**Original Research Article**

**Effect of Rice and Edible Insects Varieties on Cordycepin Production of *Cordyceps militaris***

**Running title: Rice varieties and edible insects on the growth of *Cordyceps militaris***

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**Novelty statement**

This study discovers the effect of rice varieties and edible insects on cordycepin production of *Cordyceps militaris* that can be beneficial for the *C. militaris* cultivation. This study will help the researcher to uncover the critical areas of using rice varieties and edible insect varieties for *C. militaris* cultivation to obtain high cordycepin content that many researchers were not able to explore. Thus, a new application using rice varieties and edible insect varieties on cordycepin production of *Cordyceps militaris* may be arrived at.

**Abstract**

*Cordyceps militaris* is an edible mushroom that has many medical properties including anti-microbial, anti-inflammatory, reduced blood sugar, and reduced cholesterol. This research aimed to evaluate the effect of rice varieties and edible insects on cordycepin production of *C. militaris*. Ten rice varieties and 8 edible insect species were used as ingredients for the *C. militaris* culture medium preparation. After 75 days of cultivation, the cordycepin content of each condition was determined using HPLC. **Results:** The results showed that the *C. militaris* grown with RD 43 rice and Eri silkworm (*Samia ricini* D.) were presented with the highest content of cordycepin at 1,861.98 mg/kg and 1,390.76 mg/kg. **Conclusion:** The suitable rice and edible insect for  *C. militaris* cultivation were RD 43 rice and Eri silkworm. This is a new finding about RD 43 rice and Eri silkworm are suitable for *C. militaris* culture medium composition which benefit *C. militaris* cultivation.

**Keywords:** Rice variety, Edible insect variety, *C. militaris* cultivation

**Introduction**

*Cordyceps militaris*is one of the beneficial mushrooms which belongs to the Cordycipitaceae family (Dong et al., 2014). *C. militaris* has attracted considerable attention for its benefits to human health, such as obesity-related disease prevention, antitumor (Jin et al., 2018), antioxidant, antihyperglycemic, anticancer (Uzma Sitara et al., 2022), antitumor, immunomodulatory (Lee et al., 2020), antimicrobial, anti-inflammatory, and anti-hyperlipidemic effects (Huang et al., 2022). Many bioactive compounds were presented in *C. militaris* including adenosine, cordycepin, polysaccharides, mannitol, superoxide dismutase (SOD), and carotenoids (Guo et al., 2016; Lan et al., 2022). The most important bioactive compound is cordycepin which is considered a nucleic acid antibiotic. Cordycepin (C10H13N5O3) or 3’-Deoxyadenosine is a purine nucleoside that produces by *C. militaris* (Sharma et al., 2022). Various interactions of cordycepin in biochemical processes, including antioxidant, anti-microbial, anti-inflammatory (Kopalli et al., 2022), nucleic acid synthesis, platelet aggregation, metastasis, apoptosis, cell cycle signaling (Tuli et al., 2013), enhanced immunity, inhibited the viral RNA proliferation, and suppressed cytokine storms, and potential to treat COVID-19 (Tan et al., 2021).

*C. militaris* culture medium was reported with various compositions including various C-source, Nitrogen sources, vitamins, and minerals. Mao *et al*. (2005) reported the seed culture medium of *C. militaris* consisted of glucose, 40 g/l; yeast extract, 10 g/l; KH2PO4, 0.5 g/l; K2HPO4·3H2O, 0.5 g/l and MgSO4·7H2O, 0.5 g/l (Mao et al., 2005). Lin *et al*. (2017) reported the liquid medium (GPBY) of *C. militaris*, consisting of glucose 20 g/l, peptone 5 g/l, beef extract 3 g/l, and yeast extract 1 g/l (Lin et al., 2017). Mao *et al*. (2006) presented the cultivation medium was composed of 10 g/l glucose, 0.5 g/l MgSO4·7H2O, 0.5 g/l K2HPO4·3H2O, 0.5 g/l KH2PO4, 0.1 g/l CaCl2, 0.1 g/l FeSO4·7H2O and 40mM of a nitrogen compound (Mao and Zhong, 2006). Huy, N.N. and H.A. Phuong (2021) reported the using of brown rice, fresh pupae liquid, dry pupae powder and mineral solution for *C. militaris* cultivation (Huy and Phuong, 2021).

