

Domestication of *Ganoderma leucocontextum*, *G. resinaceum*, and *G. gibbosum* Collected from Yunnan Province, China

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Ganoderma mushrooms have been used in traditional medicines for centuries and as such are highly sought after, especially in Asia. The present study is the first report of the successful cultivation of *G. leucocontextum*, *G. resinaceum*, and *G. gibbosum* collected from the wild, in Yunnan Province, China. One mature fruiting body of the laccate *G. leucocontextum*, one mature fruiting body of the laccate *G. resinaceum*, and seven non-laccate *G. gibbosum* fruiting bodies were collected and isolated into culture. These strains were cultivated using both soil casing layer and non-casing layer methods. The highest yield and biological efficiency (BE) of *G. leucocontextum* was obtained when using the soil casing layer method (60.43% BE, with 253.82 g/Kg⁻¹ of the total yield) with the non-casing layer method (13.60% BE, with 58.18 g/Kg⁻¹ of the total yield), respectively. Only one cycle of production (26.94% BE and 7.02 g/Kg⁻¹) was obtained for *G. resinaceum* KUMCC19-0001 when the soil casing layer method was applied, while a high yield of 109.26% BE, with a total yield of 27.75 g/Kg⁻¹, was obtained when the non-casing layer method was used. A BE of 73.80% and total yield of 284.15 g/Kg⁻¹ were obtained for the *G. gibbosum* KUMCC17-0005 when it was cultivated with a soil casing layer, while a BE of 40.26% and a total yield of 172.08 g/Kg⁻¹ was obtained when the non-casing layer method was used. Based on this comprehensive study, this result will be helpful for the commercial cultivation for laccate *G. leucocontextum*, *G. resinaceum*, and non-laccate *G. gibbosum*.

Keywords: Medicinal mushroom, Morphological characteristics, Mushroom cultivation, Soil casing method, White rot.

Ganoderma is one of the most important medicinal mushroom genera and has been widely used in traditional Chinese medicine systems for over 2000 years (Paterson 2006). Members of the genus *Ganoderma* are renowned medicinal mushrooms and subsequently species from this

group are highly sought after and of great economic value, especially in Asia (Dai *et al.*, 2009; Hapuarachchi *et al.*, 2018b, 2019a). *Ganoderma* was typified by *G. lucidum* (W. Curt.:Fr.) Karst, which belongs to the order Polyporales (Justo *et al.*, 2017; He *et al.*, 2019); however, Cui *et*

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al. (2019) stated that *Ganoderma* should not be included in Polyporales because their double-walled basidiospores are quite different from other members of the Polyporales. The genus *Ganoderma* is characterized by its unique laccate and non-laccate basidiocarps, double-walled basidiospores, and interwall pillars (Moncalvo and Ryvarden, 1997; Hapuarachchi *et al.*, 2019a). *Ganoderma* is a cosmopolitan genus, distributed worldwide in temperate and tropical areas (Cao and Yuan, 2013; Hapuarachchi *et al.*, 2019a, b).

Ganoderma is rich in several biologically active compounds: more than 400 bioactive compounds have been found in various *Ganoderma* species, including fatty acids, polysaccharides, steroids, and triterpenoids, providing nutrition and containing medicinal properties (Wasser and Weis, 1999; Gao *et al.*, 2003; De Silva *et al.*, 2012a, b; Li *et al.*, 2013; Richter *et al.*, 2015). These valuable natural compounds are used to treat a variety of pathological diseases (Cheng *et al.*, 2010; Teng *et al.*, 2011; De Silva *et al.*, 2013; Richter *et al.*, 2015). Commercial *Ganoderma* products are available in many forms such as dried fruiting bodies, spore capsules, dietary supplements, and cosmetic products (Shiao 2003; Hapuarachchi *et al.*, 2018a, 2019a, b).

Laccate *Ganoderma leucocontextum* was discovered on the Tibetan Plateau. It is commonly called the “white *Ganoderma*” referring to its white context (Li *et al.*, 2015). Its fruiting bodies contain novel triterpenes and meroterpenes, which are used to treat nerve system diseases (Wang *et al.*, 2015; Chen *et al.*, 2018; De Silva *et al.*, 2012a, b). *Ganoderma resinaceum* contains high numbers of triterpenoids (Chen *et al.*, 2019), with strong inhibitory effects against α -glucosidase, and is used to treat hepatoprotective activity (Chen *et al.*, 2017; Chen *et al.*, 2018). *Ganoderma gibbosum* also contains triterpenoids, which are used in clinical treatment for their hypertension and cholesterol-reducing properties (Pu *et al.*, 2017). Therefore, triterpenoids have been regarded as an important indicator in the quality evaluation of *Ganoderma* species and related products (Guo *et al.*, 2013; Hennieke *et al.*, 2016). *Ganoderma* species also show significant cytotoxic activities and contain the following properties: immunostimulatory, anticancer, antiviral, anti-inflammatory, and antioxidant (treating liver, lung, kidney, spleen,

and stomach functions), allowing for its use in the clinical treatment of various types of diseases (Niu *et al.*, 2007; De Silva *et al.*, 2012a, b; Chen *et al.*, 2017). However, a number of *Ganoderma* species remain poorly studied: for example, there are few reports on the chemistry and bioactivity of non-laccate *G. gibbosum*, and the only known pharmacologically active compounds derived from *G. gibbosum* are a few forms of lanostane triterpenes, which were found to possess immunoregulatory and anti-inflammatory activities (Pu *et al.*, 2017).

Most *Ganoderma* cultivation is carried out in China, with local raw materials such sawdust or wood chips used as substrates (Stamets 2000; Jo *et al.*, 2010; Roy *et al.*, 2015; Tan *et al.*, 2015; Æilderžīe *et al.*, 2016; Luangharn *et al.*, 2017). Non-casing layer methods are usually applied to *Ganoderma* cultivation (Zhou *et al.*, 2012; Tan *et al.*, 2015; Liu *et al.*, 2017), while soil casing methods are rarely used (Nicholas and Ogame, 2006). Although *G. leucocontextum*, *G. resinaceum*, and *G. gibbosum* have been domesticated, the use and effectiveness of a soil casing layer has not been previously reported on. Thus, the objectives of this study were to determine most effective methods of cultivation for *G. leucocontextum*, *G. resinaceum*, and *G. gibbosum*, for which most scientists report sawdust as the optimal substrate, and to introduce a new protocol in *Ganoderma* cultivation for inducing the production of fruiting bodies via soil casing and non-casing layer methods in Kunming, Yunnan Province, China.

