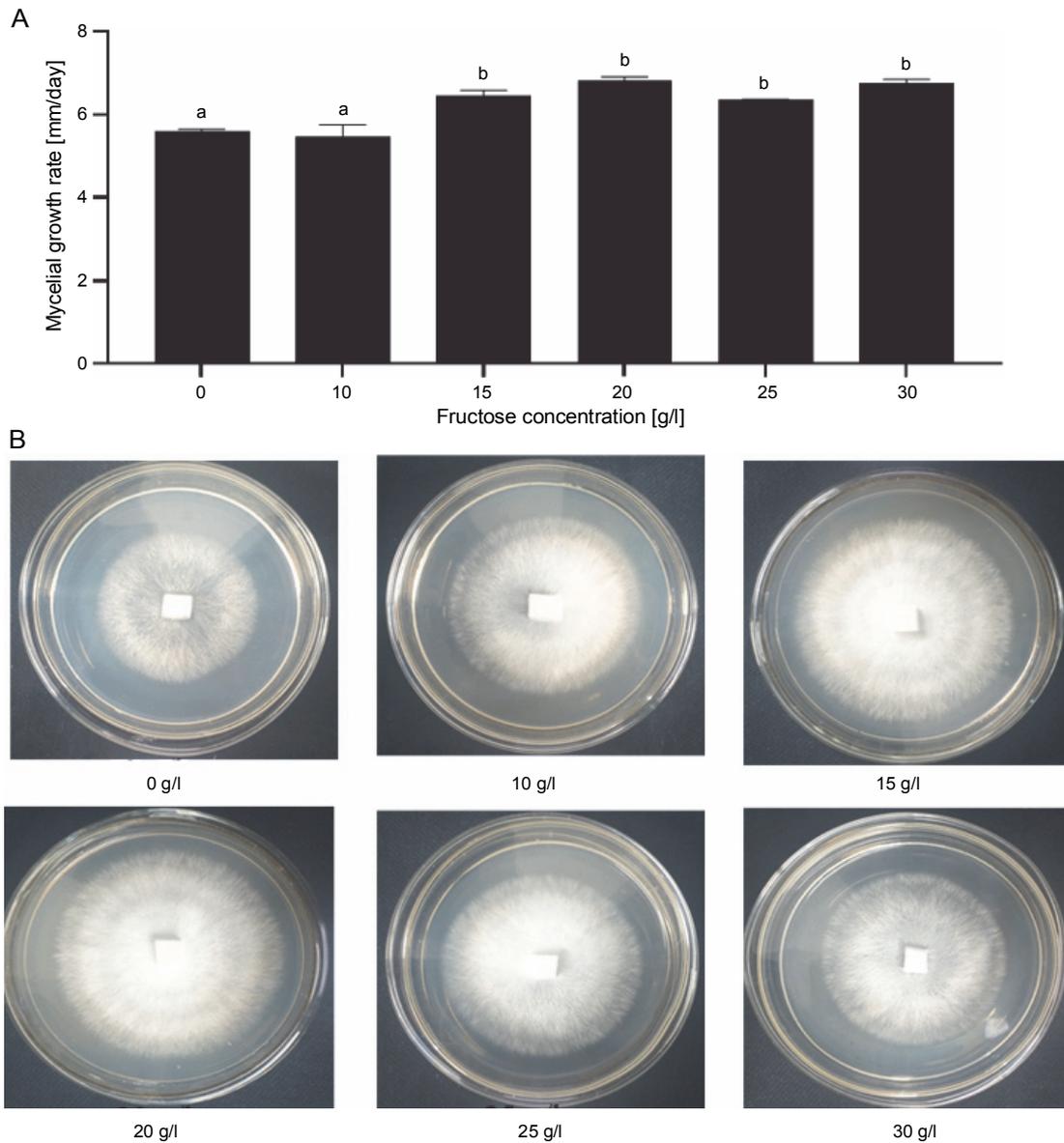


Fig. 3. Effect of fructose concentration on the mycelial growth rate (A) and mycelial morphology (B) of *Ganoderma sinense*; different letters denote statistically significant differences at  $P < 0.05$

ted with all tested nitrogen sources (Fig. 4A). The medium supplemented with yeast extract showed a higher growth rate (10.11 mm/day) than other nitrogen sources. Furthermore, the highest mycelial density (3+) was observed for yeast extract and peptone. Therefore, yeast extract was selected as an optimal nitrogen source for the mycelial growth of *G. sinense* as it grew best on a medium containing 1 g/l yeast extract ( $7.68 \pm 0.06$  mm/day) (Fig. 4B).

