International Journal of Agricultural Technology 2019 Vol. 15(1):113-126
Available online http://www.ijat-aatsea.com
ISSN: 2630-0613 (Print) 2630-0192 (Online)

The growth of *Antrodia cinnamomea* mycelia on different kinds of substrates in Thailand

Sornprasert, R.[1*], Aroonsrimorakot, S.[2], Hambananda, A.[1], Kasipar, K.[1], Sukkapan, P.[1] and Saenkamol, P.[3]

[1]Faculty of Science, Chandrakasem Rajabhat University, Bangkok 10900; [2]Faculty of Environment and Resource Studies, Mahidol University, Nakhon Pathom Province 73170; [3]Pho Thong District, Ang Thong Province 14120, Thailand.

Sornprasert, R., Aroonsrimorakot, S., Hambananda, A., Kasipar, K., Sukkapan, P. and Saenkamol, P. (2019). The growth of *Antrodia cinnamomea* mycelia on different kinds of substrates in Thailand. International Journal of Agricultural Technology 15(1):113-126.

Abstract The cultivation of *Antrodia cinnamomea* on the different kinds of substrates was investigated. Firstly, it was cultivated on sawdusts from five sources: Khae na (*Dolichandrone serrulata*), rain tree (*Samanea saman*), mango (*Mangifera indica*), Para rubber tree (*Hevea brasiliensis*) and cinnamon tree (*Cinnamomum verum*). It was found that the mushroom mycelia grew well on mango sawdust: the average initial growth time was 9.40 days and the average time the mycelia fully colonized the sawdust surface was 90.80 days. The mycelia had abundant density and orange color at 70 days, with cinnamon odor at 110 days. Secondly, it was cultivated on the barley (*Hordeum vulgare*), millet grass (*Sorghum* sp.), two varieties of rices (*Oryza sativa*): Sang yod and Hom nin, black sesame (*Sesamum indicum*), job's tears (*Coix* sp.) and mung bean (*Vigna radiata*). It was found that the mycelia grew well on millet grass with initial growth time in 9.86 days and it fully colonized the substrate in 26.14 days. The mycelia had abundant density and orange color in 50 days, with cinnamon odor in 110 days. Finally, the mycelia were cultivated on five liquid media: PD, PMD, PPD, PYD and PMPYD. It revealed that the mycelia grew well on PMPYD. It had abundant density with yellow-orange colors when it was 90 days. The mycelia fully covered the surface of the media in 60.00 days. Its colony was a thick mat and floated on the surface of the liquid media. The average dry weight of the mycelia was 0.528 g/100 ml in 90 days and the pH value of liquid media was increased during the growth of the mycelia. In addition, when the quantities of the bioactive compounds, adenosine and cordycepin, from dried mycelia which cultivated on the Mango sawdust, Millet grass seeds and the PMPYD were analyzed by the HPLC, adenosine was found at 202.23, 25.25 and 81.90 mg/100 g dried weight mycelia, respectively. However, 499.69 mg/100 g of cordycepin was found only in mycelia cultivated on Mango sawdust, but not found in dried mycelia cultivated on Millet grass seeds and the PMPYD. It revealed that the mycelia had different quantity nutrients such as protein, fat, carbohydrate, ash and moisture.

Keywords: *Antrodia cinnamomea*, Cultivation of mushroom, Bioactive, Nutritional value

[*]**Coressponding Author:** Sornprasert, R.; **Email:** sornprasert_r@hotmail.com

Introduction

It is well known that many mushrooms can be used as herbs such as *Antrodia cinnamomea*, *Cordyceps sinensis*, *Ganoderma lucidum*, *Phellinus igniarius*, *Polystictus versicolori* and *Poria cocos* (Halpern, 2007), especially *A. cinnamomea* which depends on the timber, *Cinnamomum kanehirae*, a native tree of Taiwan. *A. cinnamomea* is used for treatment against diseases for centuries. The mushroom's mycelia have many important bioactive compounds, polysaccharide, stearoids and triterpenoids which help people for treatment of many symtoms such as immunomodulatory (Kuo *et al*., 2011), diabetes (Cherng *et al*., 1996), antimalarial (Yang *et al*., 1996), exhaustion, hypertension, diarrhea, liver diseases, itchy skin (Geethangili and Tzeng, 2011). Consequently, the demand of *A. cinnamomea* has been increased. In 2005, the market price of *A. cinnamomea* was $10,000/kg and the market value will be $100,000,000/year (Wang *et al*., 2005 and Lu *et al*., 2014). Moreover, Chiu (2007) and United States Patent (2013) had reported the genus *Antrodia* grew well with many type of biomaterials such as *Acacia confuse*, *Cunninghamia osmophloeum*, *C. lanceolata*, *Machilus kusanoi*, *Picea abies*, *Psidium guajava* and *Tsuga canadensis*. Patent Application Publication (2008) had reported the *Antrodia* sp. can grow in cereal grains, namely maize, oats, rye, sorghum, wheat or other cereal grains. Moreover, the *Antrodia* sp. can grow in submerged culture with suitable amount of carbon, nitrogen and other nutrients with the control of environmental conditions such as pH, temperature and light control. (Yang *et al*., 2006).

