# Cultivation of *Ganoderma lucidum* (W. Curtis: Fr.) P. Karst on sawdust of *Brachystagia nigerica* Hoyle & A.P.D.Jones.

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***Abstract***

*This study examines the growth and cultivation of Ganoderma lucidum (Curt.) P. Karst. to fructification stage. G.lucidum is a white rot polypore mushrooms popular in the oriental traditional cultures for its pharmacological and health modulating values. Isolates of Ganoderma sp were collected from ten different locations in the tropical rainforest zone of Southern Nigeria and cultured in Potato Dextrose Agar (PDA). Guinea corn (Sorgum bicolor) grains were used as the spawn medium while the substrate was non-supplemented sawdust of Brachystagia nigerica Hoyle & A.P.D Jones. Petri dishes were colonized by Ganoderma sp in 8 days, reaching a maximum mycelium diameter of 8.40 ± 0.00cm (n= 5). Guinea corn spawn attained full mycelium colonization in 5 days. Substrate bags were colonized after 62 days of inoculation with the spawn while primordial initiation of Ganoderma sp. appeared 5 days after the bags were fully colonized. The cropping cycle was approximately 110 days with a biological yield and biological efficiency of 19.93g/bag and 9.97% respectively. Isolates (1, 4, 5) from the rural communities of Iguikhinwin (Edo State) and Oghara (Delta State) produced heavier mycelium biomass in both culture and substrates. The successful cultivation of indigenous Ganoderma and their potential for domestic as well as commercial production in Nigeria is reported.*

***Keywords: Ganoderma lucidum, Sawdust, Pin head, Mycelium, Substrate.***

## Cultivation de Ganoderma lucidum (W. Curtis: Fr.) P. Karst sur la sciure de bois de *Brachystagia nigerica* Hoyle & A.P.D.Jones.

***Résumé***

*Cette étude examine la croissance et la culture de Ganoderma lucidum (Curt.) P. Karst. à l*

*étape de la fructification. G.lucidum est un champignon blanc polypore en poudre populaire dans les cultures traditionnelles orientales pour ses valeurs pharmacologique et de modulation sanitaire. Les isolats de Ganoderma sp ont été recueillis dans dix endroits différents dans la zone de forêt tropicale du sud du Nigeria et cultivés dans l'Agar de Dextrose de Pomme de Terre (PDA). Les grains de maïs (Sorgum bicolor) ont été utilisés comme milieu de germination alors que le substrat était la sciure de bois non complétée de Brachystagia nigerica Hoyle et A.P.D Jones. boîtes de Petri ont été colonisées par Ganoderma sp en 8 jours, atteignant un diamètre maximal de mycélium de 8,40 ± 0,00 cm (n = 5). La génétique du maïs a atteint la colonisation complète du mycélium en 5 jours. Les sacs de substrat ont été colonisés après 62 jours d'inoculation avec le génome alors que l'initiation primordiale de Ganoderma sp. est apparu 5 jours après la colonisation totale des sacs. Le cycle de culture était environ 110 jours avec un rendement biologique et un rendement éfficace de 19,93g/sac et de 9,97% respectivement. Les isolats (1, 4, 5) issu des communautés rurales d'Iguikhinwin (L'état d'Edo) et Oghara (L'état de Delta) ont produit une biomasse de mycélium plus lourde à la fois dans la culture et les substrats. La cultivation réussie de Ganoderma indigène et leur potentiel pour la production domestique et commerciale au Nigeria sont rapportés.*

