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# THE EFFECT OF DIFFERENT TYPES OF Ganoderma lucidum ISOLATES ON THE GROWTH OF MYCELIUM ON THE GROWING MEDIUM (BAGLOG)

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#### **ABSTRACT**

The cultivation of Ganoderma lucidum (lingzhi) or G.lucidum mushrooms in Indonesia is still not widely developed. Factors that influence the success of lingzhi mushroom cultivation include the planting medium, environmental conditions, and the source of isolates. There has not been much research on lingzhi mushrooms to get superior lingzhi mushroom isolates. The aims of this study was to determine the effect of different sources of isolates on the growth of the mycelium of the Ganoderma lucidum on the growing medium (Baglog). This test was carried out using an experimental method with a completely randomized design (CRD) with the treatment of planting Ganoderma lucidum isolates from Tasikmalaya, Cianjur and Banyumas, on planting medium (baglog). Each treatment was replicated 30 times so that a total of 150 experimental units were obtained. The data obtained were analyzed by analysis of variance (one way ANOVA) and continued with Duncan's Multiple Range Test (DMRT) at an error rate of 5% and 1%. the study concludes is that Ganoderma lucidum isolates from Tasikmalaya, Cianjur and Banyumas affect the diameter and growth rate of mycelium vegetatively.

**Keywords:** baglog, Ganoderma lucidum, isolate, mycelium growth,.

### INTRODUCTION

Indonesia is an archipelagic country with a tropical climate and rich in flora and fauna biodiversity. The climatic conditions are very supportive for the organisms of such mushrooms that can be used as herbal medicine, several types of mushrooms that grow in the Tasikmalaya and Banyumas areas are very good due to climatic conditions and temperatures of 35°C which than are supportive for fungal growth. So many farmers are cultivating this mushroom. One type of mushroom that has begun to be cultivated is the Ganoderma lucidum mushroom.

Ganoderma lucidum (Ling Zhi or reishi mushroom,) (G.lucidum) has been an economically important mushroom,

especially in the East Asian countries (i.e. China, Japan, Korea), since 4000 years ago. The fungi is grown on a widespread commercial scale and is commonly consumed for medicinal and spiritual potency1–3. Ling Zhi cultivation under forests has great economic and ecological importance.

G.lucidum is a basidiomycete which has been used for over 2000 years in Japan, China, and Korea as a traditional medicine due to its properties associated with health and healing, long life, and happiness. The basidiocarp, mycelia, and spores of G. lucidum contain approximately 400 different bioactive compounds with polysaccharides, peptidoglycans, and triterpenes being the three major physiologically active constituents (Shah & Modi, 2018).

In the last decade fungal mycelium gained much attention academics and commercial enterprises due to its ability to upcycle agricultural and industrial wastes into sustainable The process composite materials. utilizes natural. low-energy а manufacturing process able to sequester carbon and create useful alternative materials (Jones et al., 2018). Mycelium composites comprise of networks of filamentous hyphae which can be converted into economically viable and environmentally friendly materials utilizing biological growth rather than expensive energy intensive manufacturing processes Jones et al (Jones, Huynh, Dekiwadia, Daver, & John, 2017).

Farmers cultivate mushrooms not only to be used as food, but many farmers cultivate them for medicinal raw materials, one of which is the cultivation of the Ganoderma lucidum mushroom, this mushroom usually lives on dead wood or tree trunks and acts as a parasite on infected trees and still alive. Wood mushroom is one of the potential forest resources that has not been optimal handling in forest resource management. Ecologically, fungi have an important role as a reformer that provides nutrients for other plants. Ganoderma is a wood mushroom that has better nutrition than with vegetables and fruit (Bano Z. and S. Rajarathnam, 1982).

Medicinal mushrooms have been used in various treatments from a very long time, among which, *Ganoderma lucidum* is one of the most important medicinal mushroom. It is cultivated worldwide to meet its everincreasing demand in the market (Bijalwan et al., 2021)

Farmers in cultivating Ganoderma Mushrooms generally do not pay attention to the seeds used, their orientation is more based on the productivity of the mushrooms planted,

by relying on seeds available in the market which are still not growing well. This has resulted in less than optimum development of ganoderma production, which until now the public's demand for this mushroom as a medicinal ingredient is increasing, it is necessary to optimize the production of this mushroom seed. The stages of making mushroom seeds are generally known as pure culture (F0), which is the result of isolating mushroom fruiting inoculated on a solid medium (agar) with synthetic or semi-synthetic nutrients. The mycelium was then developed to the next namely to become (F1) stage. transferring the fungal mycelium from a medium to a natural medium (generally cereals) which is rich in nutrients and used as parent seeds. Furthermore, the seeds are ready to be transferred to the mushroom growing medium (baglog) to produce fruit bodies. The planting medium also needs to be considered in terms of the nutrient content of the medium for the growth of the fruiting body.

