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Cultivation of some medicinal mushrooms species from *Ganoderma* on sawdust supplemented with water hyacinth

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Cultivation of some medicinal mushrooms species from *Ganoderma* on sawdust supplemented with water hyacinth

Abstract: This study investigated the cultivation of *Ganoderma mbrekobenum* E.C. Otto, Blanchette, Held, C.W. Barnes & Obodai, *G. sessile* Murrill, and *G. oregonense* Murrill using sawdust supplemented with water hyacinth (*Eichhornia crassipes* Mart.). *Ganoderma* species have medicinal applications, including immune modulation, antioxidants, and antimicrobial properties. Substrates with water hyacinth (SWH) consisted of 900 g sawdust, 100 g water hyacinth, 100 g rice bran, and 30 g calcium carbonate, adjusted to 65 % moisture while (SNWH) was not supplemented with water hyacinth. The number of primordia was the highest in *G. mbrekobenum* (8.2), followed by *G. sessile* (4.0) and *G. oregonense* (2.8), but *G. sessile* and *G. oregonense* fruited faster (21 days) than G. *mbrekobenum* (30 days). Fruiting body yield analysis showed that *G. mbrekobenum* had the highest mass (61.10 g) and biological efficiency (15.42 %). *G. sessile* had the fastest fruiting time (47.4 days), while *G. mbrekobenum* took 86.8 days. The biological efficiency of *G. oregonense* was the lowest at 9.99 % (supplemented) and 5.86 % (unsupplemented). Water hyacinth supplementation improved mushroom yield and *G. mbrekobenum* demonstrated superior yield, whereas *G. sessile* had a faster fruiting cycle, making both species suitable for large-scale medicinal mushroom production.

Key words: *Ganoderma*, cultivation, mushrooms, supplementation, efficiency, yield, water hyacinth

Gojenje nekaterih vrst zdravilnih gob iz rodu *Ganoderma* na žagovini, dopolnjeni z vodno hijacinto

Izvleček: Ta študija je preučevala gojenje gliv *Ganoderma mbrekobenum* E.C. Otto, Blanchette, Held, C.W. Barnes & Obodai, *G. sesile* Murrill in *G. oregonense* Murrill na žagovini, dopolnjeni z vodno hijacinto (*Eichhornia crassipes* Mart.). Vrste iz rodu *Ganoderma* imajo medicinske lastnosti, kot so imunska modulacija, antioksidativno in protimikrobno delovanje. Substrati (SWH) so vsebovali 900 g žagovine, 100 g vodne hijacinte, 100 g riževih otrobov in 30 g kalcijevega karbonata s 65 % vlage, medtem, ko (SNWH) ni imel dodatka hijacinte. Vrsti *G. mbrekobenum* in *G. sessile* sta kolonizirali petrijevke v 7 dneh, vrsta *G. oregonense* pa v 8 dneh. Število primordijev je bilo največje pri vrsti *G. mbrekobenum* (8,2), sledili sta mu *G. sessile* (4,0) in *G. oregonense* (2,8). Vrsti *G. sessile* in *G. oregonense* sta razvili trosnjake v 21 dneh, vrsta *G. mbrekobenum* pa v 30 dneh. Vrsta *G. mbrekobenum* je imela največji pridelek (61,10 g) in največjo biološko učinkovitost (15,42 %), vendar je za razvoj trosnjakov potrebovala več časa (86,8 dni). Vrsta *G. sesile* je razvila trosnjake najprej (47,4 dni). Biološka učinkovitost vrste *G. oregonense* je bila najmanjša (9,99 % in 5,86 %). Dodatek vodne hijacinte je izboljšal pridelek, vrsta *G. mbrekobenum* pa se je izkazala kot najdonosnejša, medtem ko je bila vrsta *G. sessile* primernejša za hitrejšo pridelavo.

