

## Effect of Thai Jasmine Rice on the Fruiting Body Growth of *Cordyceps militaris*

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### Abstract

*Cordyceps militaris* is a potential source of pharmacological activities including anti-inflammatory, anti-oxidant, anti-aging, anti-tumor, anti-cancer and anti-leukemic. Rice was used as carbon source and mixed with *C. militaris* culture media. The aim of this research was to study the effect of Thai jasmine rice on the fruiting body growth of *C. militaris*. Thai jasmine rice and Sangyod brown rice (control) were mixed with *C. militaris* culture media in glass bottle and sterilized for 30 min before used. Five milliliters of *C. militaris* liquid culture were inoculated into sterilized glass bottle containing rice and *C. militaris* culture media and incubated in the dark at 22 °C for 7 days, under light at 18 °C for 7 day and continued at 22 °C for 60 days. Fresh *C. militaris* fruiting body was cut from glass bottle, weighted and dried at 50 °C for 8 hr. The results found that average weight of fresh *C. militaris* fruiting body obtained from Thai jasmine rice ( $20.84 \pm 0.29$  g/bottle) was higher than that from Sangyod brown rice ( $6.26 \pm 0.26$  g/bottle). Cordycepin in *C. militaris* fruiting body obtained from Thai jasmine rice was determined using High-Performance Liquid Chromatography and higher than that obtained from Sangyod brown rice at 4080 mg/kg. Thai jasmine rice is an alternative substrate instead of Sangyod brown rice for *C. militaris* to reduce the cost of production.

**Keywords:** Thai jasmine rice, Sangyod brown rice, fruiting body, *Cordyceps militaris*

## Introduction

*Cordyceps militaris* (L.) Link (Clavicipitaceae) is an entomopathogenic fungus which has been used as herbal in traditional medicine [1-2]. *C. militaris* is an abundant source of useful natural products with various pharmacological activities including immunomodulatory, antitumor, lowering blood glucose, pro-sexual, anti-oxidant, anti-aging, anti-metastatic, anti-microbial, anti-viral, anti-fungal, anti-protozoal and other favorable effects [3-4]. The favorite grain source was used in *C. militaris* culture is brown rice [5]. Rice was mixed with carbon source (glucose, sucrose), nitrogen source (yeast extract, silkworm pupae, insect, egg etc.) Vitamin B before sterilization using autoclave [6].

Organic Sangyod brown rice is a rice geographical indication (GI) of Phatthalung province. Sangyod brown rice is small and long, slender grain, dark red pericarp, soft (low amylose) and nutritional enrich (high niacin) [7]. Sangyod brown rice was used as grain in *C. militaris* rice culture medium. Because of organic Sangyod brown rice is GI rice of Phatthalung that cannot be find in all market of Thailand and expensive. Organic Thai jasmine rice or Hom mali 105 is cheaper, easy to find in all market and alternative rice for *C. militaris* production. The aim of this research was to study the effect of Thai jasmine rice on the fruiting body growth of *C. militaris* for reduce the cost of production.

## Materials and methods

### Rice strain

Organic Thai jasmine rice was obtained from Thamuang community, Selphum, Roi Et province, Thailand. Organic Sangyod brown rice was purchased from Phatthalung province, Thailand.

### Sources of materials

Potato, corn, silkworm pupa and egg were purchased from local market, Selphum, Roi Et province, Thailand. Glucose, yeast extract, peptone, MgSO<sub>4</sub> and Vitamin B1 were purchased from HIMEDIA, HiMedia Laboratories GmbH, Germany.

### ***C. militaris* culture**

*C. militaris* inoculum agar was purchased from Ban Hed Phrasmutrcey farm, Samutprakan province. *C. militaris* inoculum agar was cultured in liquid medium at 22 °C, 150 rpm for 7 days. Thai jasmine rice and Sangyod brown rice (control) were mixed with *C. militaris* culture medium in 50 glass bottles of each rice and sterilized for 30 min before used. Five milliliters of *C. militaris* liquid culture were inoculated into sterilized glass bottle containing rice and *C. militaris* culture media and incubated in the dark at 22 °C for 7 days, under light at 18 °C for 7 day and continued at 22 °C for 60 days. Fresh fruiting body of *C. militaris* was collected and weight and dried at 55 °C for 18 hrs. Dried *C. militaris* were grinded to fine powder and was sent to Herb and Thai traditional medicine development division, Thailand for cordycepin and adenosine analysis using HPLC.

## **Results and discussion**

### ***C. militaris* culture**

*C. militaris* liquid culture was inoculated into sterilized rice medium in bottle and were incubated in the dark at 22 °C for 7 days, under light at 18 °C for 7 day and continued at 22 °C for 60 days. At day 8 (light condition), white *C. militaris* mycelium turned to yellow and day 13, many small fruiting bodies were grown (Figure 1). Fresh fruiting body of *C. militaris* was collected and weight at day 74. The results found that average weight of fresh *C. militaris* fruiting body obtained from Thai jasmine rice medium ( $20.84 \pm 0.29$  g/bottle fw) was higher than obtained from Sangyod brown rice medium ( $6.26 \pm 0.26$  g/bottle fw). Cordycepin in *C. militaris* fruiting body obtained from Thai jasmine rice medium were determined using High-Performance Liquid Chromatography and the value was higher than that obtained from Sangyod brown rice medium at 4080 mg/kg. In the other hand, adenosine content in fruiting body obtained from Sangyod

brown rice was higher than obtained from Thai jasmine rice medium at 1980 mg/kg (Table 1.) The cordycepin and adenosine value from this research were higher than [9] which was cultivated of *C. militaris* with the Black jasmine rice medium and cordycepin and adenosine value were at 2081.7 and 1567.3 mg/kg, respectively. Many researches were cultivated of *C. militaris* using different rice sources including Black jasmine rice [9], Sao Hai rice [10], brown rice, white rice, wheat, black glutinous rice Vietnam, and corn grain [6]. Some rice varieties cannot buy in all area so Thai jasmine rice is an alternative substrate instead of Sangyod brown rice for *C. militaris* to reduce the cost of production.



**Fig. 1.** Fruiting body of *C. militaris* at day 13.

**Table 1.** Fresh fruiting body weight cordycepin and adenosine of *C. militaris* obtained from Thai jasmine rice medium and Sangyod brown rice medium.

Rice strain	Average of fresh fruiting body weight (g/bottle fw $\pm$ SD)	Cordycepin content (mg/kg)	Adenosine content (mg/kg)
Thai jasmine rice	20.84 $\pm$ 0.29	4080	1810
Sangyod brown rice	6.29 $\pm$ 0.26	1130	1980

This work used incubation temperature range of 18-22 °C according to [8] that presented that suitable temperature range to obtain high cordycepin value was at 17.5-22.5 °C and [2] demonstrated that optimum temperature for mycelial growth and cordycepin production was 15 to 20°C and 25°C, respectively. Previous research [4]

used single temperature at 18 °C because the *C. militaris* inoculum broth that obtained from kasetbuddy farm, Saraburi was selected and cultured at 18 °C.

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