The provision of a source of nutrients in the planting substrate is a very important factor in fungal growth. Cultivation of Ganoderma on synthetic log needs to pay attention to several factors that directly affect its growth ability. It is very important to provide macronutrients (C, H, N, O, P, K, Ca, and Mg) and micronutrients (Fe, Cu, Zn, and Mn) as additional mineral sources in the growing medium. Good carbon sources for the mushroom G. lucidum are hemicellulose. pectin. and cellulose compounds. Vitamins, especially thiamin (B1) are also needed by mushrooms, the need for this vitamin is usually met with the addition of seeds or bran. Minerals are generally already contained in water and the basic material of the substrate. That in their growth, fungi need basic materials such as cellulose, lignin, and other elements. Substances resulting from the reshuffle of lignin and cellulose will be utilized by fungi for their growth, in addition, it also affects the growth rate of fungal mycelium. The high amount of cellulose content in the growing medium will help the growth of mycelium and fruiting bodies.

Mushroom cultivation in Indonesia generally still uses seeds propagation Imported seeds are hard to come by, so they are expensive. The media used in Mushroom cultivation is generally waste wood sawdust. Cultivation technology relatively simple so that it is easily absorbed by the community, and is very suitable if it is associated with the program environmental conservation and use of biodiversity. Example, based on studies economic feasibility on a household scale, ovster mushroom cultivation can be developed in small scale farming. In the market you can find a variety of quality of mushroom seeds that can affect crop yields. Factors that affect the growth of fungal mycelium apart from environmental conditions, media, can also be influenced by the type of isolate or the source of the isolate used. Many studies have modified the type of planting media or baglog to accelerate the growth of fungi, but not many studies have used several types of isolates to optimize the growth of Ganoderma lucidum.

In this research The best mushroom seeds were used from three sources, namely *Ganoderma lucidum* isolates from Tasikmalaya, Cianjur and Banyumas. The purpose of this research is to find out whether the different sources of the *Ganoderma lucidum* mushroom have differences in the growth of mycelium on the growing medium (baglog).

### **MATERIAL AND METHOD**

### A. Materials

This research was carried out from February 2021 to May, 2021 at CV Syahid Mushroom, Tamanjaya Village, Tamansari District, Tasikmalaya City.

The materials used in this study were isolates of *Ganoderma lucidum* from Tasikmalaya, Cianjur and Banyumas, bran, lime (CaCO3), cotton, label paper, spiritus, 70% alcohol, gas, water,

materials, polypropylene plastic bag size 25 x 15x 0, 05 cm, paralon ring, rubber band, and mask.

The tools used in this study were autoclave, petri dish, mushroom house or kumbung measuring 4 m long, 3 m high 3 m high, thermometer, soil pH-hygrometer, room hygrometer, 40 watt TL lamp.

### B. Procedure

# 1. Making mushroom agar media (F0).

Agar medium (F0) was prepared by mixing 39 grams of instant PDA into 1000 ml of distilled water. agar medium was prepared aseptically by sterilizing moist heat under pressure using an autoclave (121°C, 1 atm, for 20 minutes. Each agar medium was taken 10 ml by thawing it first and poured into a petri dish. In the middle of each agar medium, Then 1 piece of cork drill inoculum was inoculated with the rejuvenated *G. lucidum* fungal isolate with a diameter of 0.5 cm and incubated for 7 days at room temperature.

# 2. Making mushroom cereal media (F1).

The cereals used are first cleaned of dirt and moistened by boiling. A cereal made with medium was the basic ingredients of each type of seed used by mixing 84% seeds, 1% lime (CaCO3), 1% gypsum (CaSO4), 14% fine bran, and a little vitamin B complex, plus water until the humidity reaches 60 %. The mixture is then put into a bottle measuring 10 cm in diameter and 20 cm in length, filled to the brim with the top surface of the mixture, and compacted by pressing the entire top surface of the mixture to a depth of 5 cm from the top surface of the bottle (reaching medium density) and then covering it with cotton. and newsprint and tied with rubber.

