



Growth and effective inhibition of pathogens of *Cordyceps militaris* cultured on Sakon Nakhon local rice

Krit Phinetsathian¹, Nida Arbsuwan², Krongjai Somrug³, Nantiya Chaiyapet⁴, Phichamai Taikham⁴, Sawitree Chaiload⁴, Nathaporn Jirawattanasomkul^{4*}

¹ Program of Biology, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000 Thailand

² Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, 30000 Thailand

³ Department of Plant Science, Faculty of Agricultural Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000 Thailand

⁴ Program of Environmental Science, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon, 47000 Thailand

*Corresponding Author: nathaporn.j@snru.ac.th

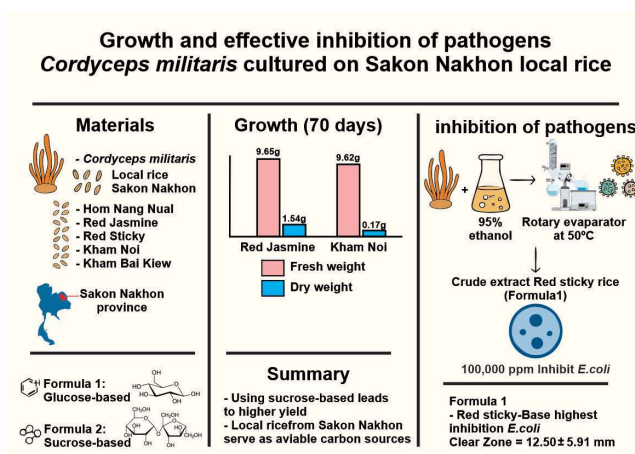
DOI: 10.55674/cs.v17i2.261051

Received: 6 March 2025 Revised: 17 April 2025; Accepted: 24 April 2025; Available online: 25 April 2025

Abstract

This study investigated the growth and antimicrobial activity of *Cordyceps militaris* cultivated on two media types—Formula 1 (glucose-based) and Formula 2 (sucrose-based)—using five local rice varieties from Sakon Nakhon Province: Hom Nang Nual, Red Jasmine, Red Sticky, Kham Noi, and Kham Bai Kiew rice. The growth experiment, spanning 70 days, was divided into three stages, during which the fresh and dry weights of fruiting bodies and bases were recorded. Results showed that Formula 2 with Red Jasmine rice supported the highest growth, yielding 9.65 ± 1.23 g (fresh) and 1.54 ± 0.05 g (dry) per bottle. In Formula 1, the highest yield was observed using Kham Noi rice, with 9.62 ± 1.01 g (fresh) and 0.71 ± 0.16 g (dry) per bottle. For antimicrobial testing, dried *C. militaris* samples were soaked in 95% ethanol for 48 h, filtered, and concentrated via rotary evaporation at 50 °C. The crude extracts were tested against *Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Escherichia coli* using the disc diffusion method at concentrations of 20,000 – 100,000 ppm. At 100,000 ppm, all extracts exhibited antibacterial activity. Notably, the extract from Red Jasmine rice in Formula 1 showed the highest inhibition against *E. coli*, with a clear zone of 12.50 ± 5.91 mm. Statistical analysis confirmed significant differences ($p \leq 0.01$) in inhibitory effectiveness. These findings indicate that a sucrose-based medium enhances the yield and quality of *C. militaris*, and nutrient-rich local rice substrates contribute to its antimicrobial potential. The study supports the application of local rice in *C. militaris* cultivation and suggests potential for developing natural antimicrobial products for future medical use.

Keyword: *Cordyceps militaris*; Pathogenic microorganisms; Local rice; Antimicrobial activity



1. Introduction

Golden cordyceps (*Cordyceps militaris*) is a mushroom that is gaining significant popularity worldwide. In ancient China, it was believed to be an elixir of life that could help nourish the body. *C. militaris* contains essential nutrients, vitamins, and two key bioactive compounds: cordycepin and adenosine [1]. In addition, there are other active compounds including polysaccharides, ergosterol, peptides, amino acids, organic acids, cinnamic acid, and both polyunsaturated and saturated fatty acids [2]. Several studies have reported that the bioactive compounds found in *C. militaris* possess diverse biological activities. These include strong antioxidant properties [3], anti-cancer activity, inhibition of cancer cell growth, and suppression of tumor cell proliferation [4]. In addition, these compounds exhibit antimicrobial effects against bacteria, fungi, and viruses. In particular, it has shown the ability to inhibit pathogenic bacteria that cause diseases in humans, include *E. coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella Typhi*, *Salmonella Typhimurium*, *Shigella dysenteriae*, *B. cereus*, *B. subtilis*, and *S. aureus* [2]. Furthermore, *C. militaris* exhibits anti-inflammatory properties [5]. Additionally, *C. militaris* may play a role in regulating blood sugar levels [6].

