Received: September 13, 2023 | Revised: November 5, 2023 | Accepted: November 6, 2023

Boosting cordycepin production through plant-based oils for vegetarian consumption

Watcharin Yuttavanichakul*, Nipaporn Kanthong and Nuntaporn Pungsungvorn

Department of Biotechnology, Faculty of Science and Technology, Rajamangala University of Technology Tawan-ok, Chonburi 20110, THAILAND

*Corresponding author: Watcharin_yu@rmutto.ac.th

ABSTRACT

Cordyceps fungi, including species such as *Cordyceps sinensis* and *Cordyceps militaris*, are known for producing bioactive chemicals, notably cordycepin. Traditional cordyceps cultivation in Thailand relies on silkworm pupae as a substrate in solid-state fermentation, posing challenges in catering to vegetarian consumers. This study aimed to develop a solid-state fermentation process for cordyceps cultivation using vegetable oils, thus modifying the cereals medium and eliminating the need for silkworm pupae while enhancing bioactive chemical production and promoting cordyceps growth. The findings demonstrate that *C. militaris* can successfully grow and produce adenosine and cordycepin when the culture medium is modified with plant oils. Plant oils, including olive, soybean, peanut, palm, sesame, coconut, and sunflower oil, proved effective for cultivating *C. militaris* on PDA. Notably, adding a 3% mixture of palm oil in PDA resulted in the most significant promotion of *C. militaris* mycelium, with a diameter of 5.93 cm. Consequently, the modified cereals medium, incorporating palm oil, was adopted for solid-state fermentation of *C. militaris*. The results demonstrate that *C. militaris* can be successfully cultured to produce fruiting bodies comparable and total yields to those obtained using traditional cereals and silkworm pupa medium. Furthermore, there is a notable increase in adenosine and cordycepin production, indicating the potential of this method to enhance *C. militaris* yield and bioactive chemical output. This research highlights the feasibility of incorporating plant oils as substitutes or additives to silkworm pupae, improving productivity and enabling the production of *C. militaris* suitable for vegetarian consumption.

Keywords: C. militaris, Adenosine, Cordycepin, Vegetable oils, Vegetarian

INTRODUCTION

Cordyceps is a mushroom that has gained significant popularity as a widely consumed and highly sought-after product in the global market. The emergence of the COVID-19 pandemic has further increased the focus on maintaining health, leading to a heightened interest in cordyceps. These mushrooms have a long history of traditional medicinal use spanning thousands of years. Scientists from various fields have studied the nutritional benefits and bioactive compounds found in cordyceps, focusing on the important compound called cordycepin. Cordycepin, also known as 3'-deoxyadenosine, is a bioactive compound abundantly present in *Cordyceps* spp. Its potential therapeutic properties have captured significant attention within the scientific community. Cordycepin exhibits various biological activities, including anti-inflammatory, anticancer, antiviral, immunomodulatory, and antioxidant effects [1-3]. The medical field has embraced the application of cordyceps to enhance immunity and treat various diseases, including cancer, kidney disease, and diabetes [4]. Moreover, they have found utilization in the beauty industry and as health supplements [5]. Cordycepin has exhibited significant antiviral activity against various viruses, including influenza, hepatitis B, and herpes simplex [6]. While the therapeutic potential of cordyceps is promising across various domains, further research is warranted to elucidate their mechanisms of action and clinical applications comprehensively. Acknowledging that the properties and active compounds may exhibit variations among different Cordyceps species and cultivation conditions is important.

Cultivating cordyceps holds immense potential for harnessing their medicinal benefits. The cultivation process involves the propagation and maintenance of fungal cultures, ultimately providing a sustainable source for cordycepin production. Numerous studies have dedicated efforts toward developing efficient and reliable cultivation techniques. For instance, [7] explored the cultivation of cordyceps using a solid-state fermentation method. They optimized growth conditions, including the choice of substrate, moisture content, temperature, and pH, to enhance cordycepin

