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## METHODS USED FOR CULTIVATION OF *CORDYCEPS*: A REVIEW

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### ABSTRACT

*Cordyceps*, a genus of entomopathogenic fungi, have garnered significant interest due to their potent bioactive compounds and applications in traditional medicine, nutraceuticals and biotechnology. However, natural harvesting is limited and unsustainable, prompting the development of various cultivation strategies to meet growing demand. This review provides a comprehensive overview of the cultivation methods employed for *Cordyceps*, including solid-state and liquid fermentation techniques and artificial insect host systems. Key parameters influencing mycelial growth and fruiting body development such as substrate composition and environmental conditions are discussed. Additionally, recent innovations in large-scale production and optimization of bioactive compound yield are highlighted. This review aims to inform future research and industrial-scale production of *Cordyceps*, contributing to their sustainable utilization and commercial exploitation.

**Keywords :** *Cordyceps*, cultivation method, solid state fermentation, liquid fermentation.

### Background Information

Humans have utilized mushrooms for centuries, both as a food source and in traditional medicine. Of the more than 14,000 known mushroom species, approximately 2,000 are considered as edible mushrooms. Among these, medicinal mushrooms hold particular significance due to their valuable pharmacological properties. Genus *Cordyceps* comprises of the most valuable fungi which are majorly used in field of medicine. It belongs to the family *Cordycipitaceae*, class Ascomycetes and phylum Ascomycota. *Cordyceps* is most important genus with over 750 identified species (Anon., 2025; Nguyen *et al.*, 2020; Krishna *et al.*, 2025).

Among all the different species most scientific researches has been conducted on *Cordyceps sinensis*, *Ophiocordyceps sinensis* and *C. militaris* because of their beneficial effects. Traditional Chinese medicine recommends the use of *Cordyceps* spp. for treating several human disorders such as cardiovascular and respiratory diseases, disorders of the liver and kidney, cancers, diabetes, infectious and parasitic diseases and sexual dysfunctions (Jedrejko *et al.*, 2021). *O. sinensis* is well known and rare in nature despite being the most well-known and costly species, it is quite uncommon

and difficult to cultivate in culture; in comparison, *C. militaris* is found all over the world and the fruiting bodies have been successfully commercialized after being raised on silk worm pupae and cereal substrate (Guo *et al.*, 2016).

*C. militaris* generally parasitizes the larva or pupae of lepidopteran insects. The use of this fungus is very popular in many countries, especially Southeast Asia due to its multiple pharmacological effects, including antitumor, anti-influenza virus, immunomodulatory and anti-inflammatory effects. It contains different bioactive compounds such as adenosine, cordycepin, mannitol, superoxide dismutase (SOD), carotenoids and polysaccharides. Cordycepin, the primary bioactive compound in *Cordyceps* fruiting bodies which was initially isolated from *Cordyceps militaris*. It was later also identified in *Cordyceps sinensis* (Cunningham *et al.*, 1951) and *Cordyceps kyushuensis* (Ling *et al.*, 2002). It was listed as Novel Food by the Ministry of Health of the People's Republic of China in 2009 (Guo *et al.*, 2016).

Natural fruiting bodies of *Cordyceps* are scarce and expensive to harvest. Moreover, *in vitro* production of fruiting bodies is inconsistent. The cordycepin content in wild *Cordyceps* is significantly

lower as compared to that in cultured mycelia (Guo *et al.*, 1998). Natural populations of key *Cordyceps* species are decreasing rapidly due to over collection, presenting the need of increased cultivation of *Cordyceps in vitro* using an artificial medium. Cultivating *C. militaris* mycelia on artificial media has resulted in higher cordycepin yields (Masuda *et al.*, 2007; Elkhateeb *et al.*, 2022). Currently there is an

increasing demand of biomass and fruiting body of *Cordyceps* spp. for obtaining cordycepin due to its health benefits but considering its scarcity there is a need to cultivate *Cordyceps* using different artificial methods. This review highlights the different cultivation methods of *Cordyceps* spp. to obtain its biomass and also large-scale production using fermentation technique.

**Table 1:** Common therapeutic effects of different *Cordyceps*

Therapeutic effects	<i>Cordyceps</i> spp.	Major bioactive compounds
Antitumor	<i>C. sinensis</i>	Cordycepin, Cordyglucans, Monosaccharide saponins, EPSF (5-enolpyruvylshikimate 3-phosphate)
	<i>C. militaris</i>	Cordycepin and mannitol
Antimicrobial activity	<i>C. sinensis</i>	Cordycepin
	<i>C. militaris</i>	Ergosterol Mannitol, trehalose, Polyunsaturated fatty acids, $\delta$ -tocopherol and p-Hydroxybenzoic acid
Anti-diabetic effects	<i>C. sinensis</i>	Cordymin
	<i>C. militaris</i>	Cordycepin, adenosine
Anti-oxidant activity	<i>C. sinensis</i>	Exopolysaccharide fraction, EPSF CPS-1 CME-1
	<i>C. militaris</i>	Polysaccharide (PSC)
Anti-inflammatory	<i>C. sinensis</i>	Cordycepin
	<i>C. militaris</i>	Adenosine $\beta$ -(1 $\rightarrow$ 3)-D-glucan