Various substrates have been used as solid-state for *C. militaris* cultivation such as husked rice (TingChi et al., 2008), bean powder, corn grain, corn cobs, cotton seed coats, jowar, millet, sorghum, fragments of sunflower floral disks, and wheat grain (Shrestha et al., 2012). Rice is the main principal carbon source ingredient for growing stromata of *C. militaris*.Various rice varieties were used such as Brown rice (Huy and Phuong, 2021), Jasmine Rice 105 (Rattanasuk et al., 2018), Rice bran, Riceberry rice, Barley, Wheat (Li et al., 2022; Pintathong et al., 2021), oats (Wu et al., 2022), glutinous rice (Wen et al., 2014), etc. Various insect pupae and larvae have been used as nitrogen source such as silkworm (*Bombyx mori*) (Lei et al., 2009), *Samia ricini* D., *Acheta domesticus* L., *Gryllus bimaculatus* De Geer, *Tenebrio molitor* L., *Rhynchophorus ferrugineus*, *Lethocerus indicus* (Wongsorn et al., 2021). A few reports about effect of rice and edible insects on cordycepin production of *C. militaris* cultivation. This research aimed to evaluate the effect of rice varieties and edible insects on cordycepin production of *C. militaris*. This report will present the suitable rice and edible insect varieties which supplement in culture medium to obtain high cordycepin content for *C. militaris* cultivation.

**Materials and methods**

**Study area:** All the experiments were performed during October 2020 to September 2021 in Microbiology Laboratory, Department of Science and Technology, Faculty of Liberal Arts and Science, Roi Et Rajabhat University, Roi Et, Thailand**.**

***Cordyceps militaris* strain**

The *C. militaris* strain BH was purchased from Farm Hed Prasamutjadee, Samut Prakan, Thailand. The *C. militaris* BH was transferred onto potato dextrose agar (PDA) before being used as a starter.

**Rice varieties**

Ten rice varieties including Jasmine Rice 105, Rice RD 79, Banlay Brown Rice, Rice RD 43, Japanese rice, Tha Heang rice, Sao Hai Rice, Red Cargo Rice, Riceberry Rice, and Tubtim Chum Phae brown ricewere used as the solid culture medium.

**Edible insect species**

Eight edible insect species including Silkworm (*Bombyx mori* L.), Mealworm (*Tenebrio molitor* L.), Eri silkworm (*Samia ricini* D.) Oriental Mole Cricket (*Gryllotalpa orientalis*), Giant water bug (*Lethocerus indicus*), Field cricket (*Gryllus bimaculatus* De Geer), House cricket (*Acheta domesticus* L.), and Sago palm weevil (*Rhynchophorus ferrugineus*) were used as the main nitrogen source.

**Chemicals and reagents**

Potato dextrose agar (PDA), yeast extract, Glucose, and Peptone were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd, India). The potato was purchased from the local market.

**Effect of rice varieties**

*C. militaris* starter was prepared by culturing in a liquid culture medium (potato 200g/L, glucose 25 g/L, Yeast extract 2 g/L and peptone 2 g/L) with shaking at 150 rpm at 22 oC for a week before use. Each rice variety was mixed with *C. militaris* liquid culture medium in a glass bottle and sterilized for 30 min at 121 oC before use. Two milliliters of inoculum starter were added to each bottle using an aseptic technique. The bottles were incubated in the dark at 22 oC for 7 days, under light at 18 oC for 7 days, and at 22 oC for 60 days. Fresh *C. militaris* was collected and dried at 50 °C for 24 hrs (Rattanasuk et al., 2018).