DOI: 10.60101/jarst.2023.254104

production. The results demonstrated a significant increase in cordycepin content in the cultivated mushrooms, thereby establishing them as a valuable source of this bioactive compound. Similarly, [8] delved into submerged fermentation of cordyceps, investigating the influence of various fermentation parameters such as carbon and nitrogen sources, pH, and agitation on cordycepin production. Their optimization efforts yielded higher yields of cordycepin, providing valuable insights into the industrial-scale production of this valuable compound. These cultivation studies not only contribute to the understanding of cordyceps biology but also present practical approaches for sustainable production. By optimizing cultivation conditions, it becomes possible to obtain cordyceps with elevated concentrations of the bioactive compound, thus maximizing their potential therapeutic benefits. It is worth noting that mushroom farmers face challenges in cultivating high-quality cordyceps due to low levels of cordycepin and low yields. Consequently, this results in low profits despite high production costs. Typically, the price of cordyceps is determined based on the number of active compounds found per gram and the mushroom size. Particularly, cordyceps exhibiting a high content of cordycepin per gram command higher prices. Environmental conditions, mushroom strains, and cultivation methods influence the quantity of active compounds. One promising approach involves cultivating cordyceps using silkworm substrate. Silkworms produce silk, rich in fatty acid, protein, and nutrients and an ideal medium for cordyceps growth. Several studies have investigated the cultivation of cordyceps using silkworm substrate, providing valuable insights into the techniques and benefits associated with this approach. For instance, [9] studied cultivating *Cordyceps militaris* using silkworm pupae as the substrate. The results demonstrated a significant increase in the production of bioactive compounds, including cordycepin and adenosine. The use of silkworm substrate not only enhanced the growth and yield of cordyceps but also enriched them with essential nutrients. The study highlighted the potential of silkworm pupae residue as a cost-effective and sustainable alternative to traditional substrates. Using this substrate resulted in a higher production of bioactive compounds, increased antioxidant activity, and improved yield of cordyceps.

While cordyceps offer numerous health benefits, it is essential to consider dietary aspects before consuming them. In particular, individuals adhering to vegetarian diets should be aware that cordyceps cannot be classified as vegetarian-friendly. Traditionally, cordyceps grow as parasitic fungi on the larvae of insects, specifically caterpillars. This natural growth process involves the fungus colonizing and eventually replacing the host organism. Consequently, the harvested cordyceps contain remnants of the caterpillar host. Due to this characteristic, cordyceps are not suitable for vegetarian consumption. However, alternative cultivation methods could be developed, such as growing cordyceps on cereal substrates, excluding the involvement of insect hosts. These cultivation methods provide a viable option for vegetarians to enjoy the benefits of cordyceps without compromising their dietary preferences. Cultivating cordyceps presents an opportunity to tap into their medicinal benefits sustainably, and it is important to ensure that their consumption is accessible. However, exploring the use of locally available plant oils in cordyceps cultivation on cereal substrates is an intriguing avenue for improving cordycepin content, developing cost-effective cultivation formulas, and making them accessible to all consumers. Research reports have indicated that adding coconut oil, corn oil, and sunflower oil in appropriate quantities can promote mycelial growth and enhance the production of exo-biopolymer in *C. militaris* [10]. Furthermore, incorporating peanut oil as a secondary carbon source in liquid static cultivation has shown a significant increase in cordycepin content, up to 5.26 grams per liter or approximately 3.17 times higher than the normal method [11]. These reports underscore the limited research on the effects of using plant oils and fatty acids to boost cordycepin content in cordyceps cultivation. Moreover, there is a lack of studies focusing on using locally available plant oils in Thailand to enhance cordycepin production in cordyceps cultivation using cereal substrates, which are widely accessible and hard to shortage. Consequently, investigating the utilization of locally available plant oils to improve cordycepin production efficiency in cordyceps cultivation on cereal substrates of solid-state fermentation holds great interest. Therefore, this study aims to investigate the effect of using locally available plant oils on the growing and cordycepin concentration of cordyceps cultivation using cereal substrates. Such endeavors will contribute to the development of a cost-effective and high-quality cultivation formula with a high cordycepin content, accessible to all consumer groups and beneficial for cordyceps farmers in Thailand.

MATERIALS AND METHODS

Isolation and culture

C. militaris was isolated from a commercial fresh cordyceps product. The cordyceps were cultured on peptone-added potato dextrose agar (PPDA) slants at 25 °C. The culture was allowed to grow for 10 days and then stored at 4 °C for stock purposes.

Liquid spawn preparation

Liquid spawn was prepared using a liquid medium modified from [8] consisting of glucose (20 gL^{-1}), peptone (5 gL^{-1}), beef extract (3 g L^{-1}), and yeast extract (1 gL^{-1}). In a 250 mL flask, 100 mL of the liquid

DOI: 10.60101/jarst.2023.254104

JOURNAL OF APPLIED RESEARCH ON SCIENCE AND TECHNOLOGY

medium was aseptically transferred. A small piece (~0.5 cm^2)of the mycelial slant from the PPDA culture was then inoculated into the flask. The flask was incubated at a temperature of 25 °C with shaking at 120 rpm. The mycelia were allowed to grow for approximately 7 days in the liquid medium. During this period, optimal growth of the mycelia was observed. After 7 days, the spawn culture was diluted by adding four volumes of sterile distilled water. The spores were obtained by observing under a microscope using a hematocytometer to obtain a 10^6 spores/mL concentration before being used to inoculate media for fruit body production.