Ključne besede: *Ganoderma*, gojenje, gobe, dodatki substratu, učinkovitost, donos, vodna hijacinta

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1 INTRODUCTION

Mushrooms, particularly those from the *Ganoderma* genus, have garnered significant attention due to their medicinal and therapeutic properties (Ekiz et al., 2023). *Ganoderma* species are widely recognized for their distinctive morphological features, including a hard, woody fruiting body, a white margin, and reddish-brown, double-walled basidiospores with interwall pillars and a thin hyaline exosporium (Konara et al., 2017). Commonly referred to as "lingzhi" in China and "reishi" in Japan, these mushrooms have been utilized in traditional medicine for centuries (Jin et al., 2012). The bioactive compounds found in *Ganoderma* species contribute to a variety of pharmacological benefits, including immune modulation, antioxidant activity, and antimicrobial properties (Hapuarachchi et al., 2018). The inclusion of *Ganoderma* in dietary and pharmaceutical products has led to its widespread application in traditional and modern medicine. However, despite their medicinal significance, some *Ganoderma* species also act as pathogens, affecting important crops such as cacao, rubber, tea, and coffee, with *G. zonatum* Murrill being particularly problematic in the Asian oil palm industry (Yang et al., 2018; Castillo et al., 2022).

The medicinal properties of *Ganoderma* species are attributed to a wide range of bioactive compounds, including polysaccharides, triterpenoids, proteins, and essential vitamins and minerals. These compounds have been shown to exhibit antitumor, anti-inflammatory, immunomodulatory, and neuroprotective effects (Klupp et al., 2015; Adebesin et al., 2019). Additionally, essential minerals such as calcium, potassium, magnesium, iron, and zinc are found in *Ganoderma mbrekobenum*, along with trace elements like cobalt, nickel, and manganese (Rana et al., 2021).

Over time, environmental factors have significantly impacted the genomic structure of *Ganoderma*, leading to the emergence of new species. These newly developed species are known to contain unique and beneficial bioactive compounds (Hapuarachchi, 2018). A notable example is *Ganoderma mbrekobenum*, which has been identified for its valuable bioactive properties. This species was first identified from Nigeria in 2014 (Ofodile et al., 2022). *G. mbrekobenum* is characterized by its distinct morphological features, though detailed descriptions are limited. *Ganoderma mbrekobenum* closely resembles *Ganoderma lucidum* (Curtis) Karst in appearance. However, molecular analyses, particularly of the ITS and LSU gene regions, have distinguished *G. mbrekobenum* as a separate species (Otto et al., 2016). Ofodile et al. (2025) reported that *G. mbrekobenum* could prevent and control uterine tumour in Wistar rats. Isolates have been described as having brownish, red coloured, size-20 cm-25 cm of fruiting body, red brown stem colour and spore print. Strong bioactive substances such as ganoderic acid A, ganoderic acid C1,ganoderic acid S, ganoderiol F, ganodermanondiol have been characterised from *G. mbrekobenum* (Parihar et al., 2021). Olmesartan medoxomil, benazeprilat, isopropamide, ramipri glucuronide, desmethyldoxepine, oleamide, phorbol myristate acetate and cepharanthine were first reported from *G. mbrekobenum* by Parihar et al. (2021). Desmethyldoxepine is used in the treatment of mild depression, ganoderic acid S has immune resporative effect and anticancer while olmesartan medoxomil is used in medicine for high blood pressure, heart failuere, diabetics and kidney related diseases (Parihar et al., 2021).

G. sessile mature fruiting bodies are laccate (shiny) with a reddish-brown hue and often exhibit a wrinkled margin when dry. *G. oregonense*, *G. sessile* have been described as from all over the world, are mainly characterized by laccate pileus. *G. oregonense* produces large, perennial, woody brackets, commonly known as conks. These fruiting bodies are leathery, with a fan-like or hoof-like appearance, and grow on the trunks of living or dead trees (Ginns, 2017). For centuries, laccate *Ganoderma* species have been integral to traditional medicine in various Asian cultures, utilized to prevent and treat a multitude of ailments (Galappaththi at al., 2022). Ganoderic acid, endopolysaccharides and polysaccharides from the mycelia of *G. oregonense* (Boromenskyi et al., 2021; Boromenskyi and Bisko, 2020; Fátima et al., 2021) and polysaccharides from the mycelia *G. sessile* (Fátima et al., 2021) produced in submerged and solid state cultivation have been reported.