The specific objectives of this research were to study: 1) the growth of *A. cinnamomea* on the sawdusts, 2) the growth of *A. cinnamomea*on the seeds, 3) the growth of *A. cinnamomea* in the liquid media and 4) the bioactive compounds analysis and the nutritional values of the cultivated *A. cinnamomea* mycelia.

Materials and methods

The growth of A. cinnamomea on the sawdusts

The culture preparation: The culture supplied from the Xin Gao Farm (No. 6-6, Ciansi Rd., Puli Township, Nantou County 545, Taiwan) was transfered on PDA (Potato Dextrose Agar), then incubated in the dark with temperature of 25^{o}C for 30 days. Agar block with the mycelia on the surface were cut by the cork borer with 0.5 cm diameter. (2) The 1,000 ml of liquid spawn contained potatoes extracted with boiled water (potato 200 g, distilled water 1,000 ml) was mixed with glucose 25 g, thiamine 1 g, peptone 5 g,

dipotassium phosphate 1 g, malt extract 3 g, magnesium sulfate 1 g and yeast extract 3 g, then place 100 ml on 320 ml culture bottles. The mixtures were sterilized with an autoclave at a temperature of 121°C for 15 min at 15 psi. The agar blocks were transferred in the liquid media, then the mixtures were shaken at 115 rpm, then incubated in the dark with temperature of 25°C for 30 days. The liquid spawns were blended in the blender for 2 min. (3) The sawdust preparation: The sawdust of Khae na (*Dolichandrone serrulata*), mango (*Mangifera indica*), para rubber tree (*Hevea brasiliensis*), rain tree (*Samanea saman*) and cinnamon tree (*Cinnamomum verum*) were mixed with magnesium sulphate 2 g, calcium carbonate 1,000 g, pumice 1,000 g, yeast extract 1,000 g and rice bran 5,000 g/100 kg of the sawdust, then the moisture were adjusted between 60-70%. Then 100 g of solid media were placed in the 480 ml of cultured bottle, and they were sterilized with a steam boiler at a temperature of 100°C for 3 hr. Finally, 5 different culture media formulas were obtained. (4) The culture transfer and incubation: the 3 ml of liquid spawn were transfered to the culture bottle, then incubated in the dark at 25°C. When the mycelia were fully covered on each formula, 2,000 lux of the fluorescence light was supplied for 8 hr/day. (5) The experimental data were collected: Day of the initial growth, day of the mycelia fully colonized, density, color and odor of the mycelia. (6) Experimental design: Complete Randomized Design (CRD) with five treatments, nine replicates and the mean values were analysed by DMRT (Duncan's New Multiple Test).

The growth of A. cinnamomea on the seeds

The preparation of the culture materials: 20 g of seeds namely; barley (*Hordeum vulgare*), millet grass (*Sorghum bicolor*), 2 varieties of rices (*Oryza sativa*) namely; Sang yod and Hom nin, black sesame (*Sesamum indicum*), job's tears (*Coix* sp.), mung bean (*Vigna radiata*) were placed to 480 ml bottle, soaked with 250 ml of the distilled water for 12 hr, and the water out was drained of the media. Then 20 ml/bottle of the supplementary materials namely; 1,000 ml of potatoes extracted, glucose 25 g, thiamine 1 g, peptone 5 g, dipotassium phosphate 1 g, malt extract 3 g, magnesium sulfate 1 g and yeast extract 3 g were added. They were sterilized with an autoclave at a temperature of 121°C for 15 min at 15 psi. Finally, seven formulas of the seeds culture media were obtained. (2) The culture transfer, the incubation method and the experimental data collection were performed as same as the sawdust's experiments. (3) Experimental design: CRD with seven treatments, nine replicatesand the mean values were analysed by DMRT.

The growth of A. cinnamomea in the liquid media

The preparation of the liquid media; 5 liquid media formulas namely; PD (Potato 200.0 g, Dextrose 20.0 g and Distilled water 1000.0 ml), PDM (Potato 200.0 g, Dextrose 20.0 g, Malt extract 5.0 g and Distilled water 1000.0 ml), PDP (Potato 200.0 g, Dextrose 20.0 g, Peptone 5.0 g and Distilled water 1000.0 ml), PDY (Potato 200.0 g, Dextrose 20.0 g, Yeast extract 5.0 g and Distilled water 1000.0 ml) and PDMPY (Potato 200.0 g, Dextrose 20.0 g, Malt extract 2.5 g, Peptone 2.5 g,Yeast extract 2.5 g and Distilled water 1000.0 ml). Then pH was adjusted to 5.0. The 100 ml of the liquid media were transfered to the 325 ml culture bottles. They were sterilized with an autoclave at a temperature of 121^{o}C for 15 min at 15 psi. (2) 1 ml of the liquid spawn was transfered into the liquid media then incubated at 25^{o}C in the dark place. (3) The experimental data were collected: the color and the mycelia density, the day of mycelia fully colonized covered the liquid media surface, the colony characteristics, the dried weight of mycelia and the pH of the liquid media. (4) Experimental design: CRD with five treatments, nine replicates and the mean values were analysed by DMRT.

