Optimization of indigenous *Ganoderma lucidum* productivity under cultivation in Namibia

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Abstract

Ganoderma lucidum is a mushroom which shows antitumor, anti-inflammatory and cytoxic activity and grows prolifically in warm climates on decaying hardwood logs and stumps. An experiment was done at the University of Namibia to cultivate the indigenous *G. lucidum*; however, compared to results from other countries the mushroom took much longer to grow. The objective of this study was to shorten the cropping cycle of the indigenous *G. lucidum* under cultivation at the University of Namibia. The indigenous mushroom was cultivated according to the established protocol. A suitable indigenous *Ganoderma* mushroom was selected to make tissue culture, which was used to make the spawn that was inoculated into the woodchips substrate. New ideas were introduced to induce fast growth and optimum yield. The grains were inoculated with more pieces of tissue culture; the substrate was inoculated with increased layers of spawn and mixed with pearl millet husk instead of wheat bran to accelerate the colonization of the substrates. In addition to the hanging bag method used in the previous study, the buried wood log method was also introduced. Temperature and moisture were closely controlled during the experiment. A major highlight of this study was the significant reduction ($p < 0.05$) in the time it took for the substrate to reach fruiting stage. The substrate mixed with pearl millet husk was completely colonized by the mycelia, two weeks faster than the substrate that was mixed with wheat bran. Since Pearl millet is readily available in Northern Namibia, individuals and communities can substitute the wheat bran with pearl millet husk, which allows the substrate to be ready for fruiting

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faster, thus shortening the cropping cycle and also reducing the production cost as they no longer have to purchase wheat bran. The buried wood logs did not yield any fruiting bodies, but the hanging bags did produce *G. lucidum*. The study was successful in producing fruiting bodies in a shorter period. There was no significant improvement in the yield obtained.

Keywords: *Ganoderma lucidum*, cropping cycle, substrate, yield, optimization, indigenous, woodchips, layers

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1 Introduction

Ganoderma lucidum is an edible basidiomycetous fungus mostly used in complementary and alternative medicine, particularly in Asian countries for thousands of years (Martinez-Montemayor et al. 2011). Species similar to this mushroom are found on different kinds of wood all over the world, although in North America, *G. lucidum* preferably grows on hardwoods (McIntosh 2010). In Namibia *Ganoderma* grows on the stumps of trees such as *Sclerocarya caffra, Colophospermum mopane, Combretum zeyheri, Combretum collinum, Acacia sieberana, Baikiaea plurijuga, Terminalia sericea, Combretum frarans, Acacia erioloba*, and *Grewia retinervis* (Kadhila–Muandingi, 2012). *Ganoderma* also grows indigenously in Southern Africa, and is reportedly found in South-Western Africa (Namibia), the southern and Eastern Cape Provinces, Kwazulu Natal and Gauteng regions of South Africa, Mozambique and in Zimbabwe. It occurs in all these areas during the summer and autumn seasons (Shikongo, 2012).

Ganoderma mushrooms are widely used as herbal remedy for a variety of diseases ranging from bronchitis, hypertension, hepatitis, and as an adjuvant treatment of cancer. According to Wagner et al. (2003) this is because *G. lucidum* produces bioactive compounds such as polysaccharides and terpenoids. Due to its potent medicinal properties, *Ganoderma* is a widely sought after mushroom and more people are consuming *Ganoderma* products for its healing qualities (Jo et al. 2013; Wachtel-Galor et al. 2011). *Ganoderma* grows annually, although its fruiting bodies are hard (Ma et al. 2011) and can last for months, demand for it is high and there is need to make it available even when it is not its usual growing season. *G.lucidum* has been cultivated on solid substrate, stationary liquid medium and by submerged cultivation which have become essential to make the mushroom more accessible and affordable (Peksen & Yakupoglu 2009). According to Peksen & Yakupoglu (2009) the need to discover a way to produce high quality *Ganoderma* mushrooms in a shorter time is urgent and new techniques need to be explored in order to meet the increasing demand for the mushroom worldwide.

A cultivation protocol for the indigenous *G.lucidum* was established at the University of Namibia, although it took a long time to yield mushrooms (Kamukwanyama, 2009). This is a follow up study with the objective to shorten the cropping cycle of the indigenous *G. lucidum* at the University of Namibia.

2 Materials and Methods

2.1 Selection of a suitable mushroom for pure culture

The choice of a good strain is a very important stage in mushroom cultivation which can determine success or failure. A fresh and young *G. lucidum* mushroom was obtained from the ZERI Project at the University of Namibia to make a pure culture of *G. lucidum*. The Major practical phases of mushroom cultivation were adapted from Kadhila-Muandingi & Mubiana (2008). A piece was pulled from the indigenous *Ganoderma* mushroom with a pair of tweezers and placed onto Potato Dextrose Agar (PDA) in the Petri dishes. The Petri dishes were sealed with Parafilm and stored at room temperature in a cardboard box until the mycelia invaded the plates fully. A pure culture of indigenous *G. lucidum* was obtained after a week. The mycelia spread over the plate like a thick carpet. The mycelia were tough to cut and it tore when cutting to make the spawn, a trait which is characteristic of *Ganoderma* mycelia.