***Mots-clés: Ganoderma, la sciure de bois, épingle, Mycelium, Substrat, Champignon***

## Introduction

Ganoderma lucidum (Curt.) P.Karst. is a white rot polypore mushroom belonging to the Family Ganodermataceae. It is popular in the oriental traditional cultures for its pharmacological and health modulating values (Ling et al., 2007). The hard texture of Ganoderma sp limited their use as culinary and masticatory mushrooms contrary to the use of other medicinal and edible mushrooms. The mushroom forming fungus is variously represented in mycological literature as "Ling-Zhi" or herb of spiritual potency in Chinese language, "Reishi" in Japanese and "Leman Kwado" in Hausa and rife in the tropical rainforests of southern Nigeria (Okhuoya et al., 2010; Shamaki *et al.,* 2012). Although, Ganoderma species have in recent time been catalogued in Europe and many other parts of the world including Africa, its cultivation is still less widespread especially in West Africa (Ueitele et al., 2014). This fungus has been reported by numerous studies to possess rich bioactive compounds and referred to in some literature as biological response modifiers (BRM). Ganoderma possess properties relating to antimicrobial (antifungal, antiviral, antibacterial), anti-tumour, antioxidant, hepatoprotective, anti-inflammatory, anti-platelet aggregation, hypotensive, longevity and other immune-modulating effects (Wasser and Weis, 1999; Leung, 2002; Kim and Kim, 2002; Tan and Vanitha, 2004; Paterson, 2006; Maiti et al., 2008). Additionally, Mshigeni et al. (2009) reported their activity in improving the quality of life of those affected with HIV/AIDS in Africa. Deepalakshmi and Mirunalini (2011) in a separate study reported their use in the treatment of headaches (migraine), hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, haemorrhoids, hyper-cholesterolaemia and nephritis. This is in addition to their recent use as probiotics in animal farming and bioprotection of tomato fruits (Ogbe *et al.,* 2009; Osemwegie *et al.,* 2010). Cultural, ecological, ethnomycological, pharmacological and economic uses of this mushroom especially in the manufacture of different products have remarkably pushed up its global demand and the international market value to over US$3

billion per annum (Mizuno *et al.,* 1995; Wachtel-Galor *et al.,* 2004; Kapoor and Sharma, 2014). This trend which though is poorly documented in Africa has consequently reinforced interests and momentum in the cultivation of Ganoderma lucidum in recent decades as evident in their production spread in Europe and America.

Domestic and commercial cultivation of G. lucidum has developed rapidly, with China controlling over 60% of its global market (Lai *et al.,* 2004). Studies on the cultivation and post-harvest processing techniques (Okhuoya et al., 2010), pests and disease management (Fernandes *et al.,* 2012) and yield assessment of various agro-based substrates for the commercial production of this mushroom are also replete in literatures (Hsu, 1994; Chang and Buswell, 1999; Stamets, 2000). In Africa, insignificant percentage of mushrooms of culinary and medicinal values has been successfully cultivated at both low and medium scale level to meet an overwhelming local consumption need. This according to Okhuoya *et al.,* (2010) is one of the direct consequences of a generational culture of hunting culinary mushrooms from the wild, most of whose identities are based on traditional knowledge heritage. Many nutritional and ethnomycologically valuable mushrooms that have featured in different African cuisines and diets are fleshy, cartilaginous or jelly-like in texture and form part of their socio-cultural heritage. Although, different species of the genus abounds in Africa especially Nigeria, they nevertheless remained unattractive as food despite featuring in many traditional herbals (Munyanziza and Oldeman, 1996). This study therefore investigates the effect of mushroom cultivation on the yield potential of Nigerian *Ganoderma lucidum* from different locations. It further seeks to identify the best strain that could enhance its utilization in this part of the world.

## Materials and Methods

Unblemished mushrooms *(G. lucidum)* were randomly harvested from non-privately owned rainforests around the University of Benin, Ugbowo Campus; Iguikhinwin and Oghara (Delta State) in Nigeria (Fig. 1). These collections were brought to the Mushroom Biology Laboratory of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State, Nigeria where they were sorted, identified in accordance with the standard descriptions of Lincoff (1981), Largent (1986), Wang *et al.,* (2012) and washed with distilled water using a brush to remove adhering soil particles. The fruiting bodies were then labeled as isolates (1-10) for the purpose of this study (Table 1).

### *Pure and tissue culture preparation*

Twenty gram (20g) of powdered Potato Dextrose Agar (PDA) was mixed with 1litre of distilled water in a pyrex conical flask, thoroughly shaken to ensure complete dissolution and corked with non-absorbent cotton wool in aluminium foil. The preparation was then steam sterilized for 45 min at 120$^{o}$ C under 58 p.s. pressure and later allowed to cool to 19 p.s.i.at 40$^{o}$C in an autoclave. The sterilized medium was thereafter poured into sterile petri dishes in a Monmouth Scientific laminar flow hood disinfected with 80% isopropanol (isopropyl alcohol). The agar medium was allowed to cool and solidify before used (Bilay *et al.,* 2000).