Cultivating *Ganoderma* mushrooms have become an essential aspect of medicinal mushroom production, as demand continues to grow for their bioactive compounds (Wachtel-Galor et al., 2011; Salichanh et al., 2025). To support large-scale production, researchers and commercial growers have explored diverse lignocellulosic substrates for optimal cultivation. Various substrates have been explored for optimal growth conditions, including sawdust, rice bran, wheat straw, sugarcane bagasse and corn husks, as they provide essential nutrients for fungal development (Rana et al., 2021; Royse et al., 2017). They provide nutrients such as cellulose, hemicellulose, and lignin and influence yield, bioactive compound synthesis, and cultivation cost-efficiency (Oke et al., 2022). Proper management of the environmental factors, pH, temperature and humidity are crucial for the best productivity and quality of mushrooms. Most mushrooms species have their best growth at pH of 5.5-7; extreme values usually restrict the development of mycelium (Atila, 2022; Nguyen et al., 2023) and Luangharn

et al. (2020) reported that temperatures 24–28 °C were ideal for fruiting body growth of *Ganoderma* species. Improvements in cultivation techniques, including tweak in the composition of the substrate and substitution of the substrate with various other agrowastes, are important to increase yield and sustainability (Rashad et al., 2019, Luangharn et al., 2020).

Supplementing sawdust with additional organic materials, such as water hyacinth (*Eichhornia crassipes*), may enhance substrate composition by increasing nitrogen content and promoting mycelial colonization (Gaitán-Hernández et al., 2011). Water hyacinth which is an invasive aquatic plant, has been successfully integrated into mushroom cultivation due to its rapid growth and rich organic matter content (Nguyen et al., 2018). According to Su et al. (2018) water hyacinth is rich in cellulose, protein, and fat. The dry matter is rich in nutrients, with crude protein of 10–20 % protein, the crude fiber content was 13.74 % and 11.04 %, minerals up to phosphorus 0.32 %, calcium 3.08 % and potassium 4.53 %. By utilizing water hyacinth as a supplement, the dual benefits of enhancing mushroom yield and managing an invasive species can be achieved.

Given the increasing interest in sustainable mushroom cultivation, exploring the use of sawdust supplemented with water hyacinth presents a viable approach for enhancing *Ganoderma* growth and productivity. This research aims at investigating the effectiveness of this substrate combination in improving the yield of the *Ganoderma* species and to compare the performance of the three species to utilize the substrate.

2 MATERIALS AND METHODS

2.1 SAMPLE COLLECTION

Fresh samples of *Ganoderma sessile* (Accession Number PQ578284), *G. oregonense* (Accession Number PQ578286), and *G. mbrekobenum* (Accession Number PQ578285) collected from the Mushroom Research and Training Laboratory, Yaba College Technology. Sawdust from obeche (*Triplochiton scleroxylon* K. Schum.) was obtained from a sawmill in the Shomolu area of Lagos, sieved, and stored for future use. Water hyacinth (*Eichhornia crassipes* Mart.) was gathered from the Lagos Lagoon in Ikorodu and Oworonshoki, Lagos, then dried using an industrial dryer at 50 °C before being shredded into particle sizes ranging from 40 to 100 mm. Additionally, *Sorghum bicolor* L. grains, rice bran, and calcium carbonate ($CaCO_3$) were sourced from a rice mill in Abeokuta and a chemical market in Ojota, Lagos, respectively.

2.1.1 Preparation mycelia culture

The mycelial cultures of *Ganoderma sessile*, *G. oregonense*, and *G. mbrekobenum* were prepared following the method described by Ofodile et al. (2022). To obtain pure cultures, the internal tissue from each fruiting body was transferred onto sterile potato dextrose agar (PDA 1.5 %) and incubated at 25 °C for 72 hours. The resulting cultures were preserved on PDA at 4 °C for subsequent studies. Agar blocks from seven-day-old cultures of each isolate were inoculated onto PDA (2 %) and incubated at 25 °C for spawn production.

2.1.2 Spawn production

A mixture of sorghum grains and calcium carbonate (33:1) with 70 % moisture content was prepared, divided into 500 g portions, and packed into jam bottles. The bottles were sterilized at 121 °C for 1 hour at 15 psi. Once cooled, each unit was inoculated with five agar blocks (1 cm in diameter) taken from a 12-day-old plate culture of each selected isolate. After a 14-day incubation period, the bottles were fully colonized and subsequently used to inoculate test substrates for cultivation studies.