2.2 Development of spawn

Wheat grains were soaked in water overnight. After removing the grains from the water and draining of excess water was the grains were mixed with pearl millet husk. Plastic honey jars were filled with the grain until half-filled and autoclaved at $121^{\circ}C$ for 15 minutes. After the grains had cooled, they were inoculated with 5 pieces of pure culture per bottle and kept in a cardboard box at room temperature to allow the grains to be completely invaded by mycelium. Mycelia growth was initially very slow and the box was briefly put outside away from direct sunlight to heat shock it into growing.

2.3 Substrate preparation

After weighing, 5kg of woodchips was soaked in water overnight. The woodchips were removed from the water and squeezed to drain off excess water. A handful of pearl millet

husk was added to the woodchips before packing it into plastic bags and sterilizing at $121^{\circ}C$ for 25 minutes. After sterilization the woodchips were cooled, followed by inoculation with 4–5 layers of spawn. The bags were sealed, labeled with the name of the mushroom and date of inoculating before incubating in cardboard boxes in a warm dry place. As a control, development of spawn and substrate preparation was repeated, substituting the pearl millet husk with wheat bran. Out of the 20 bags inoculated, 5 were contaminated and only the remaining 15 had pure mycelia and could be used for fruiting.

2.4 Fruiting phase

The fully colonized bags were transferred to the fruiting room where the temperature and humidity was controlled by using automated sprinklers to water the room. Two methods were used for fruiting, namely the buried wood log method and the hanging method. For the first method two farm crates were cleaned and filled with autoclaved dry grass at the bottom up to 5 cm. Three bags of substrate were opened and placed evenly on the grass in each tray. The bags of substrate were covered with a layer of casing soil and they were watered twice daily. Fully colonized bags were hung on the low ceiling of a traditional mushroom house and they were slashed open with a blade to allow the mushrooms to start sprouting. The buried bag method failed to yield mushrooms. The hanging bags yielded *Ganoderma* fruits after 40 days of watering.

2.5 Statistical Analyses

Data was subjected to t-test at 0.05 probability level. The statistical analysis of data was performed using GraphPad software (2014) and the data were reported as mean values $\pm$ standard deviation (SD).

3 Results and Discussion

A cultivation protocol for the indigenous *G.lucidum* was established although it took a long time to yield mushrooms (Kamukwanyama, 2009). The objectives of this study were to shorten the cropping cycle by increasing the number of culture pieces in the grain, the layers of inoculum in the substrate and substituting wheat bran with the more readily available pearl millet husk. The effect of growth parameters like temperature and humidity were also monitored. The influence of temperature on mycelia growth was observed when the grains

were kept in the mushroom house and the mycelia grew slowly due to lower temperatures. The optimum temperature for *Ganoderma* mycelia growth is 21-27°*C*. Once the grains were moved to a warmer room mycelia invasion of the grains had improved to about 75% in 4 days. This could illustrate the importance of regulating temperature when cultivating *Ganoderma* mushrooms. Alternatively, this rapid growth could be attributed to many other factors. For example, the relationships between the details of observable growth form of an individual and its underlying genetic makeup (Wu et al. 2003).

Increasing the inoculation potential for both the spawn and substrate increased the growth rate of the mycelia because there was a bigger ratio of inoculum to amount of substrate that had to be invaded. There was a significant decrease ($p < 0.05$) observed in the period of mycelia colonizing the substrate from 55 days in the previous study to 34 days. Speeding up mycelia growth around the grains and the woodchips is beneficial because it shortens the waiting period before reaching the vegetative phase. Mycelia invasion was also enhanced by using pearl millet husks. Pearl millet husk was considered a suitable substitute for wheat bran because it is soft and fine enough to absorb excess moisture and because it has fine hair structures to which the mycelia can attach and be propagated to the woodchips. It was interesting to see that the bags with pearl millet grew at the same rate as those with wheat bran even though they were planted 10 days later.

G. lucidum mushrooms were obtained in about 40 days from opening the bags. This is an encouraging result, considering that *Ganoderma* is a slow growing mushroom. The results obtained from this study are encouraging but more work needs to be done in order to develop methods of reducing the cropping cycle considerably and to improve the yield. One of the limiting factors of cultivated mycelia colonizing the substrate it is grown on is the nutrient composition of the substrate. The growth of the mushrooms as well as quantitative and qualitative yield depends on the ability of the microorganism to break down and absorb nutrients and on the physiochemical environment in the medium (Pani, 2011). The effects of substrates on mycelia growth and mushroom yield have been studied for other mushrooms such as *Agrocybe aegerita, Volvariella volvacea, Pleurotus* spp. and *Lentinula edodes* (Peksen & Yakupoglu, 2009). Similarly, different combinations and mixing ratios of agricultural wastes and supplements should be investigated in more detail to increase mushroom yield and to improve fruiting of *G. lucidum*.

4 Conclusions

A cultivation protocol for the indigenous *G.lucidum* was established at the University of Namibia, although it took a long time to yield mushrooms (Kamukwanyama, 2009). This was a follow up study with the objective to shorten the cropping cycle of the indigenous *G.*

lucidum at the University of Namibia and a significant decrease ($p < 0.05$) was observed in the period of mycelia colonizing the substrate. Producing *Ganoderma* mushrooms in a reduced time will help to meet the increasing demand for the mushroom worldwide.

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