International Journal of Natural Medicine and Health Sciences
ISSN (Online):2790-2471 ISSN (Print): 2790-2463
Volume 2 (2) Mar 2023
Journal homepage: https://journals.iub.edu.pk/index.php/ijnms

Review

A comparative study on *Cordyceps militaris* and *Ophiocordyceps sinensis*

Muhammad Hasnain[a*], Rizwan Akhtar Jamsheed[b], Zakir Hussain[c], Ranaz Latif[d]

[a*] Department of Eastern Medicine and Surgery, University of Poonch Rawalakot, Pakistan
[b] Shandong University, Jinan City, China
[c] University of Chinese Academy of Science, Beijing, China
[d] Department of Bioscience, COMSATS University Islamabad, Pakistan

Article Info.

Received: 06-11-2022
Revised: 04-03-2023
Accepted: 10-04-2023
Online: 29-05-2023

Correspondence:
muhammadhasnainshigri@gmail.com

Keywords:
Cordyceps, *Ophiocordyceps sinensis*, *Cordyceps militaris*, Cultivation, Traditional medicine, Cordycepin, Yartsa gunbu, Caterpillar fungus, DongChongXiaCao.

Abstract

Background: *Cordyceps* mushrooms have a long history of traditional use, both as a medicine and food supplement. In Asia, it has been used in ethnomedicine for centuries because of their aphrodisiac effects, tonic effects, ability to reduce fatigue, quick recovery from sickness and boosting immune system in humans. Due to high price and huge demand, the species of *Cordyceps* are very popular all over the world. This paper includes two famous and widely used species of *Cordyceps* that are *Cordyceps militaris* and *Ophio sinensis*. Aim of the study: This review aims to focus on, introduction and importance of these two species, their ecology, scientific classification, worldwide distribution, collection, natural growth, insect-fungus mechanism, medicinal uses, therapeutic effects, description of the chemical compounds, cordycepin as an anti-cancer agent, economic impact and their artificial cultivation. Methodology: All data in this paper are collected from different review and research articles from Google Scholar, PubMed, Microsoft Academic, Science.gov, ResearchGate and Worldwide Science. Conclusion: The discussed two species of *Cordyceps* are complex fungi with a unique insect-fungus relationship, having a number of important bio-active components specially cordycepin, cordycepic acid and adenosine. These components have wide range of biomedical importance from boosting human immune system to anti-cancer activity. Scientific studies, both in vitro and in vivo should be conducted to evaluate its clinical activities to provide scientific based evidence to certify its safe and effective use as a drug.

Citation: Hasnain M, Jamsheed RA, Hussain Z, Latif R. A comparative study on *Cordyceps militaris* and *Ophiocordyceps sinensis*. IJNMS. 2023; 2 (2): 1-6.

Introduction: *"Cordyceps"* is genus of ascomycete fungi (phylum Ascomycota) also known as "Sac fungi" [1]. It includes approximately 600 species. Mostly *cordyceps* species are parasitic mainly on insects of arthropoda and other fungi. Some species shows high specificity of host [2]. The meiotic or mitotic spores infect the larva and grows through the insect by hyphae and the biomass starts to accumulate and eventually kills and mummifies the host [3]. The host body is then ruptured by fungus, forming the sexual stroma which grows upward above the soil surface but connected to dead larva/host below [4]. Traditionally, the complete unique insect/fungus combination is used [5].

Cordyceps is widely used in traditional medicines for centuries specially in China and Tibet [6]. Now *cordyceps* species are also used in western countries as medicine and tonic. They advertise them as Chinese herbs with immune boosting, anti-aging and aphrodisiacs effects [7]. Many nutraceutical products in market contains *Cordyceps* species e.g. Didanosine from *Cordyceps militaris* [8]. These are thought to be stress relieving in humans, although scientific evidence are very poor. The species of *Cordyceps* are distributed all over the world. In Asia, it is notably present in China, Nepal, Japan, Bhutan, Vietnam, Korea and Thailand [9]. Out of these, two species are being used for medicinal purposes, health supplement and as pharmaceutical drug at a large scale, especially in China. These two species are *Ophiocordyceps sinensis* and *Cordyceps militaris.* The scientific classifications of both species are given in "Table 1".

