PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (7) 2207-2215 July 2024

Cultivation, Molecular Identification and Productivity Assessment of a commercial strain and two Wild Strains of *Ganoderma* mushroom Harvested from Tree Stumps in a Rainforest at Ugbowo, Benin City, Nigeria

POYERI, WR; OHIMAIN, EI*

Department of Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

**Corresponding Author Email: eohimain@gmail.com*
**ORCID: https://orcid.org/0000-0002-5491-6271*

ABSTRACT: Ganoderma mushroom is one of the most important medicinal mushrooms that has been used especially in the Orients for the treatment of diverse chronic disease conditions. Therefore, the objective of this study is cultivation, molecular identification and productivity assessment of a commercial strain (W1) and two wild strains (W2 & W3) of *Ganoderma* mushroom harvested from tree stumps in a rainforest at Ugbowo, Benin City, Nigeria using appropriate standard techniques. The molecular study revealed that all the three strains were indeed *Ganoderma lucidum*, though, results show that there were significant differences among the strains. Irrespective of the inoculum size, the number of days to maturity of the fruiting bodies was about 30 days for strain W3, 18 -22 days for strain W2 and 10-11 days for strain W1. Overall, it took 6-11 days for primordial formation and 10-30 days for maturity of the fruiting bodies. The biological efficiency (BE) of the strain ranged from 0.7 -2.4%. The results show that biological efficiency of the strains was poor, perhaps due to the infancy of Ganoderma cultivation in the country caused by poor substrates and supplements selection, colonization and fruiting conditions. Notwithstanding, the productivity of the strains could be improved through breeding.

DOI: https://dx.doi.org/10.4314/jasem.v28i7.35

Cite this Article as: POYERI, W. R; OHIMAIN, E. I. (2024). Cultivation, Molecular Identification and Productivity Assessment of a commercial strain and two Wild Strains of *Ganoderma* mushroom Harvested from Tree Stumps in a Rainforest at Ugbowo, Benin City, Nigeria. *J. Appl. Sci. Environ. Manage.* 28 (7) 2207-2215

Dates: Received: 21 May 2024; Revised: 17 June 2024; Accepted: 23 June 2024 Published: 02 July 2024

Keywords: *Ganoderma lucidum*; mushroom domestication; medicinal mushroom; performance; wild strains

Ganoderma mushroom is quite popular in the Orients where they have been used for millennia in the management of chronic conditions such as diabetes, hypertension, arthritis and heart and kidney diseases (Roy *et al*., 2015; Winska *et al.*, 2019; Ohimain 2020). They have also been used as antiaging substance, hence they are often referred to as mushroom of immortality (Sanodiya *et al.*, 2009). The mushroom extract also has immunity boosting, anti-inflammatory and antimicrobial properties (Choi *et al*., 2014; Wu *et al*., 2019; Ohimain 2020). The mushroom has been shown to be effective against various forms of cancers (Martínez Montemayor *et al*., 2019; Wu *et al*., 2018), some of which has been tried clinically (Wong *et al*., 2020). The medicinal uses of this mushroom appear to be linked to the presence of phytochemicals including ergosterols, polysaccharides and triterpenes such as ganoderic acids, ganodermanontriol and ganoderiol A (Choi *et al*., 2014; Martínez Montemayor *et al*., 2019; Sudheer *et al.*, 2019; Wu *et al*., 2019; Wong *et al*., 2020).

Ganoderma mushroom occurs naturally in the wild in several countries especially both in the tropics and temperate regions, where they perform the ecological functions of fungi especially in the biodegradation and bioconversion of biomass and cycling of nutrients in nature. However, wild Ganoderma mushroom have been implicated in the destruction of ornamental plants (Ozumba *et al*., 2018) and palm plantations due to

**Corresponding Author Email: eohimain@gmail.com*
**ORCID: https://orcid.org/0000-0002-5491-6271*

stem rot (Rashid *et al*., 2014; Jazuli *et al*., 2022), for which several counter measures have been instituted including the use of fungicides. On the other hand, Ohimain and Bawo (2021) observed the insect catching ability of this mushroom, which can be exploited in the sustainable management of mushroom farm insect pests particularly the sciarids. Ogbe *et al* (2009) used crude extracts of wild *G. lucidum* for the treatment of *Eimeria tenella* infection in broiler chickens. Shamaki *et al* (2017) established the safety of the methanol extract of indigenous *Ganoderma lucidum* when administered orally to Wistar rats.