#### Effect of temperature on mycelial growth

In mushrooms, temperature affects nitrogen and sugar assimilation, respiration, and biosynthesis (Ta et al.,

2021). This abiotic factor is one of the most significant physical factors affecting the mycelial growth and development of fruiting bodies (Nguyen et al., 2019). Different species require different temperatures for mycelial growth. For example, the most favorable temperatures for the mycelial growth of *G. lucidum*, *Laetiporus sulphureus*, *Agaricus bisporus*, and *Calocybe indicawere* 30°C (Nguyen et al., 2019), 25–30°C (Luangharn et al., 2014), 22–24°C (Ma et al., 2014), and 30–32°C (Min et al., 2020), respectively. For *G. sinense*, the mycelial growth rate was the highest at 25°C ( $10.86 \pm 0.04$  mm/day) and 30°C ( $10.85 \pm 0.02$  mm/day), followed by 20°C ( $8.72 \pm 0.15$  mm/day) (Fig. 5A). No

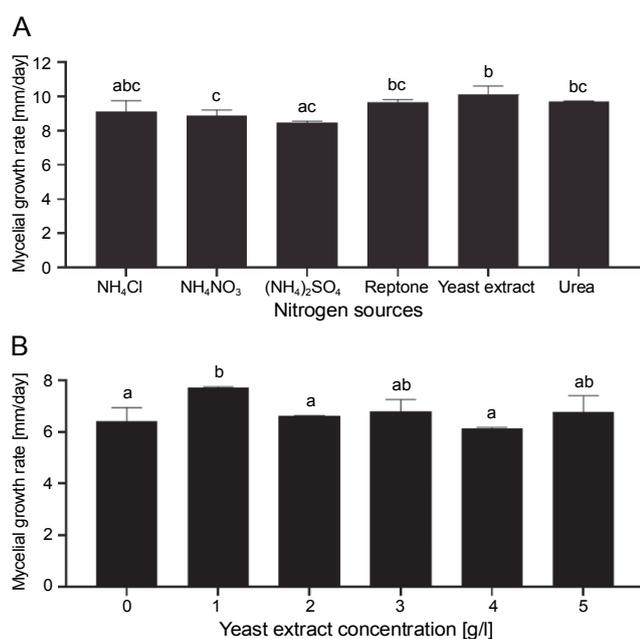


Fig. 4. Effect of nitrogen sources (A) and yeast extract concentration (B) on the mycelial growth rate of *G. sinense*; different letters denote statistically significant differences at  $P < 0.05$

mycelial growth occurred at 35°C. Basidiomycetes can be divided into three groups based on their optimal temperature range for mycelial growth: low temperature (optimum temperature: 25–30°C), intermediate (optimum temperature: 30–37°C), and high temperature (optimum temperature: 37–40°C) (Mswaka and Magan, 1999). For the low-temperature group, the ideal temperatures for mycelial growth ranged from 25 to 30°C, and they did not grow at temperatures above 37°C. Therefore, *G. sinense* was considered to belong to the low-temperature basidiomycetes group.

The mycelial density was thick (3+) at 25°C but somewhat thick (2+) at 20 and 30°C. The mycelial texture of strain GA21 was cottony, and its pigmentation was white. Taken together, *G. sinense* grew most efficiently at 25°C.