Ophiocordyceps sinensis: It was firstly described as *Sphaeria sinensis* by Berkeley in 1843 [10]. Later on it has been named as *Cordyceps sinensis* by Saccardo in 1878 and this name was used for many years [11]. But recent molecular phylogenetic studies transferred the species within a newly recognized family called Ophiocordycipitaceae and the species name is termed as *Ophiocordyceps sinensis* [12].

The *Ophiocordyceps sinensis*, previously known as *Cordyceps sinensis* (until 2007) [13] is highly prized medicinal fungus which is widely used in Chinese traditional medicine for hundreds of years and now has been officially classified as a drug in the Chinese Pharmacopoeia (2015) [14]. The fungus parasitizes the larvae of lepidoptera to form a unique insect-fungus entity [6] (Figure.1A), a welled known traditional Tibetan medicine "yartsa gunbu" (translated as "summer grass-winter worm") and in traditional Chinese medicine, "DongChongXiaCao" [15]. It is found along Himalayan plateau and in last decade its economic value has increased dramatically. It is used mostly as a tonic, immune booster and for quick recovery from illnesses [16]. *O. sinensis* firstly gained worldwide attention when record breaking Chinese runners/athletes revealed that they use *O. sinensis* as dietary supplements in their diet [17]. This fungus now has been classified as endangered species in China (China Biodiversity Red List 2018) due to overexploitation [18].

Cordyceps militaris: Another entomopathogenic fungus of genus *Cordyceps* is ''*Cordyceps militaris*'', commonly known as orange caterpillar fungus, that is parasitic to the pupa of lepidoptera species [19] (Figure.1B). It is phylogenitically related to *O. sinensis* and these tow fungi have similar bio-chemical components, there for *C. militaris* has become an important species of *Cordyceps* family [20]. However, *C. militaris* is easily searchable and less expensive and its stroma can be easily cultivated. It has worldwide distribution specially in northern hemisphere and appears from August to November in Europe [21]. *Cordyceps militaris* Contains potential bio-metabolites being used in herbal drugs for centuries. This is now started to use in modern medicines also. The main bio-chemical differences between *O. sinensis* and *C. militaris* are concentration of adenosine and cordycepin [22].

Habitat: The species *O. sinensis* is endemic to the Himalayas and Tibet, at an altitude between 3000 m to 5000 m and grows at relatively low temperature i.e. below 21 °C [23]. The wild material has been reported harvesting from Nepal, Bhutan ((Namgyel and Tshitila), India (Arunachal Pradesh, Himachal Pradesh, Sikkim, Uttarakhand) and China (eastern Qinghai, northern Yunnan, Tibet, western Sichuan and Gansu provinces) [24]. It has an altitudinal distribution ranging between 4200 and 5200 meters in Bhutan [25]. In Himalayas and Tibet, the fungus generally parasitizes the larva (moths) of hepialidae family [26]. The larva lives 15 cm underground in alpine grass land before infection. After infection by fungus, a few centimeters long, dark brown fruiting body arises from the head of cadaver and stands upright, appearing on the ground [27]. The slow growth rate and low temperature requirement is the key features that distinguish this fungus from other fungi. Over harvesting and climate changes are negatively affecting these mountain organisms. The *C. militaris* has a wide distribution in comparison to *O. sinensis*. The fungus grows out of underground cadaver and appears on ground forming 1-8 cm long orange/red fruiting body covered by stroma. The spores are septate, hyaline, long-filiform and smooth in nature.