The medium cereal was made aseptically by a sterilization process. Sterilization was carried out under pressurized moist heat using an autoclave (121°C, 1 atm, for 20 minutes). Then refrigerated for 12 hours. After the cereal medium was sterilized, it was put into the inoculation chamber which was already sterile. The finished cereal medium was

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then inoculated with 1 piece of inoculum isolate of the fungus *G. lucidum* growth results on agar medium with a length of 3.5 cm and a width of 1.5 cm which was prepared, by placing the pieces of inoculum on the top surface of the seedling medium without immersing it.

The inoculation process is carried out aseptically, which is carried out near a spirit fire to avoid contaminants. The incubation process is a process to grow the inoculated mycelium. The cereal medium was laid out horizontally in the incubation cabinet. Mycelium on the medium will grow to fill the medium within 25-30 days.

# 3. Making mushroom plant media (baglog).

The composition of the planting medium refers to the basic composition according to Suriawiria (2000), composition of the lingzhi or mushroom growing medium follows: Sawdust: 10,000 g, Rice bran: 1,000 g, CaCo<sub>3</sub>: 50 g and Gypsum: 150 g. The mixing of all the planting medium carried materials was homogeneously. The homogeneous material is then added with water little by little until it reaches a water content of approximately 75%. The medium was then packed in a polypropylene plastic bag measuring 30 cm long, 15 cm wide, and 0.05 cm thick. The ready baglog is then put into a sterilization drum with a volume of 200 liters that has been given a nest. Sterilization is carried out in moist heat without pressure for 10 (ten) hours. Then let it cool for 2 days. Baglog that has been sterilized is put into the inoculation chamber which has been sprayed with 70% alcohol. Baglog is perforated with a depth of the height of baglog using a sterile awl, then the seeds of ganoderma mushroom are inserted into the hole. The inoculated baglog was then incubated in a mushroom house. Baglogs are laid out horizontally on a shelf without stacks. Maintenance during the incubation

period includes the removal of contaminated baglog, maintenance of the the environment room and mushroom house. and keepina the temperature and humidity conditions in the room at optimal conditions for the growth of funaus. the ganoderma The dailv temperature and humidity of the room were measured.

# C. Experimental design

This test was carried out using an experimental method with a completely randomized design (CRD) with the treatment of planting *Ganoderma lucidum* isolates from Tasikmalaya, Cianjur and Banyumas, on planting medium (baglog) with the following treatments.:

GTB : Ganoderma lucidum from Tasikmalaya on Baglog

GBB : Ganoderma lucidum from Banyumas on Baglog

GCB: Ganoderma lucidum from Cianjur on Baglog

Each treatment was replicated 30 times so that a total of 150 experimental units were obtained.

# D. Data analysis

The data obtained were analyzed by analysis of variance (one way ANOVA) and continued with Duncan's Multiple Range Test (DMRT) at an error rate of 5% and 1% (Steel and Torrie, 1991).

# **RESULT AND DISCUSSION**

Based on the analysis of ANOVA variance, each type of fungal isolate had a significantly different effect on the average diameter of mycelium growth. indicates that the different sources of isolates affected growth of the the mycelium of the fungus Ganoderma lucidum. However, the mycelium growth at week 3 showed no difference from the average mycelium diameter. This indicates the presence of fluctuating mycelium growth. The results of this test were continued with the DMRT test. The results of the DMRT test showed that the best mycelium growth in week 4 was from the treatment of isolates from Cianjur (GCB) with an average mycelium diameter of 15,757 cm, followed by isolates from Tasikmalaya (GTB) of 13,247 cm and isolates from Banyumas of 11,657 cm.

The further explanation can be seen in Figure 1. Apart from the analysis of the average diameter of the mycelium of Ganoderma lucidum, an analysis of the mycelium growth rate has also been carried out. The results of the ANOVA test showed that at week 1, week 3, and week 4 there was a significant difference between the isolated source and the growth rate of Ganoderma lucidum mvcelium. This indicates that the different sources of isolates affect the growth of mycelium. These results were followed by the DMRT test. The results of the DMRT test showed that the mycelium growth rate of GTB and GBB treatments was better than that of GCB. The results of the growth rate were different from the results of the mycelium diameter.

