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Short Communications

Yield performance of *Ganoderma lucidum* (Fr.) Karst cultivation on substrates containing different protein and carbohydrate sources

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This study was conducted to investigate the effects of different dosages of molasses as a carbohydrate source and corn-gluten meal as a protein source on the yield of *Ganoderma lucidum* and to improve yield on solid state medium. Three dosages (1, 2 and 3%) of gluten meal or molasses were added to sawdust and bran based medium. Results showed that yield was increased significantly when 1% molasses and gluten meal were added to substrate media. Molasses was found to be the best supplement as a carbohydrate source compared to gluten which is rich in protein.

Key words: *Ganoderma lucidum*, Reishi, cultivation, molasses, gluten, substrate, yield

INTRODUCTION

Ganoderma lucidum (Fr.) Karst (Polyporaceae) is a spe-cies of basidiomycetes which belongs to Polyporaceae (or Ganodermaceae) of Aphyllophorales. Its fruiting body is called “Reishi” in Japanese and “Lingzhi” in Chinese (Yang and Liau, 1998; Wagner et al., 2003). *Ganoderma lucidum,* one of the most famous traditional Chinese medicinal herbs, is used as a healthy food and medicine in Far East for more than 2000 years (Fang and Zhong, 2002). Lingzhi or Reishi contains various chemical substances, including more than 119 different types of triterpenes and several types of polysaccharides (Hsieh, and Yang, 2004).

Traditionally solid cultivation technique of *G. lucidum* takes at least several months until fruit bodies are developed. This culture technique is used to obtain basi-diocarp which is used to make tonic or tea. *Ganoderma* spp. have normally been cultivated in solid substrates such as grain or other lignocellulosic materials such as straw, sawdust and supplements (Riu et al., 1997; Stamets, 2000). The advantage of SSF (solid state fer-mentation) over other techniques is that a concentrated product can be obtained from a cheap substrate, such as an agricultural residue with little pretreatment or enrichment (Wagner et al., 2003) . Supplements such as sucrose, wheat and rice bran are generally added to the mix (Chen, 1998). Gonzalez-Matute et al. (2002) reported that sunflower seed hull can be used as main energy and nutritional sources in the formulation of a substrate for cultivation of *G. lucidum* in synthetic logs with an accep-table mushroom production rate, and the addition of 5% malt to sunflower seed hulls were significantly improved the mushroom productivity.

In addition to the complex nutrient source, all workers have added sugar into medium as a major carbon source. Systematic studies to determine the best sugar source have not been conducted. But, only in a study conducted by Tang and Zhong (2002), it was found that biomass productivity was higher on maltose but lactose was the best sugar for both cell growth and production of compenents such as IPS (intracelllar polysaccharide) and ganoderic acid (Wagner et al., 2003).

Molasses has been reported to stimulate growth of many microorganisms. Molasses provides sugar, nitro-gen source and other nutrients that result in better cell growth. The approximate composition of molasses is: 17 - 25% of water, 30-40% of sucrose, 4 - 9% of glucose, 4 - 12% of fructose, 2 - 5% of starch, 7 - 15% ash, 2.5 - 4.5% nitrogen compounds, 0.5 - 4.5% of protein and 1.5 - 6% non-nitrogenous acids with varying amounts of vitamins (Paterrson-Beedle et.al., 2002).

Corn gluten is an excellent nutrient that is moderatelly high in sources of protein (20 - 25%) and in digestible fiber, while low in starch (20%) and in oil. Corn fiber-hot water soluble fraction is sufficent for promoting vegetative

Table.1. Moisture and pH values of treatments.

	Treatments*						
	Cont.	**G 1%**	**G 2%**	**G 3%**	**M 1%**	**M 2%**	**M 3%**
Moisture (%)	64	64	66	68	64	64	64
pH**	6.41	6.35	6.22	6.16	6.31	6.42	6.42

*G: Gluten, M:Molasses, ** After adjusted

Table 2. Yield performance of *G. lucidum* cultivation on substrates containing gluten and molasses**.**

	Treatments						
	Cont.	**G 1%**	**G 2%**	**G 3%**	**M 1%**	**M 2%**	**M 3%**
Yield ($g.kg^{-1}$)	61.83 bc	68.40 ab	61.23 bc	60.90 bc	73.20 a	56.00 c	47.03 d
BE (%)	17.20 bc	19.00 ab	18.00 b	17.90 b	20.37 a	15.53 c	13.07 d

G: Gluten, M:Molasses, *Means followed by the same letter are not significantly different ($p < 0.01$).

mycelial growth of edible mushrooms (Arai et al., 2003). Carbohydrate may play an important role in cell growth and polysaccharide production as a main carbon and energy source for most fungi. While molasses addition promoted higher mycelia growth rate and cell concentration, the polysaccharide production was lower than with glucose (Hsieh et al., 2005).

The aim of the present study was to determine the yield performance of some supplements such as molasses and gluten and its dosages for cultivation on solid-waste product of *G. Lucidum.*

MATER ALS AND METHODS

The strain of *G. lucidum* was collected from the Kandıra/Kocaeli forest in the Marmara region of Turkey and identified as *G. lucidum* by using conventional description method. Culture was prepared on malt extract agar (MEA) medium by tissue culture from the basidiocarp. The mycelium from MEA slant was used for spawn production on wheat grains.