Wild edible and medicinal mushroom are important in the diet and health supplements of indigenous people in West Africa (Guissou *et al*., 2015). Wild Ganoderma species have been reported by authors in Nigeria especially in the tropical rainforest belt (Ihayere and Okhuoya, 2022). Wood *et al* (2021) screened wild *Ganoderma sp* collected from Nigeria against common food-borne pathogens. Osemwegie and Oghenekaro (2010) listed *G. lucidum* among the mushrooms used as folk medicine by the people of Akoko-Edo in Edo State, Nigeria. Okigbo and Obanubi (2020) reported wild *G. lucidum* used by traditional medicine practitioners in Warri, Delta State, Nigeria. Owing to the importance of this mushroom, there has been local efforts in its domestication using available sawdust as substrates (Ihayere *et al.*, 2017; Adongbede and Atoyebi, 2021). Several species of Ganoderma mushrooms have been reported from the wild in Nigeria including *G. lucidum, G. boninense, G. resinaceum, G. praelongum, G. applanarum* (Ofodili *et al*., 2005; Wood *et al*., 2021). Among the Ganoderma mushrooms, the species of most interest is *G. lucidum*. In most of the studies carried out in Nigeria, the species were often identified phenotypically, which is prone to mistakes. However, Adongbede and Atoyebi (2021) used both morphological features and molecular method to identify *G. lucidum*, which was harvested from the wild in Lagos, Nigeria. Rashid *et al*. (2014) noticed that molecular identification of Ganoderma is more precise than morphological identification. Elsewhere, other authors have identified the mushroom using molecular methods (Rashid *et al*., 2014; Thakur *et al*., 2015; Hassan and Saadi, 2023). Hence, the objective of this study is cultivation, molecular identification and productivity assessment of a commercial strain and two wild strains of *Ganoderma* mushroom harvested from tree stumps in a rainforest at Ugbowo, Benin City, Nigeria.

MATERIALS AND METHODS

Source and Identification of Mushroom: Three different Ganoderma mushroom samples sourced from different locations in Nigeria were used for the study. Two of the mushroom samples, strain W3 and W2, were randomly harvested from tree stumps in a rainforest around the Ugbowo campus of the University of Benin in Edo State and transported to the Department of Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, where they were presumptively identified as *Ganoderma* sp. based on morphological observations. Morphological identification involved examining the features of the mushroom under a light microscope after mounting in 5% KOH solution (Wang *et al*., 2012; Adongbede and Atoyebi, 2021) followed by identification using taxonomic keys (Bhosle *et al*., 2010). The third sample was a commercially available foreign Ganoderma species (W1) that was sourced from a commercial mushroom production facility, Rohi Biotechnologies Ltd in Port Harcourt, River State, Nigeria.

Laboratory Cultivation of Ganoderma Species: Upon reaching the laboratory, the two wild strains (W3, W2) and the commercial strain (W1) were surface sterilized with 70% alcohol. Tissues were obtained aseptically from the mushroom fruiting bodies using sterile scalpel and inoculated on pre-prepared, sterilized and solidified Potato Dextrose Agar (PDA) plates under a Biobase laminar flow hood that was prior disinfected with 80% isopropanol. They were incubated at room temperature for 5 to 7 days until mycelia appeared. Pure cultures were isolated by sub-culturing 5 mm agar blocks of the mycelium into freshly prepared PDA plates and incubated at room temperature until the mycelia fully colonize the plates. Cultures were subsequently transferred and stored in a PDA slant and sealed with transparent clean films.

Spawn preparation: Guinea corn *(Sorghum bicolor)* grains were purchased from the market, cleaned and used for spawn preparation. One hundred grams (100g) of guinea corn was weighed using a top-loading balance, washed under running tap water to remove traces of insecticides, debris including dead and floating grains and was soaked overnight. The soaked grains were rewashed the next day, pasteurized at 100°C for 20 min with a water bath, allowed to cool and drained on an inclined surface for 6 hours. Calcium sulphate and calcium carbonate salts were each mixed with the drained grains constituting 2% (w/w) per dry weight according to Ihayere *et al*., (2017). The grains were then transferred into spawn jars, filled up to 2/3 of jars' capacity and plugged with cotton wool stopper before being loaded into an autoclave and sterilized at 121^{o}C for 1 hour. The grain-filled jars were then allowed to cool to room temperature, shaken to loosen the grain for even distribution of moisture. Agar blocks containing

proliferated mycelia of each strain were then inoculated into sterilized grain-filled jars aseptically under a laminar flow hood, shaken for even distributions and incubated at 25ºC in a growth room until full colonization by the mycelia of the mushroom.

Substrate preparation: Sawdust from unidentified trees was collected from saw mills within Port Harcourt, Rivers State, Nigeria and transported to the Rohi Mushroom Laboratory where they were sun-dried on a clean flat surface 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. The substrate was then aseptically and thoroughly mixed with 12g of $CaSO_4.2H_2O$ (gypsum), 3g of $CaCO_3$ and 1 g of sucrose. The substrate 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 improvised with water absorbing cotton plug, a plastic neck and rubber band (Ihayere *et al*., 2017). The substrate-filled polyethylene bags were sterilized in two-layered drum pasteurizer for 5 hours.