MATERIALS AND METHODS

Mushroom collection and isolation

One *G. leucocontextum* (KUMCC17-0007) strain, one *G. resinaceum* (KUMCC19-0001) strain, and seven non-laccate *G. gibbosum* strains were included in this study. The *G. gibbosum* strains consisted of five mature fruiting bodies (KUMCC17-0004, KUMCC17-0005, KUMCC17-0013, KUMCC18-0007, KUMCC19-0002) and two young fruiting bodies (KUMCC17-0009 and KUMCC19-0003), collected and isolated in the temperate climate of Yunnan Province, China (Figure 1). Detailed site information regarding location, climate, average monthly temperature during the rainy season, and

host trees species of the study sites are provided in Table 1. Pure cultures of the nine *Ganoderma* strains were isolated by the method described by Stamets (2000). The voucher numbers of the cultures were obtained by depositing the cultures in the Kunming Institute of Botany Culture Collection, China (KUMCC).

Grain media for inoculum production

Ganoderma spawn was prepared using wheat (*Triticum aestivum* L.) as the main spawn medium, following the procedure described in Luangharn *et al.* (2017). Broken wheat grains were avoided as a potential cause of contamination (Narh *et al.*, 2011). Wheat grains were washed and soaked for 18 hours. Next, 200 grams of wheat grain were filled into spawn bottles and autoclaved at 121 °C for 30 min. After being left to cool down, three pieces of active mycelia from agar media were cut 2–3 cm in diameter and inoculated into the spawn media bottles. The spawn media bottles were incubated at 25 ± 1 °C. 24–30 days after inoculation, and once the white mycelia fully covered the wheat grains, they were then considered to be viable mother spawn.

Bag preparation for fruiting body production

Alnus cremastogyne sawdust was obtained locally and used as the main substrate. Our basic formula for growing *Ganoderma* substrate was determined on the basis of dry weight (Smith *et al.*, 2002). The substrate was composed of sawdust (80%), wheat bran (17%), calcium carbonate (CaCO₃) (1%), magnesium sulfate (MgSO₄) (1%), and sucrose (1%). All organic and inorganic additives were mixed well. The pH was stabilized by CaCO₃ and MgSO₄ at pH 7–8 (Fang *et al.*, 2002), and levels below optimal pH values were adjusted with CaCO₃. Water was gradually added until the moisture content reached 70–75%, or until the substrate clumped when squeezed by hand. Eight hundred grams of sawdust substrate were filled into the thermo-stable polypropylene bags (6.50 × 12.50 inch). Holes (~5 cm) were punched in the top of the substrate bags, and a plastic ring was used to seal the tops of the growing bags. The substrate bags were sterilized in 121 °C for 40 min. After sterilization, the substrate bags were left to cool for 18 hrs.

Approximately 5–8 grams of active mother spawn were placed in the substrate bags under aseptic conditions, after which the bags were

Table 1. Detailed site information, including location, substrate, host tree species, and average monthly temperatures during the rainy season collection period of this study

Fungal species	Fungal strain	Location	Substrate, host species	Monthly temperature
<i>Ganoderma leucocontextum</i>	KUMCC17-0007	Baoshan, Yunnan	Decay stump of <i>Pinus</i> sp.	18 °C
<i>G. resinaceum</i>	KUMCC19-0001	Kunming Institute of Botany, Kunming	Living tree of <i>Albizia mollis</i>	20 °C
<i>G. gibbosum</i>	KUMCC17-0004	Kunming Botanical Garden, Kunming	Decay of <i>Chamaecyparis pisifera</i>	19 °C
<i>G. gibbosum</i>	KUMCC17-0005	Kunming Botanical Garden, Kunming	Living tree of <i>Albizia mollis</i>	19 °C
<i>G. gibbosum</i>	KUMCC17-0009	Kunming Botanical Garden, Kunming	Living tree of <i>Albizia mollis</i>	18 °C
<i>G. gibbosum</i>	KUMCC17-0013	Kunming Botanical Garden, Kunming	Decay wood of unknown tree	20 °C
<i>G. gibbosum</i>	KUMCC18-0007	Jinning, Yunnan	Stump of unknown tree	22 °C
<i>G. gibbosum</i>	KUMCC19-0002	Kunming Botanical Garden, Kunming	Decay wood of unknown tree	20 °C
<i>G. gibbosum</i>	KUMCC19-0003	Baoshan, Yunnan	Living tree of <i>Albizia</i> sp.	18 °C

sealed and incubated at 28 ± 2 °C for 26–30 days. All substrate bags were incubated at a relative humidity between 65–75% and maintained under dark conditions to induce mushroom mycelial formation. After the mycelia completely colonized the substrate in the bags, all bags were moved to the growing rooms to apply the two different cultivation

treatments: the soil casing and non-casing methods. The soil casing method was performed in a clean area, with sufficient ventilation and adjustable humidity. A dark sheet was used to cover the base of the growing area. Next, approximately 5–8 cm of clay soil was placed as a on top of the black sheet, acting as a ground layer. The soil was sourced from



Fig. 1. Morphological characteristics of the nine strains of *Ganoderma* recorded in the field. (a). *Ganoderma leucocontextum* strain KUMCC17-0007, (b). *G. resinaceum* KUMCC19-0001, (c). *G. gibbosum* KUMCC17-0004, (d). KUMCC17-0005, (e). KUMCC17-0009, (f). KUMCC17-0013, (g). KUMCC18-0007, (h). KUMCC19-0002, (i). KUMCC19-0003. Scale bar: a–i = 2 cm

a nearby village (Xiaoshao Village, Ciba Town, Panlong District, Kunming). The fully colonized mushroom bags were cut at top and bottom, placed on the soil bed, and finally covered with a casing layer of the same clay-based soil. For the non-casing method, the fully colonized growing bags were kept in a clean room with good ventilation. Both bags were grown at 25–28 °C, and water was sprayed daily to maintain the relative humidity at 75–85% during primordia initiation, while 70–80% humidity was maintained during the formation of mature fruiting bodies until the fruiting bodies fully developed. Mycelia growth, primordia initiation, fruiting development, and fructification periods were recorded regularly. All experiments were carried out with ten replicates.

Harvesting and yield data

The number of primordia, young primordia, and mature fruiting bodies per bag were recorded during the total harvest period of 180–185 days. Mature fruiting bodies (indicated by caps becoming completely red and the white margin disappearing) were manually harvested (Royse 1996), counted, and weighed. The biological efficiency (BE) and total yields (g/Kg⁻¹) were determined by following the protocols described by Royse (2010) and Peksen and Yakupoglu (2009).