However, in Thailand, *C. militaris* is still not widely cultivated or consumed, primarily due to its high price [7]. Another challenge in commercial cultivation is its low yield and limited production of bioactive compounds. These limitations are likely influenced by factors such as cultivation conditions, the culture medium, and the strain of *C. militaris* used. The cultivation medium plays a crucial role, as it provides essential sources of carbon, vitamins, and minerals [8]. Rice could serve as an alternative carbon source for cultivating *C. militaris*, potentially increasing future yields.

Thailand, a major rice-producing country, reported rice exports of 8.35 million tons in January 2024—a 20.32% increase in

volume and a 39.81% rise in value compared to the same period in 2023, according to the Ministry of Commerce [9]. Despite its abundance, rice remains an underutilized resource. Studies have shown that rice can also be used as a substrate for *C. militaris* cultivation [10]. Therefore, indigenous rice varieties from Sakon Nakhon Province present a promising alternative substrate for the cultivation of *C. militaris*, due to their low cost and the fact that they have not yet been widely recognized or utilized. Utilizing locally available rice to create added value aligns with the national policy framework known as the BCG Model. Specifically, the enhancement of rice value falls under the concept of the Bio-economy, which focuses on the sustainable use of biological resources to drive economic growth [11].

Therefore, this research aims to study of growth and effective inhibition of pathogens of *C. militaris* cultured on Sakon Nakhon local rice. Locally available Sakon Nakhon rice is used as an alternative raw material to enhance its value and contribute to the economic growth of Sakon Nakhon Province, Thailand.

2. Materials and Methods

Chemicals and reagents

Potato Dextrose Agar (PDA) and Nutrient Broth (NB) were purchased from HiMedia Laboratories Pvt. Ltd., India. Ethanol was purchased from QReC™, New Zealand.

C. militaris strain and pathogenic microorganisms

The *C. militaris* strain was obtained from Roi Et, Thailand. Pre-made Potato Dextrose Agar (PDA) was used to prepare the culture medium. The pathogenic microorganisms—*Bacillus subtilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Escherichia coli*—were obtained from Sakon Nakhon Rajabhat University. Nutrient agar (NA) was used to prepare the medium for bacterial cultivation.

Preparation and Cultivation of C. militaris in Potato Dextrose Broth (PDB) and the pathogenic microorganisms in Nutrient (NB)

The mycelia of *C. militaris* were transferred to the PDB medium, use corn borer to pierce the

area of the agar with fibers to a diameter of 6 mm, 2 pieces, put into the liquid medium, PDB volume 100 mL, shake at 150 rpm in the dark, temperature 22 °C, 7 days before use [12]. The pathogenic microorganisms were cultured on nutrient agar (NA) and incubated at 37 °C for 24 h. A single colony from the NA culture was then aseptically transferred into a test tube containing nutrient broth (NB). The inoculated broth was subsequently incubated at 37 °C for 18–24 h until the turbidity of the culture matched the 0.5 McFarland standard, which was used for further testing modified from Tasoon [13].

Preparation of Cultivation Medium for Growth of C. militaris

Formula 1 (glucose-based) consists of 30 g of silkworm pupae, 200 g of potato, 15 g of yeast extract, 15 g of peptone, 20 g of glucose, and 0.5 g of sodium chloride.

Formula 2 (sucrose-based) was prepared using the following components: 50 g of silkworm pupae, 200 g of potato, 0.5 g of sodium chloride, 5 g of glucose, and 15 g of sucrose. The ingredients were dissolved in distilled water and adjusted to a final volume of 1 L. For substrate preparation, 40 g of each local rice variety—Hom Nang Nual rice, Red Jasmine rice, Red sticky rice, Kham Noi rice, and Kham Bai Kiew rice—was individually weighed and transferred into separate cultivation bottles. Each bottle was then supplemented with 30 mL of medium: Formula 1 for treatments 1–5 and Formula 2 for treatments 6–10. All bottles were sterilized via autoclaving at 121 °C and 15 psi for 15–20 min, followed by cooling to room temperature, in accordance with the method described in Imtiaj *et al.* [14].

The cultivation of *C. militaris* may be achieved using two primary methods: biological substrates, such as silkworm pupae or other insect larvae, and synthetic or semi-synthetic media composed of various plant-based substrates. In the case of synthetic cultivation, cereals such as millet, oats, corn, Job's tears, and several types of rice are commonly employed as primary carbon sources. A standard growth

medium for *C. militaris* typically consists of three major components: (1) carbon sources—grains, flours, and sugars; (2) nitrogen sources—yeast extract, peptone, egg powder, silkworm pupae powder, or potato extract; and (3) micronutrients—vitamins (e.g., vitamin B1) and minerals (e.g., magnesium sulfate or Epsom salt). Therefore, the choice of raw materials serving as carbon and nitrogen sources plays a crucial role in influencing the growth and yield of *C. militaris* [15].

