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Affect of substrate on growth and development of Ganoderma lucidum (Leyss ex. Fr.) Karst cultivation in Bao Loc - Lam Dong - Vietnam

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Abstract

Purpose: The study of "the influence of substrate on growth and development of *Ganoderma lucidum* cultivation in Bao Loc - Lam Dong" was conducted at the Technology College of economy and technology of Bao Loc from May to September 2023 with the aims of selectingsuitable substrates for growth and development in Reishi, completed the processes of Reishi artificial cultivation in Bao loc.

Methodology: Database involved nine treatments: rubber tree sawdust 75% + rice bran 25% supplemented urea 0,25%; rubber tree sawdust 95% + rice bran 5% supplemente urea 0,25% and DAP 0,25%; rubber tree sawdust 100% supplemented SA 0,5% and DAP 0,25%; rubber tree sawdust 96% + 2% maize powder + 2% rice bran; rubber tree sawdust 90% + maize powder 10% ; rubber tree sawdust 75% + maize powder 10% + rice bran 15%; rubber tree sawdust 95% + rice bran 5% supplemented DAP 0,2% , SA 0,2% and urea 0,1%; rubber tree sawdust 70% + rice bran 30%; control treament (rubber tree sawdust 90% + rice bran 5% + 5% maize powder). Experimental design was a Completely Randomized Design with 3 replications. The data were analyzed by using the analysis of variance (ANOVA) and group means were compared by Duncan Multiple Range Test (DMR) using the SAS 9.1 program. Means separation were computed following Duncan's Multiple Range Test (DMRT).

Findings: Results were achieved: Significant difference was observed in biological yield, dry yield and biological efficiency of Reishi (*Ganoderma lucidum*) on differences substracts .The highest biological yield (52,11 g/packet) and biological efficiency (15,79%) was obtained from mixed rubber sawdust 70% + 30% ridce brain

Unique Contribution to Theory, Policy and Practice: Complete the process of growing Ganoderma mushrooms in Bao Loc, orienting the development of Lam Dong into a large medicinal mushroom production area in Vietnam, creating Ganoderma mushroom products for export to the world.

Keywords: *Ganoderma Lucidum, Medicinal Mushrooms, Lamdong, Vietnam*

1. INTRODUCTION

Ganoderma lucidum (Leyss ex. Fr.) Karst.) is a species of the class Basidiomycetes, which belongs to the family Polyporaceae (or Ganodermataceae) of the order Aphyllophorales. It is known as "Ling Zhi" in china and "Reishi" (Wagner *et al.* 2003). A number of other *Ganoderma* species (*G. tsugae, G. sinensis, G. applanatum, G. capense* and *G. tenus*) have been used as medicinal mushrooms in China and Southeast Asia (Hseu 1993, Chen and Chao, 997)

Ganoderma has a woody consistency and possesses chemicals which offer many health benefits. It is a popular remedy to treat conditions like chronic hepatitis, hypertension, cancer, low blood pressure, high blood pressure, diabetes, rheumatism, heart problems, paralysis, ulcers, arthritis, asthma, tiredness, hepatitis A, B, and C, sterility, psoriasis, mumps, epilepsy and alcoholism. It produces several metabolites such as polysaccharides, peptidoglycans and terpenoids, which are responsible for medicinal properties. Besides promoting longevity, *Ganoderma* has unique property of strengthening the immune system (Liu 1999, 2001). *Ganoderma* products are available in various forms such as powders, tablets, capsules and syrups. It is one of the most desired medicinal mushrooms and has been used for more than 2000 year. *G. lucidum* is most commonly cultivated in China, Taiwan, Japan, Korea, Malaysia and North America.

Ganoderma lucidum has been cultivated by using several different substrates and by maintaining growth parameters such as temperature, relative humidity, water content, air pH and light intensity (Chang and Miles, 2004). They have been cultivated on a variety of sawdust substrates (beech, *Carpinus*, oak, poplar) mixed with diverse supplements such as:bran, sugar cane bagasse, tea residues, straw, coffee by-products (Peksen, Yakupoglu, 2009).