Data collection and statistical analysis

Data analysis was carried out using the SPSS statistical program (Softonic International SA, Barcelona, Spain) with ten replicates. Data

for initiation of primordia, young primordia production, mature fruiting body production, and time period for the development of young to mature fruiting bodies were collected. All the data were compared to obtain a mean separation using Tukey’s test (*p* < 0.05), followed by post-hoc tests that were expressed in a one-way ANOVA.

RESULTS

Mushroom cultures and spawn production

Colonization of grow bags

After 14 days of incubation, *Ganoderma leucocontextum*, *G. resinaceum*, and *G. gibbosum* were fully colonized with white mycelia on a PDA medium under dark conditions. *Ganoderma leucocontextum*, *G. resinaceum*, and *G. gibbosum* mycelia were produced on wheat grain media after being incubated for 3–4 days. White mycelia of *G. leucocontextum* and *G. resinaceum* were seen on wheat grain media after being incubated at 25 ± 1 °C for 3–4 days, and the grains were fully colonized after 22 days of incubation. After only 3 days we observed colonization of the wheat grain media from the cultures derived from the mature *G. gibbosum*, white massive mycelia were seen after 2–4 days of further incubation, and the grains were fully covered after 18 days of incubation at 25 ± 1 °C. The cultures derived from the young *G. gibbosum* spawn took 5 days before we observed grain colonization, and grains were fully covered after 26 days of incubation at 25 ± 1 °C.

Table 2. Results of the total numbers of initial primordial formed, primordial, and mature fruiting bodies of *Ganoderma leucocontextum*, *G. resinaceum*, and *G. gibbosum*

Fungal species	Fungal strains	No. of initial primordial formed		No. of primordia		No. of mature fruiting bodies	
		SC	NC	SC	NC	SC	NC
<i>Ganoderma leucocontextum</i>	KUMCC17-0007	102	31	98	24	61	14
<i>G. resinaceum</i>	KUMCC19-0001	22	57	18	50	9	35
<i>G. gibbosum</i>	KUMCC17-0004	36	22	33	20	33	20
<i>G. gibbosum</i>	KUMCC17-0005	39	32	39	31	35	30
<i>G. gibbosum</i>	KUMCC17-0009	24	0	22	0	20	0
<i>G. gibbosum</i>	KUMCC17-0013	36	33	36	33	34	32
<i>G. gibbosum</i>	KUMCC18-0007	48	23	43	23	40	22
<i>G. gibbosum</i>	KUMCC19-0002	39	35	37	34	37	33
<i>G. gibbosum</i>	KUMCC19-0003	20	0	20	0	20	0

Notes: SC = Soil casing; NC = Non-casing

Table 3. Results of the fruiting cycles of *Ganoderma leucocontextum*, *G. resinaceum*, and *G. gibbosum* cultivated by both soil casing and non-casing layer methods. Values with the same column followed by the same letter are not significantly different ($p < 0.05$) by Tukey's test

Fungal species	Fungal strains	Average wet weight offruiting bodies (g)		Biological Efficiency (BE) (%)		Total yield (g/Kg ⁻¹)	
		SC	NC	SC	NC	SC	NC
<i>Ganoderma leucocontextum</i>	KUMCC17-0007	203.06 ± 0.17 ^b	46.54 ± 0.11 ^c	60.43	13.60	253.82	58.18
<i>G. resinaceum</i>	KUMCC19-0001	21.55 ± 0.8 ^g	87.41 ± 0.45 ^h	26.94	109.26	7.02	27.75
<i>G. gibbosum</i>	KUMCC17-0004	147.05 ± 0.93 ^d	119.26 ± 0.64 ^c	42.24	33.15	183.81	149.07
<i>G. gibbosum</i>	KUMCC17-0005	227.32 ± 0.71 ^a	137.67 ± 0.22 ^{ab}	73.80	40.26	284.15	172.08
<i>G. gibbosum</i>	KUMCC17-0009	88.70 ± 0.85 ^e	0 ^f	26.72	0	110.88	0
<i>G. gibbosum</i>	KUMCC17-0013	182.96 ± 0.15 ^c	125.10 ± 0.11 ^{bc}	61.20	39.09	228.70	156.37
<i>G. gibbosum</i>	KUMCC18-0007	154.54 ± 0.10 ^d	116.76 ± 0.11 ^c	45.98	30.39	193.17	145.95
<i>G. gibbosum</i>	KUMCC19-0002	183.84 ± 0.13 ^c	141.81 ± 0.41 ^a	60.66	44.45	229.80	177.26
<i>G. gibbosum</i>	KUMCC19-0003	73.02 ± 0.42 ^f	0 ^f	20.92	0	91.28	0

Notes: SC = Soil casing; NC = Non-casing

Ganoderma leucocontextum production Cultivation using a soil casing layer

Ganoderma leucocontextum (KUMCC17-0007) was successfully cultivated with the soil casing and non-casing layer methods, obtaining higher total yields when cultivated with the casing layer method. Results of the fruiting cycles of *G. leucocontextum* grown with the soil casing layer are shown in Table 2, 3, and Figure 2. Three fruiting cycles were obtained when the casing layer method was applied. In the first cycle, initial primordia were seen on day 26, developing into young fruiting bodies on day 36, and harvested as mature fruiting bodies on day 58. The second cycle began 18 days after the first cycle was harvested, and its mature fruiting bodies were harvested after 35 days. The third cycle was initiated much later than the first two cycles: primordia were seen on day 25, and mature fruiting bodies were harvested

after 41 days. A single fruiting body per bag was produced with both soil casing and non-casing methods. Fruit body sizes in the first fruiting cycle were larger than in other cycles, and generally this mushroom showed strongly laccate fruiting bodies. Morphological characteristics of the mature fruiting bodies resulting from the casing layer method were stipitate and annual, applanate, and dimidiate pilei shape, with plump pilei (Figure 2).