Medicinal Value:

Ophiocordyceps sinensis*: *O. sinensis has been used for centuries in traditional medicine as a tonic herb for general physical health, sexual performance and quick recovery from illnesses. *Cordyceps* species are recommended by traditional Chinese medicine practitioners to treat several human disorders that are, respiratory diseases, cardiovascular diseases, diabetes mellitus, renal diseases, liver diseases ,infectious diseases and sexual dysfunctions [28]. The bio-active chemical constituents present in *O. sinensis* includes, cordycepin (30-deoxyadenosine), cordycepic acid, polysaccharides, ergosterol, peptides containing aminoisobutyric acid and glycoproteins [9].

The pharmacological activities of these compounds includes, anti-depression, hypoglycemic, hypolipidemic, anti-osteoporotic, anti-metastatic, anti-arteriosclerosis, anti-oxidant, anti-microbial, insecticidal, anti-inflammatory, anti-aging, anti-tumour, anti-diabetic, neuroprotective, reno-

protective, pneumo-protective, immunomodulatory effects, aphrodisiac effects, memory improving effects, treatment of bowel injury, photo-protection, stimulate lymphocyte proliferation and accelerates leukocyte recovery [29].

The main and important constituent is Cordycepin. Its chemical structure is shown in Figure 2. It is an adenosine nucleoside analogue (3-deoxyadenosine) initially extracted from *Cordyceps*, but now it is produced synthetically [30]. It is chemically different from adenosine by lacking oxygen molecules in its ribose entity. Due to its similarity to adenosine, some enzyme cannot differentiate between cordycepin and adenosine, hence participating in some important reactions such as, it may cause termination of RNA molecules synthesis by incorporating in it [31]. Cordycepin is marked as an anti-cancer compound [32].

Cordyceps militaris: All mushrooms (fungi) are not edible, even some of edible fungi may contain toxic compounds. As early in 1970, a toxic protein called "flammutoxin" was found in "*Flammulina velutipes"* and "volvatoxin" in "*Volvariella volvacea"* despite that both are edible mushrooms. In 2006, it is found that an edible mushroom called *"Pleurotus ostreatus"* contains a toxin protein "ostreolysin" [33]. In this regard, many studies have been done on *C. militaris* to check its toxicity towards human beings but no evidences were found [34]. In-fact researchers mostly used mixed extractions instead of using pure compounds in their experiments. Howbeit, it is being used in many countries as a traditional medicine with surprising results. *Cordyceps militaris* contains many important bio-active metabolites which revitalize the various systems of the body. It is considered the oldest source, having useful chemical compounds among all species of *Cordyceps* and has been used as a tonic medicine since ancient times. Now it is widely used in modern medicine as well. The potential of this fungus is now gearing up the traditional treatments techniques and brought a revolution in traditional system of medicine. Evidences showed that, the active constituents of *C. militaris* are responsible for, immunomodulatory, aphrodisiac, anti-oxidant, anti-cancer, anti-inflammatory, anti-leukemic, anti-metastatic, anti-proliferative, anti-bacterial, anti-fungal, anti-viral, anti-protozoal, larvicidal, insecticidal, anti-fibrotic, anti-angiogenetic, hypolipidaemic, hypoglacaemic, hepatoprotective, renoprotective and pneumo-protective effects [35]. Some research showed that apoptosis of cancer cells is induced by water extraction of *C.militaris*. The bio-activity and cyto-toxicity of *C. militaris* towards cancerous cells have been numerously investigated. A new protein "18-kDa protein" is found to induce apoptotic death of "Murine Primary Cells" and other cell lines [33]. Furthermore, by alkalization and heat treatment the apoptosis-inducing ability of this protein can be degraded.