The bioactive compounds analysis and the nutritional values of the cultivated A. cinnamomea mycelia

Analysis of the bioactive compounds: the well growth mycelia from the sawdust, grains and liquid media were used for analysis of the bioactive contents namely; adenosine and cordycepin using the modified method of Huang *et al*. (2009) by High Performance Liquid Chromatography (HPLC) (Waters 600 controller model) with Waters 717 plus autosampler, Waters 2996 photodiode array detector with the wave length of 254 nm, the mobile phase of methanol: water (15:85) with 1 ml/min, the 10 µl of sample were injected, the retention time was 15 min and Phenomenex Luna C18(2) with 4.6×150.0 mm column. The adenosine and cordycepin from Sigma-Aldrich were used as standards. (2) The nutrition analysis: protein content was analyzed by the AOAC Official Methods (2012d), fat content was analyzed by the AOAC Official Methods (2012b), carbohydrate content was analyzed by the Laboratory of Central Laboratory Thailand (2013), ash content was analyzed by the AOAC Official Methods of Analysis (2012a) and moisture content was analyzed by the AOAC Official Methods (2012c)

Results

The growth of A. cinnamomea on the sawdusts

The average initial growth time of the mycelia were different among the sawdust formulas with the statistically significant with 95% confidential. The mycelia were fastest growth at 9.40 days on mango sawdust, followed by 14.80 and 1 8 . 8 0 days in the cinnamon tree sawdust and the Khae na sawdust respectively. However, no mycelia growth was found in the rain tree sawdust and the para rubber tree sawdust. The average day of mycelia fully colonized on the sawdust were different among the sawdust formulas with the statistically significant 9 5 % . The mycelia were fastest fully colonized on the mango sawdust at the average of 90.80 days, followed by 100.40 and 111.40 days when grow on the cinnamon tree sawdust and the Khae na sawdust, respectively (Figure 1 and Table 1).

Figure 1. The growth of *A. cinnamomea* mycelia on the Khae na sawdust (A), mango sawdust (B) and cinnamon tree sawdust (C)

The density of mycelia growing on the mango sawdust were abundant (+++) in 70 days. However, in 90 and 70 days, only moderate density (++) was found in mycelia growing on the Khae na sawdust and the cinnamon tree sawdust. The color of mycelia growing on the Khae na sawdust was white (#) in 50 days and then became orange-yellow (×) in 70 days. The mycelia growing on the mango sawdust were orange-yellow in 50 days and then changed to orange (✓) in 70 days. The mycelia growing on the cinnamon tree sawdust were orange-yellow in 70 days and then became orange in 90 days. The odor of mycelia growing on the Khae na sawdust, mango sawdust and cinnamon tree sawdust resembled the cinnamon odor in 110 days (Table 1).

The growth of A. cinnamomea on the seeds

For the mycelia growth on the seeds, the average initial growth dates of the mycelia were different with 95% significant level. The mycelia were growing on the millet grass in 9.86 days, followed by the Sang yod, Hom nin,

barley and job's tear in 11.14, 11.14, 11.86 and 12.29 days, respectively. However, the mycelia were not grow in the bBlack sesame and the mung bean. The mycelia fully colonized the seeds at the different date with 95% significant level. The fastest mycelia fully colonized on the millet grass in 26.14 days, followed by the Sang yod, Hom nin, barley and job's tear in 31.00, 31.57, 37.29 and 41.29 days, respectively (Figure 2 and Table 2).

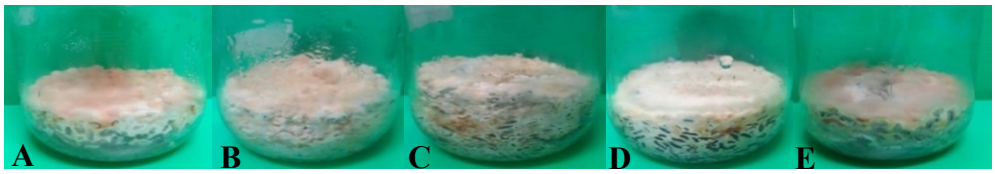

Figure 2. The growth of *A. cinnamomea* mycelia on the barley (A), millet grass (B), Sang yod (C), Hom nin (D) and job's tear (E)

The density of mycelia of *A. cinnamomea* growing with different seeds were observed. The mycelia had abundant density in the millet grass in 50 days and in Sang yod and Hom nin in 90 days, while moderate density was found in the barley and Job's tear in 90 days. The mycelia growing on the barley were orange-yellow in 50 days and then became orange in 70 days. The mycelia growing on Sang yod were orange-yellow in 70 days and then became orange at 90 days. The mycelia growing on Hom nin were yellow-orange (۞) in 50 days and then became orange in 70 days. Finally, The mycelia growing on the millet grass and Job's tear were orange in 50 days. The odor of mycelia was observed in 110 days. The barley, millet grass, Sang yod, Hom nin and job's tear mycelia had cinnamon odor (Table 2).

The growth of A. cinnamomea in the liquid media

The growth of *A. cinnamomea* on five liquid media were observed. The results revealed that the mycelia in all medias were white (#) in 15 days, white-yellow (€) in 30 days, yellow (£) in 45 days and then became yellow-orange in 60 days. The mycelia density in 90 days on the PMPYD had abundant density and medium moderate density when grow on the PD, PMD, PPD and PYD media. The fully surface colonized time of the mycelia on the PD, PPD, PMPYD, PMD and PYD were 6 0 .00, 60.00, 60.00, 60.33 and 75.33 days, respectively. The statistical test of means showed that the PD, PMD, PPD and PMPYD encouraged the mycelia grew faster than the PYD at 95% confidential level. The mycelia colony were mat and floated in 135 days (Table 3 and Figure3).