**Effect of edible insect species**

The Jasmine Rice 105 was added to a glass bottle and mixed with *C. militaris* liquid culture medium containing each edible insect. Two milliliters of inoculum starter were added to each bottle using an aseptic technique and incubated at mention above.

**Cordycepin and adenosine content analysis**

 Dried *C. militaris* was sent to the Institute of Product Quality and Standardization, Maejo University, Chiang Mai, Thailand for cordycepin and adenosine content analysis using HPLC.

**Data Analysis**

In this study, it was used experimental design followed by descriptive analysis

**Results and discussion**

 **Effect of rice varieties on cordycepin and adenosine content**

Ten rice varieties including Jasmine Rice 105, Rice RD 79, Banlay Brown Rice, Rice RD 43, Japanese Rice, Tha Heang Rice, Sao Hai Rice, Red Cargo Rice, Riceberry, and Tubtim Chum Phae Brown Rice were used as substrate for cultivation. Seventy days after incubation, all fresh *C. militaris* were collected, dried, and sent for cordycepin and adenosine content analysis at the Institute of Product Quality and Standardization, Maejo University, Chiang Mai, Thailand. The result indicated that the highest cordycepin content at 1,861.98 mg/kg was presented in dried *C. militaris* cultured using Rice RD 43 as substrate culture medium. Follow by *C. militaris* cultured using Japanese Rice (1,753.77), and Red Cargo Rice (1,753.50), respectively (Table 1.). The highest adenosine content at 1,391.51 mg/kg was obtained from using Riceberry.

 From this study, it was seen that the *C. militaris* cultured with RD 43 rice had the highest amount of cordycepin. This may be because RD 43 rice that has sufficient sugar or carbon source and is suitable for the production of cordycepin. RD43 rice is a rice with moderate amylose content. Amylose is a linear polymer α-linked glucose units which resulting in a slow release of sugar (Nilkamheang, 2021). This will result in *C. militaris* being able to use nutrients continuously and for a long time, thus affecting the production of active ingredients as well. It was reported that glucose was chosen as the most suitable carbon source. Its concentration was determined to be 1-4%(Lee et al., 2019). The cordycepin content from this research was higher than report from Patthanajuck and Bunnag that used Mun-pu rice as solid state for *C. militaris* cultivation (Patthanajuck and Bunnag, 2021). As for adenosine content, it was seen that the adenosine content of *C. militaris* cultured with Riceberry rice had the highest adenosine content.

 **Effect of edible insect varieties on cordycepin and adenosine content**

Eight edible insect varieties including Silkworm (*Bombyx mori* L.), Eri silkworm (*Samia ricini* D.), Field cricket (*Gryllus bimaculatus* De Geer), House cricket (*Acheta* *domesticus* L.), Mealworm (*Tenebrio* *molitor* L.), Giant water bug (*Lethocerus* *indicus*), Oriental Mole Cricket (*Gryllotalpa* *orientalis*), and Sago palm weevil (*Rhynchophorus* *ferrugineus*) were used as a nitrogen source for *C. militaris* cultivation using Jasmine Rice 105. The resulted showed that the highest cordycepin content at 1583.10 mg/kg was presented in *C. militaris* cultured with Eri silkworm (*Samia ricini* D.) followed by Silkworm (*Bombyx mori* L.) and House cricket (*Acheta domesticus* L.) at 1,390.76 and 1,190.30 mg/kg, respectively. (Table 2.). The highest adenosine content at 1,840.23 mg/kg was obtained from cultured with Mealworm (*Tenebrio molitor* L.).