For a long time, the source of Ganoderma mushrooms used as medicine has mainly been wild mushrooms in nature. Wild lingzhi is rare, and in the years before it was cultivated, only the nobility could afford it. It was believed that the sacred fungus grew in the home of the immortals on the "three aisles of the blest" off the coast of China (McMeekin, 2005). However, its reputation as a panacea may have been earned more by virtue of its irregular distribution, rarity, and use by the rich and privileged members of Chinese society than by its actual effects. Nevertheless, the *Ganoderma* species continue to be a popular traditional medicine in Asia and their use is growing throughout the world (Wachtel-Galor, Buswell, 2004; Lindequist, Niedermeyer, and Jülich 2005), so it is necessary to cultivate Ganoderma mushrooms under artificial conditions to meet domestic and export demand as well as preserve the diversity of Ganoderma mushrooms in the wild. Nature. In Vietnam, people often grow Ganoderma mushrooms on two main types of substrates: on logs and on synthetic substrates. The nutritional composition of the substrate plays an important role in the growth,

development and quality of Ganoderma lucidum (Vu and Hoa, 2009).

Mushroom farming technology on synthetic substrates is more convenient and economical, allowing the use of waste by-products in agriculture and forestry such as sawdust, straw, rice husks, bagasse, coffee husks, etc. Peanut shells are mixed with rice bran and corn bran as a substrate for mushroom cultivation. The remaining problem is the ratio, mixing technique, and sterilization of the substrate; therefore, it is necessary to research and find suitable substrates for Ganoderma mushrooms to grow and develop well, suitable to the conditions of each locality. There have been a few studies on the growing substrate of Ganoderma lucidum, however, systematic construction from spawning to production in Lam Dong has been carried out. Starting from the above actual situation, the topic: "Effect of substrate on the growth and development of Ganoderma lucidum mushroom grown in Bao Loc - Lam Dong" will be carried out.

Target: Determine suitable production substrates for the growth and development of Ganoderma mushrooms.

During the research, it is necessary to monitor suitable substrates for reproduction. Growth and development of Ganoderma mushrooms. The experiment was carried out at the mushroom technology room of Bao Loc College of Economics and Technology.

2. MATERIALS AND METHODS

The single-factor experiment was arranged in a completely randomized design the experiment consisted of 9 treatments with 3 repetitions, each treatment contained 30 bags of mushrooms, randomly selected 10 bags in each treatment to monitor the criteria.

Total number of base cells: 9 x 3= 27 plots

Total number of substrate bags: 30 x 27= 810 substrate bags

Experiment Substrate environment

T1: Sawdust 75% + rice bran 25% added urea 0.25% (Tran Van Mao, 2004)

T2: Sawdust 95% + rice bran 5% supplement (urea 0.25% + DAP 0.25%)

T3: Sawdust 100% supplement (SA 0.5% + DAP 0.25%)

T4: Sawdust 96% + 2% corn bran + 2% rice bran (Erkel, 2009)

T5: Sawdust 90% + corn bran 10% (Saleh Ahmed, 2009)

T6: Sawdust 75% + corn bran 10% + rice bran 15% (Le Xuan Tham, 1998)

T7: Sawdust 95% + rice bran 5% supplement (DAP 0.2% +SA 0.2%+urea 0.1%) (Nguyen Minh Khang, 2005)

T8: Sawdust 70% + rice bran 30% (Saleh Ahmed, 2009)

T9: (90% sawdust + 5% corn bran + 5% rice bran)

Dry rubber sawdust is supplemented with lime powder at a rate of 0.2% (according to the dry weight of the sawdust), water is added, mixed well and incubated overnight. The moisture content of the sawdust substrate that has been limed overnight is about 45%. Mix the above synthetic substrates thoroughly with clean water (with added nutrients) to reach 65 - 70% moisture and pack into PP bags measuring 22.5cm x 30cm, each substrate bag has a weight of 1 kg. Then we put these bags into an autoclave for sterilization at 1 atm, temperature 121^0C for 1.5 hours.

After the substrate cools, transplant the seeds from the seed medium to the sawdust medium in a sterile incubator, under the flame of an alcohol lamp. The most suitable age for spawn is 20 days. Each bag of inoculum contains 10 - 15 g of spawn. The amount of seed added is just enough.

After inoculating the mushroom spawn, the substrate bags are incubated in the laboratory at a temperature of 25-28 0C, suitable for mushroom mycelium to grow.When the mushroom mycelium completely covered the substrate bag, transfer the substrate bag to the mushroom growing room. The fruiting body formation stage keeps the humidity in the mushroom growing room from 85-90%, temperature 30-32^0C. Spray mist directly into the bag of mushrooms, spray 1-2 times/day.

Growth criteria:

- Growth of mushroom mycelium length (cm): monitor mycelium length from the time the spawn is transplanted into the embryo bag until the mycelium eats the entire embryo bag. Measure from the neck of the bag to the longest thread, monitor every 5 days.