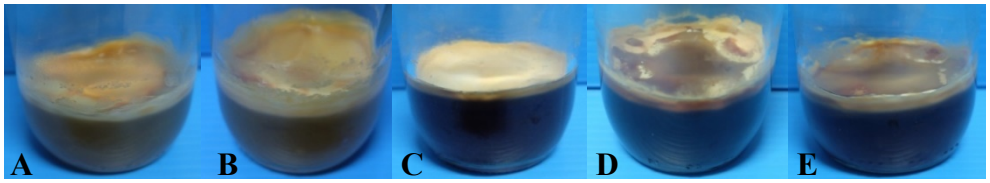

Figure 3. The growth of *A. cinnamomea* mycelia in the PD (A), PMD (B), PPD (C), PYD (D) and PMPYD (E)

The mycelia dried weight on liquid media in 15, 30, 45, 60, 75, 90, 105, 120 and 135 days were observed. The results showed that, since 105 days the PMPYD media gave the highest weight of dried mycelia, followed by the PMD>PD>PPD>PYD. The mycelia dried weight of the PMPYD were 0.048, 0.091, 0.269, 0.367, 0.472, 0.528, 0.540, 0.560, 0.561g/100 ml, respectively, the PMD were 0.062, 0.095, 0.264, 0.350, 0.425, 0.486, 0.506, 0.517, 0.533g/100 ml, the PD were 0.050, 0.093, 0.241, 0.318, 0.379, 0.467, 0.477, 0.483, 0.500 g/100 ml, the PPD were 0.044, 0.070, 0.253, 0.307, 0.426, 0.438, 0.451, 0.479, 0.501g/100 ml and the PYD were 0.037, 0.077, 0.267, 0.337, 0.384, 0.437, 0.440, 0.455, 0.462g/100 ml (Figure 4). The statistical test of means showed that the PMPYD gave the dried weight of mycelia greater than the others with 95% confidential level. The pH in five liquid media in 15, 30, 45, 60, 75, 90, 105, 120 and 135 days were observed. The results showed pH value of the liquid media were increased while the culture time increased: pH 5.23 to 7.00 for the PD, pH 5.13 to 6.91 for the PMD, pH 5.40 to 7.11 for the PPD, pH 5.50 to 7.10 for the PYD and pH 5.36 to 7.18 for the PMPYD (Figure 5).

The bioactive compounds analysis and the nutritional values of the cultivated A. cinnamomea mycelia

The adenosine and cordycepin content were analyzed from the dried mycelia growing on the mango sawdust, millet grass and PMPYD with HPLC. The results revealed that the adenosine was found in the mycelia from the mango sawdust, millet grass and PMPYD at retention time (R_t) 7.12, 7.23, 7.19 (R_t of adenosine standard 7.19 min) with 202.23, 25.25 and 81.90 mg/100g dried weight, respectively. The cordycep was found in the mycelia growing on the mango sawdust at R_t 9.21 (R_t of cordycepin standard 9.25 min) with 499.69 mg/100 g dried weight. Nevertheless, the cordycepin were not found in the millet grass and PMPYD (Figure 6).

Moreover, the protein, fat, carbohydrate, ash and moisture were analyzed. The results showed the mycelia growing on the mango sawdust had highest

protein content, followed by the PMPYD and the millet grass with 32.62, 15.68 and 15.54 g/100 g dried weight, respectively. The millet grass had highest fat content, followed by the PMPYD and the mango sawdust with 6.77, 6.05 and 3.14 g/100 g dried weight, respectively. The mycelia from the PMPYD had highest carbohydrate, followed by the millet grass and the mango sawdust with 63.93, 63.21 and 46.61g/100 g dried weight, respectively. The mycelia from mango sawdust had highest amount of the ash content, the next were the PMPYD and the millet grass with 6.80, 2.41 and 1.86g/100 g dried weight, respectively. The mycelia from the millet grass had highest amount of the moisture content, followed by the PMPYD and the mango sawdust with 12.62, 11.93 and 10.83 g/100 g dried weight, respectively (Table 4).