Cultivation without a casing layer

The non-casing layer method resulted in two fruiting cycles (Figure 3). The highest yield was obtained from the first fruiting cycle. The initial primordia appeared at the top of the grow bags after 18 days of incubation at 25 ± 1 °C; the mushroom primordia developed into young fruiting bodies within 34 days; and the mature fruiting bodies were harvested after 60 days. The second cycle was initiated 24 days after the first harvest,



Fig. 2. *Ganoderma leucocontextum* strain KUMCC17-0007 cultivated by soil casing method. (a–c). The production of young fruiting bodies after 26–32 days of soil casing, (d–f). Moderately mature fruiting bodies after 50–60 days of soil casing, (g–i). Mature fruiting bodies after 65–72 days of soil casing. Scale bars: a–h = 2 cm, i = 4 cm

and mature fruiting bodies were harvested after 54 days. Only one out of four bags produced a fruiting body, and six bags out of the total used in the experiment produced primordia. This mushroom strain required 180 days to complete three cycles

via the casing layer method, while it took 138 days to complete two cycles with the non-casing method. Morphological characteristics of the mature fruiting bodies resulting from the non-casing layer method formed a long stipe with non-pilei when



Fig. 3. *Ganoderma leucocontextum* strain KUMCC17-0007 cultivated by non-casing method. (a–b). The production of young fruiting bodies after 36–38 days of incubation, (c–d). Moderately mature fruiting bodies after 55–60 days of incubation, (e). Mature fruiting bodies after 80–85 days of incubation. Scale bars: a–e = 2 cm

young that became pilei when mature, with some wrinkled pilei (Figure 3).

***Ganoderma resinaceum* production**
Cultivation using a soil casing layer

Laccate *Ganoderma resinaceum* (KUMCC19-0001) was cultivated successfully, with one fruiting cycle obtained using the soil casing method, and two fruiting cycles using the non-casing method (Table 2, 3). *G. resinaceum* had

a lower yield when cultivated using the casing layer method. On day 36 after the soil casing was applied, initial primordia were observed. The primordia developed to the young stage within 26 days, and mature fruiting bodies were harvested on day 30. Although 1–4 initial primordia were observed on the surface, only 1–3 mature fruiting bodies per bag developed and were collected. Morphological developments of the fruiting bodies exposed to

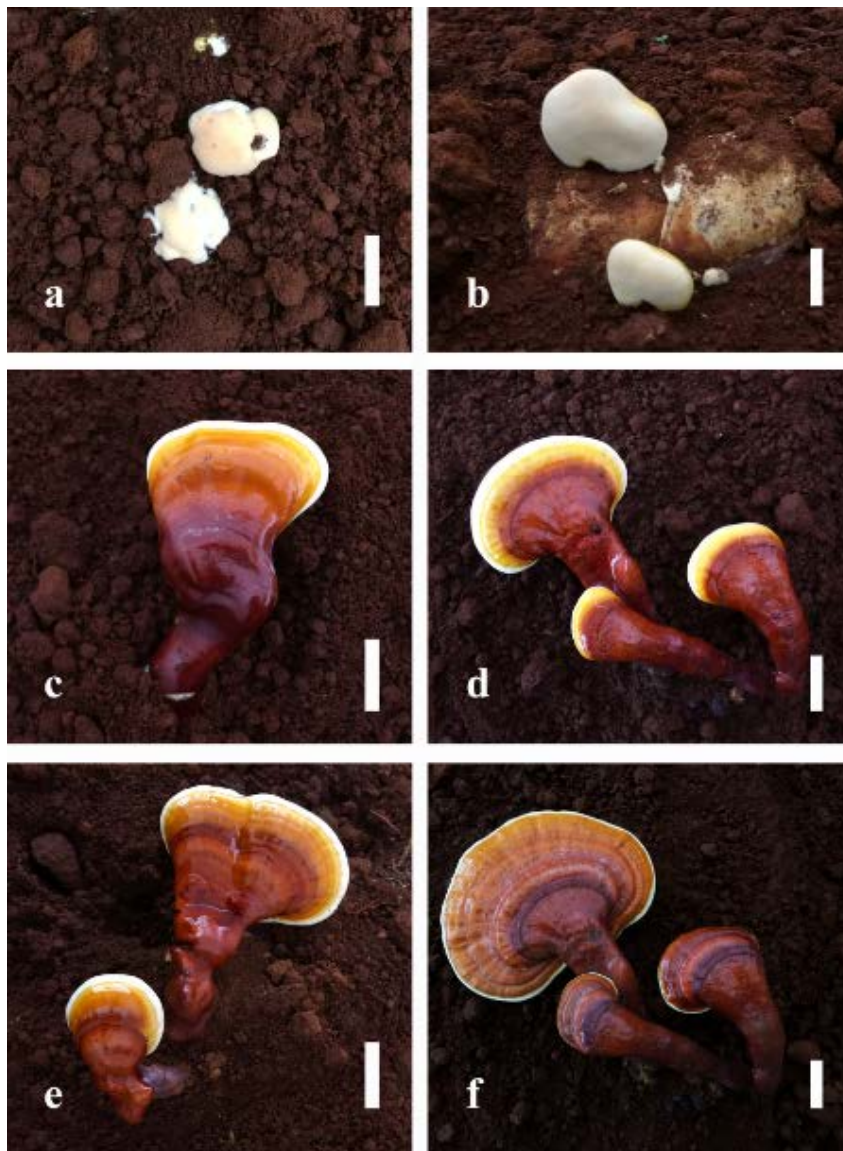


Fig. 4. *Ganoderma resinaceum* strain KUMCC19-0001 cultivated by the soil casing method. (a). Primordia formation after 38 days of soil casing, (b). The production of young fruiting bodies after 42 days of soil casing, (c–d). Young fruiting bodies are developing into mature fruiting bodies after 50–60 days, (e–f). Mature fruiting bodies were seen after 80–85 days. Scale bars: a–f = 2 cm

the soil casing layer method are shown in Figure 4. Based on the morphological characteristics of *G. resinaceum*, when the soil casing was used, *G. resinaceum* were generally composed of white and strongly laccate fruiting bodies, whereas with non-casing treatment, the fruiting bodies were dull and pale when young, weakly laccate when mature, and strongly laccate when old.

Cultivation without a casing layer

The morphological developments of its fruiting bodies produced in the non-casing

layer treatment are shown in Figure 5. Primordia formation was seen 22 days after incubation at 25 ± 1 °C. Young fruiting bodies were seen on day 33, and mature fruiting bodies were harvested on day 54. The primordia of the second cycle were observed 24 days after the first cycle was harvested, and mature fruiting bodies of the second cycle were harvested after 41 days. However, no primordia were produced after the second fruiting cycle. We obtained a single mature fruiting body per growing bag. Primordia were not observed in



Fig. 5. *Ganoderma resinaceum* strain KUMCC19-0001 cultivated without a casing layer. (a). Young fruiting bodies 32 days after induction by the non-casing layer, (b). Young fruiting bodies were observed to be developing into mature fruiting bodies after 48–50 days, (c–d). Mature fruiting bodies were seen after 60–65 days, (e–f). Old fruiting bodies were seen after 72–75 days. Scale bars: a–f = 2 cm

one of the growing bags, and one of the harvested fruiting bodies was abnormal. Morphological characteristics of the mature fruiting bodies resulting from the non-casing layer method were weakly laccate when young and strongly laccate when old.