- Mushroom mycelium growth time (days): monitor the time it takes for mycelium to start growing, time for mycelia to cover 1/3 of the bag, time for mycelia to cover 1/2 of the bag, time for mycelia to cover the whole bag of substrate .

- Growth rate of mycelium (cm/day): monitor the length of mycelium from the time the spawn is transplanted into the embryo bag until the mycelium eats the entire embryo bag and calculate the average for one day.

- Time for the mycelium to fill the mushroom bag (days): calculated from the time the spawn is transplanted until the bags measure the white mycelium are full.

- Number of fruiting body clusters/bag: count all the number of fruiting body clusters on the monitored bags, take the average for each bag.

- Mushroom stem length: use a ruler to measure from the base of the mushroom stem to the part of the mushroom stem adjacent to the mushroom cap.

- Canopy thickness of *Ganoderma lucidum* : cut lengthwise from the top to the bottom of the mushroom canopy thickness at the position through the stem and use a ruler.

Diameter of canopy Ganoderma *lucidum* (cm): measure the mushroom Diameter of canopy with a ruler.

Productivity targets:

- Fresh mushroom yield = average weight of fresh mushrooms/1 bag of substrate when harvested.

- Dry mushroom yield = average weight of dried mushrooms/1 bag of substrate when harvested.

- Biological efficiency = dry weight of mushroom/dry weight of substrate * 100%

Statistical analysis. All experiments followed a completely randomized design. Data were subjected to analysis of variance, and average values were compared using the multiple LSD/DUNCAN classification test with SAS 9.1 software.

3. RESULTS AND DISCUSSION

3.1. Effect of substrate on Ganoderma mushroom mycelium growth time

Mushroom growth time is decisive in mushroom cultivation. Usually this indicator is based on breed factors, substrate, embryo bag size, external conditions and a number of other factors. The shorter the growth time, the more meaningful it is to shorten the harvest time. Therefore, it is necessary to investigate the influence of substrates on mycelial growth. Under the same conditions, different substrates will be the basis for evaluating the growth time of mycelia mycelial growth.

The time it takes for Ganoderma mycelial colonization 1/3 of the bag is relatively slow. The time to reach this stage in different treatments had a statistically significant difference ($p < 0.01$). The time it takes for mycelial to cover 1/3 of a bag of T1: sawdust 75% + rice bran 25% supplemented with 0.25% urea and T5: sawdust 70% + rice bran 30% is nearly equal at 10.2 respectively. Days and 10.4 days compared to the control 13.1 days, there is a very significant difference with the remaining treatments. While in T8: sawdust 90% + corn bran 10%, the time for mushroom silk to cover 1/3 of the bag is the longest (13.4 days).

Time taken for Ganoderma mycelial colonization ½ bag: between treatments there was a statistically significant difference ($p < 0.01$). Among them, 95% sawdust + 5% rice bran supplemented with 0.25% urea + 0.25% DAP has the longest mycelial colonization time (19.8 days), 70% sawdust + 30% rice bran. % has the fastest silk spreading time (14.8 days).

Time taken for Ganoderma mycelial colonization the bag: the time to reach this stage on different substrates has a statistically significant difference ($p < 0.01$). In particular, in the 70% sawdust + 30% rice bran treatment, after 22.9 days the mycelium had spread to cover the substrate bag compared to 25.8 days in the control, while in the 95% sawdust + 5% rice bran treatment,

supplemented Adding urea 0.25% + DAP 0.25% to cover the bag with mycelium takes 27.9 days (Table 1).

Table 1: Effects of substrates on growth time of Ganoderma Mycelial colonization

Treatment	Mycelial colonization 1/3 bag (days)	Mycelial colonization 1/2 bag (days)	Mycelial colonization all bag (days)
sawdust 75% + CG 25% + Urea 0,25%	**10,4 c**	**15,4 e**	**23,6 ef**
sawdust 95%+CG5% + Urea 0,25%+DAP 0,25%	12,8 ab	19,8 b	27,9 a
Sawdust 100% + SA 0,5% + DAP 0,25%	12,6 b	18,6 b	26,7 b
Sawdust 96% + Rice bran 2% + CB2%	13,2 ab	18,3 bc	26,2 bc
Sawdust 90% + corn bran 10%	13,4 ab	17,4 cd	25,2 cd
Sawdust 75% + corn bran 10% + Rice bran 15%	12,8 ab	16,8 d	24,4 de
Sawdust 95% + Rice bran 5% + Urê 0,1% + DAP 0,2% SA 0,2%	13,6 a	18,2 bc	26,4 b
Sawdust 70% + Rice bran 30%	**10,2 c**	**14,8 e**	**22,9 f**
Sawdust 90% + corn bran 5% + Rice bran 5%	13,1 ab	16,4 d	25,8 bc
CV(%)	2,89	2,48	1,69
F	42,33**	41,55**	41,38**

* *Different letters at the same column indicate significant differences between mean values according to the multiple range test of LSD/***DUNCAN** *with* $\alpha = 0.01$.