Unblemished *G. Lucidum* fruit bodies each representing an isolate from the different sample locations in their early primordial stage was selected, surface sterilized with 70% alcohol and excised aseptically with a sterile scalpel near the white growing zone of the fruit body. Pieces of these were each centrally placed on medium (PDA) in a Monmouth Scientific laminar flow hood

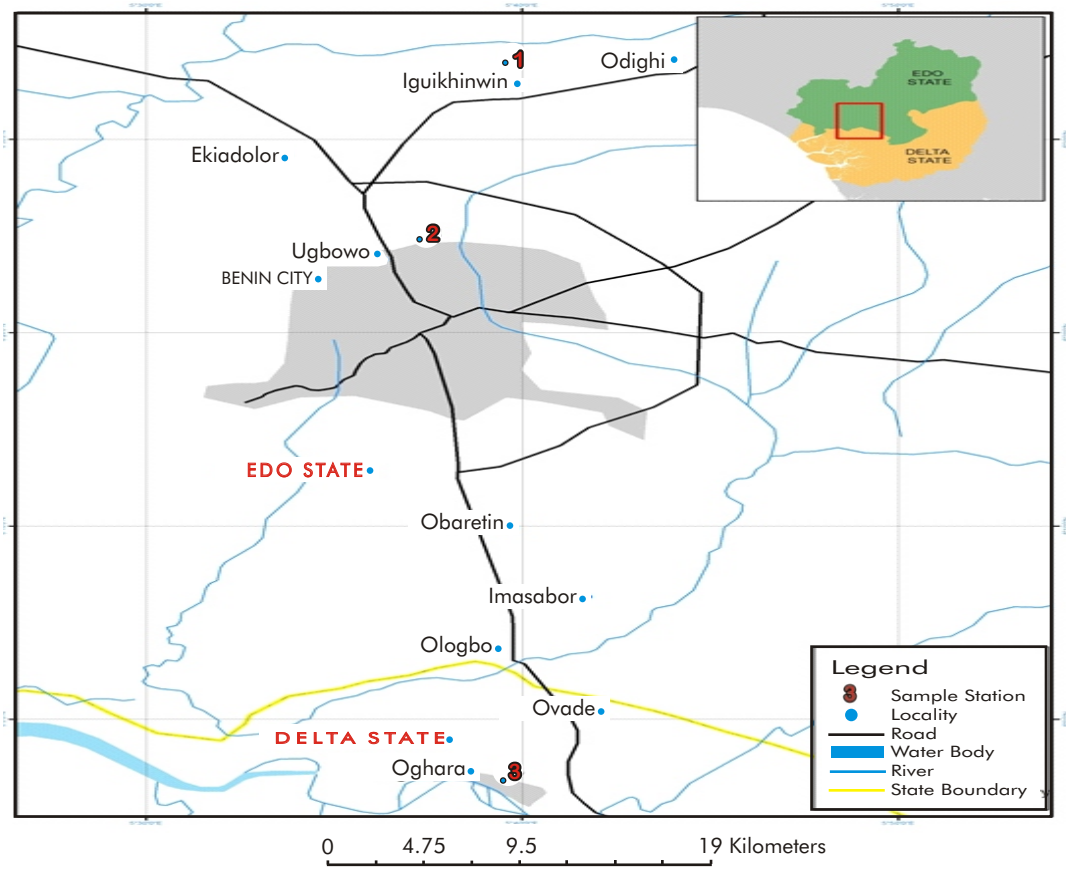


Fig 1: Study map of sample location from Edo and Delta States

using a sterilized forceps and then incubated at 25°C according to Anakalo *et al.* (2008) and Curvetto *et al.* (2002). Each of the extracted pieces per isolate was inoculated in triplicates in a completely dark room.

The pure culture on appearance was multiplied by aseptically cutting mycelium-based blocks using a 5 mm cork borer and transferred to freshly prepared potato dextrose agar plates. These were later sealed with transparent tapes to prevent contamination and incubated at 25°C as stock culture (Stamets, 2000).

***Spawn preparation***

One hundred grams (100g) of guinea corn *(Sorgum bicolor)* grains was weighed using a top-loading balance, washed under running tap water to remove traces of insecticides, debris as well as dead and floating grains and soaked overnight. The soaked grains were rewashed the next day, pasteurized at 100°C for 20 min with an autoclave, allowed to cool and drain on an inclined surface for 6hrs. Calcium sulphate and calcium carbonate salts were each mixed with the drained grains constituting 2% (w/w) per dry weight according to Anakalo *et al.* (2008) and Smith