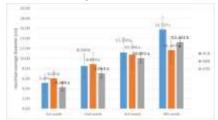
In addition to testing the average mycelium diameter. growth of observations and analyzes were also carried out on the growth rate of mycelium every week of growth. The results of the ANOVA test on the mycelium growth rate of several G. lucidum isolates showed а significant difference in the growth of weeks 1, 3, and 4. The mycelium growth rate at week 2 showed no difference in mycelium from several types of isolates. To find out the best growth rate of several isolates per week, the DMRT test was carried out, the results of the DMRT test showed the growth rate in week 1 of the treatment of g.lucidum

isolates from Cianjur (GCB) with the best growth rate from other treatments, namely 1.768 cm/week, followed by the growth of isolates from Banyumas (GBB) and isolates from Tasikmalaya (GTB) which were 1.581 cm/week and 1.423 cm/week.

The growth rate at week 3 was inversely proportional to the growth rate at week 1, the treatment of isolates from Tasikmalaya (GTB) with the best growth rate was 0.362 cm/week, followed by the treatment of isolates from Banyumas (GBB) isolates from Cianjur (GCB) with a growth rate of 0.276 cm/week and 0.187 cm/week. The growth rate at week 4 of the treatment of isolates from Banyumas (GBB) had the same good growth rate ability as isolates from Tasikmalaya (GTB) of 0.360 cm/week and 0.279 cm/week while the treatment of isolates from Cianjur (GCB) with the smallest growth rate and different from the other treatments, namely 0.830 cm/week. The results of the growth rate analysis can be seen in table 1 and figure 2. After the incubation period for vegative growth for 4 weeks, primordia growth occurred at week 5 and 6.

The results are shown in Figure 3. In addition to observing the diameter of mycelium growth. temperature and humidity observed in were also the incubation The results room. of observations show that there are differences in the average temperature and humidity each week, with an average temperature between 27- 29°C and an average humidity between 84 - 88 %. The overall results for each week are as in table 2.

1. The result of growth in diameter of the mycelium of Ganoderma lucidum some isolates.



Note: The numbers followed by the same letter indicate that the treatment is not significantly different based on the DMRT test at a level of 0.05%.

GTB: Ganoderma lucidum from Tasikmalaya on Baglog GBB: Ganoderma lucidum from Banyumas on Baglog GCB: Ganoderma lucidum from Cianjur on Baglog

Table 1. The result of average mycelium growth rate of Ganoderma lucidum some isolates

Treatments -	Average mycelium growth rate (cm/week)				
	1	2	3	4	
GBB	1,423 a ± 0,346	$0,517 \text{ a} \pm 0,303$	$0,276 \text{ b} \pm 0,161$	$0,360 \text{ b} \pm 0,262$	
GTB	1,581 b ± 0,302	0,518 a ± 0,144	$0,362 c \pm 0,066$	$0,279 \text{ b} \pm 0,043$	
GCB	1,768 c ± 0,232	0,388 a ± 0,264	$0,187 \text{ a} \pm 0,092$	0,083 a ± 0,091	

Note: The numbers followed by the same letter indicate that the treatment is not significantly different based on the DMRT test at a level of 0.05%.

GTB: Ganoderma lucidum from Tasikmalaya on Baglog

GBB: Ganoderma lucidum from Banyumas on Baglog GCB: Ganoderma lucidum from Cianjur on Baglog

Table 2. The average temperature and humidity for growth mycelium

	at 09.00 am		at 15.00 pm	
Week	Average Tempetarure (°C)	Average Humidity (%)	Average Tempetarure (°C)	Average Humidity (%)
1	28	86	27	87
2	29	87	28	84
3	28	85	29	87
4	27	84	28	88

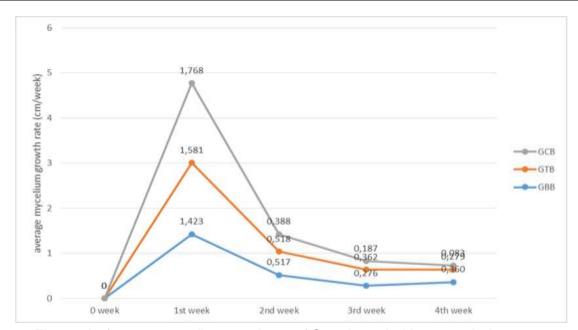


Figure 2. The result of average mycelium growth rate of Ganoderma lucidum some isolates.

Note: The numbers followed by the same letter indicate that the treatment is not significantly different based on the DMRT test at a level of 0.05%.