Economic Impact: In rural Tibet, farmers economy now mainly dependant on *O. sinensis* and it is an important source of income [36]. About 40% of locals annual cash income is meet from harvesting this fungus (*O. sinensis*) and it made 8.5% of GDP in 2004 [13]. Since the late 1990s, the price of fungus has been continuously increasing. In Tibet, one kilogram fungus was sold for about $3,000 for lowest quality and $ 18,000 for best quality (largest larvae) in 2008. The annual production of *O. sinensis* was estimated 80–175 tons on the Tibetan Plateau in 2009 [25]. The Himalayan production is limited to a few tons only. In 2004, one kilogram of yartsa gunbu was sold-out about Rs 100,000 in India and 30,000 to 60,000 NPR in Nepal [37]. The value of fungus rapidly increased and 1 KG of yartsa gunbu was sold for NPR 350,000 to 450,000 in 2011 [38]. According to an article of BBC in 2012, a single fungus has a worth value of $ 3 in north Indian villages that counts more income than daily laboring [39]. The price of Tibetan yartsa gunbu has increased about 900% between 1998 and 2008 in Tibet [40]. However, larger ones are more expensive than the small ones, regarded as low quality. The *Cordyceps* mushrooms are being sold for $52 per pound in china as of March 2023 as shown in Figure 3. This continuous increase in price and demand of fungus is due to the increasing interest in traditional medicine by Western consumers [41]. Due to its high value, inter-village conflicts have aroused to-take over the control of grasslands, habitat to fungus. Several people were killed in this regard, and it has become a big problem for local government. In November 2011, 19 villagers were convicted by a court in Nepal in accusation of murdering a group of people during a fight for highly valuable fungus. In June 2009, 7 people were killed in Manang after going for search of yartsa gunbu [42]. In 2001, for the first time, the Nepali government legalized the collection of yartsa gunbu [43]. In 2002 the fungus value increased to 1,435 USD and local government has charged a royalty of 280 USD per kilogram in Nepal. In Bhutan, the fungus was kept on Schedule 1 of the "Forest and Nature Conservation Act" and before 2004, its harvest was illegal in Bhutan [41]. Now the demand is highest in China, Vietnam, Thailand, Japan and Korea.

Cultivation of *Cordyceps militaris:* A number of different cultural techniques have been noticed for *C. militaris* growth, such as pre-culture, storage culture, indigenous culture (husked rice culture, saw dust culture, spawn culture) and laboratory culture (submerged culture, surface liquid culture, shaking culture, continuous/repeated batch culture) using a variety of media such as rice, silkworm and liquid nutrition [44].

Cultivation on Wheat Medium: First of all, confirm the fungal strain by DNA sequencing then compare it with the data set for sure identification of species. The identity of strain should be verified both molecularly and morphologically. Incubate the strain on PDA medium (potato dextrose agar) at 20°C for 10 days in a Petri dish. Transfer this into a seed culture medium. Grow this culture in a flask containing potato dextrose liquid medium (150 ml). Put it on a "rotary shaker incubator" for 3 days at 20°C with 150 rpm. Cultivate this seed in a glass

bottle with "12-hour light and 12-hour dark" conditions at 20°C. Intensity of light should be 500 lx and humidity should be less than 80%. Harvest the grown fungus (fruiting body), known as wheat FB and air-dry it at 40°C for 48 hours [45].

Cultivation on Pupae: Grind the fruiting body as obtained above, to produce seed culture and filter it with gauze (sterilized) to separate large hyphae. Inoculate the pupa (*Bombyx mori*) with seed culture (0.5 ml) with the help of a syringe [45]. Culture the pupa at 20°C until the fungus proliferates in host and mummifies it. For fruiting body growth, the pupa should keep at a temperature of 20°C under "12-hour light and 12-hour dark" condition with 500 lx light intensity and humidity less than 80%. Then air-dry the obtained "fruiting body" for 48 hours at 40°C. Grind it to produce a powder mixture containing sclerotium with fruit bodies.

Cultivation in Corncob Biochar Media: Obtain the *C. militaris* strain from an authentic source, cultivate it on potato dextrose agar (PDA) and incubate it for 7 to 10 days. Now transfer the grown mycelium in a flask (500 ml) containing potato dextrose broth (PDB). Incubate it for 7 days at 18Ċ and 80% humidity with the help of an incubator shaker. Grow a little portion of seed culture in a solid medium containing rice berry mixed and corncob biochar at different concentration. Measure the height of fruiting body. Harvest and measure the fresh and dried (for 2 days at 30Ċ) weight of mycelium. Higher the mass biochar, higher will be the yield [46].