Table 1: Location of Ganoderma lucidum isolates used for cultivation

| *Ganoderma* sp. Isolates | Community | State |
|---|---|---|
| 1, 2, 10 and 4 | Iguikhinwin community via Nifor, Edo State | Edo State |
| 5,6 and 8 | Oghara, Delta State | Delta State |
| 7, 9 and 3 | University of Benin, Ugbowo campus, Edo State | Edo State |

*et al.* (2002). The grains were then transferred into spawn jars, filled up to 2/3 of jars' capacity with each filled jar plugged with cotton wool stopper before being loaded into an autoclave at 121°C for 1 hour. The grain-filled jars were then allowed to cool, shaken to loosen for even distribution of wet grains. Proliferated mycelia from previously prepared tissue culture plates of each *G. lucidum* isolates was then injected into the grain-filled jars aseptically with a 5 mm radius cork borer in an enclosed hood with minimal illumination, shaken for even distributions and incubated at 25°C in a growth room (Anakalo *et al.,* 2008; Curvetto *et al.,* 2002).

***Substrate preparation***

Unmixed sawdust of *Brachystagia nigerica* Hoyle & A.P.D.Jones (common name; naga) was collected directly in sterile bags from a saw mills within Benin City, Edo State, Nigeria. This was transported to the Mushroom Biology Laboratory of the Department of Plant Biology and Biotechnology where it was sun-dried on a washed flat surface for 5 hrs each day for 5 days. One kilogram (1.0 kg) of the dried sawdust was later boiled in 1.5 litres of water for 15 min, left to cool for additional 15 min before draining off excess water. This preparation was then aseptically and thoroughly mixed with 12 g $CaSO_4.2H_2O$ (gypsum), 3 g $CaCO_3$ and 1 g of sucrose. The preparation was later set on wire sieves to drain and stabilize for 2 days at 28°C. On the third day, the substrate was bagged in polyethylene bags (Fig. 2c) improvised with water absorbing cotton plug, a plastic neck and rubber band (Anakalo *et al.*, 2008). Each of the substrate-filled polyethylene bags was centrally holed and sterilized in locally made two-layered custom built drum pasteurizer for 5 hrs for vaporization. On cooling the substrates to 25°C, each bag was inoculated with the mushroom spawn (five replicates) from each sample location, using 1/3 of the spawn per bag and placed on shelves in a dark windowless grown room (Adebayo *et al.*, 2009). The humidity of the growth room was maintained at about 90% during primordial induction by moistening the grown room thrice daily, 70 - 80% during cap formation by wetting the room twice daily and 30 - 40% during the final stage of fruit body development by spraying only once daily. The relative humidity was monitored by means of a battery hydrometer and temperature slightly stabilized below 28°C by wetting the floor twice daily. The bags were partially punctured and ventilation restored to the growing chamber when the substrate bags were fully colonized (Fig. 2d).

**Statistical analysis**

The experimental design was a Complete Randomized Block and data were presented as mean ± standard error means (SEM).

Statistical analyses were performed using SPSS 16.0 software and Duncan Multiple Range (DMR) for the one-way analysis of variance (ANOVA) for means separation. Significant differences between means were indicated by the p-value ($P<0.05$).

**Results and Discussion**

*G. lucidum* mycelium grew, reaching a radius of 8.40 ± 0.00 cm on the 8th day. The radial growth pattern increased consistently in rate when incubated between temperatures of 25°C - 28°C (Table 2). The ten isolates were significantly different ($p < 0.05$) in growth rate per day with observed marginal growth rate pattern from the 2nd to 5th day of incubation which may be the physiological lag phase of development (Fig 2a). Comparatively, the results as well as the growth temperature used in the current study are in agreement with Jo *et al.* (2009). All the *G. lucidum* isolates showed healthy mycelia growth especially between the 6th to 7th day of inoculation and short growth period (8 days) which according to You *et al.* (2013) are traits that validates their use in the exploitation of ganoderic acids and other biological principles. Consequently, further studies may be required to determine as well as understand the extent of their exploitable socio-economic values, the richness of active biological and medically useful health modifiers of Nigerian Ganoderma sp. It was observed that PDA culture optimizes the vegetative growth of isolates from the rural communities of Iguikhinwin of Edo State and Oghara in Delata State compared to isolates from the more urban Ugbowo communities of Edo State.. While it could be assumed that these strains exhibited better vegetative agility which transcends substrates, the underlying reason for this remains unclear. Osemwegie *et al.,* (2016) linked optimally healthy mycelium development to environmental nourishment and biodiversity effects. This study has therefore showed that rural isolates are