#### Effect of pH on mycelial growth

pH has a significant impact on cell membrane function, the absorption of nutrients, cell morphology, enzyme activity, and product biosynthesis (Ta et al., 2021). *G. sinense* grew at a pH range of 3.0–10.0 (Fig. 5B). This finding is consistent with those of previous studies showing that *Ganoderma* species can grow in a wide pH range (Jayasinghe et al., 2008; Nguyen et al., 2019). The optimal pH for enhancing *Ganoderma* mycelial growth

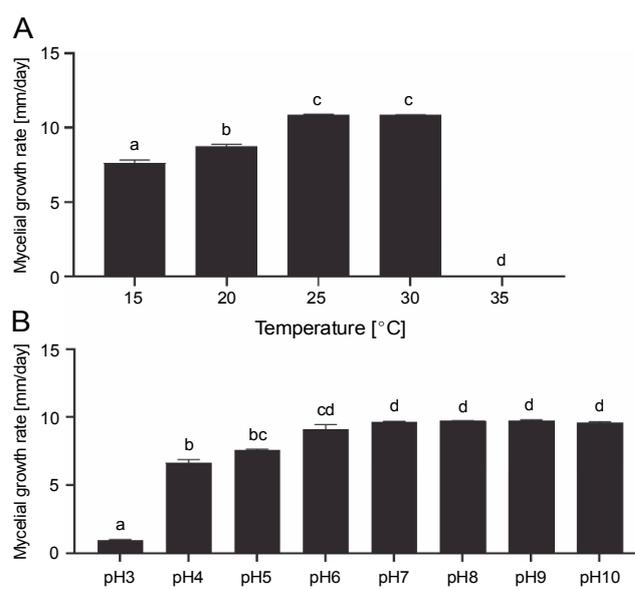


Fig. 5. Effect of temperature (A) and pH (B) on the mycelial growth rate of *Ganoderma sinense*; different letters denote statistically significant differences at  $P < 0.05$

varies from one species to another. For example, the maximum mycelial growth of *G. lucidum* was observed at pH 4.5–5.5 (Jayasinghe et al., 2008; Subedi et al., 2021), whereas *Ganoderma resinaceum* and *G. australe* grew fastest at pH 7 and pH 7–8, respectively (Kim et al., 2006; Luangharn et al., 2017). For the growth of *G. sinense*, pH 7 proved to be optimal (Fig. 5B). At this pH level, the mycelial growth rate was 9.63 mm/day, and the density was high (3+).

#### Mycelial growth on spawn substrates

Spawn plays an important role in determining the biological efficiency of mushrooms. Using grains (e.g., wheat, barley, sorghum, brown rice) in spawn production causes high contamination rates due to their high nutrition (W.R. Zhang et al., 2019). Furthermore, the use of grains increases manufacturing costs (W.R. Zhang et al., 2019). Using a mixture of sawdust and rice is an effective method for reducing production costs and contamination rates (Nguyen et al., 2021). For example, the optimal substrate for *T. versicolor* spawn production is a mixture of 20% rice grains, 79% sawdust, and 1% calcium carbonate (Nguyen et al., 2021). In the present study, sawdust was mixed with rice at different ratios and used as spawn substrates. Spawn grain substrates had a significant influence on the mycelial growth rate of *G. sinense* (Fig. 6). *G. sinense* began to colonize spawn

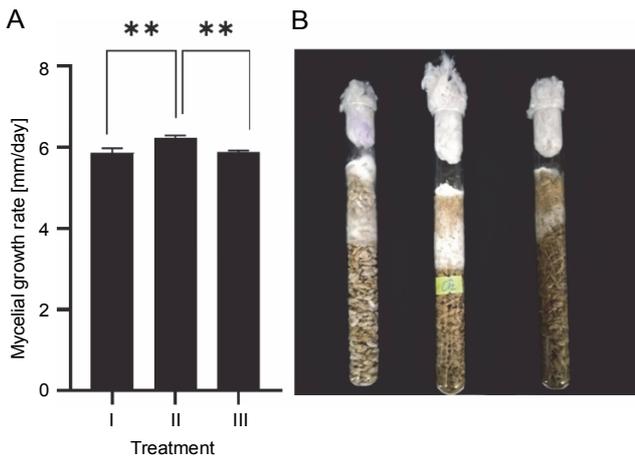


Fig. 6. Effect of various spawning materials on the mycelial growth rate (A) and density (B) of *Ganoderma sinense*; treatment I (99% rice grains + 1% calcium carbonate), treatment II (69% rice grains + 30% sawdust + 1% calcium carbonate), and treatment III (39% rice grains + 60% sawdust + 1% calcium carbonate); Asterisks show significant differences (\*\* $P < 0.01$ )

