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Original Article

Effects of palm oil sludge as a supplement on *Ganoderma lucidum* (Fr.) Karst. cultivation

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Abstract

The objective of this study was to determine the optimum rate of palm oil sludge for *Ganoderma* mushroom (*Ganoderma lucidum* (Fr.) Karst) cultivation. Different concentrations of palm oil sludge (5-20% by dry weight) were mixed with rubber sawdust and used to grow the *Ganoderma* mushroom in plastic bags. Growth rate of the mycelia, number of basidiocarp, cap diameter, thickness of pileus and yield were compared with the control (not supplemented) and with 5% rice bran (standard formula). The results showed that use of 5% palm oil sludge was optimal for *Ganoderma* cultivation. Yield when grown on 850 g/bag of rubber sawdust with 5% palm oil sludge, 2% $Ca(OH)_2$ and 0.2% $Mg(SO_4)$ was 74.82 g/bag (B.E.=22.01%), which does not differ significantly from that with 5% rice bran in the sawdust (66.31 g/bag, B.E.=19.50%).

Keywords: biological efficiency, lingzhi, mushroom cultivation, formula, waste

1. Introduction

Ganoderma lucidum (Fr.) Karst. is a basidiomycetes mushroom belonging to the family Polyporaceae. In Thailand and around the world, it is called by its Chinese names that are Lingzhi, or more commonly Reishi. There is a bioactive ingredient in the reishi fruiting body that has been known in China since ancient times to be medically active. It is alleged to have several therapeutic effects such as anti-tumor, anti-inflammatory, anti-bacterial and anti-parasitic effects (Hsieh, Hsu, & Yang, 2005; Siew, Chung, & Poh, 2011). *G. lucidum* has been used widely in alternative medicine, such as naturopathy, to treat various human diseases.

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Successful cultivation of *G. lucidum* on substrates such as sawdust or solid pieces of wood has been reported. Lignocellulosic materials such as rubber sawdust are low in protein, and are therefore insufficient to commercially cultivate mushrooms. Therefore, the substrate materials require various supplements or additives for sufficient amounts of nitrogen, phosphorus, potassium and vitamins, for stimulating growth and improving the yield of mushrooms. Various supplements have been investigated for *G. lucidum* cultivation, such as wheat bran, rice bran, corn flour, gram flour, rice husks, coconut fiber, peanut hulls, sorghum, sugarcane bagasse, sucrose, molasses, gluten meal, and tea waste, as additives in the substrate to improve *Ganoderma* cultivation (Bernabé-González *et al.*, 2015; Chen, 1998; Erkel, 2009a, 2009b; Gurung, Budhathoki, & Parajuli, 2012; Paterson-Beedle, Kennedy, Melo, Lloyd, & Medeiros, 2002; Peksen & Yakupoglu, 2009; Triratana, Thaithatgoon, &

Gaugla, 1991). Particularly rice bran and wheat bran provide nitrogen for enhanced growth of primordia of mushrooms (Choi, 2004).

Palm oil sludge (POS), including decanter cake type, is a by-product from the dehydration of palm oil mill effluent (POME). During its processing, most solids in the effluent are decanted in a decanter and passed on to a filter press, before drying in a rotary drier (Devendra, Yeong, & Ong, 1981). Regarding its nutrients, palm oil sludge contains 45.00% carbon, 2.18% nitrogen, 1.40% phosphorus, 2.55% potassium, 0.74% calcium, 14.10% protein, 10.52% fat, 12.30% fiber and 14.47% ash (Kananam, Suksaroj, & Suksaroj, 2011). Presently, palm oil sludge is used as a fertilizer, soil cover material in oil palm plantations, or in biogas production (Chavalparit, Rulken, Mol, & Khaodhair, 2006; Paepatung, Nophatatana, & Songkasiri, 2009) or in animal feed (Seephueak, Ngampongsai, & Chanjula, 2011).

Palm oil sludge has a high nutrient content that might make it a useful supplement in mushroom cultivation. Moreover, the beneficial use of agro-industrial wastes, such as of palm oil sludge in mushroom cultivation, is good for sustainable farming (Oei, 2005; Seephueak, Phadungmas, Kaewmano, & Seephueak, 2014; Seephueak, Preecha, & Seephueak, 2016). This research aimed to study the efficacy of palm oil sludge as a supplement when culturing mushrooms (*G. lucidum*) in plastic bags.