3.2. **Effect of substrate on the fruiting body development stage of *Ganoderma lucidum***

After opening the bag, the mycelium continues to grow. Primary filaments combine to form secondary filaments. The growth of secondary filaments leads to the formation of fruiting bodies (Nguyen Dung, 2003). This time is long or short depending on the breed characteristics and growing conditions. When the mycelium senses the sawdust environment, the white mycelium will grow and spread into the environment. These mycelium become thicker and deeper and spread deeper into the environment. When the mycelium has spread 2/3 of the bag or the entire bag of sawdust has spread, we remove the cotton plug, then spray moisture in the form of mist with an air humidity of 80-90%. At this stage, the mycelium at the top of the bag will curl

and form small white knobs. From these small knobs, the fungal filaments continue to curl up to form larger knobs, growing around the neck of the bag. When this knob is large enough (to the old stage) to form fruit body buds, they begin to spread to create a complete fruit body. The purpose of dispersal is for them to have a layer of spores, from which they can spread spores to maintain the species.

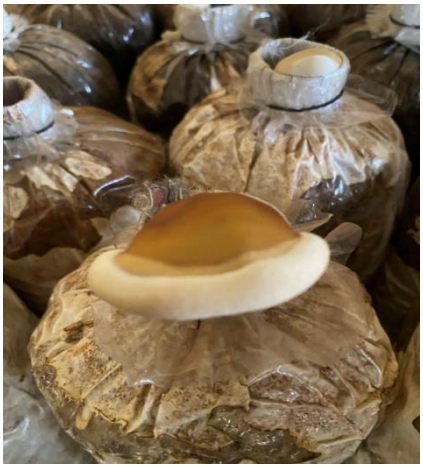
When the mycelium completely covers the bag (growth stage), they begin to move into the growth stage. In the new stage, the fungal mycelium intertwines and fruiting bodies begin to appear. The time for fruiting bodies to appear in treatments Sawdus 75% + CG25% supplemented with 0.25% urea, Sawdus70% + Rice bran 30% and Sawdus 90% + corn bran 5% + Rice bran 5% is about 27-29 days, only for the remaining treatments. The time for canopy appear is about 30-32 days (Table 2). In general, fruiting body sprouts appear after seeding in the environmental bags, and the time for sprouting between environments varies from 1 to 5 days. According to the results of statistical analysis, the time to appear fruiting bodies in the treatments had a very significant difference ($p < 0.01$).

The number of fruiting bodies on Ganoderma growing substrates varied from 1.37 to 1.43 fruiting bodies. The results of statistical analysis showed that the number of fruiting bodies among the treatments was not significantly different.

Table 2: Effect of substrate on fruiting body appearance time and number of *Ganoderma* fruit body clusters/bag of substrate

Treatment	Time to appear canopy (days)	Number of mushroom fruit bodies/bag
sawdust 75% + CG 25% + Urea 0,25%	27,60 d	1,43
sawdust 95%+CG5% + Urea 0,25%+DAP 0,25%	32,33 a	1,37
Sawdust 100% + SA 0,5% + DAP 0,25%	31,2 ab	1,37
Sawdust 96% + Rice bran 2% + CB2%	31,93 a	1,33
Sawdust 90% + corn bran 10%	29,73 bc	1,40
Sawdust 75% + corn bran 10% + Rice bran 15%	29,07 c	1,37
Sawdust 95% + Rice bran 5% + Urê 0,1% + DAP 0,2% + SA 0,2	30,40 bc	1,40
Sawdust 70% + Rice bran 30%	26,90 d	1,40
Sawdust 90% + corn bran 5% + Rice bran 5%	29,20 c	1,40
CV(%)	2,03	0,23
F(value)	27,86**	8,65 ns

Different letters at the same column indicate significant differences between mean values according to the multiple range test of LSD/DUNCAN with α= 0.01. ns: non significant