Table 2: Daily radial growth measurement (cm) of isolates (1-10) in culture

| Isolates | Mycelia Growth | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| 1 | 0.65 ± 0.02c | 1.47±0.07b | 3.07 ± 0.04b | 4.6 ±0.04c | 6.48 ± 0.06d | 7.21 ± 0.06b | 8.40 ± 0.00b | 8.40 ± 0.00b |
| 2 | 0.62 ± 0.01ab | 1.15 ± 0.07a | 2.09 ± 0.03a | 3.55 ± 0.02ab | 4.98 ± 0.01ab | 6.05 ± 0.03a | 7.07 ± 0.19a | 7.07 ± 0.19a |
| 3 | 0.60 ± 0.00a | 1.05 ± 0.02a | 2.86 ± 0.05b | 4.06 ± 0.22bc | 5.40 ± 0.29bc | 6.24 ± 0.27a | 7.34 ± 0.45a | 7.34 ± 0.45a |
| 4 | 0.60 ± 0.00a | 1.14 ± 0.02a | 2.98 ± 0.07b | 4.32 ± 0.11c | 6.00 ± 0.27cd | 7.08 ± 0.05b | 8.32 ± 0.08b | 8.32 ± 0.08b |
| 5 | 0.60 ± 0.00a | 1.07 ± 0.05a | 3.03 ± 0.03b | 4.50 ± 0.00c | 6.50 ± 0.00d | 7.11 ± 0.03b | 8.32 ± 0.08b | 8.32 ± 0.08b |
| 6 | 0.64 ± 0.01bc | 1.48 ± 0.15b | 2.93 ± 0.06b | 4.40 ± 0.10c | 6.20 ± 0.27d | 7.07 ± 0.03b | 8.31 ± 0.08b | 8.31 ± 0.08b |
| 7 | 0.60 ± 0.00a | 1.09 ± 0.08a | 2.33 ± 0.38a | 3.47 ± 0.48a | 4.68 ± 0.53ab | 5.62 ± 0.60a | 6.95 ± 0.75a | 6.95 ± 0.75a |
| 8 | 0.60 ± 0.00a | 1.09 ± 0.04a | 2.11 ± 0.04a | 3.66± 0.07ab | 4.72± 0.12ab | 5.55± 0.16a | 6.69± 0.12a | 6.69± 0.12a |
| 9 | 0.60±0.00a | 1.01± 0.02a | 2.31 ± 0.12a | 3.71 ± 0.10ab | 4.84 ± 0.12ab | 5.77 ± 0.24a | 7.04 ± 0.21a | 7.04 ± 0.21a |
| 10 | 10.60 ± 0.00a | 1.08 ± 0.04a | 2.08 ± 0.03a | 3.57 ± 0.04ab | 4.62 ± 0.18a | 5.60 ± 0.21a | 6.43 ± 0.26a | 6.43 ± 0.26a |

Mean values ± standard error of mean (SEM) along the same column with different superscript alphabets are significantly different ($P < 0.05$) from each other, n=5

innately more vegetative and virile than their urban counterparts with the possibility for commercial production.

The spawns prepared with the different isolates all attained full mycelium run on the 5th day after inoculation (Fig. 2b). The stages involved in the spawning and cultivation of the *G. lucidum* samples are as shown in Figs. 2. It was observed that the sawdust substrate derived from Brachystegia nigerica supported mycelium run 19 days after inoculation and complete colonization of the substrate bags in 62 days (Fig. 2d) of all isolates studied. The total cropping cycle from vegetative growth to fruiting body maturation was 110 days. While this observation compares favourably with the cultivation cycle (approximately 42 to 105 days) of other edible and medicinal mushrooms *(Auricularia auricula, Lentinus edodes, Pleurotus tuberregium, Volvariella volvacea)* dominating both national and international mushroom trades, it however contradicts with Ueitele *et al.,* (2014) on the length of maturation (approximately 150 days) of Ganoderma lucidum. Similarly, Azizi *et al.* (2012) and Carvalho *et al.* (2015) working with Ganoderma from a different geographical zone reported a cropping cycle of 90 days. Although, the aforementioned studies used corn cobs and sawdust as substrates in their respective studies, the variation in the maturation length from the different studies may have been affected by the chemical nature or nutrient availability of the substrate used, geographical differences, method of cultivation (supplementation) and genetic factor. Other studies on the artificial cultivation of *Ganoderma lucidum* on