Ganoderma gibbosum production

Cultivation using a soil casing layer

Three fruiting cycles of *G. gibbosum* with well-developed fruiting bodies were obtained with the casing layer method. Five strains of *G. gibbosum* gave similar results with the casing layer method. Results of the fruiting cycles are shown in Table 2, 3. *G. gibbosum* KUMCC17-0005 was the most successful strain of this species, giving the highest total yield after the first cycle, followed successively by the strains KUMCC19-0002, KUMCC17-0013, KUMCC18-0007, and KUMCC17-0004 (Table 3). The initial primordia of these five *G. gibbosum* strains formed on the entire soil surface 22–24 days after the soil casing was applied. The production of the first cycle was harvested after 33–35 days. 25–27 days after the

first cycle was harvested, the initial primordia of the second cycle were seen, and mature fruiting bodies were harvested after 46–48 days. The primordia of the third cycle were seen 25–28 days after the fruiting bodies of the second cycle were harvested, and mature fruiting bodies were again harvested after 40–42 days. However, in the fourth cycle, all *G. gibbosum* strains produced initial primordia with the soil casing layer method, but fruiting body development was not observed. The morphological development of the fruiting bodies of these five *G. gibbosum* strains are shown in Figures 6–7, 9–10. The two younger *G. gibbosum* strains (KUMCC17-0009 and KUMCC19-0003) were also cultivated successfully using the soil casing layer method. However, only two fruiting cycles were obtained from these two strains (Table 3). The morphological development of the two younger *G. gibbosum* fruiting bodies under the soil casing method are shown in Figures 11–12. The initial primordia of these two strains of *G. gibbosum* formed over the entire soil surface 36 days after the soil casing was applied. Production

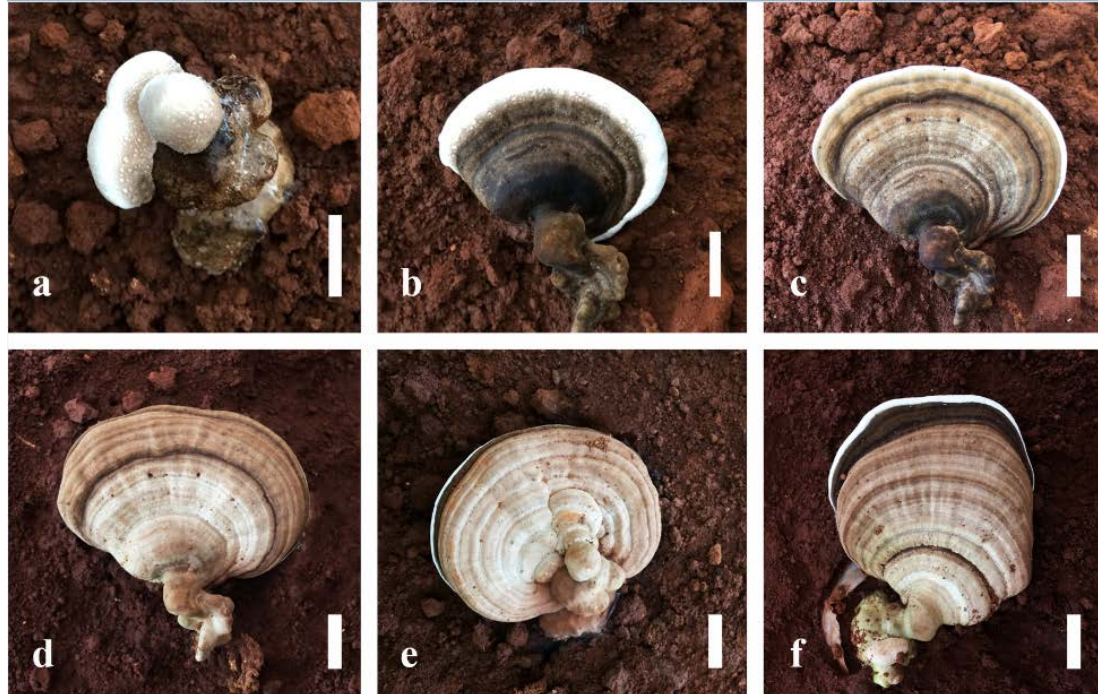


Fig. 6. *Ganoderma gibbosum* strain KUMCC17-0004 cultivated using a soil casing layer. (a). The production of young fruiting bodies after 26 days of soil casing, (b). The production of young fruiting bodies after 36 days of soil casing, (c). Young fruiting bodies developing into mature fruiting bodies after 45 days, (d–f). Mature fruiting bodies seen after 45–50 days. Scale bars: a–f = 2 cm

from the first cycle was harvested after 42 days. The initial primordia of the second cycle were seen 40 days after the fruiting bodies of the first cycle were harvested, and mature fruiting bodies were harvested after 56 days. Small primordia were seen in the third cycle, but the development of fruiting bodies was not seen.

Cultivation without a casing layer

Using the non-casing cultivation method, the following total yields were obtained for the listed strains: KUMCC19-0002 (177.26 g/Kg⁻¹), KUMCC17-0005 (172.08 g/Kg⁻¹), KUMCC17-0013 (156.37 g/Kg⁻¹), KUMCC17-0004 (149.07 g/Kg⁻¹), and KUMCC18-0007 (145.95 g/Kg⁻¹) (Table 3). The formation of primordia initiation was seen after 18–22 days of incubation at 25 ± 1 °C; fruiting bodies were harvested after 23–26 days. After the first harvest the primordia of the second cycle were observed after 20–24 days, and their mature fruiting bodies were harvested after 36–40 days. Initial primordia of the third cycle were seen 35–42 days after the second cycle was harvested, and mature fruiting bodies were harvested after 48–50 days. The morphological developments of

the *Ganoderma gibbosum* fruiting bodies under the non-casing method are shown in Figures 13, 14. The five strains of *G. gibbosum* showed non-laccate pilei when immature and a slightly dull or pale surface when developing to full maturity and into old age. They usually grew as single fruiting bodies, although a few formed chains of fruiting bodies (Figure 9). A single fruiting body produced 1–3 pinheads (Figures 6a, 9a, 12, c), and each substrate bag produced 1–3 single fruiting bodies.