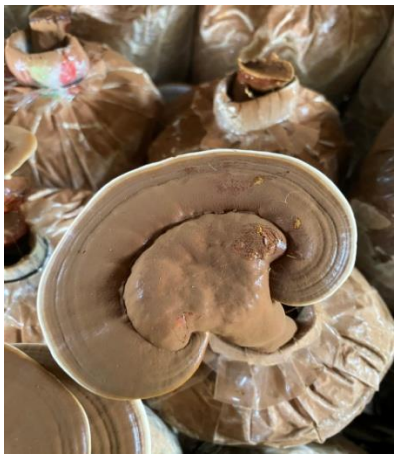


Figure 1: Canopy of *Garnoderma lucidum*

3.3. Effect of substrate on the size of *Ganoderma lucidum*

The fruiting body of the mushroom is the final result of mycelium growth. When the mycelium clumps together, fruiting bodies begin to form and develop into mushroom stalks and mushroom caps. The influence of the substrate on the growth of the mushroom stalks.

The length of the mushroom stem in the substrate 75% sawdust + corn bran 10% + rice bran 15% has the largest value (9.45 cm) and the substrate Sawdust 95% + rice bran 5% supplemented with urea 0 .1% + DAP 0.2% + SA 0.2% has the smallest value and the difference between treatments is very statistically significant ($p < 0.01$) (Table 3).

Table 3: Effect of substrate on mushroom stem length, diameter and canopy thickness of Ganoderma lucidum (cm)

Treatment	Length stem (cm)	Diameter of ofcanopy (cm)	Thickness of canopy (cm)
sawdust 75% + CG 25% + Urea 0,25%	7,46 e	8,79 ab	2,01 a
sawdust 95%+CG5% + Urea 0,25%+DAP 0,25%	8,48 b	8,14 bc	1,48 c
Sawdust 100% + SA 0,5% + DAP 0,25%	8,60 b	7,60 c	1,62 bc
Sawdust 96% + Rice bran 2% + CB2%	8,76 b	8,2 bc	1,55 c
Sawdust 90% + corn bran 10%	8,42 b	7,58 c	1,82 ab
Sawdust 75% + corn bran 10% + Rice bran 15%	9,45 a	8,58 ab	1,90 a
Sawdust 95% + Rice bran 5% + Urê 0,1% + DAP 0,2% + SA 0,2%	7,75 de	8,33 b	2,02 a
Sawdust 70% + Rice bran 30%	7,97 cd	9,2 a	2,06 a
Sawdust 90% + corn bran 5% + Rice bran 5%	8,31bc	8,67 ab	1,89 a
CV(%)	1,87	3,35	5,48
Ftính	41,59**	11,01**	14,14**

Different letters at the same column indicate significant differences between mean values according to the multiple range test of ***LSD/DUNCAN*** *with α= 0.01.*

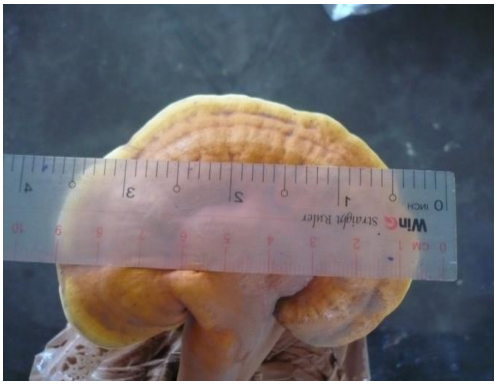


Figure 2: Diameter of canopy

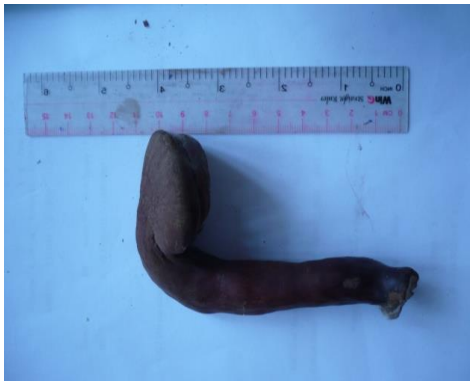


Figure 3: Length of stem

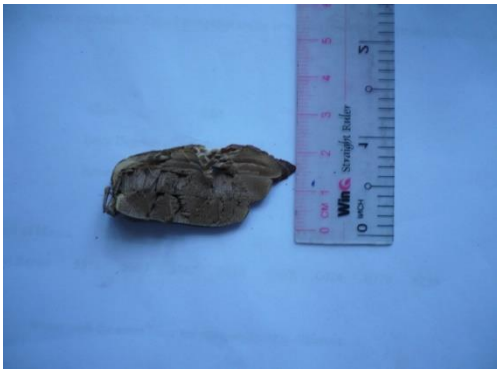


Figure 4: Thickness of canopy

Table 4: Effect of substrate on fresh weight and dry weight of *Ganoderma licidum*