Mushroom inoculation: Upon cooling the substrates to 25ºC, the bags were inoculated under the laminar flow hood with grain spawn containing the different mushroom strains. Ten replicates of each strain were inoculated at 5%, 10%, 15% and 20% of the wet weight of the spawn bags and incubated on shelves in a dark fruiting room. The spawn bags were partially punctured while the growing chamber was ventilated when the substrate bags were fully colonized and matured with evidence of pinning. The humidity of the growth room was maintained at different stages of the growth of the mushroom. It was maintained at about 90%, 70 - 80% and 30 - 40% during primordial induction, cap formation and maturation of fruit body development by water spraying thrice daily, twice daily and once daily respectively. The relative humidity was monitored using a battery-powered hygrometer and temperature meter. Upon maturity, the fruiting bodies were then harvested, cleaned, measurements were carried out, dried and stored. Measurements carried out included extent of substrate colonization, days to pinning and maturation of the fruiting bodies, number of fruiting bodies per bag, fresh and dry weight of fruiting bodies, biological efficiency, BE (100 x weight of fruiting bodies/weight of wet substrate) and major and minor axis of the fruiting bodies.

Molecular Identification: The molecular identification of the strains involved DNA extraction, amplification of the internal transcribed spacer (ITS) region, sequencing and phylogenetic analysis.

DNA extraction and quantification: The extraction of the DNA of the mushroom followed the instructions of the manufacturer of the kits. It was carried out using a ZR fungal DNA mini prep extraction kit supplied by Inqaba South Africa. The extraction was carried out by suspending a dense growth of mycelia of the mushroom pure culture in 200 microliters of isotonic buffer in a ZR Bashing Bead Lysis tube and 750 microliter of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes and the ZR bashing bead lysis tube were centrifuged for one minute.

Four hundred (400) microliters of the supernatant were transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrifuged at 7000*g* for 1 minute before adding 1200 microliters of fungal deoxyribonucleic acid binding buffer bringing the final volume to 1600 microliter. Of the 1600 microliter, about 800 microliter was transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000*g* for 1 minute. The collection tube served as a medium through which the flow through was discarded. Thereafter, the remaining volume was transferred to the same Zymo-spin and was spun. The Zymo-spin IIC received 200 microliter of the DNA Pre-Wash buffer in a new collection tube which was spun at 10,000*g* for 1 minute followed by the addition of 500 microliter of fungal DNA Wash Buffer and centrifuged at 10,000*g* for 1 minute.

A clean 1.5 microliter centrifuge tube received the transferred Zymo-spin IIC column.100 microliter of DNA elution buffer was added to the column matrix and centrifuged at 10,000*g* for half a minute (30 seconds) to elute the deoxyribonucleic acids. The extracted genomic deoxyribonucleic acid was quantified using the Nanodrop 1000 spectrophotometer. The ultra-pure DNA was then stored at -20 degree Celsius for further analysis.

ITS amplification: The ITS region of the strains was amplified using the primers
ITSF: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'- TCCTCCGCTTATTGATATGC-3, on a ABI 9700 Applied Biosystems thermal cycler at a final volume of thirty (30) microlitres for 35 cycles. The polymerase chain reaction (PCR) mix included: the X2 Dream Taq Master mix (Taq polymerase, DNTPs, MgCl) supplied by Inqaba, South Africa, the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial

denaturation, 95ºC for five (5) minutes; denaturation, 95ºC for thirty (30) seconds; annealing, 53ºC for thirty seconds; extension, 72ºC for thirty seconds for 35 cycles and final extension, 72ºC for five minutes. The product was resolved on a 1% agarose gel at 120V for fifteen (15) minutes and visualized on a blue light transilluminator.

Sequencing: Sequencing of the samples were done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological located in Pretoria, South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul BigDye® terminator v1.1/v3.1, 2.25ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-10ng PCR template per 100bp. The conditions for sequencing were: 32 cycles of 96°C for 10 seconds, 55°C for 5 seconds and 60°C for 4min.

Phylogenetic analysis: The obtained sequences from the *Ganoderma* mushroom analysis were edited using the bioinformatics algorithm Trace edit, while similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. Alignment of these sequences were done using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) was taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

RESULTS AND DISCUSSION

Productivity assessment: The productivity assessment of the three strains of the mushroom is presented in Table 1. In terms of the colonization of the substrate by the fungi mycelia, there seemed to be no discernable pattern among the various strains and inoculation rates. The number of days to pinning decreases with increasing concentration of the inoculum, but results also show that the wild strain W3 has the longest day to pinning followed by W2, while W1 is the fastest. Irrespective of the inoculum size, the number of days to maturity of the fruiting bodies was about 30 days for W3, 18 -22 days for W2 and 10-11 days for W1. Again, showing that strain W3 is the slowest. The number of fruiting bodies per bag was generally less than 3 across all strains irrespective of inoculum size. The fresh weight and biological efficiency of the mushrooms indicated poor performance, though with W2 performing best followed by the W1 strain. Along the major and minor axis of the fruiting body, that of W1 was significantly wider than others and tend to increase with increasing inoculum size.