substrates on day 2. Treatment II showed the highest fungal growth rate ( $6.23 \pm 0.06$  mm/day), followed by treatment III ( $5.9 \pm 0.11$  mm/day) and treatment I ( $5.8 \pm 0.03$  mm/day). A higher mycelial density was observed in treatments I and II than in treatment III (Fig. 6B). In brief, treatment II (69% rice grains + 30% sawdust + 1% calcium carbonate) was the most suitable culture media for the mycelial growth of *G. sinense*.

#### Cultivation characteristics

Due to its medicinal and cultural importance, lingzhi has huge economic value (Bishop et al., 2015). Temperature, light, and nutrient availability play crucial roles in mushroom fructification. Under warm conditions, lingzhi mushrooms may develop fruiting bodies earlier (Bijalwan et al., 2021). The yield performance and quality of the mushrooms are strongly dependent on substrate cultivation. The substrates used in mushroom cultivation are generally selected based on locally available waste. In this study, sawdust was used as a basal substrate. Since sawdust has a low nutrient content and may be insufficient for the development of fruiting bodies, it was enriched with wheat bran, which acts as a nitrogen reservoir for mushrooms (Nguyen et al., 2021). However, high supplementation with wheat bran can increase the risk of contamination, inhibit the mycelial growth, and reduce the yield of mushrooms (Nguyen et al., 2021). The highest mycelial growth rate

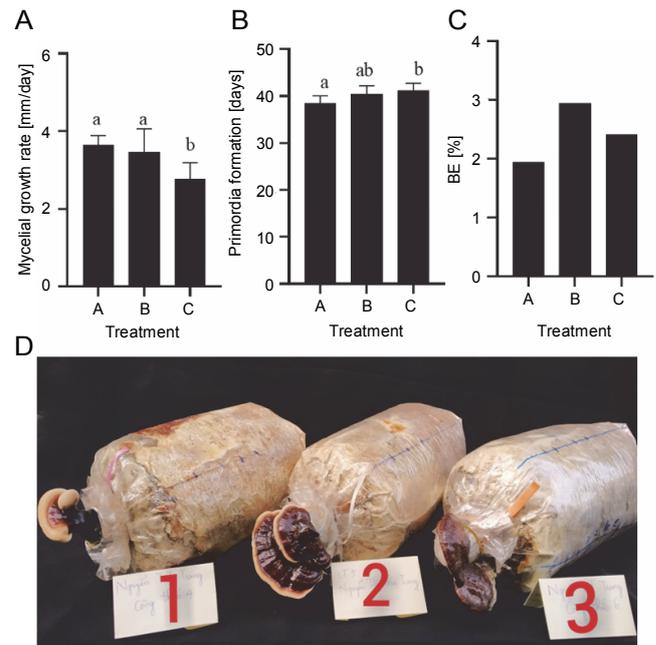


Fig. 7. Effect of different cultivation substrates on the mycelial growth rate (A), primordia formation (B), and BE (C) of *Ganoderma sinense*; treatment A (99% sawdust, 0% wheat bran, 1% calcium carbonate), treatment B (98% sawdust, 1% wheat bran, 1% calcium carbonate), and treatment C (96% sawdust, 3% wheat bran, 1% calcium carbonate); different letters denote statistically significant differences at  $P < 0.05$

of *G. sinense* was observed in treatment A (99% sawdust, 1% calcium carbonate) ( $3.65 \pm 0.24$  mm/day) and treatment B (98% sawdust, 1% wheat bran, 1% calcium carbonate) ( $3.47 \pm 0.60$  mm/day). In contrast, compared with treatments B and A, treatment C (96% sawdust, 3% wheat bran, 1% calcium carbonate) showed a lower mycelial growth rate ( $2.76 \pm 0.43$  mm/day).