Inoculation and cultivation

The inoculation was performed by transferring 10 mL of the prepared *C. militaris* culture into the prepared cultivation bottles (treatments 1-10). The bottles were then shaken to ensure even distribution of the culture throughout the medium. The *C. militaris* cultivation was incubated and observed for growth, which was divided into three stages. During the Initial Growth Stage, the bottles were incubated in a culture room at a temperature ranging from 20-24 °C for approximately 3 weeks. The mycelium of *C. militaris* began to grow and spread, covering the substrate with white mycelial growth modified from Dang *et al.* [16].

Fruiting Body Initiation Stage

Once the mycelium has fully colonized the substrate, the bottles were moved to an incubator where the temperature was adjusted to 15-18 °C, and light was provided at 300-500 lux for 12-14 h per day.

Fruiting Body Growth Stage

When the *C. militaris* began to produce fruiting bodies approximately 1 cm in length, the temperature in the incubator was raised to 20-24 °C, and light was provided at 600-1000 lux. Ventilation holes were made on the plastic cover to allow for air exchange. The cultivation was then continued for a period of 70 days.

Record the experimental results

The experimental results were recorded by observing changes in the mycelium characteristics and color. The fruiting bodies and bases of *C. militaris* were harvested and

dried at 50 °C for 24 h. Both the fresh and dry weights were then recorded adapted from Tasoon [13]. Harvesting of Fresh and Dried of *C. militaris*, the harvesting *C. militaris* was carried out when the fruiting bodies had fully developed in the cultivation bottles. The fruiting bodies were carefully separated from the substrate base using sterilized forceps, ensuring that the fruiting structures remained as intact as possible. The fresh weight of the fruiting bodies and base was recorded prior to drying. The drying process was performed using a hot air oven. *C. militaris* was dried at 50 °C for 6 h, while base was dried at the same temperature for 12 h. After drying, the dry weights were measured.

Crude extract preparation from C. militaris

The *C. militaris* to be tested for antimicrobial activity. The fruiting bodies and base is washed thoroughly with water, then dried at a temperature of 50 °C for 48 h. The samples were dried and ground into a fine powder before use. One gram sample was extracted by soaking in 10 mL of 95% ethanol for 48 h. After the extraction period, the mixture is filtered through sterile cheesecloth to collect the first filtrate. The sample is then soaked again for another 48 h, followed by a second filtration to separate the residue. The two filtrates are combined, and the solvent is evaporated using a rotary evaporator at 50 °C until a viscous extract is obtained with no remaining condensed solvent. The crude extract was transferred into a dark glass bottle and stored in a refrigerator at 4 °C before use.

The effective inhibition of pathogens of C. militaris cultured on Sakon Nakhon local rice

The efficacy of crude extracts on the growth of four pathogenic microorganisms was tested using the paper disc diffusion method. The bacterial pathogens were prepared with an initial cell concentration of 1×10^8 CFU mL⁻¹, which was adjusted by comparing turbidity with the McFarland standard No. 0.5. The swab plate technique was then applied to NA agar plates [17]. Using a micropipette, 10 µL of the *C. militaris* crude extract, extracted with ethanol at

concentrations of 20,000, 40,000, 60,000, 80,000, and 100,000 ppm, were applied to 6 mm diameter paper discs. For the control group, DMSO was used instead of the crude extract. The plates were incubated at 37 °C for 48 h, and the test was repeated three times. The inhibition results were assessed by measuring the diameter of the clear zones, and the effectiveness of the crude extracts in inhibiting the pathogenic bacteria was compared. Statistical analysis was performed using ANOVA (Analysis of Variance), and differences in mean values were compared using SPSS software, with a confidence level of 99% ($P < 0.01$).

3. Results and Discussion

Growth of C. militaris cultured on Sakon Nakhon local rice

Stage 1: The growth of *C. militaris* was compared on two culture media formulations: Formula 1 (glucose-based) and Formula 2 (sucrose-based). Results showed that Formula 2 promoted better mycelial growth, with white mycelium covering the substrate more effectively. In Formula 2, mycelium fully covered the substrate in an average of 14 days for Red Jasmine rice and 16 days for Kham Bai Kiew rice.

The next best results were observed with Kham Noi rice, Hom Nang Nual rice and Red sticky rice, where mycelium fully covered the substrate in 17, 18, and 21 days, respectively. In contrast, Formula 1 resulted in full mycelial coverage in 17 and 18 days for Kiew rice and Kham Bai rice, respectively. Red sticky rice and Red Jasmine rice achieved full coverage in 19 days, while Hom Nang Nual rice took an average of 21 days, as shown in Fig. 1. This is consistent with the study by Liang *et al.* [18], which found that the duration for mycelial colonization of the substrate was similar to that observed in the present study. Brown rice (Br), plumule rice (Pr), wheat (W), and pearl barley (Pb) were used for the production of fruiting bodies and bioactive compounds. The results showed that the mushroom strains fully colonizing this substrate within an average of 12 day.