2. Materials and Methods

2.1 Culture and its maintenance

The culture of *G. lucidum* was prepared by tissue culture technique in a laboratory (Seephueak *et al.*, 2016). The culture was grown on potato dextrose agar media (PDA) and maintained on slants at 10°C for further studies.

2.2 Cultivation technique

Sorghum (*Sorghum bicolor*) grain spawn was prepared using a standard method (Seephueak *et al.*, 2016). The palm oil sludge used as a supplement was collected from a palm oil mill belonging to Univanich Palm Oil Public Company Limited, Krabi, Thailand. The samples were sun dried and smashed. Nutrient contents of the palm oil sludge and other substrate components were analyzed at the Scientific Equipment Center, Prince of Songkla University, Hat Yai, Songkhla, Thailand. The nitrogen content was determined by the micro-Kjeldahal method. Phosphorus content was determined using a spectrophotometer. Potassium content was determined by flame photometry. Calcium and magnesium contents were determined by atomic absorption spectrophotometry (Association of Official Analytical Chemists, 1998).

Rubber sawdust was used as the main substrate for cultivation of *G. lucidum*, on a dry weight basis mixed with 2% $Ca(OH)_2$ and 0.2% $Mg(SO_4)$ w/w. The alternative substrates were packed in plastic bags (850 g/bag) and the openings of the bag were plugged with cotton and secured with plastic rings. The bags were sterilized in an autoclave at 121 °C for 30 minutes, and after cooling they were inoculated with 15 grains of spawn. The inoculated substrate bags were kept at room temperature (28-32 °C). After they were fully colonized with mycelia, the bags were exposed in the growth room by opening the top of the plastic bags.

The alternative substrates compared were:
Treatment 1: sawdust + 5% palm oil sludge
Treatment 2: sawdust + 8% palm oil sludge
Treatment 3: sawdust + 10% palm oil sludge
Treatment 4: sawdust + 15% palm oil sludge
Treatment 5: sawdust + 20% palm oil sludge
Treatment 6: sawdust + 5% rice bran
Treatment 7: sawdust (control)

2.3 The experimental design

The experimental design was a Completely Randomized Design (CRD) with 4 replications. Ten bags were done for each replication. The following data were collected; number of days for full colonization by the mycelia, number of basidiocarp, thickness of pileus, cap diameter, and yield. Basidiocarps were harvested when mature (45 days/flush). The data were analyzed according to standard methods, and means were compared using Duncan's multiple range test. Biological efficiency (B.E.) was calculated as follows:

$$\text{Biological efficiency or B.E. (\%)} = \frac{\text{Weight of fresh mushroom basidiocarps}}{\text{Weight of dry substrate}} \times 100$$

3. Results and Discussion

3.1 Nutrient content in palm oil sludge and substrates

It was expected that palm oil sludge has a high nutrient content beneficial as a supplement in mushroom culture, because especially materials rich in nitrogenous materials give high mushroom yields and fast mycelial growth. Nitrogen, phosphorus and potassium contents in the palm oil sludge were 2.20%, 0.25% and 0.22 %, respectively (Table 1). The ratio of carbon to nitrogen (C:N) plays an important role in spawn running, while the nitrogen content is also a main factor for developing fruiting bodies on mushroom mycelia. Erkel (2009a) reported that artificial application of a nitrogen source during the mycelial growth stage gave very good results regarding early primordial imitation. Choi (2004) suggested that if cotton waste is chosen as the main substrate material for oyster mushroom cultivation, a nitrogen source such as rice bran should be added. Bayer and Wilkinson (2002) found a correlation between substrate ammonia content and subsequent growth of mushrooms (Alam, Amin, Khair, & Lee, 2010; Pokhrel, Kalyan, Budathoki, & Yadav, 2013).

Nitrogen, phosphorus and potassium are chemical species important to have in the substrate for mushroom cultivation. N, P and K contents of sawdust supplemented with palm oil sludge varied in the ranges 0.53%-0.95%, 0.02 %-0.04% and 0.26%-0.53%, respectively, and increased with the proportion of palm oil sludge in the mixture. On using 5% rice bran the N, P and K contents were 0.54%, 0.05% and 0.22%, respectively.

C:N ratio in the substrate decreased with the amount of palm oil sludge and ranged from 83.87 to 45.21. The C:N ratio influenced the time to beginning of fruiting and the

Table 1. The nutrient contents (%) in palm oil sludge, sawdust, sawdust supplemented with various percentages of palm oil sludge, and supplemented with 5% rice bran.