The optimal growth differences in plant oil concentrations

Cork Borer cut the cutter to a 5-mm disc. Subsequently, the disc was transferred to the central of a fresh Petri dish containing potato dextrose agar (PDA) supplemented with various plant oils, modified from [10] including olive oil, soybean oil, peanut oil, palm oil, sesame oil, coconut oil, and sunflower oil, at different concentrations of 0%, 1%, 3%, and 5% (v/v). The cultures were then incubated at a temperature of 25 °C with a light-dark cycle of 12 hours each, under white fluorescent light with an intensity of 1600 lx. The relative humidity was maintained above 70% throughout the week-long incubation period. The growth of the colonies was assessed by measuring their diameter, allowing for the determination of the growth rate. To ensure reliable results, each experiment was conducted with six replicates.

The fruiting body formation medium with plant oil with solid-state fermentation

The fruiting body formation medium was modified by introducing a plant oil treatment, while the control medium (CM) consisted of a 500 mL glass bottle (17 oz) containing 60 g of brown rice and 80 mL of distilled water. For the positive control cereals and silkworm pupa medium (CSM), a 500 mL glass bottle contained 60 g of brown rice, 20 mL of silkworm pupa spinning juice, and 60 mL of distilled water. In contrast, the modified cereals medium (MCM) included 60 g of brown rice, 2.4 mL of palm oil, and 77.6 mL of distilled water in the same 500 mL glass bottle. Both mixtures were sterilized at 121 °C for 20 minutes. After cooling, 25 mL of liquid inoculum was added to each fruiting medium. The incubation process took place at a temperature of 25 °C with a light-dark cycle of 12 hours each, under white fluorescent light with an intensity of 1600 lx for 60 days and with a relative humidity maintained between 70%. The formation of fruiting bodies was assessed about the grains. Total yields of fruiting body production were calculated as follows:

$$\text{Total yields} = F + S$$

Where, F = mg dry weight of fruiting body part

S = mg dry weight of fruiting body part

Bioactive compound analysis

Fruit bodies obtained from each sample were dried in the oven to a constant weight at 60 °C for 3 days. The dried samples were then pulverized to a particle size smaller than 20-mesh using a hammer mill. To extract adenosine and cordycepin, 0.5 g of the dry powder was suspended in 50 mL of doubly deionized water and sonicated for 3 hours in an ultrasonic bath operating at 50 kHz and 400 W. The resulting supernatant, obtained by centrifuging at 1740 × g for 15 minutes, was filtered through a 0.45 µm membrane filter. High-performance liquid chromatography (HPLC) analysis was conducted using an Agilent 1100 Series HPLC system from Agilent Technologies Inc. in Santa Clara, CA, USA. The method was modified from [1]. The separation and detection conditions for adenosine and cordycepin were as follows: a C18 column (4.6 mm × 150 mm, with a particle size of 5 µm) from Agilent, a mobile phase composed of 10 mmol L^{-1} KH_2PO_4 buffer (pH 6.0) and methanol in a ratio of 85:15 (v/v), a flow rate of 0.8 mL min^{-1}, a column temperature of 30 °C, and detection at a wavelength of 260 nm using a UV-visible detector. The analysis of cordycepic acid from the extracts was carried out at a column temperature of 35 °C and a refractive index detector temperature of 35 °C. The mobile phase for Cordycepic acid analysis consisted of deionized water, with a flow rate of 0.6 mL min^{-1}. The injection volume in the HPLC system was set to 20 µL.

Statistical analysis

Data were analyzed with SPSS Software Package v.18. All variables were tested for normality using the Kolmogorov-Smirnov. Normally distributed data were analyzed using ANOVA. Variance homogeneity was confirmed by Levene's test for variance homogeneity ($P > 0.05$). Significant differences between treatment effects were determined by one-way ANOVA analysis, followed by Duncan's tests, and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSIONS

Growth of C. militaris on different plant oil

In order to explore the potential utilization of these plant oils as components in the solid medium to replace silkworm pupa for cultivating *C. militaris*, the mycelial growth was assessed by cultivating *C. militaris* on PDA mixed with each type of plant oil at various concentrations. The findings revealed that the mycelial growth of *C. militaris* on PDA medium mixed with palm oil at a concentration of 3% exhibited the most favorable outcomes among all the tested. This outcome was statistically significant, as shown in Table 1. Likewise, the mycelial growth on PDA medium mixed with olive oil at a concentration of 3% demonstrated superior