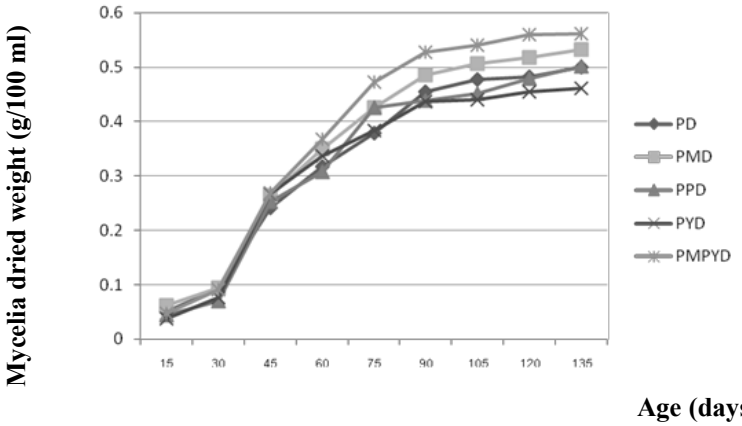

Figure 4. The dried weight of *A. cinnamomea* mycelia in liquid media

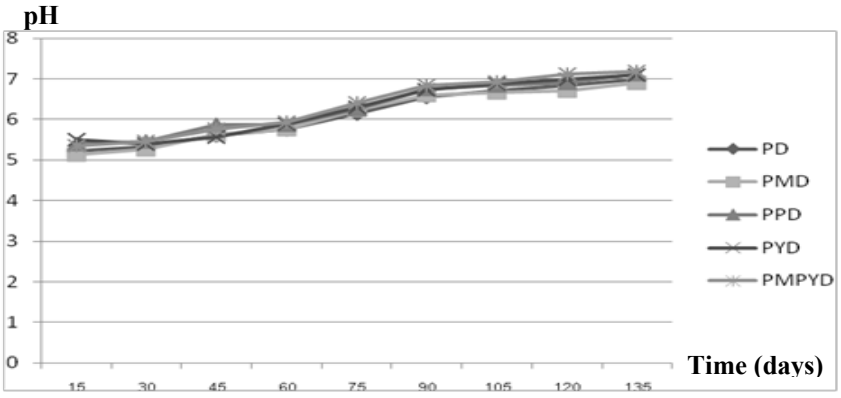

Figure 5. The pH change of liquid media culturing *A. cinnamomea* mycelia

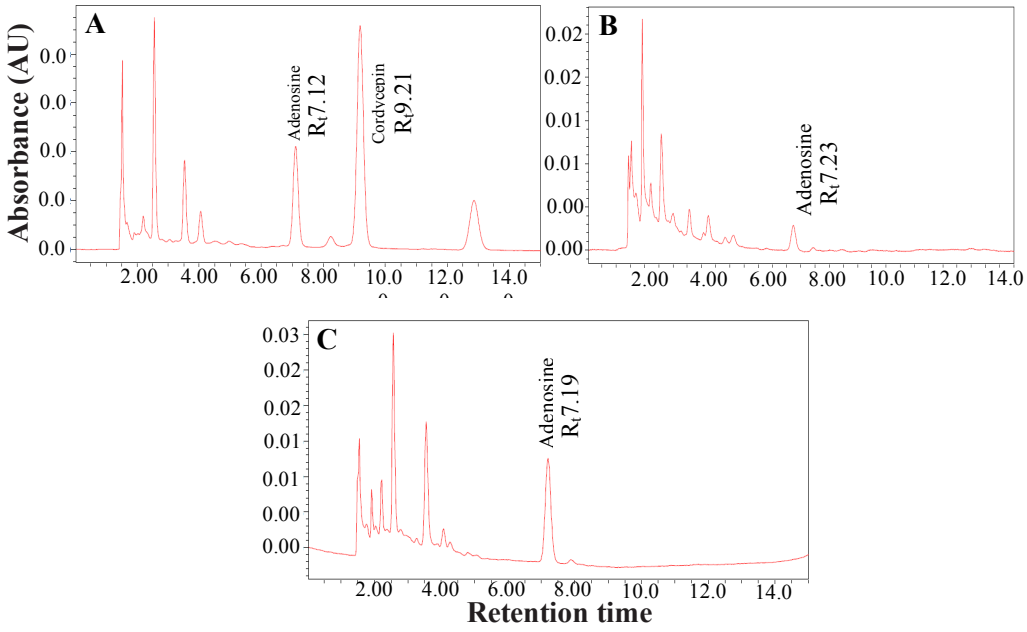

Figure 6. Chromatogram of the adenosine and cordycepin in *A. cinnamomea* mycelia on the mango sawdust (A), millet grass (B) and PMPYD (C). R_t of adenosine standard 7.19 min and cordycepin standard 9.25 min

Table 1. The growth of *A. Cinnamomea* mycelia on sawdust

Sawdust	*Mycelia growth time (days)		Mycelia characteristic				
			Density, Color				Odor at 110 days
	Initial growth	Fully colonized	50 days	70 days	90 days	110 days	
Khae na sawdust	18.80^{c} ±1.84	111.40^{c} ±1.94	+, #	+,×	++,×	++,×	cinnamon
Rain tree sawdust	-	-	-	-	-	-	-
Mango sawdust	9.40^{a} ±0.54	90.80^{a} ±2.28	++,×	+++,✓	+++,✓	+++,✓	cinnamon
Para rubber tree sawdust	-	-	-	-	-	-	-
Cinnamon tree sawdust	14.80^{b} ±0.83	100.40^{b} ±6.22	+,×	++,×	++,✓	++,✓	cinnamon

Note: * = average from 5 replicates, + = poor density, ++ = moderate density, +++ = abundant density, - = no growth of mycelia, ✓ = orange mycelia, × = orange-yellow mycelia, # = white mycelia
The same alphabet in each column means not statistical significantly different at 0.05 level of probability.