2.1.3 Substrates preparation

On a dry mass basis, a substrate mixture consisting of 900 g sawdust and 100 g water hyacinth (SD+WH), along with 100 g rice bran and 30 g calcium carbonate ($CaCO_3$), was prepared, with the moisture content adjusted to 65 %. The substrate (1130 g dry mass) was packed into polyethylene bags (30 × 43.2 cm in diameter) and pasteurized in a locally fabricated pasteurizer at 65 OC-70 °C for six hours, then left to cool overnight. The bags were partially opened under aseptic conditions and the spawns were evenly distributed on top of the substrates, the necks twisted and the sterile PVC pipes were inserted, covered with clean tissue papers and secured with rubber bands. Each bag was inoculated with 50 g of grain spawn per substrate bag. The inoculated substrates were incubated at room temperature, ranging from 20-25 °C at night and 25-30 °C during the daytime. Upon completion of the incubation period, the fully colonized substrate bags were opened and transferred to fruiting rooms maintained at 30±2 °C with 90-95 % relative humidity, where they were monitored for fruiting. The fruiting bodies were harvested at maturity when the colour of the cap was uniform without the whitish tips. Data collected during the fruiting experiment were the spawn run period, the number of days before primordia formation, and the number of primordia per bag. Also recorded were the number of days from commencement of

fruiting body induction to first flush, mass and number of basidiocarp per flush. The biological efficiency (BE), which was calculated as the mass of fresh mushroom harvested/dry mass of substrate × 100 %.The experiment followed a randomized design and was conducted over two growing cycles, with five replications per cycle.

2.2 STATISTICS ANALYSIS

The mushroom cultivation experiment followed a randomized complete block design. Data from all experiments are presented as the mean ± SD, and analysis of variance (ANOVA) with Tukey's post hoc test was conducted at a 5 % significance level.

3 RESULTS AND DISCUSSION

3.1 PRIMORDIAL DEVELOPMENT

The results of primordial emergence for the three *Ganoderma* species are presented in Figure 2. Primordia formation and fruiting body yield were assessed in *G. mbrekobenum*, *G. sessile*, and *G. oregonense* cultivated on a sawdust alone and sawdust supplemented with water hyacinth substrates. For substrate with water hyacinth, the number of primordia produced by *G. mbrekobenum* (8.2) was significantly higher than that of *G. sessile* (4.0) and *G. oregonense* (2.8) ($p < 0.05$). However, the primordia of *G. sessile* (21 days) and *G. oregonense* (21 days) emerged significantly earlier ($p < 0.05$) compared to *G. mbrekobenum* (30 days). The number of primordia produced by *G. mbrekobenum* was also significantly higher than that of the other species for the substrate without water hyacinth but the primordia of *G. mbrekobenum* (21.2 days). and *G. sessile* (30 days) emerged significantly ($p < 0.05$) faster than that of *G. sessile* (68.4 days)

Similar patterns have been reported in other *Ganoderma* cultivation studies. Under artificial cultural conditions, *G. lucidum* forms primordia after inoculation for 43–48 days (Ngo et al., 2019). *Ganoderma sinense* J.D. Zhao, L.W. Hsu & X.Q. Zhang, demonstrated that primordia formation occurred between 38 to 41 days after inoculation, with variations attributed to different substrate compositions. The study found that substrates enriched with wheat bran accelerated primordia initiation and enhanced biological efficiency (Nguyen et al., 2023). This suggests that substrate composition plays a crucial role in the timing of primordia emergence.

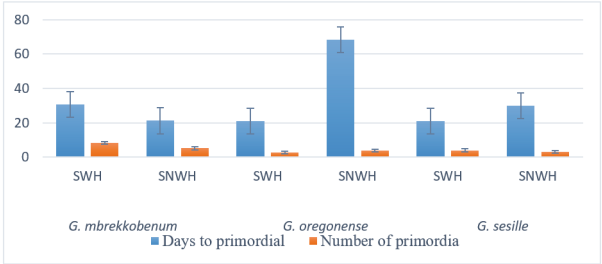

Figure 2: Time to primordia development and number of primordia during cultivation of *Ganoderma* species Key: SWH = substrate with water hyacinth; SNWH = substrate without water hyacinth, Days to primordial = days taken before primordia was formed, show significant difference at 5 % ($p < 0.005$) between species.

3.2 FRUITING BODY MASS AND BIOLOGICAL EFFICIENCY

The primordia were monitored until full fruit body development which is the matured basidiocarp, with the resulting fruiting bodies displayed in Figure 3. Fruiting body yield and biological efficiency are documented in Table 2.