This is consistent with the research by Sornprasert *et al.* [19], who studied the cultivation of *C. militaris* (L.) Link BCC 18247 using cereal grains as substrates. They found that brown sweet potato rice, Red Jasmine rice, and glutinous rice G6 exhibited a high density of mycelium after 15 days of cultivation. In contrast, black rice, jasmine brown rice, jasmine rice, glutinous rice D, and glutinous rice Luea Phua showed a medium density of mycelium.

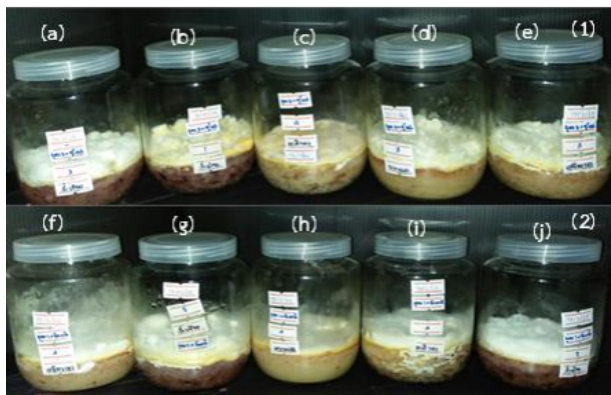


Fig. 1 Mycelial development after 3 weeks of dark incubation. (1) Formula 2: (a–e) Kham Bai Kiew, Kham Noi, Red Jasmine, Hom Nang Nual, and Red sticky rice, respectively. (2) Formula 1: (f–j) Kham Bai Kiew, Kham Noi, Red Jasmine, Hom Nang Nual, and Red sticky rice, respectively.

Stage 2: The light exposure stage triggers the color change of the fibers from white to yellow-orange and the formation of flower buds. The experimental results showed that the Formula 2, nutrient medium formula promoted a faster color change and flower bud development compared to the Formula 1. With the Formula 2, Kam Noi rice and Red sticky rice underwent the color change from white to yellow-orange and developed flower buds the fastest, in an average of 29 and 32 days, respectively. In contrast, the Formula 1 showed that Kam Bai Kiew rice and Kam Noi rice took longer to change color, with an average of 36 days for both, as shown in Fig. 2. This is consistent with Sornprasert *et al.* [20], who studied the cultivation of *C. militaris* using local grains and insects. The experimental results indicated that the wheat seed formula significantly outperformed other grain formulas

in promoting the fastest development of the mycelium into flower buds, with an average of 35.14 days. The next fastest were Black Jasmine rice, white Red sticky rice, millet seeds, and scented rice, which developed the mycelium into flower buds in 38.92, 38.57, 38.57, and 39.57 days, respectively. After 40 days, it was observed that Black Jasmine rice, wheat, and millet seeds had a moderate number of flower buds.

Stage 3: In this stage, increased light exposure from fluorescent lamps at 600 - 1,000 lux was used to promote the development of *C. militaris* fruiting bodies from the primordia into mature mushrooms after 70 days of incubation. The experimental results showed that the Formula 2, which used Red Jasmine rice and Hom Nang Nual rice, promoted the fastest development into *C. militaris*, with an average of 18 and 21 days, respectively.



Fig. 2 Fiber color change from white to yellow-orange and bud formation. (1) Formula 2: (a–e) Kham Bai Kiew, Kham Noi, Red Jasmine, Hom Nang Nual, and Red sticky rice, respectively. (2) Formula 1: (f–j) Kham Bai Kiew, Kham Noi, Red Jasmine, Hom Nang Nual, and Red sticky rice, respectively.

The next fastest were Kam Noi rice, Red sticky rice, and Kam Bai Kiew rice, with averages of 21, 22, and 23 days, respectively. In the Formula 1, Kam Noi rice and Red sticky rice developed into *C. militaris* with averages of 21, 23, and 25 days. This is consistent with the research by Ratthaphol *et al.* [20], which

studied the cultivation of golden cordyceps using local grains and insects. The research found that the development of the primordia into the mature fruiting body of *C. militaris* was statistically significantly different ($p < 0.05$) when comparing the average times. Black jasmine rice developed into the *C. militaris* fruiting body the fastest, with an average of 39.14 days, followed by white sticky rice, wheat, scented rice, and millet, with averages of 41.14, 41.71, 42.71, and 45.14 days, respectively. *C. militaris* was harvested after cultivation using both nutrient medium formulas.