Table 2. The growth of *A. Cinnamomea* mycelia on seeds

Seeds	*Mycelia growth time (days)		Mycelia characteristic				Odor at 110 days
			Density, Color				
	Initial growth	Fully colonized	50 days	70 days	90 days	110 days	
Barley	11.86^{b}±1.34	37.29^{c}±1.79	+, ×	+,✓	++,✓	++,✓	cinnamon
Millet grass	9.86^{a}±1.06	26.14^{a}±0.90	+++,✓	+++,✓	+++,✓	+++,✓	cinnamon
Sang yod	11.14^{b}±1.06	31.00^{b}±0.81	++,×	++,×	+++,✓	+++,✓	cinnamon
Hom nin	11.14^{b}±1.06	31.57^{b}±1.61	++,۞	++,۞	+++,✓	+++,✓	cinnamon
Black sesame	-	-	-	-	-	-	-
Job's tear	12.29^{b}±1.89	41.29^{d}±2.36	+,✓	+,✓	++,✓	++,✓	cinnamon
Mung bean	-	-	-	-	-	-	-

Note: * = average from 7 replicates, + = poor density, ++ = moderate density, +++ = abundant density, - = no growth of mycelia, ✓ = orange mycelia, × = orange-yellow mycelia, ۞ = yellow-orange mycelia

The same alphabet in each column means not statistical significantly different at 0.05 level of probability.

Table 3. The growth of *A. Cinnamomea* mycelia in liquid media

Liquid medias	Growth and development of mycelia							
	Density, Color						*Fully surface colonized date (days)	Colony characteristic, Odor at 135 days
	15 days	30 days	45 days	60 days	75 days	90 days		
PD	+, #	+, €	+, £	+,۞	++,۞	++,۞	60.00^{a}±1.00	§, cinnamon
PMD	+, #	+, €	+, £	+,۞	++,۞	++,۞	60.33^{a}±0.57	§, cinnamon
PPD	+, #	+, €	+, £	+,۞	++,۞	++,۞	60.00^{a}±2.00	§, cinnamon
PYD	+, #	+, €	+, £	+,۞	++,۞	++,۞	75.33^{b}±0.57	§, cinnamon
PMPYD	+, #	+, €	+, £	+,۞	++,۞	+++,۞	60.00^{a}±1.00	§, cinnamon

Note: * = average from 3 replicates, # = white mycelia, € = white-yellow mycelia, £ = yellow mycelia, ۞ = yellow-orange mycelia, + = poor density, ++ = moderate density, +++ = abundant density, § = mat and floated

The same alphabet in each column means not statistical significantly different at 0.05 level of probability.

Table 4. Amount of nutrients from *A. cinnamomea* mycelia

Substrates	Nutrients (g/100 g dried weight)				
	Protein	Fat	Carbohydrate	Ash	Moisture
Mango sawdust	32.62	3.14	46.61	6.80	10.83
Millet grass	15.54	6.77	63.21	1.86	12.62
PMPYD	15.68	6.05	63.93	2.41	11.93

Discussion

Growth of *A. cinnamomea* could be grown on mango sawdust, cinnamon tree sawdust and the Khae na sawdust but not on the rain tree sawdust and the para rubber tree sawdust. The mycelia were fastest fully colonized on the mango sawdust, followed by the cinnamon tree sawdust and the Khae na sawdust, respectively. This was consistent with Chiu (2007) and Lu *et al*. (2013) who reported that *A. cinnamomea* can grow on *C. kanehirai*. In addition, United States Patent (2013) reported that *A. cinnamomea* can grow on *C. camphora* and *C. osmophloeum*.

The density of mycelia growing on the mango sawdust were abundant but, only moderate density was found in mycelia growing on the Khae na sawdust and the cinnamon tree sawdust. In 90 days, the mycelia growing on the cinnamon tree sawdust, as well as the mango sawdust, were orange. This was consistent with Chang and Chou (2004) and Chang and Wang (2005) who reported *A. cinnamomea* mycelia had orange-red color and *A. salmonea* mycelia had cream-pink color because of the accumulation of carotenoid (Harding and Shropshire, 1980). Friederichsen and Engel (1958) and Shrestha *et al*. (2 0 06) reported that carotenoid affect the orange color of *C. militaris* mycelia. The odor of mycelia growing on the Khae na sawdust, mango sawdust and cinnamon tree sawdust resembled the cinnamon odor in 110 days consistent with Chang and Wang (2005).

For the mycelia growth on the seeds, the fastest mycelia growth was found in millet grass, followed by the Sang yod, Hom nin, barley and job's tear. However, the mycelia were not grew on the black sesame and the mung bean. This is corresponding with Patent Application Publication (2008) that *A. cinnamomea* can grow on seeds: maize, oats, rye, sorghum, wheat or other cereal grains.

The mycelia had abundant density in the millet grass, Sang yod and Hom nin, while moderate density was found in the barley and job's tear. In all formulas, the mycelia became yellow with cinnamon odor, corresponding with Chang and Chou (2004) and Chang and Wang (2005) who reported *A. cinnamomea* had orange-red color and cinnamon odor.

The growth of *A. cinnamomea* on five liquid media were found in all formulars which revealed yellow-orange mycelia. The mycelia in all media were white in 15 days, white-yellow in 30 days, yellow in 45 days and then became yellow-orange in 60 days. The results were corresponded to Chang and Chou (2004) who reported the *A. salmonea* mycelia had cream-pink color when grew on the MEA media. Only the PMPYD had abundant density while other formula had medium moderate density. The fastest growth were found in PD, PPD and PMPYD, followed by PMD and PYD. The mycelia colony were mat

and floated in 135 days, similarly to Wanthongkham (2014) who reported the *Cordyceps militraris* mycelia were mat.