*Ganoderma* species can be artificially cultivated using many substrates, such as sawdust, tea waste, cotton seed husk, corn cobs, and sunflower seed hulls (Hapuarachchi et al., 2018). All analyzed treatments induced the primordia formation of *G. sinense* (Fig. 7B), suggesting the possibility of *G. sinense* cultivation using rubber wood sawdust. In *G. sinense*, primordia formation was observed on the 38th day in treatment A, the 40th day in treatment B, and the 41st day in treatment C (Fig. 7B). Under artificial cultural conditions, *G. lucidum* forms primordia after inoculation for 43–48 days (Ngo et al., 2019). Therefore, the cropping cycle of *G. sinense* was similar to that of *G. lucidum*. A previous study reported that the yield of *G. lucidum* was increased when grown on a cultivation substrate supple-

mented with wheat bran (Mehta et al., 2014). Similarly, in the present study, the wheat bran content was found to affect the yield of *G. sinense*. The highest BE was achieved in treatment B (3.0%), followed by treatment A (2.4%) and treatment C (1.9%) (Fig. 7C and D). Thus, treatment B (98% sawdust, 1% wheat bran, 1% calcium carbonate) can be considered the optimal substrate mixture for *G. sinense* cultivation.

## Conclusions

Despite being a well-known medicinal macrofungus, little is known about the optimal cultivation conditions of *G. sinense*. In this study, the optimal cultural conditions for the mycelial growth of *G. sinense* were as follows: fructose (15 g/l), yeast extract (1 g/l), pH 7, and 25–30°C. This study demonstrated for the first time that *G. sinense* can form primordia and develop fruiting bodies on sawdust as the base substrate. Sawdust enriched with 1% wheat bran could be a suitable and inexpensive substrate for *G. sinense* cultivation. The findings of this study contribute to the industrialization of *G. sinense* cultivation. Further studies are needed to optimize the temperature and humidity for *G. sinense* cultivation and assess its medicinal value.

## Acknowledgments

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## Conflict of interest

The authors declare that there are no conflicts of interest.

## Ethical statement

This article does not contain any studies related to human participants and/or animals.