A low productivity and BE were observed among all the strains including the commercial strains. Several factors could be responsible, which are due to the infancy of *Ganoderma* cultivation in the country. We suspect poor substrates and supplements, and colonization and fruiting conditions. For instance, available sawdust at the sawmills that was produced from diverse unidentified trees, was used for the cultivation. This might have affected the biomass yield, because the percentage substrate colonization varied from 50 -89%, with the lowest values obtained among the W1 stains, which did not follow the pattern of substrate inoculation. Several authors have shown that different substrates affect the performance and BE of *G. lucidum* (Gurung *et al*., 2012; Lisiecka *et al*., 2015). Gurung *et al*. (2012) reported significant differences in the mushroom yield among varieties of sawdust types and supplements. They reported mean yield and BE of 15.05g/400g and 15.69% when *Alnus nepalensis* sawdust was used as substrate which significantly decreased to 0.65g/400g and 0.51% respectively when *Shorea robusta* sawdust was used as substrate. Roy *et al.* (2015) used five sawdust types from readily available woods and observed different BE with some wood species including *Tectona grandis, Michelia champaca* and *Gmelina arborea*, causing stunted growth of *Ganoderma* mushroom. They also found that *Swietenia mahagoni* sawdust supplemented with 8% wheat bran provided the best mushroom yield of 235.2 g/kg and BE of 7.6% among the substrates, which took 6 days for the mycelial growth, 33 days for primordial formation and 60 days to maturity. In our study, we observed significant difference among the strains, being 6-11 days for primordial formation and 10-30 days for maturity of the fruiting bodies and BE of 0.7 - 2.4%.

Adongbede and Atoyebi (2021) domesticated and cultivated *G. lucidum* obtained from the wild using sawdust of six hardwood species; *Avecennia germinans*, *Mansonia altissima*, *Lophira alata*, *Uapaca guineensis*, *Triplochiton scleroxylon*, and *Nauclea diderrichii* supplemented with rice and wheat bran. They found that depending on the wood species used as substrate, the spawn run period varied from 28 to 40 days when sawdust was used alone, but was considerably shorter when supplemented with rice (22-31 days) and wheat bran (20-30 days). Primordia formation took 38 – 45 days when sawdust was used alone, but was 31- 43 days with rice bran and 29 - 44 days with wheat bran supplementation. The number of days to first harvest was 44- 59 days when sawdust was used alone, but 38-61 days and 35-60 days when

supplemented with rice and wheat bran respectively. The BE varied based on the type of wood that was used for cultivation and supplements used. Among the six hard woods used, the highest BE of 31% was obtained when *Mansonia altissima* sawdust was supplemented with wheat bran, while the least value of 3.4% was obtained when *Nauclea diderrichii* was supplanted with rice bran. Overall, the sawdust without supplementation generally recorded the lowest BE.

Sudheer *et al* (2018) showed that different substrates result in different mushroom yields. They reported diverse yields when they prepared their substrates from sawdust, oil palm processing wastes and their combinations; mesocarp fiber (16%), empty fruit bunch fiber (19%), sawdust (26%), mesocarp fiber with sawdust (19%) and empty fruit bunch fiber with sawdust (27%).

Azizi *et al* (2012) show that the sawdust of different woods and their supplementation affects the yield and BE of mushrooms. They tested three types of sawdust substrates (beech, poplar, and hornbeam) mixed with two levels of supplements, malt extract (2.5% and 5% w/w) and wheat bran (5% and 10% w/w) for *Ganoderma* cultivation and found that mycelia growth rate, fruiting body yield and BE were significantly different, with the best recipes for high yield of 142.44 g/kg and BE of 18.68% obtained from the sawdust of poplar wood supplemented with 10% wheat bran and 5% malt extract, whereas the fastest mycelia growth rate of 10.6 mm/day was gotten from the sawdust of beech supplemented with 10% wheat bran and 2.5% malt extract.

Lisiecka *et al* (2015) compared the yield and productivity of four wild strains and 4 cultivated strains of *G. lucidum* produced with supplemented sawdust and observed that the wild strains had faster mycelium growth and higher BE irrespective of the type of substrate and additive. Atila (2020) used different agricultural wastes as basal substrates to cultivate *G. lucidum* including cottonseed meal, wheat straw, sunflower meal, bean straw, soybean straw, oak sawdust and poplar sawdust and reported average spawn run period of 14.2 - 18.2 days, yields of 28.6 g/kg to 86.1 g/kg and BE of 8.9%–24.7%, with the best performance reported for oak sawdust.