Since 105 days the PMPYD media gave the highest weight of dried mycelia, followed by the PMD>PD>PPD>PYD. The results revealed that *A. cinnamomea* could grow on the liquid media, agreed with Yang *et al*. (2010), Lu *et al*. (2011), Liu *et al*. (2012) and Geng *et al*. (2013). Therefore, it is interesting in culture of *A. cinnamomea* mycelia in batch bioreactor. Asaduzzaman (2007) and Youpensuk (2009) showed that the growth curve of many mycelia were sigmoid curve. The pH value of the liquid media were increased while the culture time increased, probably because the extracellular organic compounds were bases. This was corresponding to Wanthongkham (2014) who reported the pH value of liquid media for *C. militaris* were increased during cultivation.

For the adenosine and cordycepin content analysis of the dried mycelia growing on the mango sawdust, millet grass and PMPYD, the adenosine was found in the mycelia from the mango sawdust, millet grass and PMPYD while the cordycepin was found only in the mycelia growing on the mango sawdust. This is similar to Lu *et al*. (2006) and Chen *et al*. (2015) who reported the mycelia and fruiting body of *A. cinnamomea* had adenosine and cordycepin.

The mycelia growing on the mango sawdust had highest protein content, followed by the PMPYD and the millet grass. The millet grass had highest fat content, followed by the PMPYD and the mango sawdust. The mycelia from the PMPYD had highest carbohydrate, followed by the millet grass and the mango sawdust. The mycelia from mango sawdust had highest amount of the ash content, followed by the PMPYD and the millet grass. The mycelia from the millet grass had highest amount of the moisture content, followed by the PMPYD and the mango sawdust. The nutrient content were found nearly to the nutrient content of *A. camphorate* by Chang *et al*. (2001) who reported the amount of protein, fat, carbohydrate, ash and moisture were 9.49, 9.79, 56.70, 3.92 and 6.65%, respectively. Besides, Chiu *et al*. (2014) reported *A. cinnamomea* had protein, carbohydrate and amino acid content.

However, the adenosine, cordycepin and nutrient content of *A. cinnamomea* may depends on the culture media, pH, the culture conditions, the culture time, the harvest method and the extraction method.

Acknowledgement

This work would not havebeen possible without the financial support of Dr. Sonthaya Klomplien (Director of Smithipol Co., Ltd.) to Chandrakasem Rajabhat University. We also would like to show our gratitude to the Xin Gao Mushroom Farm (Taiwan) for donation of *A. cinnamomea* TT Chang. & WN Chou.

References

AOAC Official Methods of Analysis (2012a). AOAC Official Method 920.153 Ash of Meat. Association of Official Analytical Chemists.

AOAC Official Methods of Analysis (2012b). AOAC Official Method 922.06 Fat in Flour Acid Hydrolysis Method. Association of Official Analytical Chemists.

AOAC Official Methods of Analysis (2012c). AOAC Official Method 920.153 Ash of Meat. Association of Official Analytical Chemists.

AOAC Official Methods of Analysis (2012d). AOAC Official Method 981.10 Crude Protein in Meat Block Digestion Method. Association of Official Analytical Chemists.

Asaduzzaman, M. D. (2007). Standardization of Yeast Growth Curves from Several Curves with Different Initial Sizes. Department of Mathematical Sciences. Chalmers University of Technology and G¨oteborg University. Sweden.

Chang, J. M., Lee, Y. R., Hung, L. M., Liu, S. Y., Kuo, M. T., Wen, W. C. and Chen, P. (2011). An extract of *Antrodia camphorata* mycelia attenuates the progression of nephritis in systemic lupus erythematosus-prone NZB/W F1 mice. Evidence-Based Complementary and Alternative Medicine. 2011:1-7.

Chang, T. T. and Chou, W. N. (2004). *Antrodia cinnamomea* reconsidered and *A. salmonea* sp. nov. on *Cunninghamia konishii* in Taiwan. Botanical Bulletin Academia Sinica. 45:347-352.

Chang, T. T. and Wang, W. R. (2005). Basidiomatal formation of *Antrodia cinnamomea* on artificial agar media. Botanical Bulletin Academia Sinica. 46:151-154.

Chen, Y. Y., Liu, F. C., Wu, T. S. and Sheu, M. J. (2015). *Antrodia cinnamomea* inhibits migration in human hepatocellular carcinoma cells: the role of ERp57 and PGK-1. American Journal of Chinese Medicine. 43:1671-1696.

Cherng, I. H., Wu, D. P. and Chiang, H. C. (1996. Triterpenoids from *Antrodia cinnamomea*. Phytochemistry. 41:263-267.

Chiu, C. H., Peng, C. C., Ker, Y. B., Chen, C. C., Lee, A., Chang, W. L., Chyau, C. C. and Peng, R. Y. (2014). Physicochemical characteristics and anti-inflammatory activities of antrodan, a novel glycoprotein isolated from *Antrodia cinnamomea* mycelia. Molecules. 19:22-40.

Chiu, H. H. (2007). Phylogenetic analysis of *Antrodia* species and *Antrodia camphorata* inferred from internal transcribed spacer region. Antonie Van Leeuwenhoek. 91:267-276.

Friederichsen, L. and Engel, H. (1958). Der Farbstoff von Cordyceps militaris L. Archives of Microbiology. 30:393-395.