## References

- Bijalwan A., Bahuguna K., Vasisht A., Singh A., Chaudhary S., Dongariyal A., Kumar Thakur T., Kaushik S., Javed Ansari M., Alfarraj S. et al. (2021) *Growth performance of Ganoderma lucidum using billet method in Garhwal Himalaya, India*. Saudi J. Biol. Sci. 28(5): 2709–2717.
- Bishop K.S., Kao C.H.J., Xu Y., Glucina M.P., Paterson R.R.M., Ferguson L.R. (2015) *From 2000 years of Ganoderma lucidum to recent developments in nutraceuticals*. Phytochemistry 114: 56–65.
- Dulay R.M.R., Damaso E.J. (2020) *The successful cultivation of Philippine wild mushroom Pycnoporus sanguineus (BIL7137) using rice straw and sawdust-based substrate*. J. Appl. Biol. Biotechnol. 8(5): 72–77.
- Felsenstein J. (1981) *Evolutionary trees from DNA sequences: a maximum likelihood approach*. J. Mol. Evol. 17(6): 368–376.
- Han W., Chen H., Zhou L., Zou H., Luo X., Sun B., Zhuang X. (2021) *Polysaccharides from Ganoderma sinense – rice bran fermentation products and their anti-tumor activities on non-small-cell lung cancer*. BMC Complement Med. Ther. 21(1): 1–10.
- Hapuarachchi K. (2019a) *Ganodermataceae (Polyporales): diversity in Greater Mekong subregion countries (China, Laos, Myanmar, Thailand and Vietnam)*. Mycosphere 10(1): 221–309.
- Hapuarachchi K. (2019b) *High phenotypic plasticity of Ganoderma sinense (Ganodermataceae, Polyporales) in China*. Asian J. Mycol. 2(1): 1–47.
- Hapuarachchi K.K., Elkhateeb W.A., Karunarathna S.C., Cheng C.R., Bandara A.R., Kakumyan P., Hyde K.D., Daba G.M., Wen T.C. (2018) *Current status of global Ganoderma cultivation, products, industry and market*. Mycosphere 9(5): 1025–1052.
- Jayasinghe C., Imtiaj A., Hur H., Lee G.W., Lee T.S., Lee U.Y. (2008) *Favorable culture conditions for mycelial growth of Korean wild strains in Ganoderma lucidum*. Mycobiology 36(1): 28–33.
- Jo W.-S., Cho Y.-J., Cho D.-H., Park S.-D., Yoo Y.-B., Seok S.-J. (2009) *Culture conditions for the mycelial growth of Ganoderma applanatum*. Mycobiology 37(2): 94.
- Junior Letti L.A., Destéfani Vítola F.M., Vinícius de Melo Pereira G., Karp S.G., Pedroni Medeiros A.B., Ferreira da Costa E.S., Bissoqui L., Soccol C.R. (2018) *Solid-state fermentation for the production of mushrooms*. [in:] *Current advances in solid-state fermentation*. Ed. Pandey A., Larroche C., Soccol C.R. Elsevier: 285–318.
- Kim H.M., Paik S.-Y., Ra K.S., Koo K.B., Yun J.W., Choi J.W. (2006) *Enhanced production of Exopolysaccharides by fed-batch culture*. J. Microbiol. 44(2): 233–242.
- Kumar S., Stecher G., Li M., Knyaz C., Tamura K. (2018) *MEGA X: Molecular evolutionary genetics analysis across computing platforms*. Mol. Biol. Evol. 35(6): 1547–1549.
- Luangharn T., Karunarathna S., Khan S., Xu J., Mortimer P., Hyde K. (2017) *Antibacterial activity, optimal culture conditions and cultivation of the medicinal Ganoderma australe, new to Thailand*. Mycosphere 8(8): 1108–1123.
- Luangharn T., Karunarathna S.C., Dutta A.K., Paloi S., Promputtha I., Hyde K.D., Xu J., Mortimer P.E. (2021) *Ganoderma (Ganodermataceae, basidiomycota) species from the greater mekong subregion*. J. Fungi. (Basel) 7(10): 819.
- Luangharn T., Karunarathna S.C., Hyde K.D., Chukeatirote E. (2014) *Optimal conditions of mycelia growth of Laetiporus sulphureus sensu lato*. Mycology 5(4): 221–227.
- Ma Y., Guan C.Y., Meng X.J. (2014) *Biological characteristics for mycelial growth of Agaricus bisporus*. Appl. Mech. Mater. 508: 297–302.
- Mehta S., Jandaik S., Gupta D. (2014) *Effect of cost-effective substrates on growth cycle and yield of lingzhi or reishi medicinal mushroom, Ganoderma lucidum (Higher Basidiomycetes) from Northwestern Himalaya (India)*. Int. J. Med. Mushrooms 16(6): 585–591.