The experimental results showed that *C. militaris* cultivated with Formula 1, which included Hom Nang Nual rice, Red Jasmine rice, Kham Noi rice, and Kham Bai Kiew rice as cultivation materials, did not grow fruiting body. This may have been due to the insufficient light intensity from the incubation chamber, which did not meet the light requirements for the growth of *C. militaris*, resulting in incomplete development of the fruiting bodies. This is consistent with the research by Srijumpa *et al.* [21], which found that light is a key factor influencing the growth of the mycelium of *C. militaris*. The growth characteristics of *C. militaris* are shown in Fig. 3.



Fig. 3 Characteristics of *C. militaris* cultivated on five rice types. (1) Formula 1: (a–e) Kham Bai Kiew, Kham Noi, Red Jasmine, Hom Nang Nual, and Red sticky rice. (2) Formula 2: (f–j) Kham Bai Kiew, Kham Noi, Red Jasmine, Hom Nang Nual, and Red sticky rice.

Determination of Fresh and Dry Weight of C. militaris

The growth of *C. militaris* cultivated on two nutrient media (Formulas 1 and 2) using five local rice types—Hom Nang Nual, Red Jasmine, Red Sticky, Kham Noi, and Kham Bai Kiew—was evaluated based on fresh and dry weights of fruiting bodies and bases. Formula 2 (sucrose-based) showed superior performance, supporting growth in all rice types. The highest average fresh fruiting body weight was observed with Red Jasmine rice (9.66 ± 1.23 g/bottle), followed by Kham Bai Kiew (9.44 ± 1.37 g) and Kham Noi (7.37 ± 1.68 g). Corresponding dry weights were 1.54 ± 0.05 g, 1.05 ± 0.23 g, and 0.78 ± 0.43 g, respectively.

In contrast, Formula 1 (glucose-based) only supported substantial fruiting on Red Sticky (2.77 ± 0.91 g fresh, 0.45 ± 0.27 g dry) and Kham Noi rice (9.62 ± 1.01 g fresh, 0.71 ± 0.16 g dry). Base weights also varied. Formula 1 produced the highest fresh base weight with Kham Bai Kiew rice (24.08 ± 2.97 g) and highest dry base weight with Red Sticky (4.92 ± 0.03 g). For Formula 2, Red Jasmine rice yielded the highest base fresh and dry weights at 19.57 ± 0.18 g and 5.17 ± 0.82 g, respectively. These findings are summarized in Table 1.

The experiment demonstrated that *C. militaris* cultivated on five types of local rice using nutrient medium formula 2 (sucrose-based) grew well, particularly on Red Jasmine rice, which yielded the highest fresh and dry fruiting body weights at 9.65 ± 1.23 g and 1.54 ± 0.05 g per bottle, respectively. In comparison, formula 1 (glucose-based) showed the highest growth on Kham Noi rice, with fresh and dry weights of 9.62 ± 1.01 g and 0.71 ± 0.16 g, respectively.

The superior performance of formula 2 aligns with previous of Moa *et al.* [22], findings which suggest that sucrose is a more effective carbon source than glucose for *C. militaris* cultivation. This is further supported by Palakawong [23], where the sucrose-containing medium yielded the highest fiber content (2.45 ± 0.03 g per bottle), reinforcing the

conclusion that sucrose promotes greater biomass accumulation.

The success of formula 2 can be attributed to its comprehensive nutrient composition, which supports fungal growth. Additionally, Red Jasmine rice, known for its high levels of copper, iron, beta-carotene, vitamin E, and lutein, likely contributed to the enhanced development of *C. militaris*. However, for optimal growth, supplementation of the medium with additional nutrients remains

essential. These include carbon sources (e.g., glucose), nitrogen sources (e.g., peptone, yeast extract), minerals (e.g., calcium salts), and vitamins (e.g., B1), which are crucial for supporting mycelial development and fruiting body formation. Overall, the results confirm that Hom Nang Nual rice, Red Jasmine rice, Red Sticky rice, Kham Noi rice, and Kham Bai Kiew rice are all suitable substrates for *C. militaris* cultivation.

Table 1 Comparison of the growth of *C. militaris*.

Formula	Types of local rice	Fresh weights of fruiting bodies (g)	Dry weights of fruiting bodies (g)	Fresh weights of base (g)	Dry weights of base (g)
Formula 1	Hom Nang Nual	-	-	16.97 ± 1.60	4.15 ± 0.48
	Red Jasmine	-	-	18.53 ± 0.85	2.82 ± 0.80
	Red Sticky	2.77 ± 0.91	0.45 ± 0.27	19.12 ± 0.87	4.92 ± 0.03
	Kham Noi	9.62 ± 1.01	0.71 ± 0.16	9.51 ± 0.27	2.23 ± 0.46
	Kham Bai Kiew	-	-	24.08 ± 2.97	4.49 ± 0.67
Formula 2	Hom Nang Nual	7.13 ± 0.49	0.74 ± 0.42	16.32 ± 1.79	5.50 ± 0.95
	Red Jasmine	9.65 ± 1.23	1.54 ± 0.05	19.57 ± 0.18	5.17 ± 0.82
	Red Sticky	3.15 ± 0.96	0.46 ± 0.02	15.05 ± 1.47	3.74 ± 0.29
	Kham Noi	7.37 ± 1.68	0.78 ± 0.48	16.73 ± 0.61	0.61 ± 0.81
	Kham Bai Kiew	9.44 ± 1.37	1.05 ± 0.23	12.88 ± 1.26	3.38 ± 0.21