The mass of fruiting bodies (g) produced by *G. mbrekobenum* (61.15 ± 1.01 g) was significantly higher ($p = 0.05$) than that of *G. sessile* and *G. oregonense* on the substrate with water hyacinth (SWH). Additionally, *G. sessile* produced significantly heavier fruiting bodies than *G. oregonense* ($p < 0.05$). Similar results were produced on substrates without water hyacinth (SNWH), although the mass of fruiting bodies was significantly higher on SWH than without water hyacinth SNWH, for *G. mbrekobenum* and *G. oregonense*. The biological efficiency (BE) of *G. mbrekobenum*, *G. sessile*, and *G. oregonense* in utilizing the substrate SWH were 15.42 %, 9.99 %, and 10.44 %, respectively while in SNWH, BE were 12.62 %, 10.20 % and 5.86 %. During cultivation, *G. sessile* developed four fruiting bodies, while *G. mbrekobenum* and *G. oregonense* each produced approximately two fruiting bodies on the substrate supplemented with water hyacinth. On the other hand, these species had longer time to fruitbodies production than on substrate supplemented with water hyacinth but were only significant for *G. oregonense* and *G. sessile*.

In this study, *G. sessile* produced fruiting bodies approximately 47.4 days post-inoculation, whereas *G. mbrekobenum* required about 86.8 days, indicating a significantly slower development. *G. oregonense* also exhibited a slower fruiting process compared to the other two species. Similar disparities in fruiting times have been documented in other *Ganoderma* species. *Ganoderma resinaceum* Boud. was reported to produce fruiting bod-

Table 3: Fruiting body production of Ganoderma species on sawdust with supplements

Ganoderma species	Substrates	Average weight of fruiting body (g)	Number of fruiting body	Days to fruiting bodies	Biological Efficiency (%)
G. mbrekobenum	SWH	61.10C 1.08	1.80D ± 0.84	86.80Z ± 1.10	15.42
	SNWH	50.30BC ± 0.90	1.20D ± 0.45	81.20YZ ± 4.15	12.62
G. oregonense	SWH	39.58B ± 12.40	1.20D ± 0.45	50.80G ± 2.95	9.99
	SNWH	23.20A ± 2.05	4.20E ± 0.45	76.60XY ± 6.43	5.86
G. sessile	SWH	41.34B ± 12.28	4.00E ± 0.71	47.40G ± 6.47	10.44
	SNWH	40.40B ± 0.55	2.20D ± 0.84	68.60X ± 0.55	10.20

Key: SWH = substrate with water hyacinth; SNWH = substrate without water hyacinth, show significant difference at 5 % ($p < 0.005$) between species.

ies approximately two months of post-inoculation, while another unidentified *Ganoderma* species required three months under similar conditions (El-Fallal et al., 2015). These differences highlight the influence of species-specific genetic factors and environmental conditions on the developmental timelines of *Ganoderma* fruiting bodies (Makarenkova et al., 2021; De Oliveira Campos et al., 2024). Additionally, research on MYB transcription factors in *Ganoderma* species indicates that these genes play crucial roles in various developmental and physiological activities, suggesting that genetic differences among species can impact their development (Wang et al., 2020).

A study investigated the effects of various supplements, including rice bran and oilseed cakes, on the cultivation of *Calocybe indica* Purkay. & A. Chandra. The findings revealed that supplementing rice straw with these additives increased both the yield and quality of the mushrooms (Alam et al., 2010).