Table 1: Productivity of two wide strains of mushroom compared with a commercial strain

Spawn inoculation rate	Strain	Substrate colonization (%)	Days to Pinning	Days to fruiting body maturity	No. of Fruiting bodies per bag	Fresh weight of fruiting body (g)	Dry weight of fruiting body (g)	Biological efficiency, %	Major axis (cm)	Minor axis (cm)
5%	W3	50.40±6.80a	11.90±1.05cd	30.00±0.00d	1.90±0.23abc	9.30±0.84a	4.55±0.41b	0.93±0.08a	9.13±0.31cd	5.79±0.40bcd
	W2	79.50±2.52cd	7.70±0.70ab	18.90±0.90b	2.30±0.26abc	9.18±0.64a	4.61±0.35b	0.92±0.06a	6.05±0.27a	3.82±0.25a
	W1	83.50±4.95cd	7.80±0.51ab	10.90±0.7a4	2.40±0.37bc	7.16±1.31a	2.12±0.27a	0.72±0.13a	6.33±0.44a	3.95±0.20a
10%	W3	83.50±2.76cd	13.90±1.07d	30.00±0.00d	1.50±0.22ab	8.66±0.65a	5.00±0.39bc	0.87±0.06a	9.57±0.34cd	5.72±0.19bcd
	W2	80.00±4.47cd	7.40±0.27ab	18.70±0.40b	1.70±0.21abc	15.80±0.75b	9.30±0.38d	1.58±0.08b	10.15±0.62d	6.42±0.30d
	W1	69.00±7.67bc	8.10±0.92ab	11.30±0.82a	2.70±0.56c	9.46±1.12a	4.12±0.31b	0.95±0.11a	7.95±0.58bc	5.10±0.38bc
15%	W3	89.50±3.06d	10.10±1.33bc	30.00±0.00d	1.40±0.16ab	6.97±0.83a	4.25±0.43b	0.70±0.08a	7.31±0.70ab	4.99±0.55b
	W2	54.00±7.77ab	8.80±0.61ab	22.60±0.79c	2.30±0.26abc	20.88±2.14c	14.04±1.48e	2.09±0.21c	9.44±0.67cd	5.91±0.39bcd
	W1	54.00±3.40ab	6.30±0.65a	11.00±0.65a	2.00±0.37abc	13.64±1.53b	4.96±0.66bc	1.36±0.15b	10.03±0.41d	6.15±0.29cd
20%	W3	82.50±4.96cd	10.00±1.69bc	30.00±0.00d	1.30±0.15a	8.51±0.81a	4.94±0.49bc	0.85±0.08a	8.61±0.71bcd	5.70±0.48bcd
	W2	55.00±5.22ab	7.50±0.17ab	20.10±0.78b	2.60±0.16c	23.76±1.85c	15.68±1.18e	2.38±0.18c	9.21±0.56cd	5.79±0.31bcd
	W1	50.00±3.65a	6.60±0.70a	11.10±0.78a	2.70±0.45c	17.25±1.33b	7.01±0.64c	1.73±0.13b	10.19±0.56d	6.43±0.28d

mean ± standard error (n=10) with the same alphabets are not significantly different (P>0.05)

Molecular Identification of Ganoderma Mushroom: The obtained ITS sequence (Figure 1) from the isolates produced an exact match during the megablast search for highly similar sequences from the National Center for Biotechnology Information (NCBI) non-redundant nucleotide (nr/nt) database. The ITS region of the strains showed a 100% similarity to previously identified *G. lucidum*. The evolutionary distances computed using the Jukes-Cantor method were in agreement with the phylogenetic placement of the ITS

of the strains within the *Ganoderma* sp. and revealed a close relatedness to *Ganoderma lucidum.* The results of the phylogenetic analysis therefore show that all the tested mushrooms were *Ganoderma lucidum* of different strains (Figure 2). Adongbede and Atoyebi (2021) similarly used morphological and molecular techniques to identify wild isolates of *Ganoderma lucidum* sourced from the dead tree stump of *Albizia zygia* in Lagos, Nigeria. Rashid *et al.* (2014) observed that there were significant variations within and between *Ganoderma* species found in the wild especially in terms of their morphology and through molecular studies they identified diverse species of *Ganoderma* including *G. zonatum, G. boninense* and *G. miniatocinctum*, which they regarded as pathogens of oil palm. Other authors have also used molecular method to identify *G. lucidum* (Thakur *et al*., 2015; Hassan and Saadi, 2023).