DOI: 10.60101/jarst.2023.254104

performance compared to 1% and 5%, respectively (Table 1 and Figure 1 a-d). Similarly, the mycelial growth on PDA medium mixed with sunflower oil at 1% yielded better results than concentrations of 3% and 5% (Table 1 and Figure 1 e-h). The mycelial growth on PDA medium mixed with soybean oil at a concentration of 1% exhibited better results compared to concentrations of 3% and 5% (Table 1 and Figure 1 i-l). Similarly, the mycelial growth on PDA medium mixed with peanut oil at a concentration of 1% demonstrated better results compared to concentrations of 3% and 5% (Table 1 and Figure 1 m-p). Additionally, the mycelial growth on PDA medium mixed with sesame oil at a concentration of 1% showed better results compared to concentrations of 3% and 5% (Table 1 and Figure 1 q-t). Furthermore, the mycelial growth on PDA medium mixed with palm oil at a concentration of 3% exhibited better results compared to concentrations of 1% and 5% (Table 1 and Figure 1 u-x). Moreover, the mycelial growth on PDA medium mixed with coconut oil at a concentration of 3% demonstrated better results than concentrations of 1% and 5% (Table 1 and Figure 1 aa-ad). These results indicate that palm oil, olive oil, and coconut oil at a concentration of 3% and sunflower oil, peanut, and sesame oil at 1% are the most effective concentrations for promoting the mycelial growth of *C. militaris* in PDA medium. The addition of optimum palm oils significantly promoted the mycelial growth of *C. militaris* in static culture. Oils, in general, can be partially incorporated into the cell membrane, facilitating the immediate uptake of nutrients from the culture medium and stimulating mycelial growth [12]. However, the growth of the fungus was completely inhibited by short-chain fatty acids with a carbon chain length shorter than 10, such as capric acid, caproic acid, propionic acid, and acetic acid [13, 14]. The main components of plant oil, such as corn oil, cottonseed oil, palm oil, and soybean oil, are linoleic acid and palmitic acid, which are believed to benefit mycelial growth [15]. It is worth noting that palm oil contains 45% palmitic acid and 44% linoleic acid, which appear to play a role in stimulating mycelial growth. Additionally, palmitic acid is the most stimulating factor for mycelial growth, resulting in an 18.3-fold increase during a 3-week incubation of *Hericium erinaceum* [16].

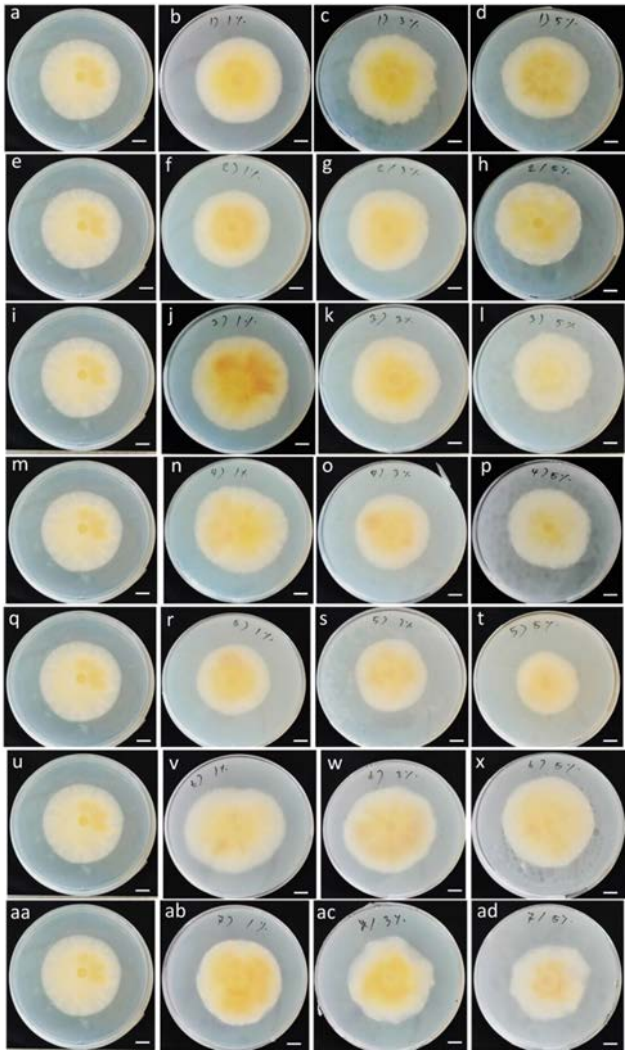

Figure 1 Colony of *C. militaris* on PDA agar medium under 0, 1, 3, 5, (V/V) of each plant oil; olive oil (a-d); sunflower oil (e-h); soybean oil (i-l); peanut oil (m-p); sesame oil (q-t); palm oil (u-x) and coconut oil (aa-ad). - white bar scale reference value = 1 cm.

Table 1 Growth of *C. militaris* on agar medium under 0, 1, 3, and 5% of plant oils.