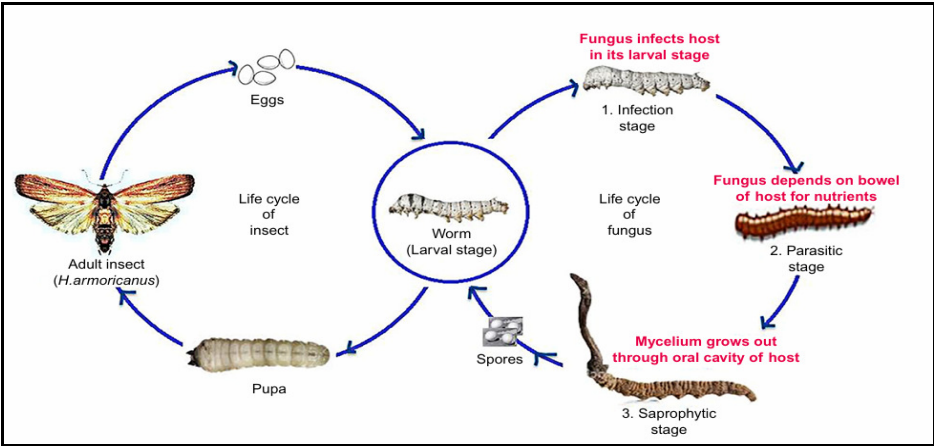
Source: Elkhateeb *et al.*, 2022

### Methods of Cultivation

#### ➤ Conventional method:

To cultivate the fungi conventionally it requires to mimic the habitat of both host insect and the fungus which is very hard to achieve. In its natural environment the fungi go through many growth phases which needs unique growth conditions. In late October, *Ophiocordyceps sinensis* infects subterranean larvae, with the highest infection rates occurring in fourth to fifth instar stages as they moult. Early-stage larvae are

less susceptible due to low activity and feeding, while post-5th instar larvae show greater resistance. This infection period aligns with the fungus's spore release. The mycelium invades the larval hemocoel, proliferates via yeast-like budding, and eventually kills the larva 2–5 cm below the soil, head-upward. In spring, a stroma bud emerges from the head, developing into the stalked fruiting body (Yang *et al.*, 1989).



**Fig. 1:** Developmental stages of *Cordyceps sinensis* in the host caterpillar *Hepialus armoricanus* (Pal and Misra, 2018)

### ➤ Complete artificial cultivation mode:

It involves whole process under the artificial condition from rearing of host larvae, culturing fungi to infecting the host larvae with fungi. It takes approximately 2 years for the mushroom to arise (Munir *et al.*, 2023). Development of *Cordyceps*

fruiting bodies was outstanding on *Bombyx mori* and *Tenebrio molitor* when grown for 35 days whereas highest cordycepin content (89.5mg/g DW) was in *Cordyceps* developed on *Allomyrina dichotoma* (Turk *et al.*, 2022).

**Table 2:** Host range of *Cordyceps*

Different genera	Host family	Host genus/ species
<b>Lepidoptera</b>		
<i>C. longdongensis</i>	Saturniidae	<i>Actias artemis</i>
<i>C. michaelisii</i>	Bombycidae	<i>Bombyx mori</i>
<i>C. militaris</i>	Noctuidae	<i>Arcte coerula</i> , <i>Panolis flammea</i>
<b>Coleoptera</b>		
<i>Cordyceps brongniartii</i>	Scarabaeidae	<i>Anomala cuprea</i>
<i>C. pseudoinsignis</i>	Scarabaeidae	<i>Melolontha sp.</i>
<i>Ophiocordyceps dovei</i>	Cerambycidae	<i>Oemona hirta</i>
<b>Hemiptera</b>		
<i>O. takaoensis</i>	Cicadidae	<i>Tanna japonensis</i>
<i>O. nutans</i>	Pentatomidae	<i>Pentatoma japonica</i> , <i>P. Rufipes</i> , <i>Plautia stali</i>
<i>O. toriharamontanum</i>	Cicadidae	<i>Tibicen bihamatus</i>
<i>Cordycpes javanica</i>	Delphacidae	<i>Nilaparvata lugens</i>
<b>Hymenoptera</b>		
<i>O. oxycephala</i>	Apidae	<i>Bombus equestris</i>
<i>O. ditmarii</i>	Ichneumonidae	<i>Amblyteles armatorius</i>

Source: Shrestha *et al.*, 2017; Bunsap *et al.*, 2024

### ➤ Semi-natural cultivation mode:

In this mode same concept is applied for rearing and infection of larvae but the successfully infected larvae are released into the wild to grow at their own. The caterpillar-fungus complex formed upon maturity in 3-5 years in the nature.

### ➤ Fermentation:

Large scale fermentation is used for the generation of huge quantities of fungal strains.