DISCUSSION AND CONCLUSION

The soil casing layer method has been investigated for some mushroom species (Zhed *et al.*, 2012; Thongklang *et al.*, 2014; Subbiah and Balan, 2015; Martos *et al.*, 2017; Thongbai *et al.*, 2017), but very few studies have reported on its application for *Ganoderma* species (Chen 2012; Hapuarachchi *et al.* 2018a). This study documents on the first successful cultivation of laccate and non-laccate *Ganoderma* by using both soil casing layer and non-casing layer methods.



Fig. 7. *Ganoderma gibbosum* strain KUMCC17-0005 cultivated using a soil casing layer. (a–b). The production of young fruiting bodies after 21–24 days of soil casing, (c–d). Young fruiting bodies developing into mature fruiting bodies after 30–33 days, (e–f). Mature fruiting bodies seen after 52–54 days. Scale bars: a–f = 2 cm

Using the soil casing layer method in *Ganoderma* cultivation is the most suitable way to induce the production of fruiting bodies, since the soil casing layer helps maintain the moisture content in the growing substrate, an important

factor also noticed by Cho *et al.*, (2008). Colauto *et al.* (2010) stated that moisture content is a factor of great importance to promoting mushroom formation, and the soil casing layer traps additional water that can be accessed by *Ganoderma* spawn.

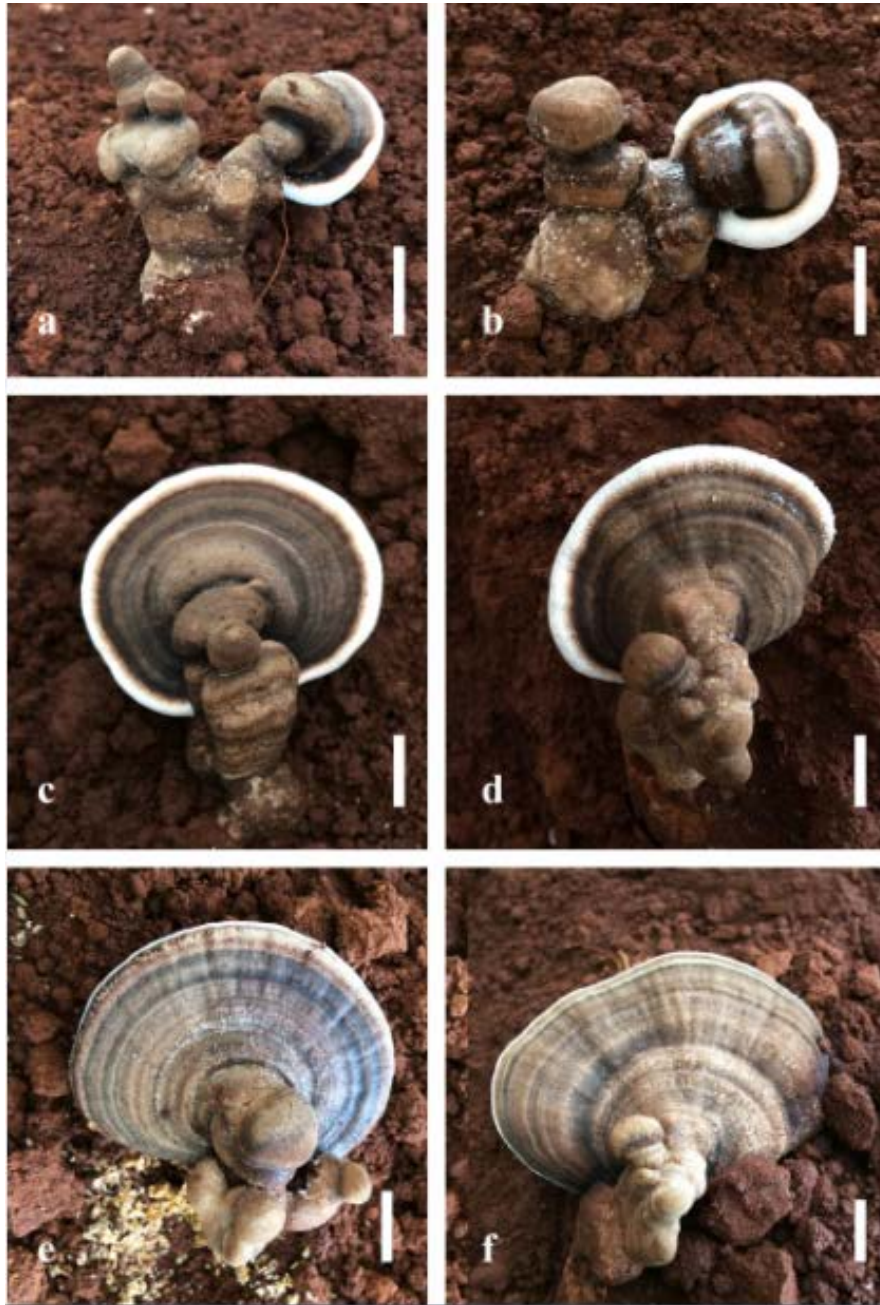


Fig. 8. *Ganoderma gibbosum* strain KUMCC17-0013 cultivated using a soil casing layer. (a–b). The production of the young fruiting bodies production after 24–28 days of soil casing, (c–d). Young fruiting bodies developing into mature fruiting bodies after 35–38 days, (e–f). Mature fruiting bodies seen after 65–68 days. Scale bars: a–f = 2 cm