Nghiệm thức	Weight of fresh mushrooms (g/bag)	Weight of dry mushrooms (g/bag)	Biological efficiency (%)
Sawdust 75% + CG 25% + Urea 0,25%	50,23 b	17,92 b	15,22 b
Sawdust 95%+CG5% + Urea 0,25%+DAP 0,25%	41,53 d	13,76 e	12,58 d
Sawdust 100% + SA 0,5% + DAP 0,25%	38,51 e	12,48 g	11,67 e
Sawdust 96% + Rice bran 2% + CB2%	40,19 de	13,87 e	12,18 ed
Sawdust 90% + corn bran 10%	40,24 de	14,18 d	12,19 ed
Sawdust 75% + corn bran 10% + Rice bran 15%	48,29 c	16,36 c	14,63 c
Sawdust 95% + Rice bran 5% + Urê 0,1% + DAP 0,2% + SA 0,2%	38,65 e	12,88 f	11,71 e
Sawdust 70% + Rice bran 30%	52,11 a	18,66 a	15,79 a
Sawdust 90% + corn bran 5% + Rice bran 5%	47,45 c	16,23 c	14,38 c
CV(%)	1,71	0,6	1,71
F(value)	150,4**	1808,5**	150,04**

Different letters at the same column indicate significant differences between mean values according to the multiple range test of ***LSD/DUNCAN*** *with α= 0.01.*

According to the results of Table 4, the weight of fresh and dried Ganoderma mushrooms in the substrates participating in the experiment has a statistically significant difference ($p < 0.01$). In sawdust substrate mixed with 70% sawdust and 30% rice bran, Ganoderma mushroom yield was highest compared to the remaining treatments: fresh mushroom yield reached 52.11g/bag of substrate, dry mushroom yield reached 18.66g/bag of substrate. Next, the substrate is 75% sawdust + 20% rice bran supplemented with 0.25% urea, yielding fresh and dried Ganoderma mushrooms is 50.23g/bag and 17.92g/bag, respectively. 100% sawdust substrate supplemented with SA 0.5% + DAP 0.25% obtained the lowest weight of fresh and dried Ganoderma mushrooms.

According to Nguyen Minh Khang (2005), rubber sawdust substrate mixed in the ratio: sawdust 95% + rice bran 5% supplemented with urea 0.1% + DAP 0.2% + SA 0.2% is very good. Suitable for the growth and development of black Ganoderma mushrooms. However, when cultivating Da Lat Ganoderma strain on this substrate, the mushroom yield was lower than in the remaining treatments. The yield of fresh mushrooms obtained on this substrate is only 38.51g of fresh mushrooms/bag and 12.54g of dried mushrooms/bag.

According to the research results of Le Tran Hoai Vu and Tran Dang Hoa, 2009. Ganoderma mushrooms were grown on a substrate of 96.5% rubber sawdust + 2% cornstarch + 1.5% rice bran. The yield of fresh mushrooms is 47.90 g of fresh mushrooms/bag of substrate, the yield of dried mushrooms is 15.86 g/bag of substrate.

The statistical results show that the biological efficiency obtained from the substrates in the experiment has a very significant difference ($p < 0.01$). Thus, the biological efficiency of the substrates ranges from 11.76 -15.79%, the substrate 70% rubber sawdust + 30% rice bran is the highest (15.57%). While sawdust substrate 100% + SA 0.5% + DAP 0.25%, the biological yield obtained was the lowest (11.67%) and the biological yield achieved in all experimental environments was high. more than 11%.

4. CONCLUSION AND RECOMMENDATIONS

4.1. Conclusion:

Highly efficient production substrate: Sawdust 70% + rice bran 30% and sawdust 75% + rice bran 25% + urea 0.25%. The yield of fresh Ganoderma mushrooms reached 52.11g/bag of substrate, the yield of dried mushrooms reached 18.66g/bag of substrate and the biological efficiency of this substrate reached 15.79%.

4.2. Suggestion:

It is recommended to do more research on: Ganoderma cultivation substrates to determine the most optimal environments for the growth and development of Ganoderma mushrooms and soon complete the mushroom growing process. advanced in Bao Loc - Lam Dong

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