Treatments	Colony or hyphae diameter (centimeters)			
	Plant oil concentration (%v/v)			
	0 %	1 %	3 %	5 %
PDA (control)	5.43 ^{abcd} ± 0.05	-	-	-
PDA + olive oil	-	5.26 ^{bcde} ± 0.23	5.66 ^{ab} ± 0.09	5.00 ^{cde} ± 0.00
PDA + sunflower oil	-	5.06 ^{bcde} ± 0.23	4.90 ^{de} ± 0.06	5.00 ^{cde} ± 0.12
PDA + soybean oil	-	5.50 ^{abc} ± 0.25	5.46 ^{abcd} ± 0.15	5.16 ^{abcde} ± 0.09
PDA + peanut oil	-	5.66 ^{ab} ± 0.17	5.00 ^{cde} ± 0.29	5.16 ^{abcde} ± 0.17
PDA + sesame oil	-	5.16 ^{abcde} ± 0.33	4.66 ^{e} ± 0.17	4.13 ^{f} ± 0.09
PDA + palm oil	-	5.73 ^{ab} ± 0.15	5.93 ^{a} ± 0.12	5.33 ^{abcd} ± 0.17
PDA + coconut oil	-	5.60 ^{ab} ± 0.10	5.66 ^{ab} ± 0.09	5.23 ^{abcde} ± 0.15
C.V. (%)	0.00	0.05	0.08	0.08

Letters denote significant differences between mean using a post hoc Duncan's multiple comparisons test. ($P < 0.05$) after one-way ANOVA. Values are means (±SEM, n = 6).

DOI: 10.60101/jarst.2023.254104

JOURNAL OF APPLIED RESEARCH ON SCIENCE AND TECHNOLOGY

Fruit body production and bioactive compound of C. militaris on different fruiting body formation medium

Through the experimental use of a 3% mixture of palm oil in the PDA medium, evidence suggests a tendency to enhance the growth of *C.militaris* fruiting body. In order to culture *C. militaris*, MCM was utilized and compared with the CM and CSM over 60 days. The results revealed that the MCM significantly impacted the dry weight of *C. militaris* fruiting bodies, solid-based residues, and total yields, which measured 9.75, 20.67, and 30.42 mg, respectively. These values did not differ significantly from cultivating in the CSM, where the dry weight of *C. militaris* fruiting bodies, solid-based residues, and total yields were 9.88, 20.74, and 30.58 mg, respectively.

Table 2 Fruiting body production of *C. militaris* on fruiting body formation medium.

Fruiting body formation medium	Fruiting body production of *C. militaris*		
	Fruiting body (mg dry weight)	Solid-based residues (mg dry weight)	Total yields (mg dry weight)
Cereals (CM)	3.23 ^{b} ±0.87	17.65 ^{b} ±1.75	20.88 ^{b} ±1.25
Cereals and silkworm pupa (CSM)	9.83 ^{a} ±1.19	20.74 ^{a} ±1.94	30.58 ^{a} ±1.57
Modified fruiting body (MCM)	9.75 ^{a} ±0.81	20.67 ^{a} ±2.55	30.42 ^{a} ±1.88
C.V. (%)	0.40	0.07	0.16

Letters denote significant differences between mean using a *post hoc* Duncan's multiple comparisons test ($P < 0.05$) after one-way ANOVA. Values are means (±SEM, n=3).

Figure 2 Fruiting body of *C. militaris* on fruiting body formation medium; a: CM; b: CSM and c: MCM.

Table 3 Bioactive compound of *C. militaris* extracted from different fruiting body formation medium.

Fruiting body formation medium	Bioactive compound (mg/g)		Bioactive compound (ug) per Total yields or Production	
	Adenosine	Cordycepin	Adenosine	Cordycepin
Cereals (CM)	0.17 ^{ns} ±0.00	0.74 ^{ns} ±0.00	3.55 ^{b} ±0.21	16.07 ^{c} ±0.92
Cereals and silkworm pupa (CSM)	0.22 ^{ns} ±0.00	1.03 ^{ns} ±0.00	6.72 ^{a} ±0.34	31.49 ^{a} ±1.67
Modified fruiting body (MCM)	0.20 ^{ns} ±0.00	0.81 ^{ns} ±0.00	6.08 ^{a} ±0.37	24.64 ^{b} ±1.56
C.V. (%)	0.15	0.12	0.27	0.26

Letters denote significant differences between mean using a *post hoc* Duncan's multiple comparisons test ($P < 0.05$) after one-way ANOVA. Values are means (±SEM, n=3). ns = non-significant

However, MCM and CSM mediums exhibited notable differences when cultivating with CM, yielding statistically significant results. The dry weight of the fruiting bodies, solid-based residues, and total yields were 3.23, 17.65, and 20.88, respectively (Table 2). The culture of *C. militaris* on different fruiting body formation mediums at 60 days is shown in figure 2.