The effective inhibition of pathogens of C. militaris cultured on Sakon Nakhon local rice

This study investigated the antimicrobial activity of crude ethanol extracts from *C. militaris* cultivated on various local rice varieties from Sakon Nakhon Province, including Fragrant Nang Nual rice, Red Jasmine rice, Red Sticky rice, and Khao Kham Noi rice. The extracts exhibited a deep brick-yellow color and viscous texture. Their antimicrobial activity was tested against four pathogenic bacterial strains: *S. aureus*, *S. epidermidis*, *E. coli*, and *B. subtilis*, using extract concentrations of 20,000–100,000 ppm.

The crude extract from *C. militaris* cultivated on Hom Nang Nual rice demonstrated broad-spectrum antimicrobial activity, inhibiting all four pathogens with an average inhibition zone diameter of 7.00 mm. Similarly, the extract from Kham Noi rice showed inhibition against all tested strains, with an average zone diameter of 8.00 mm. In contrast, extracts from Red Jasmine rice and Red Sticky rice inhibited only

E. coli, *S. aureus*, and *B. subtilis*, with average inhibition zones of 5.00 mm and 12.50 mm, respectively.

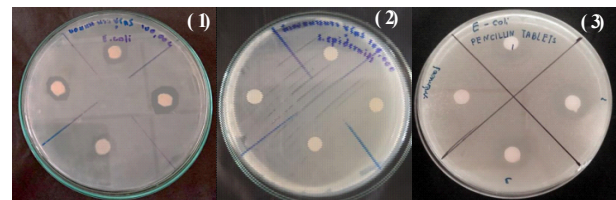


Fig. 4 Fig. 4. Representative inhibition zones of pathogenic microorganisms by crude extracts of *C. militaris*. (1) Inhibition of *E. coli* growth by crude extract from *C. militaris* cultivated on Hom Nang Nual rice (Formula 2) at a concentration of 100,000 ppm. (2) Inhibition of *S. epidermidis* growth by crude extract from *C. militaris* cultivated on Hom Nang Nual rice (Formula 2) at a concentration of 100,000 ppm. (3) Positive control: Inhibition of *E. coli* and *S. epidermidis* growth by Penicillin.

Statistical analysis indicated significant differences in inhibition efficacy between treatments ($p < 0.01$), as shown in Table 2 and Fig. 4. These findings are consistent with previous reports. For instance, Ratnasajja *et al.* [17] reported that *C. militaris* crude extract (ethanol, 48-h extraction) inhibited *E. coli* with a zone of 13.30 ± 1.32 mm, while Deshmukh *et al.* [24] reported a methanol extract achieving 15.96 ± 0.68 mm against *E. coli*.

Our findings also align with those of [13], who cultivated *C. militaris* on cereal grains and insect media and reported antimicrobial activity from extracts containing 103.47 mg of adenosine and 209.42 mg of cordycepin per 100

g dry weight. They observed inhibition of *Trichophyton rubrum* and *S. aureus* at concentrations similar to this study.

Notably, in our experiment, crude extracts from *C. militaris* grown on Hom Nang Nual and Kham Noi rice did not inhibit *S. epidermidis*. This may be attributed to the organism's ability to form biofilms and its resistance to penicillinase and cephalixin, making it more resilient to crude bioactive compounds. Fig. 4 illustrates an example of inhibition zones produced by the crude extract from *C. militaris* fruiting bodies.

Table 2 The average diameter of the inhibition zone against pathogenic bacteria using crude extracts of *C. militaris*.

Types of crude extract	Crude extract (ppm)	Clear zone (mm)			
		<i>E. coli</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>S. epidermidis</i>
Red sticky (Base) Formula 1	20,000	0	0	0	0
	40,000	0	0	0	0
	60,000	0	4.00±0.00	0	0
	80,000	9.00±4.24	5.00±0.00	6.00±0.82	0
	100,000	12.5±5.91	6.33±0.47	6.60±0.94	0
Kham Noi rice (fruiting bodies) Formula 1	20,000	0	0	0	0
	40,000	0	0	0	0
	60,000	0	6.5±3.09	0	0
	80,000	5.00±2.36	7.70±2.05	0	0
	100,000	6.00±2.83	8.00±3.78	4.00±1.89	5.00±0.81
Hom Nang Nual (Base) Formula 2	20,000	0	0	0	0
	40,000	3.00±1.41	0	0	0
	60,000	4.33±0.62	0	0	0
	80,000	5.00±0.70	0	0	0
	100,000	7.00±0.47	4.00±0.70	5.00±2.36	5.00±0.00
Red Jasmine (fruiting bodies) Formula 2	20,000	0	0	0	0
	40,000	0	0	0	0
	60,000	0	0	0	0
	80,000	4.00±1.89	4.00±0.00	4.50±2.16	0
	100,000	5.00±2.36	5.00±0.40	4.00±0.23	0
	Control	0	0	0	0