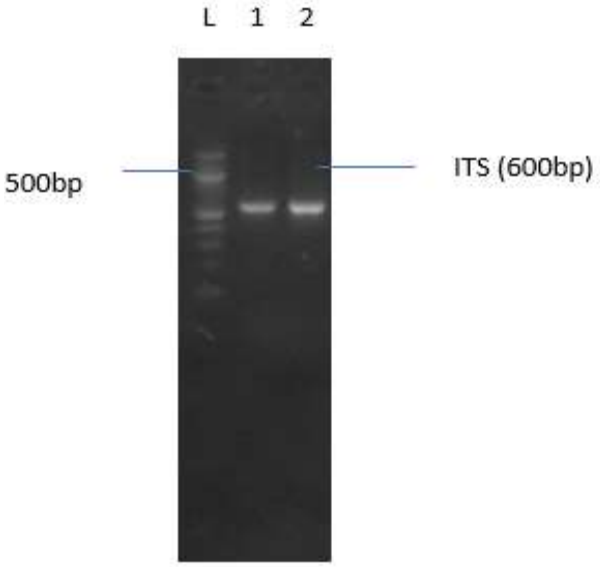

Fig 1: Agarose gel electrophoresis showing the amplified ITS of the fungal isolates.
Lane 1 and 2 represent the ITS bands at 600bp while lane L represents the 100bp molecular ladder

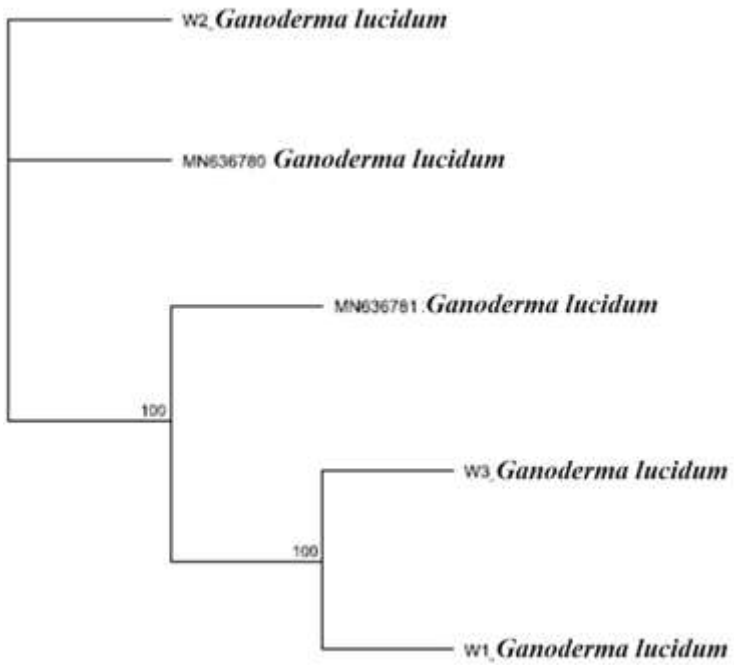

Fig 2: Phylogenetic tree showing the evolutionary distance between the fungal isolates

Conclusion: Two wild strains of mushroom that was collected from the wild in Edo State, Nigeria, was presumptively identified as *Ganoderma* mushroom. The wild strains and a commercial strain of the mushroom were subjected to molecular analysis and found to be closely related to *Ganoderma lucidum*. The growth characteristics of the three strains were assessed in sawdust substrate. The results revealed poor biological efficiency of all the three strains, but with strain W3 significantly slower than the others. The poor biological efficiency might be as a result of the infancy of *Ganoderma* mushroom cultivation in the country. However, the performance of the wild strains could be improved through mushroom breeding processes.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data from which this manuscript was extracted are available upon request from the corresponding author.

REFERENCES

Adongbede, EM; Atoyebi, AF (2021). Isolation of *Ganoderma lucidum* (Curtis) P. Karst. from the wild in Lagos through Tissue Culture Techniques and Cultivation on Sawdust of six Nigerian Hardwoods. *J. Appl. Sci. Environ. Manage.* 25(8), 1497-1503. doi:10.4314/jasem.v25i8.33

Atila, F (2020). Comparative study on the mycelial growth and yield of *Ganoderma lucidum* (Curt: Fr.) Karst. on different Lignocellulosic wastes. *Acta Ecologica Sinica*, 40(2), 153-157. DOI:10.1016/j.chnaes.2018.11.007

Azizi, M; Tavana, M; Farsi, M; Oroojalian, F (2012). Yield Performance of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst. (Higher Basidiomycetes), using different waste materials as substrates. *Int. J.Med. Mushr.*, 14(5), 521–527. https://doi.org/10.1615/intjmedmushr.v14.i5.110

Bhosle, S; Ranadive, K; Bapat, G; Garad, S; Deshpande, G; Vaidya, J (2010). Taxonomy and diversity of Ganoderma from the Western parts of Maharashtra (India). *Mycosphere*, 1(3), 249-262. ID: 135128093. http://mycosphere.org