Substrate	Nutrient content (%)					
	C	N	P	K	Ca	C/N ratio
1. palm oil sludge	48.00	2.20	0.25	0.22	1.64	21.82
2. sawdust	44.10	0.39	0.01	0.20	1.28	113.07
3. sawdust + 5% palm oil sludge	44.45	0.53	0.02	0.26	1.35	83.87
4. sawdust + 8% palm oil sludge	44.10	0.54	0.02	0.31	1.38	81.67
5. sawdust + 10% palm oil sludge	43.83	0.55	0.03	0.38	1.54	79.83
6. sawdust + 15% palm oil sludge	43.35	0.89	0.04	0.52	1.63	48.35
7. sawdust + 20% palm oil sludge	43.21	0.95	0.04	0.53	1.65	45.21
8. sawdust + 5% rice bran	43.94	0.54	0.05	0.22	1.60	81.3 7

Table 2. Number of days it took for full colonization by mycelia of *G. lucidum* on sawdust supplemented with various percentages of palm oil sludge and with 5 % rice bran.

Substrate (850 g/bag)	No. of days for full colonization of mycelia
1. sawdust + 5% palm oil sludge	45.68 ± 2.109 cd
2. sawdust + 8% palm oil sludge	45.68 ± 1.826 cd
3. sawdust + 10% palm oil sludge	45.73 ± 2.251 bc
4. sawdust + 15% palm oil sludge	42.85 ± 0.933 d
5. sawdust + 20% palm oil sludge	45.25 ± 0.866 cd
6. sawdust + 5% rice bran	47.63 ± 1.090 cd
7. sawdust (control)	54.69 ± 3.469 a
CV (%)	4.20
Significant difference	**

Means within a column followed by the same letter are not significantly different (P>0.01) according to DMRT.

mushroom yield on synthetic media in the case of *G. lucidum* (Alam *et al.*, 2010). In many compost systems, N supplementation can be used to increase mushroom yields by adjusting the C:N ratio to between 80:1 and 10:1. In the solid substrate cultivation of *G. lucidum*, the C:N ratio is a crucial factor affecting the growth rate of mycelia and also the formation of fruiting bodies (Alam *et al.*, 2010). Calcium content of the substrates varied within 1.35% - 1.65% for substrates with palm oil sludge supplement and was 1.60% when 5% rice bran was added. When no supplement was used, the nutrient contents of N, P, K and Ca were the lowest at 0.39%, 0.01%, 0.20% and 1.28%, respectively, and the C:N ratio was 113.07. Therefore, this experiment was based on the hypothesis that palm oil sludge contains nutrients suitable for mushroom cultivation, as indicated by previous studies using palm oil sludge as a supplement similarly for *Pleurotus pulmonarius* and *Auricularia polytricha* cultivation (Seephueak *et al.*, 2014, 2016).

3.2 Days of mycelial growth

The delay until full colonization by spawn on the substrates had statistically significant differences between the treatments (P<0.01). The periods are shown in Table 2. The case in which *G. lucidum* substrate was rubber sawdust supplemented with 15% palm oil sludge gave the fastest mycelial growth. The growth period was 42.85 days, followed by rubber sawdust supplemented with palm oil sludge at 20%, 5%, 8% and 10% in this order, giving 45.25, 45.68, 45.68 and 45.73 days, respectively, without statistically significant difference to the 47.63 days with 5% rice bran. Non-supplemented substrate gave the slowest mycelial growth taking 54.69 days.

The results indicate 15% palm oil sludge supplement in the sawdust was the most effective for mycelium development. The average time for full colonization by *G. lucidum* was 42.85 days, while with 5% rice bran (standard formula) the average time was 47.63 days. A previous study of Seephueak *et al.* (2014, 2016) reported that 5% or 8% of palm oil sludge was suitable for the mycelial growth of phoenix mushroom (*P. pulmonarius*) and for jew's ear (*Auricularia polytricha* Mont Sacc.) cultivation. The delay for full colonization by the mycelia of jew's ear was 39.38 days, and this was significantly different (P<0.01) from the average with 5 % rice bran or with non-supplemented sawdust, for which the delays were 45.80 and 47.28 days. The delay to full colonization by mycelia of phoenix was 20.00 days, and this was significantly different (P<0.001) from the cases with 5% rice bran or no supplement, with 24.48 and 27.55 days respective delays.