- Mei R.Q., Zuo F.J., Duan X.Y., Wang Y.N., Li J.R., Qian C.Z., Xiao J.P. (2019) *Ergosterols from Ganoderma sinense and their anti-inflammatory activities by inhibiting NO production*. *Phytochem. Lett.* 32: 177–180
- Miles P.G., Chang S.-T. (1997) *Mushroom biology: concise basics and current developments*. [in:] *Mushroom biology: concise basics and current developments*. Ed. Miles P.G. World Scientific Publishing Company: 41.
- Min G.J., Park H.S., Lee E.J., Lee C.J. (2020) *Culture characteristics and optimal conditions for mycelial growth of Calocybe indica*. *Korean J. Mycol.* 48(3): 273–284.
- Mswaka A.Y., Magan N. (1999) *Temperature and water potential relations of tropical Trametes and other wood-decay fungi from the indigenous forests of Zimbabwe*. *Mycol. Res.* 103(10): 1309–1317
- Ngo N.X., Nguyen B.T.T., Le V. Van, Nguyen L.T., Nguyen T.T., Nguyen Q.D. (2019) *Morphological characteristics, yield performance, and medicinal value of some lingzhi mushroom (Ganoderma lucidum) strains cultivated in Tam Dao, Vietnam*. *Vietnam J. Agric. Sci.* 2(1): 321–331.
- Nguyen B.T.T., Le V. Van, Nguyen H.T.T., Nguyen L.T., Tran A.D., Ngo N.X. (2021) *Successful rescue of wild Trametes versicolor strains using sawdust and rice husk-based substrate*. *Pakistan J. Biol. Sci.* 24(3): 374–382.
- Nguyen B.T.T., Le V. Van, Nguyen H.T.T., Nguyen L.T., Tran T.T.T., Ngo N.X. (2021) *Nutritional requirements for the enhanced mycelial growth and yield performance of Trametes versicolor*. *J. Appl. Biol. Biotechnol.* 9(1): 1–7.
- Nguyen B.T.T., Ngo N.X., Le V. Van, Nguyen L.T., Kana R., Nguyen H.D. (2019) *Optimal culture conditions for mycelial growth and fruiting body formation of Ling Zhi mushroom Ganoderma lucidum strain GA3*. *Vietnam J. Sci. Technol. Eng.* 61(1): 62–67.
- Schoch C.L., Seifert K.A., Huhndorf S., Robert V., Spouge J.L., Levesque C.A., Chen W., Bolchacova E., Voigt K., Crous P.W. et al. (2012) *Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi*. *Proc. Natl. Acad. Sci.* 109(16): 6241–6246.
- Subedi K., Basnet B.B., Panday R., Neupane M., Tripathi G.R. (2021) *Optimization of growth conditions and biological activities of Nepalese Ganoderma lucidum strain Philippine*. *Adv. Pharmacol. Pharm. Sci.* 2021: 4888979.
- Ta K., Vyu B., As S. (2021) *Review of the basic cultivation conditions influence on the growth of basidiomycetes*. *Curr. Res. Environ. Appl. Mycol.* 11(1): 494–531.
- Teseo S., Houot B., Yang K., Monnier V., Liu G., Tricoire H. (2021) *G. sinense and P. notoginseng extracts improve healthspan of aging flies and provide protection in a Huntington disease model*. *Aging Dis.* 12(2): 425–440.
- Thongbai B., Wittstein K., Richter C., Miller S.L., Hyde K.D., Thongklang N., Klomklung N., Chukeatirote E., Stadler M. (2017) *Successful cultivation of a valuable wild strain of Lepista sordida from Thailand*. *Mycol. Prog.* 16(4): 311–323.
- Wang D., Wang Y.L., Zhang P., Li X.Z., Li H., Chen L.X. (2020) *New sesquiterpenoid derivatives from Ganoderma sinense with nitric oxide inhibitory activity*. *Phytochem. Lett.* 35: 84–87.
- White T.J., Bruns T., Lee S., Taylor J. (1990) *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. [in:] *PCR protocols, a guide to methods and applications*. Ed. Ma I.: 315–322.
- Yalcin O.U., Sarikurkcu C., Cengiz M., Gungor H., Ćavar Zeljković S. (2020) *Ganoderma carnosum and Ganoderma pfeifferi: metal concentration, phenolic content, and biological activity*. *Mycologia* 112(1): 1–8.
- Zhang W.R., Liu S.R., Kuang Y.B., Zheng S.Z. (2019) *Development of a novel spawn (Block Spawn) of an edible mushroom, Pleurotus ostreatus, in liquid culture and its cultivation evaluation*. *Mycobiology* 47(1): 97–104.
- Zhang Y., Jiang Y., Zhang M., Zhang L. (2019) *Ganoderma sinense polysaccharide: An adjunctive drug used for cancer treatment*. *Prog. Mol. Biol. Transl. Sci.* 163: 165–177.
- Zheng M., Tang R., Deng Y., Yang K., Chen L., Li H. (2018) *Steroids from Ganoderma sinense as new natural inhibitors of cancer-associated mutant IDH1*. *Bioorg. Chem.* 79: 89–97.
- Zhou X.-W. (2017) *Cultivation of Ganoderma lucidum*. [in:] *Edible and medicinal mushrooms*. Ed. Diego C.Z., Pardo-Gimenez A.: 385–413.
- Zhou X.-W., Su K.Q., Zhang Y.M. (2012) *Applied modern biotechnology for cultivation of Ganoderma and development of their products*. *Appl. Microbiol. Biotechnol.* 93(3): 941–963.