Geethangili, M and Tzeng, Y. M. (2011). Review of pharmacological effects of*Antrodia camphorata* and its bioactive compounds. Evidence-based Complementary and Alternative Medicine. 2011:1-17.

Geng, Y., He, Z., Lu, Z. M., Xu, H. Y., Xu, G. H., Shi, J. S. and Xu, Z. H. (2013). *Antrodia camphorata* ATCC 200183 sporulates asexually in submerged culture. Applied Microbiology and Biotechnology. 97:2851-2858.

Halpern, G. M. (2007). Healing Mushrooms: Ancient Wisdom for Better Health. Square one Publishers. U.S.A.

Harding, R. W. and Shropshire, W. Jr. (1980). Photocontrol of carotenoid biosynthesis. Annual Review of Plant Physiology. 31:217-238.

Huang, L., Li, Q., Chen, Wang, Y. X. and Zhou, X. (2009). Determination and analysis of cordycepin and adenosine in the products of *Cordyceps* spp. African Journal of Microbiology Research. 3:957-961.

Kuo, J. T., Lin, E. S. and Yang, C. T. (2011). Effect of cultivating conditions on the superoxide and free radical-scavenging activities of *Antrodia cinnamomea*. Journal of Food Biochemistry. 35:1493-1500.

Laboratory of Central Laboratory Thailand (2013) . In-House Method TE-CH-169 Based on Compendium of Methods for Food Analysis Thailand 1st Edition.Bureau of Laboratory Quality Standards. The Laboratory of Central Laboratory Thailand CO., LTD.

Liu, C. J., Chiang, C. C. and Chiang, B. H. (2012) . The elicited two-stage submerged cultivation of *Antrodia cinnamomea* for enhancing triterpenoids production and antitumor activity. Biochemical Engineering Journal. 64:48-54.

Lu, M. C., Shazly, M. E., Wu, T. Y., Du, Y. C., Chang, T. T., Chen, C. F., Hsu, Y. M., Lai, K. H., Chiu, C. P., Chang, F. R. and Wu, Y. C. (2013). Recent research and development of *Antrodia cinnamomea*. Pharmacology & Therapeutics. 139:124-156.

Lu, M. K., Cheng, J. J., Lai, W. L., Lin, Y. R. and Huang, N. K. (2006) . Adenosine as anbioreactive component of *Antrodia cinnamomea* that prevents rat PC12 cells from serum deprivation-induced apoptosis through the activation of adenosine A2A receptors. Life Sciences. 79:252-258.

Lu, M. Y. J., Fan, W. L., Wang, W. F., Chen, T., Tang, Y. C., Chu, F. H., Chang, T. T., Wang, S. Y., Li, M. Y., Chen, Y. H., Lin, Z. S., Yang, K. J., Chen, S. M., Teng, Y. C., Lin, Y. L., Shaw, J. F., Wang, T. F. and Li, W. H. (2014). Genomic and transcriptomic analyses of the medicinal fungus *Antrodia cinnamomea* for its metabolite biosynthesis and sexual development.Proceedings of the National Academy of Sciences. 111:4743-4752.

Lu, Z. M., Lei, J. Y., Xu, H. Y., Shi, J. S. and Xu, Z. H. (2011) . Optimization of fermentation medium for triterpenoid production from *Antrodia camphorata* ATCC 200183 using artificial intelligence-based techniques. Applied Microbiology and Biotechnology. 92:371-379.

Patent Application Publication (2008) . Mycellated Grain and Other Mycellated Agricultural Materials to be Used as Animal Food Supplement. Publication No.US 2008/0187574 A1.

Shrestha. B., Lee, W. H., Han, S. K and Sung, J. M. (2006). Observations on Some of the Mycelial Growth and Pigmentation Characteristics of Cordyceps militaris Isolates. Mycobiology. 34:83-91.

United States Patent (2013) . Culture Method of a *Antrodia camphorata*. Patent No.2 US. 8,524,486 B2.

Wang, W. M., Wu, R. Y. and Ko, W. H. (2005) . Variation and segregation following nuclear transplantation in *Antrodia cinnamomea*. Botanical Bulletin of Academia Sinica. 46:217-222.

Wanthongkham, T. (2014). Cultivation of *Cordyceps militaris* (L.)Link Mycelium in Different Liquid Medium. Bachelor of Science. Chandrakasem Rajabhat University. Bangkok.

Yang, C. T., Kuo, J. T. and Lin, E. S. (2010). Screening of medium composition for the free radical-scavenging properties by *Antrodia cinnamomea*. International Journal of Food Science & Technology. 45:305-311.

Yang, H. L., Hseu, Y. C., Chen, J. Y., Yech, Y. J., Lu, F. J., Wang, H. H., Lin, P. S. and Wang, B. C. (2006). *Antrodia camphorata*in submerged culture protects low density lipoproteins against oxidative modification. The American Journal of Chinese Medicine. 34:217-231.

Yang, S. W., Shen, Y. C. and Chen, C. H. (1996) . Steroids and triterpenoids of *Antrodia cinnamomea* a fungus parasitic an *Cinnamomum micranthum*. Phytochemistry. 41:1389-1392.

Youpensuk, S. (2009). Mycology. Pongsawat Printing. Chiang Mai Province. Thailand.

(Received: 20 October 2018, accepted: 28 December 2018)