Mycelial growth is a preliminary step that creates suitable conditions for the fruiting bodies (Pokhrel, Yadav, & Ohga, 2009). In the experiments, among the seven alternative substrates for cultivating *G. lucidum*, 15% palm oil sludge gave the fastest mycelial growth. However, the obtained yields were in the reverse order and 5% palm oil sludge resulted in the highest yield. Liang, Wu, and Wang (2005); Noonsong *et al.* (2016); Obodai, Cleland-Okine, and Vowotor. (2003); and Seephueak *et al.* (2014, 2016) reported that the period for spawn to fully colonize the substrate does not relate well to mushroom number and yield. The results indicate that palm oil sludge supplement may have excessive nitrogen and fat contents, which can adversely affect the number and yield of the fruiting bodies (Fakoya, Adjumo, & Akinyele, 2014; Noonsong *et al.*, 2016). Moreover, Lelley and Janssen (1993) mentioned that high doses of N rich supplements cause temperature increases sufficient to kill the mycelia. According to Heleno *et al.* (2012); Regina (2001); and Rolim, Sales-Compos, Cavalcanti, and Urben (2014) excess nitrogen tends to disable the degradation of lignin, retarding or even inhibiting the mycelial growth, and lessening the production of basidiocarp. So, the nutrient composition of the supplement is one of the most important factors limiting saprophic colonization by cultivated mushrooms, and particularly the fruiting of *G. lucidum.*

3.3 Number of basidiocarps and yield

The number of basidiocarps of *G. lucidum* on 850 g substrate in a plastic bag at the 4^{th} flush harvesting is shown in Table 3, with significant differences between the treatments indicated (P<0.01). From the first flush to the 3^{rd} flush there were significant differences between supplemented cases and no use of supplement. However, in the 4th flush the number of basidiocarps showed no significant differences. The *G. lucidum* on rubber sawdust supplemented with palm oil sludge gave 4.52-5.04 basidiocarps/bag, which does not significantly differ from the case of sawdust with 5% rice bran giving 5.12 basidiocarps/bag. Non-supplemented sawdust had the lowest average number of basidiocarps at 1.78 basidiocarps/bag (Table 3).

3.3.1 Diameter of pileus

The average diameters of pileus were significantly different (P<0.01) between cases with supplement and that without. The diameter of pileus tended to decrease between flushes. The average diameter of pileus obtained on rubber sawdust supplemented with 15% palm oil sludge was 5.84 cm, which was not significantly different from the case of 5% rice bran in sawdust substrate (5.03 cm). The smallest diameter of pileus was for non-supplemented sawdust at 1.34 cm (Table 4).

3.3.2 Thickness of pileus

The average thickness of pileus was the highest at 1.10 cm./basidiocarp on non-supplemented substrate, followed by the case of rubber sawdust supplemented with 5% rice bran at 1.04 cm./basidiocarp. Using 5-20% palm oil sludge resulted in 0.89-0.97 cm./basidiocarp, without significant differences (Table 5).

3.3.3 Yield

The highest yield was 74.82 g/ bag (B.E.=22.01%) obtained on rubber sawdust supplemented with 5% palm oil sludge, followed by 8%, 10%, 15% and 20% of palm oil sludge supplement with the yields 74.75 g, 71.88 g, 69.44 g and 65.06 g/bag, respectively. The biological efficiencies were 21.99%, 21.14%, 20.42% and 19.14%, in the same order. No significant differences were found from 5% rice bran sawdust with 66.31 g/bag yield and 19.50% biological efficiency. The lowest yield was with the non-supplemented sawdust at 33.37 g/bag (B.E. =9.81%) (Table 6).

The highest yield was obtained on substrate supplemented with 5% palm oil sludge while the other 8-20% dose levels were inferior. The yield of mushroom tended to decrease with palm oil sludge proportion over the range tested. The dosage of palm oil sludge correlated negatively with both yield and B.E. According to Erkel (2009b), the highest yield was obtained with 1% molasses while higher dosages performed worse. This inverse relationship means that *G. lucidum* does not need a high dosage of protein and fat for growth.