Choi, S; Nguyen, VT; Tae, N; Lee, S; Ryoo, S (2014) Antiinflammatory and heme oxygenase-1 inducing activities of lanostane triterpenes isolated from mushroom *Ganoderma lucidum* in RAW264.7 cells. *Toxicol. Appl. Pharmacol.* 280(3): 434-442.DOI:https://doi.org/10.1016/J.TAAP.2014.09.007

Felsenstein, J (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39:783-791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Guissou, KML; Yorou, NS; Sankara PH; Guinko, S (2015). Assessing the toxicity level of some useful mushrooms of Burkina Faso (West Africa). *J. Appl. Biosci.*, *85*,7784-7793.DOI:10.4314/jab.v85i1.5

Gurung, OK; Budathoki, U; Parajuli, G (2012). Effect of different substrates on the production of *Ganoderma lucidum* (Curt.: Fr.) Karst. *Our nature*, 10(1): 191-198

Hassan, AA; Saadi, MA (2023). Isolation, Identification and Cultivation of Ganoderma spp. And Evaluation of their Efficiency in Inhibiting Plant Pathogenic Fungi *Fusarium oxysporum* and *Alternaria tenuissima*. In *IOP Conference Series: Earth and Environmental Science* Vol. 1158, No. 7, p. 072021). IOP Publishing. DOI 10.1088/1755-1315/1158/7/072021

Ihayere, CA; Okhuoya, JA (2022). Phytochemical analysis of cultivated medicinal mushroom-*Ganoderma sp.* Nigerian Journal of Botany Journal/Nigerian Journal of Botany 35 No.1: 11-18. DOI:10.4314/njbot.v35i1.2

Ihayere, CA; Okhuoya, JA; Osemwegie, OO (2017). Cultivation of Ganoderma lucidum (W. Curtis: Fr.) P. Karst on sawdust of Brachystagia nigerica Hoyle & APD Jones. *Agricul. Food Sci. J. Ghana*, 10(1), 852-868.. eISSN: 2821-9023. ISSN: 0855-5591

Jazuli, NA; Kamu, A; Chong, KP; Gabda, D; Hassan, A;Abu Seman, I; Ho, CM (2022). A review of factors affecting Ganoderma basal stem rot disease progress in oil palm. *Plants*, *11*(19), 2462. DOI:10.3390/plants11192462

Jukes, TH; Cantor CR (1969). Evolution of Protein Molecules. In Munro HN, editor, Mammalian Protein Metabolism, pp. 21-132, Academic Press, New York. http://dx.doi.org/10.1016/B978-1-4832-3211-9.50009-7

Lisiecka, J; Rogalski, J; Sobieralski, K; Siwulski, M; Sokół, S; Ohga, S (2015). Mycelium growth and biological efficiency of *Ganoderma lucidum* on substrate supplemented with different organic additives. *J. Fac. Agr.*, Kyushu Univ., 60 (2), 303–308. https://doi.org/10.5109/1526342

Martínez Montemayor, MM; Ling, T; Suarez Arroyo, IJ; Ortiz Soto, G; Santiago Negrón CL; (2019). Identification of Biologically Active Ganoderma lucidum Compounds and Synthesis of Improved Derivatives that confer Anticancer Activities in vitro. Front *Pharmacol* 10: 115. https://doi.org/10.3389/fphar.2019.00115

Ofodile, LN; Uma, NU; Kokubun, T; Grayer, RJ; Ogundipe, OT; Simmonds, MSJ (2005). Antimicrobial activity of some Ganoderma species

from Nigeria. *Phytotherapy Res.* 19(4), 310-313. DOI: 10.1002/ptr.1641

Ogbe, AO; Atawodi, SE; Abdu, PA; Sannusi, A; Itodo, AE (2009). Changes in weight gain, faecal oocyst count and packed cell volume of Eimeria tenella-infected broilers treated with a wild mushroom (Ganoderma lucidum) aqueous extract. *J. South Afri. Vet. Asso.* 80(2), 97-102. DOI: 10.4102/jsava.v80i2.179

Ohimain, EI; Bawo DDS. (2021). Preliminary Studies of *Ganoderma lucidum* and *Pleurotus ostreatus* Trapping of a Sciarid Insect. *EC Microbio.*17.4: 29-34.

Ohimain, EI (2020). Possible use of Ganoderma mushroom extracts for the treatment of some chronic risk factors associated with severe Covid-19. *Virol. Immun. J.* 4(3): 000254. DOI:10.23880/vij-16000254

Okigbo, RN; Obanubi, SI (2020). A Study on the *Ganoderma lucidum* of Warri South Local Government Area. *J. Food Tech. Nutr. Sci*. SRC/JFTNS/103. DOI: doi.org/10.47363

Osemwegie, O; Oghenekaro, A (2010). Folk uses of mushrooms by the Akoko-Edo people of Nigeria. *Asian J. Microbio. Biotech. Environ. Sci. 12*(4), 709-714.