Note: 0 means no clear zone of growth inhibition of the pathogenic microorganism

4. Conclusion

This study investigated the growth performance and antimicrobial activity of *C. militaris* cultivated on two culture media (Formula 1: glucose-based and Formula 2: sucrose-based), using five local rice varieties from Sakon Nakhon Province: Hom Nang Nual,

Red Jasmine (Mali Dang), Red Sticky, Kham Noi, and Kham Bai Kiew.

C. militaris successfully developed mycelia and fruiting bodies on all rice types in both formulations. Formula 2, with sucrose and Red Jasmine rice, yielded the highest fresh weight of fruiting bodies (9.65 ± 1.23 g/bottle). In contrast, Formula 1 supported significant

fruiting only in Kham Noi and Red Sticky rice, with Kham Noi yielding 9.62 ± 1.01 g/bottle. Regarding base weight, Formula 1 with Kham Bai Kiew rice showed the highest value (24.08 ± 2.97 g/bottle), while Formula 2 with Red Jasmine rice produced the highest in its group (19.57 ± 0.18 g/bottle). These results highlight the influence of both culture medium and rice substrate on fungal biomass and productivity.

Antimicrobial assays revealed that crude ethanol extracts from both fruiting bodies and bases inhibited the growth of *B. subtilis*, *S. epidermidis*, *S. aureus*, and *E. coli* at 100,000 ppm. Notably, the extract from Red Sticky rice (Formula 1) showed the strongest inhibition against *E. coli* (12.50 ± 5.91 mm), while the extract from Kham Noi rice (fruiting body) was most effective against *B. subtilis* (8.00 ± 3.78 mm). Significant differences ($p < 0.01$) in antimicrobial activity were observed among the different rice substrates.

In summary, local rice varieties from Sakon Nakhon serve as viable carbon sources for the cultivation of *C. militaris*, enhancing both growth and antimicrobial properties. This adds value to traditional Thai rice strains and supports the development of health-related applications. The crude extracts show potential for use in dietary supplements, functional foods, or pharmaceuticals. Further research on safety, cytotoxicity, and clinical efficacy is recommended to support future therapeutic use.

5. Suggestions

- Future research should quantify active compounds in *C. militaris* and identify optimal growth conditions (e.g., light, nutrients).
- Studies should determine the minimum inhibitory concentration (MIC) of crude extracts against pathogens.
- Further investigation is needed into the potential use of *C. militaris* extracts in cosmetics, pharmaceuticals, and healthcare products.

6. Acknowledgement

We would like to express our sincere gratitude to Asst. Prof. Dr. Surachai Rattanasuk from Roi Et Rajabhat University for providing the *C. militaris* used in this study. Our heartfelt thanks also go to the Program of Environmental Science, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, for generously providing the facilities and chemicals essential for this research.

7. References

- [1] S.K. Das, M. Masuda, A. Sakurai, M. Sakakibara, Medicinal uses of the mushroom *Cordyceps militaris*, *Fitoterapia*. 81(8) (2010) 961-968. 10.1016/j.fitote.2010.07.010.
- [2] P. Ratnasajja, S. Taikhaw, Effects of crude extract in stroma of *Cordyceps militaris* for human enteropathogenic bacterial inhibition, *J. Res. Unit Sci. Technol. Environ.*, 15 (1) (2024) 96-113.
- [3] C.H. Dong, T. Yang, T. Lian, A comparative study of the antimicrobial, antioxidant, and cytotoxic activities of methanol extracts from fruit bodies and fermented mycelia of caterpillar medicinal mushroom *Cordyceps militaris* (Ascomycetes), *Int J Med Mushrooms*. 16(5) (2014) 485-495. 10.1615/intjmedmushrooms.v16.i5.70.
- [4] S.L. Qu, S.S. Li, D. Li, P.J. Zhao, Metabolites and Their Bioactivities from the Genus *Cordyceps*, *MDPI*. 10(8) (2022) 1-20. 10.3390/microorganisms10081489.
- [5] D. Zhang, Q. Tang, X. He, Y. Wang, G. Zhu, L. Yu, Antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of *Cordyceps militaris* spent substrate, *PLoS ONE*. 18(9) (2023) 1-23. 10.1371/journal.pone.0291363.
- [6] L. Ma, S. Zhang, M. Du, Cordycepin from *Cordyceps militaris* prevents hyperglycemia in alloxan-induced diabetic mice, *Nutr Res*. 35(5) (2015) 431-9. 10.1016/j.nutres.2015.04.011.
- [7] Y. Tongaram, A comparative study of *Cordyceps militaris* (Tung Chao) culture from liquid and solid inoculation on growth medium