Poplar sawdust and wheat bran were mixed at a ratio of 4:1 based on their dry weight (w/w) (Yang et al., 2003; Smith et al., 2002). This mixture was taken as a control treatment. Other treatments were formulated including the addition of 1, 2 and 3% molasses and corn gluten meal dosages based on substrate dry weigth (w/w). The formulas, their moistures and pH values of treatments were given in Table 1. Moisture contents of treatments were determined before substrate sterilized (Kacar, 1994). pH was measured in 1 : 2.5, soil : water (v/v) suspension. The subtract mediums were mixtured homogeneously. The pH was stabilised by gypsum ($CaSO_4$, $2H_2O$) and $CaCO_3$ at 5.5 - 6.5 (Fang et al., 2002, Smith et al., 2002). pH levels of treatments below from the optimal value were adjusted with $CaCO_3$ (1 - 1.5%) and others which have optimal pH value were treated with 4:1 (w/w) gypsum:$CaCO_3$.

Substrates were wetted to increase moisture content approximately to 60 - 70% (Yang et al., 2003; Chen, 2004). The wetted substrates were filled into the polypropylene bags with 1.0 kg per bag. Bags were plugged with cotton plug by using PVC ring and autoclaved at 121^{o}C for 1.5 h. After cooling, sterilized bags were inoculated in the laminar flow cabinet and incubated at 25 ± 2^{o}C without light exposure. Spawn run period completed 12 days later. When the mycelium had colonized on the substrate completely, bags were transferred to cropping room at 30 – 32^{o}C, 85 - 90% relative humidity (RH) with a 10 h light exposure for the formation of fruting bodies. Bags were opened and water was sprinkled twice in a day on the bags.

The cap formation of *G. lucidum* initiated in 2 - 3 days after opening the bags. Fruiting bodies were harvested according to Royse (1996), when the caps become completely red and the white margin disappeared. Total yield ($g.kg^{-1}$) was obtained from two flushes in a harvesting period of 45 days. The biological efficiency (BE) percentage ([fresh weight of harvested mushrooms/dry matter content of the subtrate] x 100) was calculated according to Royse (1985).

Experimental design was a Completely Randomized Block with three replicates. Each block was placed with three plastic bags containing total of 3 kg substract. The data were analyzed using the analysis of variance (ANOVA), and group means were compared by Duncan Multiple Range Test (DMR) using the MINITAB program.

RESULTS AND D SCUSS ON

The effect of different dosages of molasses and corn gluten meal on yield performance of *G. lucidum* was shown in Table 2. Results revealed that only 1% molasses and corn gluten meal dosages sigificantly affected on the yield and BE ($p < 0.01$). The highest yield was obtained by in substrates added with 1% molasses and gluten meal than that of other dosages. However, the yield of mushroom decreased with increasing dosages of molasses and glutens.

The application of 1% molasses provided by the highest yield with 73.20 $g.kg^{-1}$ and BE 20.37% compared to control and other treatments. This result was 11.37 g kg^{-1} more than the control treatment. Increasing the dosage of molasses was negatively effected on the yield and BE.

These findings confirms with some authors who reported that molasses has stimulative growth effect and when

the 1% of various sugars were added to PDA plates, the highest mycelial growth was found on the plate with molasses addition (Paterrson-Beedle et.al., 2000; Hsieh et al., 2005).

Similarly 1% molasses application, 1% gluten treatment (yield; 68.40 $g.kg^{-1}$ and BE;19.00%) was obtained the same results. The 1% dosage of corn gluten meal as a good protein source gave the best yield and BE among the three dosages of gluten meal. On the other hand, while the treatment of 1% dosage of gluten gave higher yield than the control treatment and other gluten dosages, the yield of mushroom decreased with increasing dosages of gluten meal. This inverse relationship means that the *G. lucidum* does not need high dosage of protein in the growing medium.

When molasses compared with gluten, 1% molasses more effective on yield and BE than 1% gluten meal. On the other hand, significant decreases in the yield at 2 and 3% dosages of molasses were obtained while the negative effect of the same dosages of gluten were only slightly lower than control. It is possible that the addition of gluten as a protein source was not effective on yield compared with carbohydrate sources.

This is the first study about the effect of gluten and its dosages on *G. lucidum* cultivation. Most of the studies have focused on carbohydrate sources such as malt, glucose in the formulation of submerged media in obtaining mycelial biomass. Some scientists have compared media containing complex carbon and nitrogen sources such as yeast extract, peptone or malt extract with completely defined media, containing only a single carbon source, a single nitrogen sourge and salts (Wagner et al., 2003). Gonzales-Matute et al., (2002) reported that sun flower seed hull can be used as a main energy source and addition of 5% malt to the substrate improved mushroom growth rate.

Conclusions

Gluten meal's starch content is low and protein content is high, although, molasses has high carbohydrates such as sucrose, glucose and fructose. It seems that the carbon source is more necessary as an energy source than protein sources for fruiting formation. It can be concluded from this study's result that molasses can be used as a main energy and nutritional source in the formulation of a substrate for cultivating *G. lucidum* in synthetic logs.

According to Yang et al. (2003), high levels of supplementation should result in higher yields. The effect of corn gluten meal and molasses combination on yield of *G. lucidum* has not been investigated before. Further studies should be needed for this matter.

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