For the examination of bioactive compounds of *C. militaris* extraction from different fruiting body formation medium, the chromatograms obtained from the analysis of each sample of *C. militaris* extraction solution using HPLC technique were compared with the chromatograms of standard solutions containing cordycepin and adenosine. It was found that in the chromatograms of CM, CSM, and MCM, there were peaks at retention times matching those of the peaks found in the standard solutions (figure 3). This indicates the ability of *C. militaris* to produce adenosine and cordycepin in each fruiting body formation medium. By calculating the area under the peaks on the chromatogram, the quantities of important substances in *C.militaris* were determined (Table 3). It was found that the highest amounts of adenosine and cordycepin were present in *C. militaris* obtained from CSM, with quantities of 0.22 mg/g and 1.03 mg/g, respectively. The next highest amounts were found in *C. militaris* from MCM, with 0.20 mg/g and 0.81 mg/g, respectively. The lowest amounts of adenosine and cordycepin

DOI: 10.60101/jarst.2023.254104

were found in *C. militaris* obtained from CM, with 0.20 mg/g and 0.81 mg/g, respectively. The data presented in Tables 2 and 3 demonstrate that utilizing MCM and CSM in a 500 mL glass bottle (17 oz) resulted in significantly higher yields from one crop of *C. militaris* than CM. The crop produced approximately 1.45 and 1.46 times more bioactive compounds, with adenosine levels about 1.8 and 1.7 times higher and cordycepin levels about 1.95 and 1.53 times higher, respectively. These findings indicate the potential benefits of using MCM and CSM for enhanced production of bioactive compounds in *C. militaris*. In terms of fruit body production and the production of bioactive compounds of *C. militaris*, the common use of glucose as a carbon and energy source for microorganisms generates growth and secondary metabolites [17].

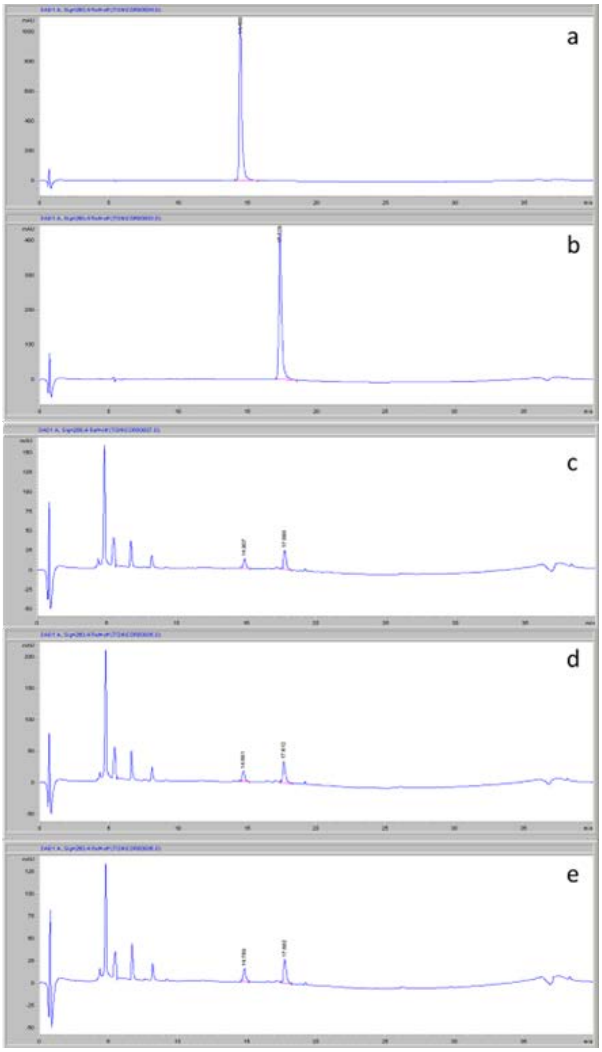

Figure 3 HPLC Chromatogram of bioactive ingredients. Peaks are indicated as follows: (a) Standard adenosine (RT=14.48), (b) Standard cordycepin (RT=17.82), (c) Cereals, (d), Cereals and silkworm pupa, and (e) Modified fruiting body.