Ozumba, C; Ilondu, EM; Ozumba, B (2018). A survey on the menace of linghzi mushrooms on Trees in Shell Residential Area, Port Harcourt. *Int. J. Sci. Res. Environ. Sci. Toxicol.* 3(3), 1-10. DOI:10.15226/2572-3162/3/3/00124

Rashid, M; Choon-Fah, MR; Bong, J; Khairulmazmi, A; Idris, AS (2014). Genetic and morphological diversity of Ganoderma species isolated from infected oil palms (*Elaeis guineensis*). *Int. J. Agri. Bio.*, 16(4). 691-699. http://www.fpublishers.org

Roy, S; Jahan, MAA; Das, KK; Munshi, SK; Noor, R (2015). Artificial cultivation of *Ganoderma lucidum* (Reishi medicinal mushroom) using different sawdusts as substrates. *Am. J. Biosci*, *3*(5), 178-82. DOI: 10.11648/j.ajbio.20150305.13

Saitou N; Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Bio. Evol.* 4:406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Sanodiya, BS; Thakur, GS; Baghel, RK; Prasad, GB; Bisen, PS (2009). *Ganoderma lucidum*: a potent pharmacological macrofungus. *Curr. Pharm. Biotechnol.* 10(8): 717-742. DOI: 10.2174/138920109789978757

Shamaki, BU; Sandabe, UK; Abdulrahaman, FI; Ogbe, AO; Hassan, ZI; Yusuf, IL; Isikhuemhen, OS (2017). Toxicity studies and body weights changes in Wistar rats following oral administration of methanol extract from indigenous Ganoderma sp. *MedCrave Onl. J. Bio. Med.*, *1*(5), 138-141. DOI: 10.15406/mojbm.2017.01.00030

Sudheer, S; Alzorqi, I; Manickam, S; Ali A (2019). Bioactive Compounds of the Wonder Medicinal Mushroom "*Ganoderma lucidum*". In: Mérillon JM, et al. (Eds.), Bioactive Molecules in Food. Reference Series in Phytochemistry. Springer pp: 1863-1893. ISBN: 978-3-319-78029-0

Sudheer, S; Alzorqi, I; Ali, A; Cheng, PG; Siddiqui, Y; Manickam, S (2018). Determination of the Biological Efficiency and Antioxidant Potential of Lingzhi or Reishi Medicinal Mushroom, Ganoderma lucidum (Agaricomycetes), Cultivated Using Different Agro-Wastes in Malaysia. *Int. J. Med. Mushr. 20*(1), 89–100. https://doi.org/10.1615/IntJMedMushrooms.2017024588

Thakur, R; Kapoor, P; Sharma, PN; Sharma, BM (2015). Assessment of genetic diversity in *Ganoderma lucidum* using RAPD and ISSR markers. *Indian Phytopathol*, *68*, 316-320. http://epubs.icar.org.in

Wang, XC; Xi, RJ; Li, Y; Wang, DM; Yao, YJ (2012). The species identity of the widely cultivated Ganoderma, 'G. *lucidum*. (Ling-zhi), in China. *PLoS One*, *7*(7), e40857. https://doi.org/10.1371/journal.pone.0040857

Winska, K; Maczka, W; Gabryelska, K; Grabarczyk, M (2019). Mushrooms of the Genus Ganoderma used to treat Diabetes and Insulin Resistance. *Molecules* 24(22): 4075. https://doi.org/10.3390/molecules24224075

Wong, JH; Ng, TB; Chan, HHL; Liu, Q; Wai Man, GC (2020) Mushroom extracts and compounds with suppressive action on breast cancer: evidence from studies using cultured cancer cells, tumor-bearing animals, and clinical trials. *Appl. Microbiol.*

Biotechnol. 104(11): 4675-4703. DOI: 10.1007/s00253-020-10476-4

Wood, TT; Rowaiye, AB; Okwu, CO; Popoola, OA; Unaeze, CH; AkienAlli, IJ; Iheka, SC; Braide, W (2021). Phytochemical screening and antibacterial effects of wild *Ganoderma* species on selected foodborne bacteria. *Int. J. Adv. Res. Bio. Sci*. 8 (1): 128- 137. DOI: 10.22192

Wu, K; Na, K; Chen, D; Wang, Y; Pan H (2018). Effects of non-steroidal anti-inflammatory drug-activated gene-1 on *Ganoderma lucidum* polysaccharides-induced apoptosis of human prostate cancer PC-3 cells. *Int. J. Oncol.* 53(6): 2356-2368. doi: 10.3892/ijo.2018.4578

Wu, Y; Han, F; Luan, S; Ai, R; Zhang, P (2019). Triterpenoids from *Ganoderma lucidum* and their potential Anti-inflammatory effects. *J. Agric. Food Chem.* 67(18): 5147-5158. DOI: 10.1021/acs.jafc.9b01195