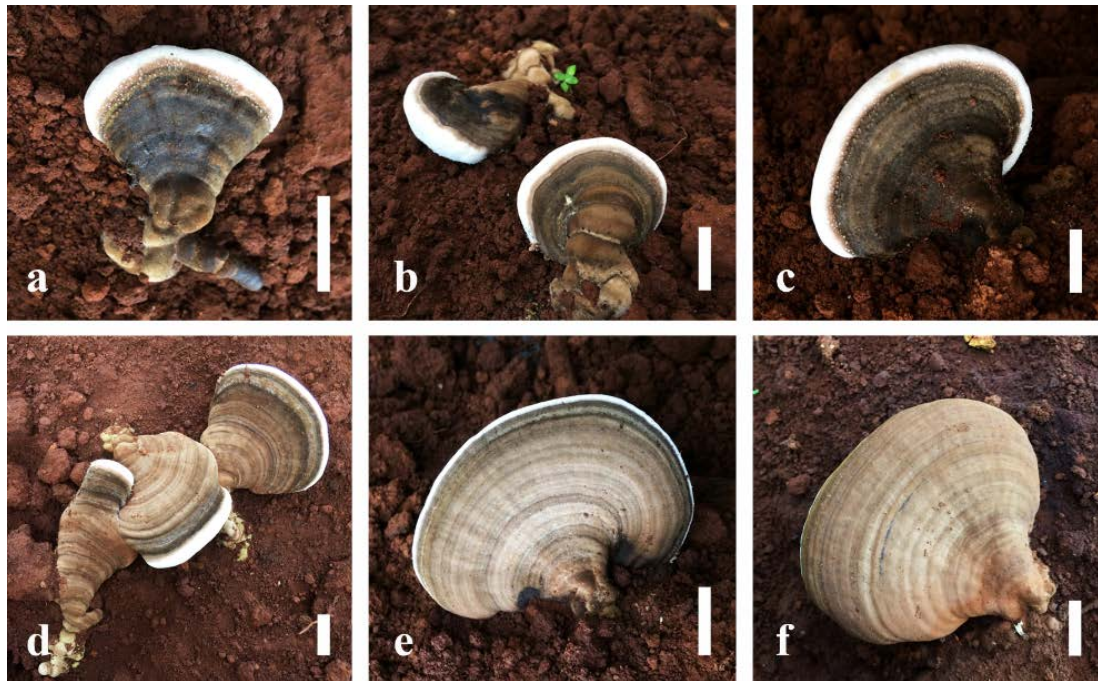


Fig. 9. *Ganoderma gibbosum* strain KUMCC18-0007 cultivated using a soil casing layer. (a). The production of young fruiting bodies after 21 days of soil casing, (b–c). Young fruiting bodies developing into mature fruiting bodies after 32–34 days, (d–e). Mature fruiting bodies seen after 45–50 days, (f). Old fruiting bodies seen after 60 days. Scale bars: a–f = 2 cm

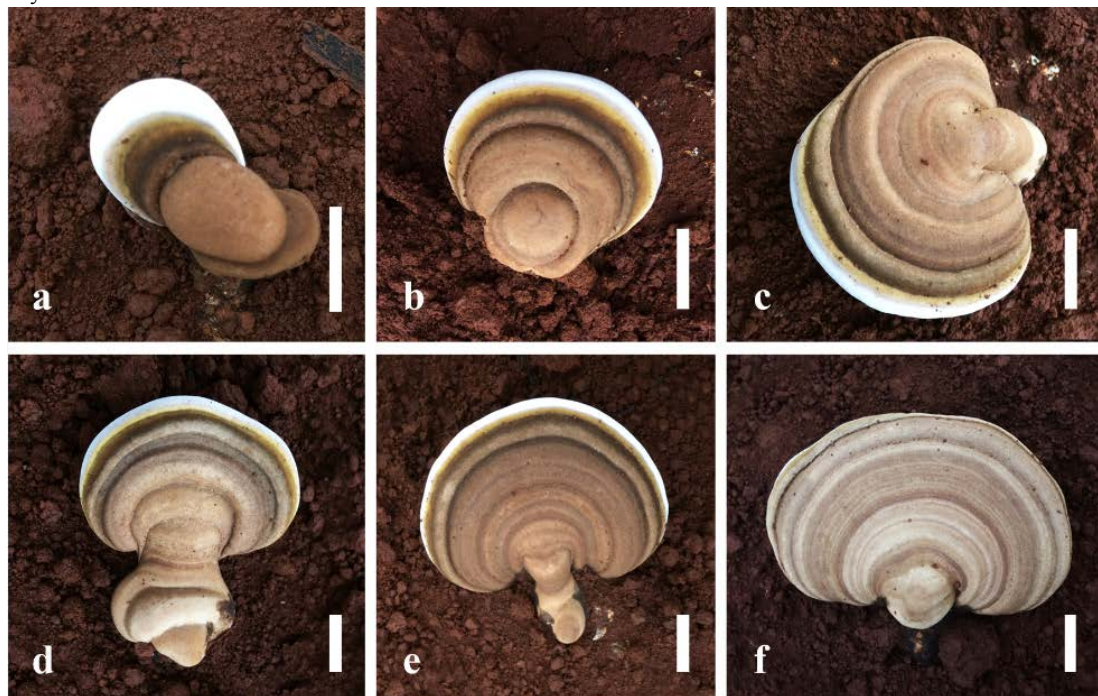


Fig. 10. *Ganoderma gibbosum* strain KUMCC19-0002 cultivated using a soil casing layer. (a). Production of young fruiting bodies production after 20 days of soil casing, (b). Young fruiting bodies developing into mature fruiting bodies after 27 days of soil casing, (c–e). Mature fruiting bodies seen after 42–45 days, (f). Old fruiting bodies seen after 55 days. Scale bars: a–f = 2 cm

However, selecting the appropriate soil, whether it be clay, loam, or sand, is dependent on the specific needs of the mushroom species (Amin *et al.*, 2010; Martos *et al.*, 2017). In this study, we selected clay soil, which is considered a suitable substrate material with a reasonable water-holding capacity that also retains good air flow to facilitate gaseous exchanges in the mushroom-growing substrate (Smerdon 1983).

Our new casing layer method had the higher number of growth cycles, highest yield within a short period of time when compared to the non-casing method for *Ganoderma* species. Cultivation using the soil casing method led to a higher number of growth cycles and yields (Table 3) by which we determined that these three *Ganoderma* species reached higher production levels than the non-casing method used for *G. lucidum* (Erkel