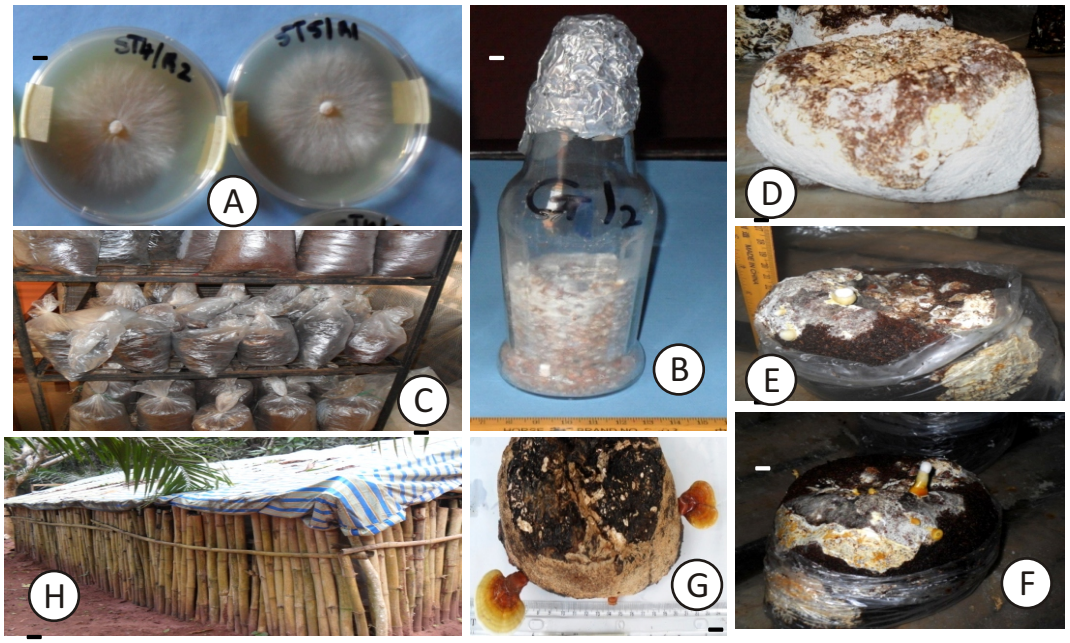


Fig.2: Methodical processes in the cultivation of *Ganoderma lucidum* during the study (a) pure culture of isolates; (b) spawn and spawn jar; (c) inoculated sawdust bags in layered arrangement; (d) colonized sawdust substrate; (e) button emergence; (f) primordial stage; (g) fruiting stage; (h) growth chamber/room.

variable basal sawdust wastes and their modulation through the use of protein-rich supplements for increased yield have been reported in Asia and the USA (Erkel, 2009; Gurung *et al.,* 2012; Subarna *et al.,* 2015). Monitoring the humidity and temperature of the growth room (Fig. 2h) through daily controlled wetting was observed to facilitate mycelium running and maturity of the fungus confirming the influence of ambient climate on the cropping cycle of *Ganoderma.*

*Ganoderma lucidum* has enjoyed a long history in native traditional medicine of the Chinese for close to 5,000 years but its artificial cultivation even though scarce in the continent of Africa has only just been accomplished in Nigeria under controlled temperature, light intensity and humidity (Subarna *et al.,* 2015). While the limited utility of the mushroom is influenced by its non-edible value, this is contrary to the decades of its domestication and development of production technology aboriginal to China (Gurung *et al.,* 2012). The high presence of this mushroom in the rainforest region of tropical West Africa may have been due to their perennial phenology, less pressure from human foraging and their zero attraction to many rural communities in Nigeria as food or masticatory. Suffice to say that cultural and culinary mushroom preferences preclude this mushroom-forming fungus from the list of edible mushrooms regularly hunted for foods or low income trade in many mycophilic (mycophagous) communities (Osemwegie *et al.*, 2014). Conversely, *Ganoderma* cultivation is reinforced by this study despite the need for further screening of several organic substrates and various supplementation materials for improved yield as well as reduced cropping cycle. This may revolutionize the utility and demand for the fungus in many native traditional folk medicine and pharmaceuticals. Furthermore, the result from the study strongly proved that the naturally occurring fungus *Ganoderma* strains from heavily forested regions are potentially more productive in terms of yield. This is in addition to being genetically malleable for modification and potentially capable of becoming a sustainable foreign exchange earner.

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