Table 3. Number of basidiocarps of *G. lucidum* obtained on sawdust supplemented with various concentrations of palm oil sludge and with 5% rice bran

Substrate (850 g/bag)	No. of basidiocarp/bag				Total
	1st flush	2nd flush	3rd flush	4th flush	
1. sawdust + 5% palm oil sludge	1.50 a	1.23 a	1.00 a	1.00	73.4 a
2. sawdust + 8% palm oil sludge	1.52 a	42.1 a	1.10 a	1.00	04.5 a
3. sawdust + 10% palm oil sludge	47.1 a	1.20 a	1.00 a	1.10	77.4 a
4. sawdust + 15% palm oil sludge	1.50 a	1.27 a	1.00 a	0.75	52.4 a
5. sawdust + 20% palm oil sludge	1.52 a	37.1 a	1.00 a	0.75	64.4 a
6. sawdust + 5% rice bran	1.70 a	1.22 a	1.10 a	1.10	12.5 a
7. sawdust (control)	78.0b	0.50 b	0.50 b	-	78.1 b
CV (%)	60.19	19.22	94.23	53.34	26.26
Significant difference	**	**	**	ns	**

Means within a column followed by same letter are not significantly different (P>0.01) according to DMRT.
- No fruiting body

Table 4. Diameter of pileus of *G. lucidum* obtained of sawdust supplemented with various concentrations of palm oil sludge and with 5% rice bran.

Substrate (850 g/bag)	Diameter of pileus (cm/basidiocarp)				
	1st flush	2nd flush	3rd flush	4th flush	Aver.
1. sawdust + 5% palm oil sludge	7.28 a	5.46 a	5.26 a	3.75	5.44 a
2. sawdust + 8% palm oil sludge	7.18 a	5.48 a	4.93 a	4.23	5.46 a
3. sawdust + 10% palm oil sludge	7.63 a	5.16 a	4.75 ab	3.37	5.23 a
4. sawdust + 15% palm oil sludge	7.26 a	5.88 a	5.86 a	4.36	5.84 a
5. sawdust + 20% palm oil sludge	7.32 a	6.02 a	5.33 a	3.71	5.60 a
6. sawdust + 5% rice bran	6.53 a	5.51 a	5.87 a	4.21	5.03 a
7. sawdust (control)	1.81 b	1.09 b	2.47 b	-	1.34 b
CV (%)	10.76	12.57	23.83	42.60	26.02
Significant Difference	**	**	**	ns	**

Means within a column followed by same letter are not significantly different (P>0.01) according to DMRT.
- No fruiting body

Table 5. Pileus thickness of *G. lucidum* obtained on sawdust supplemented with various concentrations of palm oil sludge and with 5% rice bran.

Substrate (850 g/bag)	Thickness of pileus (mm/bag)				
	1st flush	2nd flush	3rd flush	4th flush	Aver.
1. sawdust + 5 % palm oil sludge	0.95	0.95 b	0.96	0.97	0.96
2. sawdust + 8 % palm oil sludge	0.97	1.06 ab	0.98	0.88	0.97
3. sawdust + 10 % palm oil sludge	0.71	1.00 ab	0.96	0.89	0.89
4. sawdust + 15 % palm oil sludge	0.92	0.91 b	1.00	0.73	0.89
5. sawdust + 20 % palm oil sludge	0.88	0.95 b	0.94	0.86	0.91
6. sawdust + 5 % rice bran	0.90	0.95 b	0.95	1.37	1.04
7. sawdust (control)	1.34	1.34 a	0.62	-	1.10
CV (%)	44.72	24.38	31.81	114.54	38.71
Significant Difference	ns	**	ns	ns	ns

Means within a column followed by same letter are not significantly different (P>0.01) according to DMRT.
- No fruiting body

Table 6. Yield of *G. lucidum* on sawdust supplemented with various concentrations of palm oil sludge or with 5% rice bran.

Substrate (850 g/bag)	Yield (g/bag)				Total	B.E. (%)
	1st flush	2nd flush	3rd flush	4th flush		
1. sawdust + 5% palm oil sludge	30.16	22.72 a	14.25 ab	7.69	74.82 a	22.01 a
2. sawdust + 8% palm oil sludge	28.76	19.57 a	16.63 a	9.79	74.75 a	21.99 a
3. sawdust + 10% palm oil sludge	30.87	22.92 a	11.83 abc	6.26	71.88 a	21.14 a
4. sawdust + 15% palm oil sludge	28.00	22.89 a	12.14 a	6.41	69.44 a	20.42 a
5. sawdust + 20% palm oil sludge	29.46	21.10 a	9.50 bc	5.00	65.06 a	19.14 a
6. sawdust + 5% rice bran	22.76	20.05 a	15.98 ab	7.52	66.31 a	19.50 a
7. sawdust (control)	20.63	6.54 b	6.20 c	-	33.37 b	9.81 b
CV (%)	22.61	17.21	25.43	97.03	54.49	76.84
Significant difference	ns	**	**	ns	**	**