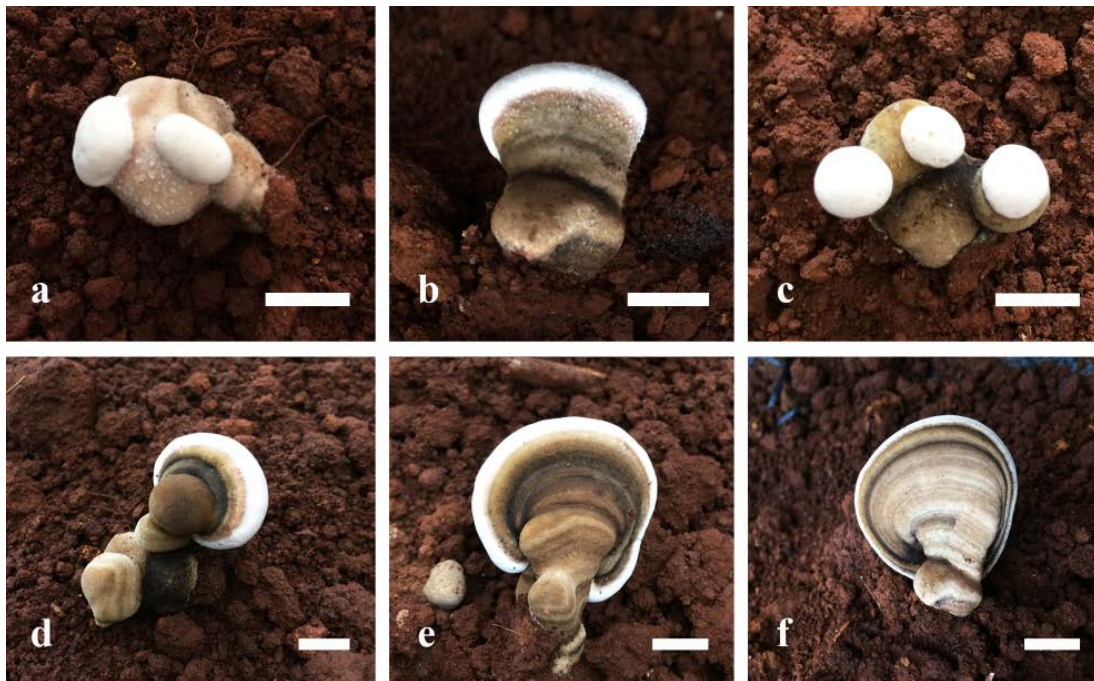


Fig. 11. *Ganoderma gibbosum* strain KUMCC17-0009 cultivated using a casing layer. (a-c). The production of young fruiting bodies after 22–25 days of non-casing, (d). Young fruiting bodies developing into mature fruiting bodies after 34 days of non-casing, (e). Mature fruiting bodies seen after 45 days, (f). Mature fruiting bodies seen after 60 days of soil casing. Scale bars: a–f = 2 cm.



Fig. 12. *Ganoderma gibbosum* strain KUMCC19-0003 cultivated using a casing layer. (a). The production of young fruiting bodies after 28 days of non-casing, (b). Young fruiting bodies developing into mature fruiting bodies after 45 days of non-casing, (c). Mature fruiting bodies seen after 70 days. Scale bars: a–c = 2 cm

2009; Roy *et al.*, 2015), and *G. neojaponicum* (Jo *et al.*, 2010). Previous studies reported that total mushroom yield was obtained from one or two cycles in a harvest period of 90 days (Peksen and Yakupoglu, 2009), while our *Ganoderma* strains required approximately 180–185 days to complete all cycles and reach their harvest period. In addition, morphological characteristics found in the strains *G. leucocontextum* and *G. resinaceum* on which the casing layer method was used were strongly laccate when compared to the non-casing method. *G. gibbosum* resulted in a darker-colored and larger-sized pilei when compared to the non-casing method. In the first of the three cycles of *G. gibbosum*, large pilei dark in color were seen, whereas in the third cycle, smaller, lighter-colored pilei were observed.

We recommend isolating the original mature fruiting bodies to achieve a high yield of production, rather than isolating the originals from young fruiting bodies. For example, under the soil casing method, three cycles of *G. gibbosum* production were achieved when the originals were isolated from mature fruiting bodies, whereas only two cycles were achieved when the originals were isolated from young *G. gibbosum* fruiting bodies. Under the non-casing layer method, all *G. gibbosum* strains produced initial primordia, whereas using the non-casing layer method they did not produce initial primordia. Thus, this study also showed that strain type affects total yield and quality, i.e., strains originating from mature fruiting bodies had better yields than strains originating from young fruiting bodies, which



Fig. 13. *Ganoderma gibbosum* strain KUMCC17-0005 cultivated using a soil casing layer. (a–b). The production of young fruiting bodies after 18–20 days of soil casing, (c–d). Young fruiting bodies developing into mature fruiting bodies after 27–29 days, (e–g). Mature fruiting bodies seen after 38–42 days, (h–i). Mature fruiting bodies seen after 50–52 days. Scale bars: a–f = 2 cm.



Fig. 14. *Ganoderma gibbosum* strain KUMCC19-0002 cultivated without a casing layer. (a). Production of young fruiting bodies production after 21 days of non-casing, (b). Young fruiting bodies developing into mature fruiting bodies after 29 days of non-casing, (c–d). Mature fruiting bodies seen after 35–42 days, (e–f). Mature fruiting bodies seen after 62–65 days. Scale bars: a–f = 2 cm.

has also been noted in other studies (Sakamoto 2018). Strains originating from young fruiting bodies (KUMCC17-0009 and KUMCC19-0003) had lower yields, longer incubation times, and required more time to produce mature pileus than the other five strains that originated from mature fruiting bodies. Thus, we agree with Sakamoto (2018) that in order to obtain a good yield, the original mushroom strain should be isolated from the mature fruiting body.

The conditions of our mushroom-growing are very similar to those of Zhou *et al.* (2012), whose study mentioned the temperature range for optimal *Ganoderma* mycelia growth to be between 15–35 °C. Jo *et al.* (2009), by contrast, stated it to be 25–27 °C (Jo *et al.*, 2009). According to Cao and Yuan (2013), a temperature range of 20–25 °C is suitable for primordia formation, while a temperature range of 24–28 °C is suitable for fruiting body development. We agree with Cao and Yuan (2013) that ideal spawn-running temperature ranges between 28–30 °C. Our results suggest that a temperature range of 18–20 °C or lower affects the production of fruiting bodies, leading to slower development and ultimately stopping

growth altogether. Chen *et al.* (2017) mentioned that temperatures higher than 30 °C negatively affect the growth and development of *Ganoderma*. In our study, we maintained the optimum relative humidity at 70–75% to promote the formation of mushroom mycelia; 80–85% during the formation of primordia initiation; and 70–80% during the formation of mature fruiting bodies. Our results resemble Peksen and Yakupoglu (2009), who have stated that the moisture content for promoting *G. lucidum* mycelia growth should be above 70%. Habijanac and Berovic (2000) showed that a moisture content less than 55% is not appropriate for mycelia growth, while Luangharn *et al.* (2017) also reported that humidity below 40% can cause slow mycelia growth in non-laccate *G. australe*.

Our study also revealed that wild laccate *Ganoderma* cultivation via the casing method has a higher yield than the non-casing method. Results from this study should prove valuable for the commercial production of *G. leucocontextum*, *G. resinaceum*, and non-laccate *G. gibbosum*. It should be noted that *Ganoderma* species cause disease and even death of trees (Hapuarachchi *et al.* 2019b). Therefore, growers need to be careful when disposing of spent *Ganoderma* compost.

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