Therefore, *Cordyceps* with high content of cordycepin can be secured by the cultivation on insects/ insects as excellent substrates for *Cordyceps* cultivation with high content of cordycepin/insects with a high content of oleic acid are suitable substrates for *Cordyceps* cultivation (Turk et al., 2022). From this study, the *C. militaris* cultured with Erie silkworm pupae had higher cordycepin content than other edible insects. This is because the Ery silkworm pupa was a good source of protein (amino acid), fat (fatty acid) and minerals (Longvah et al., 2012; Longvah et al., 2011). This may be due to the fact that the mealworm contains high amounts of protein, fatty acids, amino acids nutrients that suitable for the production of active compounds (Zhao et al., 2016). Insects are classified as a source of organic nitrogen, which plays an important role in the growth and production of active substances of cordyceps mushrooms. The use of organic nitrogen in culture medium yields higher yields of cordyceps than inorganic nitrogen (Sung et al., 2010). In addition to the type of Carbon and Nitrogen that affect the bioactive content, the carbon:nitrogen ratio is also effective. Raethong et al. (2020) reported that rationally design and optimal synthetic medium with C:N ratio of 8:1 for enhancing 3.5 fold increase in cordycepin production (Raethong et al., 2020). In comparison to grains, insects contain high amounts of protein, which is known to serve as a source of carbon and nitrogen and is necessary for the synthesis of cordycepin (Tao et al., 2020). The number of bioactive compounds obtained depends on many factors. including mushroom species Cultivated food recipes acidity-base of food Cultivation environment period of cultivation and harvesting including methods for analyzing substances (Hsieh et al., 2005; Lei et al., 2009).

**Conclusions**

Ten rice varieties and eight edible insect varieties were used for *C. militaris* cultivation to evaluate the effect on cordycepin production. The results indicated that the suitable rice, and edible insect that present the highest cordycepin content were obtained from *C. militaris* cultured with RD 43 rice, and Eri silkworm (*Samia ricini* D.).

**Author Contributions**

Conceptualization validation investigation DW, RB SR, writing and editing SR. All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**Data Availability**

Information presented in this study will be available upon request to the corresponding author

**Ethics Approval**

Not applicable

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**Table 1. The Cordycepin and adenosine content of *C. militaris* cultured with different 10 rice varieties.**

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| --- | --- | --- |
| **Rice varieties** | **Cordycepin (mg/kg)** | **Adenosine (mg/kg)** |
| Jasmine Rice 105 | 1,536.68 | 833.83 |
| Rice RD 79 | 1,276.74 | 828.36 |
| Banlay Brown Rice | 1,226.39 | 1,128.50 |
| Rice RD 43 | **1,861.98** | 1,035.73 |
| Japanese Rice | 1,753.77 | 1,182.07 |
| Tha Heang Rice | 1,637.19 | 768.97 |
| Sao Hai Rice | 1,551.40 | 974.11 |
| Red Cargo Rice | 1,753.50 | 795.22 |
| Riceberry Rice | 767.17 | 1,391.51 |
| Tubtim Chum Phae Brown Rice | 953.62 | 1,260.63 |

**Table 2. The Cordycepin and adenosine content of *C. militaris* cultured with different 8 edible insect species.**

|  |  |  |
| --- | --- | --- |
| **Edible insect species** | **Cordycepin (mg/kg)** | **Adenosine (mg/kg)** |
| Silkworm (*Bombyx mori* L.), | 1,390.76 | 1,478.70 |
| Eri silkworm (*Samia ricini* D.) | **1,583.10** | 1,235.97 |
| Field cricket (*Gryllus bimaculatus* De Geer) | 1,143.97 | 1,190.09 |
| House cricket (*Acheta domesticus* L.), | 1,190.30 | 1,374.31 |
| Mealworm (*Tenebrio molitor* L.) | 963.73 | **1,840.23** |
| Giant water bug (*Lethocerus indicus*) | 789.30 | 1,236.97 |
| Oriental Mole Cricket (*Gryllotalpa orientalis*) | 1,032.24 | 1,491.85 |
| Sago palm weevil (*Rhynchophorus ferrugineus*) | 736.88 | 1,156.47 |