Means within a column followed by same letter are not significantly different (P>0.01) according to DMRT.
- No fruiting body

In the experiments, the number of basidiocarps, the average pileus diameter, thickness of pilei and the yield of *G. lucidum* showed similar trends. In the first flush *G. lucidium* gave high reproduction that tended to decrease as the nutrients in the substrate were consumed, which is common in mushroom cultivation. This clearly shows that using palm oil sludge or rice bran to supplement sawdust was better than having no supplemen, with significant differences (P<0.01). Yield of *G. lucidum* varied widely depending on the kind of sawdust and supplement (Erkel, 2009b; Gurung *et al.*, 2012; Rolim *et al.*, 2014; Roy, Jahan, Das, Munshi, & Noor, 2015). In the results, 5% palm oil sludge was the preferred treatment as substrate mixture for *G. lucidum* cultivation. The present study gave 22.01% average yield, which is higher than those in previous investigations. The yields of *G. lucidum* on substrate supplemented with rice bran reportedly can have 12.89% biological efficiency (Azizi, Tavana, Farsi, & Oroojalian, 2012), or 10.09% (Gurung *et al.*, 2012) or 4.30% (Karma & Bhatt, 2013). The yield on wheat bran supplemented sawdust can have biological efficiency 16.67% (Peksen & Yakupoglu, 2009), or 7.6% (Karma & Bhatt, 2013), 7.6% and 6.8% (Roy *et al.*, 2015), or 7.27 % (Gurung *et al.*, 2012); and on molasses supplemented sawdust it was 20.37% (Erkel, 2009a). However, the yields in this current study were lower than when using 20% tea waste to supplement sawdust, giving 34.90% biological efficiency (Peksen & Yakupoglu, 2009). Tea waste is highly nutritious, and it could be used alone or in combination with other wastes as a substrate for *A. bisporus* (Peker, Baysal, Yigitbasi, Simsek, Colak, & Toker, 2007), *P. sajor-caju* (Dogan & Peksen, 2003), and *G. lucidum* (Yakupoglu, 2007; Peksen & Yakupoglu, 2009). In contrast, palm oil sludge is only suitable for use as a supplement, not as the sole substrate.

There are a few reports about the use of palm oil mill wastes as supplement. Petcharat and Thongwiset (2003) studied the use of oil palm kernel meal as a supplement for *A. polytricha* and *Lentinus squarrosulus*. The results showed that 5% oil palm kernel meal in sawdust was suitable for cultivation of *A. polytricha* (B.E.=114.50%) and 15% oil palm kernel in sawdust was suitable for cultivation of *Lentinus squarrosulus* (B.E.=37.20 %). Petcharat and Thongwiset (2004) studied the abalone mushroom *P. cystidiosus* suggesting that 10% oil palm kernel meal mixed with sawdust was the optimal mixture (B.E.=60.79%). Also, Seephueak *et al.* (2014, 2016) reported that 5% and 20% palm oil sludge in rubber sawdust are suitable for *A. polytricha* and *P. pulmonarius* cultivation. However, oil palm kernel is a more expensive supplement for mushroom cultivation than oil palm sludge. This current study confirmed that palm oil sludge is a competitive alternative supplement in rubber sawdust for the commercial production of *G. lucidum*.

4. Conclusions

The effects of palm oil sludge supplement on growth and yield of *G. lucidum* were investigated in this study. 5% of palm oil sludge was a suitable dose level for *G. lucidum* cultivation. The mushroom yield on rubber sawdust was 74.82 g/bag (B.E.=22.01%), which did not significantly differ (P>0.01) from that with 5% rice bran in the substrate (66.31 g/bag, B.E.=19.50%). The latter is a common substrate formulation, used as reference baseline in this study. Palm oil sludge could be used as an alternative supplement in commercial production of *G. lucidum*. As palm oil sludge is an industrial by-product and is cheaper than other supplements, it could be economically competitive especially in low income countries.

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