Conclusion: The above discussed species of *Cordyceps* are complex fungi with a unique insect-fungus relationship, having a number of important bio-active components specially cordycepin, cordycepic acid and adenosine. These components have wide range of biomedical importance from boosting human immune system to anti-tumour activity. Scientific studies, both in vitro and in vivo should be conducted to evaluate its clinical activities to provide scientific based evidence to validate its safe and effective use as a drug. *Cordyceps* species are very expensive especially *O. sinensis*, usually referred as soft gold. Developing new methods and technologies in cultivation of c*ordyceps* species other than *Cordyceps militaris* can produce important bio-active compounds such as cordycepin in large quantity and it may help to bring lower the price of fungus.

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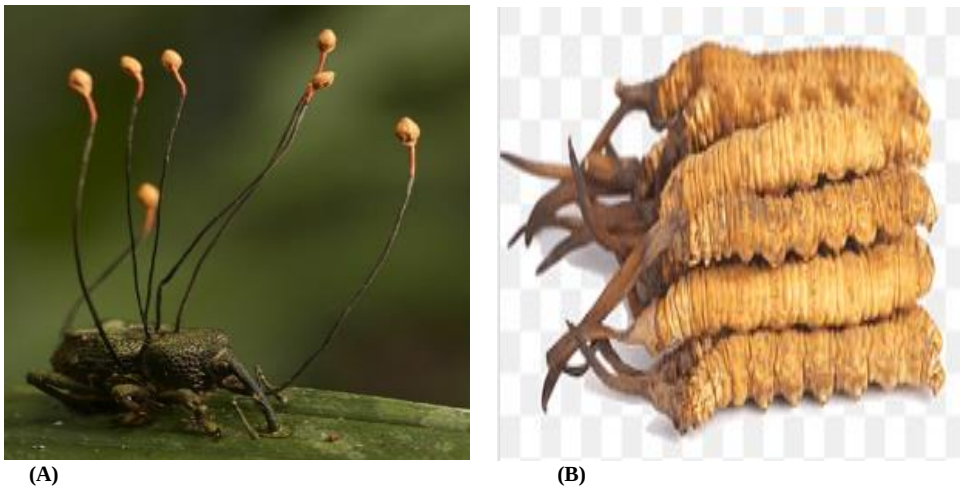
Table. 1. Scientific classification of *Ophiocordyceps sinensis* and *Cordyceps militaris*

Ophiocordyceps sinensis		***Cordyceps militaris***	
Kingdom	**Fungi**	**Kingdom**	**Fungi**
Division	**Ascomycota**	**Division**	**Ascomycota**
Class	**Sordariomycetes**	**Class**	**Sordariomycetes**
Order	**Hypocreales**	**Order**	**Hypocreales**
Family	**Ophiocordycipitaceae**	**Family**	**Cordycipitaceae**
Genus	***Ophiocordyceps***	**Genus**	***Cordyceps***
Species	***Ophiocordyceps sinensis***	**Species**	***Cordyceps militaris***



(A) **(B)**

Fig.1. (A) Wild *Cordyceps sinensis* (https://www.pngwing.com/en/free-png-dhckl). (B) *Cordyceps militaris* on dead insect (https://soundcloud.com/ronsen-799096964/cordyceps).

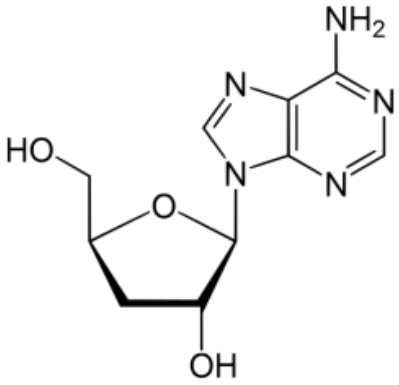


Fig.2. Chemical Structure of Cordycepin (PubChem).

Fig.3. Price of Cordycep fungus (i.e., $51.99/lb) in China, in March, 2023. (https://i.redd.it/uiqa6rzf8foa1.jpg).