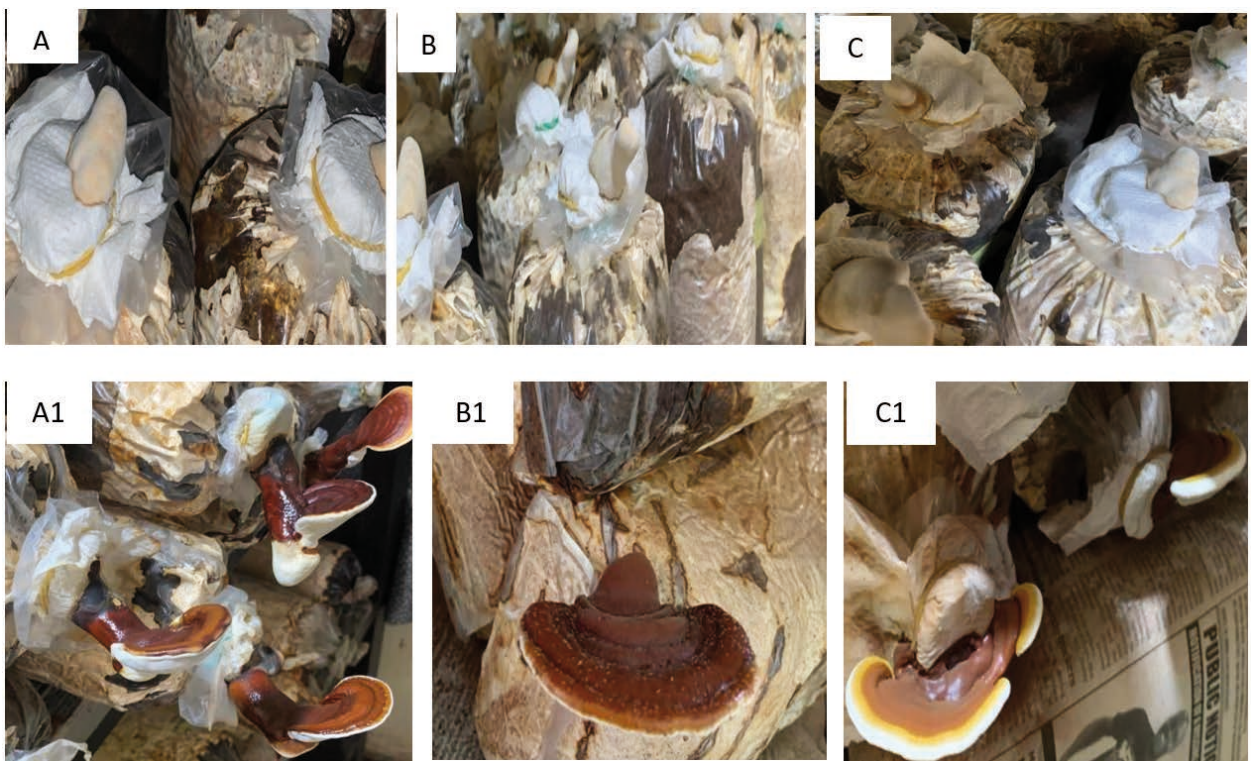

Figure 3: Top Left -Right A, B, C Pin heads of *G. oregonense*, *G. mbrekobenum* and *G. sessile*; pin head formation on substrate supplemented with water hyacinth. Lower Left –Right A1, B1, C1 *G. oregonense*, *G. mbrekobenum* and *G. sessile* carpophore formation on the substrate.

Gurung et al. (2012) reported the effects of using supplements of rice bran, wheat bran, corn flour and gram flour on the yield of *G. lucidum*. The results showed that supplementation played a positive role in the mycelia growth and yield of *G. lucidum*, demonstrating the significance of substrate composition in mushroom cultivation.

These findings are in line with previous research indicating that BE and fruiting body mass can vary significantly among *Ganoderma* species and are influenced by substrate composition and environmental factors. A previous study reported that the yield of *G. lucidum* was increased when grown on a cultivation substrate supplemented with wheat bran (Mehta et al., 2014). Also, the composition of the substrate has a significant effect on mycelium growth and biological efficiency of Reishi mushroom (Lisiecka et al., 2015).

During cultivation, *G. sessile* developed an average of four fruiting bodies per substrate bag, whereas *G. mbrekobenum* and *G. oregonense* each produced approximately two. This variation in fruiting body count may be attributed to inherent species differences and their interaction with the cultivation substrate. Optimizing factors such as substrate composition, moisture content, and environmental conditions can influence the number of fruiting bodies produced, as demonstrated in various studies on *Ganoderma* cultivation (Nguyen et al., 2023). Evaluating these three species of *Ganoderma*, *G. sessile* is the most economically viable species due to its fast growth rate marked by the shortest fruiting time (~47.4 days), decent yield and produced more fruiting bodies (~4 per bag). Also the potential for multiple cultivation cycles per year. *G. mbrekobenum* offers the highest single-batch yield and BE but has limited annual output due to its long fruiting time, increasing operational costs per unit mass. *G. oregonense* was the least efficient species economically, due to low yield and poor biological efficiency. Soriano et al. (2022) reported that supplementation was important in *G. lucidum* cultivation since it increased the biological efficiency and its subsequent economic value than that of un-supplemented substrate. It has been previously shown that *G. sessile* is equipped with a superior machinery of degrading enzymes allowing colonization of lignocellulosic biomass in solid state (Loyd et al. 2019)

4 CONCLUSIONS

This study demonstrates that substrate composition and species specificity significantly influence *Ganoderma* cultivation. Water hyacinth supplementation enhanced yield, offering sustainable use for this invasive plant. *G. mbrekobenum* exhibited superior biological efficiency, while *G. sessile* fruited fastest. These findings provide insights into optimizing medicinal mushroom production and environmental management.

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Declaration

Authors declare no Conflict of Interest

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