- mixed with Thai millet, CRMA Journal. 13(1) (2015) 87-99.
- [8] M.Y. Chang, G.J. Tsai, J.Y. Houg, Optimization of the medium composition for the submerged culture of *Ganoderma lucidum* by Taguchi array design and steepest ascent method, Enzyme and Microb. Technol. 38(3-4) (2006) 407-414. 10.1016/j.enzmictec.2005.06.011.
- [9] Thai Rice Exporters Association, Rice export report, <http://www.thairiceexporters.or.th/>, 22 January 2025.
- [10] T. Thapingkae, M. Yachai, S. Sritiwong, K. Upalsilp, A. Phonpanawit, A. Thongthap, W. Thapingkae, Study on the cultivation of Golden Cordyceps mushroom and its applications, Research report, Chiang Mai Rajabhat University, Chiang Mai, 2014.
- [11] BCG model, <https://www.doae.go.th/en/>, 21 January 2025.
- [12] B. Shrestha, W. Zhang, Y. Zhang, X. Liu, The medicinal fungus *Cordyceps militaris*: Research and development, Mycol. Prog. 11 (2012) 599-614.10.1007/s11557-012-0825-y.
- [13] S. Tasun, Optimization condition for *Cordyceps militaris* cultivation on spent coffee grounds, Master's thesis, Mahasarakham University, Mahasarakham, 2023.
- [14] A. Imtiaj, T. S. Lee, Screening of antifungal activities from Korean wild mushrooms, World J. Agric. Sci. 3(3) (2007) 316-321.
- [15] S. Awarun, Suitable Media for Cultivation of *Cordyceps militaris*, JVIA. 3(2) (2019) 15-23.
- [16] H.N. Dang, C.L. Wang, H.L. Lay, Effect of nutrition, vitamin, grains, and temperature on the mycelium growth and antioxidant capacity of *Cordyceps militaris* (strains AG-1 and PSJ-1), J RADIAT RES APPL SC. 11 (2) (2017) 130 - 138 . 10.1016/j.jrras.2017.11.003
- [17] P. Ruttanasutja, S. Taikhao, Effects of crude extract in stroma of *Cordyceps militaris* for human enteropathogenic bacterial inhibition, J. Res. Unit Sci. Technol. Environ. 15 (1) (2024). 96-113.10.14456/jstel.2024.8.
- [18] Z.C. Liang, C.H. Liang, C.Y. Wu, Various Grain Substrates for the Production of Fruiting Bodies and Bioactive Compounds of the Medicinal Caterpillar Mushroom, *Cordyceps militaris* (Ascomycetes), Int. J. Med. Mushrooms. 16 (6) (2014) 569-578.10.1615/IntJMedMushrooms.v16.i6.60.
- [19] R. Sornprasert, P. Saenkamon, C. Chanbamrung, M. Sanguankaew, A. Hambananda, Cultivation of *Cordyceps militaris* (L.) Link BCC 18247 mycelium with different grains, Chandrakasem Rajabhat University Journal. 18 (35) (2012) 83-91.
- [20] R. Somprasert, A. Hambananda, S. Aroonsrimorakot, Cultivation of Cordyceps militaria using different cereal grains and local insects and inhibitory efficiency against *Trichophyton rubrum* and *Staphylococcus aureus*, The Journal of KMUTNB, 26 (2) (2016) 239-248.
- [21] N. Srijumpa, R. Norarat, S.N. Nan, T. Maneerat, Effect of Different LED Color on Yield and Cordycepin Production of *Cordyceps militaris*, Thai J. Agric. Sci. 40 (1) (2022) 81-89.
- [22] X.B. Mao, T. Eksriwong, S. Chauvatcharin, J. J. Zhong, Optimization of carbon source and C:N ratio for cordycepin production by submerged cultivation of medicinal mushroom *Cordyceps militaris*, Process Biochem. 40(5) (2005) 1667-1672. 10.1016/j.procbio.2004.06.046.
- [23] W. Palakawong, Effect of carbon and protien sources in liquid to static cultivation of *Cordyceps militaris*, Bachelor of Science in Biotechnology, Thammasat University, Bangkok, 2018.
- [24] N. Deshmukh, L. Bhaskaran, Comparative analysis of the antioxidant and antibacterial activity of methanolic mycelium extract from *Cordyceps militaris*: A comprehensive study, Biological Forum. 15(5) (2023) 1681-1686.