GTB: Ganoderma lucidum from Tasikmalaya on Baglog GBB: Ganoderma lucidum from Banyumas on Baglog



Figure 3. Primordia growth of some Ganoderma lucidum isolates Note: GTB: Ganoderma lucidum from Tasikmalaya on Baglog

GBB : Ganoderma lucidum from Banyumas on Baglog GCB : Ganoderma lucidum from Cianjur on Baglog

The difference in mycelium diameter and mycelium growth rate is possible due to genetic factors of the type of isolate that affect the metabolic ability to process the nutrients used. One of the nutritional needs in the medium is the cellulose content found in the sawdust used. The mechanism of cellulose degradation begins when the mycelium is in direct contact with cellulose, so that the mycelium is induced to produce cellulase enzymes (Celobiohydrolase (C1), Endogluconase, and -glucosidase) into the growing medium. The first enzyme to work is endogluconase. The enzyme breaks down the cellulose molecule, thereby paving the way for the enzyme cellobiohydrolase to further hydrolyze it. Both reactions will produce cellobiose. The next process glucosidase will decompose cellobiose into glucose (Enari, 1983). During the degradation process, glucose will be used by the fungus as an energy source. Glucose will be broken down into pyruvic acid. The breakdown of glucose into pyruvic acid in two ways, namely hexose monophosphate (HMP) and hexose diphosphate (HDP). The pyruvic acid produced from the HDP system will be oxidized with the help of decarboxylase enzymes, producing acetyl Co-A and CO2. Acetyl Co-A will then enter the Krebs cycle so that it will produce CO2, H2O and ATP. ATP is used energy source as an

metabolism. (Achmadi, Jayadi, & Panji, 2002)

Based on the results of research that has been carried out for 4 weeks, it shows that the mycelium growth of the fungus G. lucidum on baglog medium both from Cianjur, Tasikmalaya, and Banyumas has advantages disadvantages its growth. in difference in the mycelium growth of the G. lucidum fungus was caused by several factors including the suspected nutritional needs, competitive ability, and antibiosis between bacterial isolates derived from the G. lucidum. This is following the statement of Ratnasari et (2014)wherein the test the antagonism was caused by the need for nutrients in the growth media. The nutrients contained in the media include protein carbohydrates, amino acids, minerals, and microelements such as phosphorus. magnesium, potassium to inhibit the germination of pathogenic fungal spores. Another opinion explains that the most important factors that determine the activity of antagonistic microorganisms are having a high growth rate to compete in terms of food and space control to suppress the growth of pathogenic fungi. It was further explained that the factors that influence the growth and development of fungi include: nutrients including sugars, polysaccharides, organic acids, lipids, nitrates, ammonia, amino acids,

polypeptides, and proteins as nitrogen sources: hydrogen, oxygen, sulfur., phosphorus, magnesium, potassium (Nasution, Periadnadi, & Nurmiati, 2017). Elements C, H, and O are three important elements that are available in organic components. The main function of nutrition is as an energy source, cell-forming material, and electron acceptor in action to produce energy (Uruilal, Kalay, Kaya, & Siregar, 2018)

The difference in the results of growth on the mycelium is possible differences in the absorption of nutrients the form of ions and simple molecules, the nutrients needed for mycelium growth are already contained in baglog. This is in line with the opinion of (Gandjar, I., 2006) which states that the growth of mycelium with a ratio of sawdust and bran 50:50 has the fastest growth, this is because this composition is the best composition for the growth of Ganoderma sp. 27.01%), P content (0.69%), K content (1.92%), and N (0.65%) required for G lucidum growth in addition to the addition of sawdust as a contributor to element C.

According to (Dabamona, May, & Worabai, 2019) the ability of bacteria to inhibit the development of the mycelium of pathogenic fungi may be related to the presence of enzymatic activity bacteria, hydraulic produced by enzymes produced by bacteria can degrade the cell walls of pathogenic fungi. Another opinion explains that the ability of bacteria as biological agents is related to their ability to compete for food substances, producing secondary metabolites such antibiotics. as extracellular siderophores, and enzymes (Habazar, 2006)