However, the rapid breakdown of glucose can reduce the biosynthesis rate of various secondary metabolites [18] and hinder the utilization of alternative carbon sources [19, 20]. Vegetable oils have been introduced as carbon sources to counter carbon catabolite repression due to their low solubility in the culture medium [21]. Additionally, vegetable oils, commonly employed as defoamers in submerged fermentation [22], have demonstrated their ability to stimulate mycelial growth in several medicinal mushrooms, thereby augmenting the production of bioactive metabolites [23, 24]. The incorporation of plant oils, fatty acids, and other carbon sources has been observed to induce the accumulation of exo-polysaccharides and facilitate cell differentiation in various mushrooms, including *Grifola frondosa* [25], *Inonotus obliquus* [26], *C. militaris* [10], and *Ganoderma lucidum* [27]. Notably, [28] reported that oil in the medium promotes the elongation of filamentous fungi. It has been postulated that oils or fatty acids can modify the cell membrane, thereby increasing permeability and stimulating the production of biopolymers [29]. Furthermore, [11] observed positive effects on the up-regulation of the glyoxylate, pentose phosphate, and cordycepin biosynthesis pathways upon adding peanut oil to *C. militaris* culture. Experimental evidence suggests that cordycepin precursor can derive from two sources: adenosine from the purine biosynthesis pathway and 2′,3′-cyclic monophosphate (2′,3′-cAMP) from mRNA degradation. Both compounds can be converted to adenosine-3′-monophosphate (3′-AMP). Subsequently, 3′-AMP is dephosphorylated to 2′-carbonyl-3′-deoxyadenosine (2′-C-3′-dA) by Cns2, ultimately leading to cordycepin through the oxidoreductase Cns1 [30]. Combining plant oil and glucose as dual carbon sources seems to be a promising strategy for enhancing intracellular carbon accumulation and cordycepin production in static culture.

CONCLUSION

In conclusion, the study investigated two aspects related to the cultivation of *C. militaris*. Firstly, in terms of the growth of *C. militaris* on different plant oils, it was found that palm oil at a concentration of 3%, olive oil at a concentration of 3%, sunflower oil at a concentration of 1%, peanut oil at a concentration of 1%, sesame oil at a concentration of 1%, and coconut oil at a concentration of 3% were the most effective concentrations for promoting the mycelial growth of *C. militaris* on PDA medium. Secondly, regarding the fruit body production of *C. militaris* on different fruiting body formation mediums, the use of MCM and CSM resulted in similar outcomes concerning the dry weight of the fruiting bodies, solid-based residues, and total yields, which were comparable to cultivating in the CSM medium. However, both MCM and CSM mediums significantly differed from cultivating with CM, indicating the importance of choosing a fruiting body formation medium for optimal results. These findings suggest that palm oil, olive oil, sunflower oil,

DOI: 10.60101/jarst.2023.254104

peanut oil, sesame oil, and coconut oil can be considered potential components in the solid medium for cultivating *C. militaris*, and that MCM and CSM are effective mediums for fruit body production.

Further research and optimization of these mediums can contribute to the efficient cultivation and production of *C. militaris* for vegetarian consumption, which has potential applications in bioactive compound production and health-related industries.

ACKNOWLEDGEMENT

This research was supported by the Faculty of Science and Technology, Rajamangala University of Technology Tawan-ok.