Due to difficulty in production of complete fruiting body of fungi researchers opt to the large-scale production of mycelial biomass using artificial culture media. There is to type of fermentation method are being used: liquid culture fermentation and solid-state fermentation.

**(a) Liquid culture:** Liquid culture or submerged fermentation refers to the introduction of fungal culture to the sterilized liquid medium which includes all the essential nutrients for the rapid growth of mycelium. This technique proved to be ideal because growth conditions can be controlled and modified to produce higher mycelial growth. Although there is drawback in using this technique that most of the bioactive compounds are lost during the harvesting process. So, it can be

proposed that the bioactive compounds generated in a liquid medium be separated and sold separately without the mycelium present in order to reduce loss (Munir *et al.*, 2023). It was found that 80 g, glucose; 10 g, yeast extract; 0.5 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; and 0.5 g,  $\text{KH}_2\text{PO}_4$  in 1 L distilled water were most suitable carbon, nitrogen, and mineral sources, respectively for the mycelial biomass and exo-polysaccharides of *C. militaris* in liquid culture (Kwon *et al.*, 2009). By the addition of peanut oil in liquid medium of *C. militaris* enhanced the cordycepin production 3.17 times higher than that of the control (Tang *et al.*, 2018). Jaggery Yolk broth found to be best for the growth of Indian strain of *C. militaris* followed by Jaggery sucrose peptone yolk broth in comparison to Potato dextrose broth (Javaid *et al.*, 2023).

**(b) Solid state fermentation:** It uses different solid substrates like grains on which mycelium will grow. The mycelium is gathered together with the remaining grain after some period of growth. This is low-cost method and it is able to produce mycelium with maximum bioactive compounds. However, mycelium produce using this method has higher content of grain than fungi. Commonly this technique is used for production of fruiting body of fungi (Munir *et al.*, 2023). Highest yield can be

obtained from wheat substrate while the concentration of cordycepin and adenosine in fruiting bodies were higher on rice substrate (Feng *et al.*, 2018). When the mycelial growth, primordia formation, yield performance and cordycepin content of five strains (DT1, DT2, DT3, DT4 and DT5) under artificial cultivation conditions were studied it was found that all strains can be successfully cultivated on brown rice medium and required 18 (strain DT3) to 25 days (strain DT5) to form primordia (Nguyen *et al.*, 2020). Oat grains were proved to be the best grain for the spawn production of *C. militaris* with growth rate of 9.80mm/day (Singh *et al.*, 2020). Solid state medium composed of rice yielded highest fumosorinone content (798.50mg/L), followed by a medium composed of wheat and oats (640.50 and 450.50mg/L), respectively at 30 days (Khan *et al.*, 2023).

### Obstacles in Artificial cultivation of *Cordyceps*

- a) **Cross-contamination:** Cross-contamination is a common challenge in facilities where multiple strains or species of *Cordyceps* are cultivated simultaneously. It can occur through various routes such as airborne spores, contaminated equipment, or improper handling by personnel. This contamination can severely impact cultivation outcomes by compromising product quality, reducing yields, and even causing the loss of genetically valuable strains. To minimize these risks, cultivation units must enforce strict hygiene protocols, including the use of sterilized tools, proper disinfection of workspaces, and implementation of physical barriers to prevent cross-transfer. Additionally, training staff in aseptic techniques and maintaining controlled environments are essential for preserving the integrity of each *Cordyceps* culture (Krishna *et al.*, 2025).
- b) **Degeneration:** Degeneration presents a major obstacle in the artificial cultivation of *Cordyceps*, limiting the industry's growth and sustainability. It typically arises from repeated subculturing and improper preservation methods. Over time, degeneration leads to several negative outcomes, including slower mycelial growth, decreased conidial formation, fewer primordia, extended cultivation periods, and a significant decline in the production of valuable secondary metabolites. DNA methylation and genes linked to degeneration play a key role in the decline of *Cordyceps militaris* cultures, with genetic variation being the

main driving factor behind this issue. To combat degeneration and promote long-term stability in cultivation, genetic engineering offers a promising solution. By precisely modifying the *C. militaris* genome, it is possible to develop high-quality, stable strains, thereby supporting the sustainable advancement of the *C. militaris* industry (Krishna *et al.*, 2025).

### Conclusion

The cultivation of *Cordyceps* spp. has evolved significantly, transitioning from exclusive reliance on natural harvesting to more controlled and scalable artificial methods. This review has outlined the major approaches used in cultivating *Cordyceps*, including natural, semi-natural, complete artificial, and fermentation-based systems. Each method offers unique advantages and limitations in terms of yield, cost, bioactive compound content, and sustainability. While natural and semi-natural methods retain ecological authenticity, they are limited by seasonality and host dependence. In contrast, artificial and fermentation techniques offer greater control and scalability, with fermentation emerging as a promising approach for mass production and commercial applications. Continued advancements in biotechnology, substrate optimization, and strain selection are essential to improving productivity and maintaining the pharmacological integrity of cultivated *Cordyceps*. Future research should focus on integrating traditional knowledge with modern cultivation technologies to meet the growing demand for *Cordyceps* in medicine, nutrition, and functional foods.

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