The growth of mycelium in Ganoderma sp gives varied production. The addition of nutrients in the form of a solution resulted in the fungus growing media being less good which was marked by a change in the color of the media to blackish brown. Baglog that is exposed to water, let alone water enters baglog, makes the the media contaminated. That fungal cells need six-chain carbon (C6) for growth. The carbon needs have been met by processing sawdust and bran which have become the main ingredients of mushrooms (Sugianto, 2010). According to the journal (Riyati, 2002) the provision of nutrients with a ratio to a certain level will be able to supply nutrients. but The increasing administration resulted in a decrease in the total lignocellulose content required by fungal growth. The resulting wet weight tends to be better and more efficient the if nutrients in mushrooms combined are not (Shifriyah, Afina, Kaswan badami, 2014). In addition to the media, the growth of mycelium is also influenced by the quality of the seeds. This is related to the ability of seeds to decompose compounds complex into simple compounds (Maulidina, Eko, & Nawawi, 2015). The growing medium supports the growth of mycelium well can also produce reproductive growth (formation of fruiting bodies) of fungi as well. Mycelium growth is influenced by organic compounds available in the growing medium. The initial conditions the appropriate medium of accelerate the growth of the mycelium.

According to (Tjokrokusumo, Hendritomo, & Widyastuti, 2004) element C is mostly used as a source of energy as well as growth, while nitrogen is required for growth through the synthesis of proteins, purines and pyrimidines. According to (Chang, S. T., 1989) a high C content in the growing medium is needed for fungi to grow mycelium (somatic), while high N is needed for fruiting body development (reproductive). According to (Leatham, G. F., 1989), excessive nitrogen content can also inhibit fungal growth.

The basic factors that become the main problem in the cultivation and maintenance of mushrooms are raw materials as planting medium. (Moerdiati, E., R. B. Ainurrasyid, 1999). The raw materials used determine the C/N ratio, mineral and vitamin content which have a very large influence as a

source of nutrition for fungal growth. The C/N ratio is the ratio between the C and N content in the medium. The C/N ratio is generally expressed as an important chemical factor that determines the rate of decomposition and mineralization of N organic matter.

The cause of mycelium growth in *G lucidum* fungus is not optimal due to the density of baglog, this is in line with the opinion of (Sa'adah, Nafwa, & Purnomo, 2016) which states that during the compaction process, baglog with a medium composition that is too dense so that it can inhibit the growth of mycelium.

The chemical composition and physiological features of the wood material play an important role in the of Ganoderma success spp. colonisation and degradation ability (Baietto & Dan Wilson, 2010) carried out several wood decaying tests using nine tree species and found higher decay rates of G. lucidum when hardwood blocks were used. The same occurred with other Ganoderma spp. (Adaskaveg, Gilbertson, & Blanchette, 1990). Despite the fact that the mycelial growth rate does not reflect the wood decay ability of fungi, the differences observed in this study could be justifed by the chemical and physiological characteristics of the wood used. Differences in the fungal growth response to the wood species could be due to the extensive genetic variability between and within populations of strains (Urbanelli, S., Rosa, V. D., Fanelli, C., Fabbri & M, 2003). For instance, (Wymelenberg et al., 2011) found that gene expression patterns of white rot and brown rot fungi (Phanerochaete chrysosporium and Postia placenta, respectively) were signifcantly influenced by hardwood (Populus grandidentata) and softwood (Pinus strobus) substrates. Understanding the genetic information of G. lucidum strains is essential to select the most suitable for commercial production.

The growth of *G. lucidum* is influenced by the location of growth, the

temperature determines the growth of the mycelium as well as the fruiting body (Bijalwan et al., 2021). Optimal temperature for mycelium growth for both fungal strains, P. ostreatus and G. lucidum was obtained at 22 °C(Fletcher, Freer, Ahmed, & Fitzgerald, 2019), and the growth of the G. lucidum from wild sources, shows varied growth, it is also possible to match the potential content of the drug produced. As the results of research by (Noverita, Sinaga, & Setia, 2017) namely from the number of macrofungi found in potential research as a medicinal ingredient more than with potential as food ingredients. existence of this difference is closely related to the substrate for growth and body environment of the macrofungi. In Mushrooms that have the general, potential as medicinal ingredients generally can grow on substrates wider, and even able to grow on extreme environmental conditions for macrofungi in general. It can seen from the index of mushroom diversity potential as an ingredient in this drug compared to the potential as food ingredients at each location research always dominates.

### CONCLUSION

Based on the results, the conclusion of this study is that *Ganoderma lucidum* isolates from Tasikmalaya, Cianjur and Banyumas affect the diameter and growth rate of mycelium vegetatively.

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