REFERENCES

1. Chaicharoenaudomrung N, Jaroonwitchawan T, Noisa P. Cordycepin induces apoptotic cell death of human brain cancer through the modulation of autophagy. In Vitro Toxicol. 2018;46:113-21.
2. Ashraf SA, Elkhalifa AEO, Siddiqui AJ, Patel M, Awadelkareem AM, Snoussi M, et al. Cordycepin for health and wellbeing: a potent bioactive metabolite of an entomopathogenic medicinal fungus Cordyceps with its nutraceutical and therapeutic potential. Molecules. 2020;25(12):2735.
3. Das G, Shin HS, Leyva-Gómez G, Prado-Audelo MLD, Cortes H, Singh YD, et al. *Cordyceps* spp.: A review on its immune-stimulatory and other biological potentials. Front Pharmacol. 2021;11: 2250.
4. Elkhateeb WA, Daba G. Cordyceps more than edible mushroom a rich source of diverse bioactive metabolites with huge medicinal benefits. Res J Environ Sci. 2022;3(5):566-74.
5. Kanlayavattanakul M, Lourith N. *Cordyceps militaris* polysaccharides: preparation and topical product application. Fungal Biol Biotechnol. 2023;10(1): 1-6.
6. Lee HH, Park H, Sung GH, Lee K, Lee T, Lee I, et al. Anti-influenza effect of *Cordyceps militaris* through immunomodulation in a DBA/2 mouse model. J Microbiol. 2014;52:696-701.
7. Adnan M, Ashraf SA, Khan S, Alshammari E, Awadelkareem AM. Effect of pH, temperature and incubation time on cordycepin production from *Cordyceps militaris* using solid-state fermentation on various substrates. CYTA - J Food. 2017;15(4): 617-21.
8. Xie CY, Gu ZX, Fan GJ, Gu FR, Han YB, Chen ZG. Production of cordycepin and mycelia by submerged fermentation of *Cordyceps militaris* in mixture natural culture. Appl Biochem Biotechnol. 2009; 158:483-92.
9. Jiaojiao Z, Fen W, Kuanbo L, Qing L, Ying Y, Caihong D. Heat and light stresses affect metabolite production in the fruit body of the medicinal mushroom *Cordyceps militaris*. Appl Microbiol Biotechnol. 2018;102:4523-33.
10. Park JP, Kim SW, Hwang HJ, Cho YJ, Yun JW. Stimulatory effect of plant oils and fatty acids on the exo-biopolymer production in *Cordyceps militaris*. Enzyme Microb Technol. 2002;31(3): 250-5.
11. Tang J, Qian Z, Wu H. Enhancing cordycepin production in liquid static cultivation of *Cordyceps militaris* by adding vegetable oils as the secondary carbon source. Bioresour Technol. 2018;268:60-7.
12. Melhuish Jr JH, Hacskaylo E, Bean GA. Fatty acid composition of ectomycorrhizal fungi in vitro. Mycol. 1975;67(5):952-60.
13. Martynova J, Kokina A, Kibilds J, Liepins J, Scerbaka R, Vigants A. Effects of acetate on Kluyveromyces marxianus DSM 5422 growth and metabolism. Appl Microbiol Biotechnol. 2016;100:4585-94.
14. Melin P, Stratford M, Plumridge A, Archer DB. Auxotrophy for uridine increases the sensitivity of *Aspergillus niger* to weak-acid preservatives. Microbiology. 2008;154(4):1251-7.
15. Andrikopoulos NK. Triglyceride species compositions of common edible vegetable oils and methods used for their identification and quantification. Food Rev Int. 2002;18(1):71-102.
16. Kang H, Hwang S, Lee H, Park W. Effects of high concentrations of plant oils and fatty acids for mycelial growth and pinhead formation of *Hericium erinaceum*. Trans ASABE. 2002;45(1):257.
17. Ruiz B, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, et al. Production of microbial secondary metabolites: Regulation by the carbon source. Crit Rev Microbiol. 2010;36(2):146-67.
18. Park YS, Momose I, Tsunoda K, Okabe M. Enhancement of cephamycin C production using soybean oil as the sole carbon source. Appl Microbiol Biotechnol. 1994;40:773-9.
19. Lane S, Xu H, Oh EJ, Kim H, Lesmana A, Jeong D, et al. Glucose repression can be alleviated by reducing glucose phosphorylation rate in *Saccharomyces cerevisiae*. Sci Rep. 2018;8(1):1-12.
20. Unrean P, Khajeeram S. Model-based optimization of *Scheffersomyces stipitis* and *Saccharomyces cerevisiae* co-culture for efficient lignocellulosic ethanol production. Bioresour Bioprocess. 2015;2: 1-11.

DOI: 10.60101/jarst.2023.254104

21. Choi DB, Park SS, Ahn BK, Lim DH, Lee YW, Moon JH, et al. Studies on production of gentamicin from Micromonosporas purpurea using crude vegetable oils. Process Biochem. 2008;43(8): 835-41.
22. Kougias P, Boe K, Tsapekos P, Angelidaki I. Foam suppression in overloaded manure-based biogas reactors using antifoaming agents. Bioresour Technol. 2014;153:198-205.
23. Conti E, Stredansky M, Stredanska S, Zanetti F. γ-Linolenic acid production by solid-state fermentation of Mucorales strains on cereals. Bioresour Technol. 2001;76(3):283-6.
24. Kagliwal L, Survase S, Singhal R. A novel medium for the production of cephamycin C by *Nocardia lactamdurans* using solid-state fermentation. Bioresour Technol. 2009;100(9):2600-6.
25. Hsieh C, Wang HL, Chen CC, Hsu TH, Tseng MH. Effect of plant oil and surfactant on the production of mycelial biomass and polysaccharides in submerged culture of *Grifola frondosa*. Biochem Eng J. 2008;38(2):198-205.
26. Xu X, Quan L, Shen M. Effect of chemicals on production, composition and antioxidant activity of polysaccharides of *Inonotus obliquus*. Int J Biol Macromol. 2015;77:143-50.
27. Yang FC, Ke YF, Kuo SS. Effect of fatty acids on the mycelial growth and polysaccharide formation by *Ganoderma lucidum* in shake flask cultures. Enzyme Microb Technol. 2000;27(3-5):295-301.
28. Kendrick A, Ratledge C. Cessation of polyunsaturated fatty acid formation in four selected filamentous fungi when grown on plant oils. JAOCS. 1996;73: 431-5.
29. Stasinopoulos S, Seviour R. Stimulation of exopolysaccharide production in the fungus *Acremonium persicinum* with fatty acids. Biotechnol Bioeng. 1990;36(8):778-82.
30. Xia Y, Luo F, Shang Y, Chen P, Lu Y, Wang C. Fungal cordycepin biosynthesis is coupled with the production of the safeguard molecule pentostatin. Cell Chem Biol. 2017;24(12):1479-89.e4.

DOI: 10.60101/jarst.2023.254104