Enhanced production of *Cordyceps militaris* fruiting bodies using brown rice substrates for therapeutic purposes

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Abstract

Cordyceps militaris commonly known as 'Kida jadi' in India is a rare entomopathogenic fungus that is typically found at high elevations on the Himalayan plateau. It is widely recognised as a therapeutic mushroom in traditional Chinese medicine. Cordyceps possesses wild range of bioactive constituents, with cordycepin being particularly noteworthy for its significant medicinal and nutraceutical properties. Fruiting bodies in industrial production are grown on synthetic substrates, however, their productivity and values are typically influenced by the quality of the fungal strains. This study examined the impact of the growth rate of fungal strains and repeated subculturing effect was measured.

Prepared brown rice substrates were infested with C. militaris inoculum and subsequently cultivated using optimised culture patterns. Throughout the cultivation process, the harvested samples, which are the fruiting bodies, were subjected to drying, resulting in a substantial yield. The findings indicated that the use of brown rice substrate promotes the growth of Cordyceps militaris fruiting bodies, resulting in a remarkable yield increase of 313%.

Keywords: *Cordyceps militaris,* Kida jadi, Entomopathogenic Fungus, Cordycepin, Nutraceutical Properties.

Introduction

Since ancient period, plants have served as vital resources for human sustenance and everyday needs. They have been utilised for a wide range of purposes, including the production of food, paper and infrastructure. Additionally, plants have been used to extract fragrances and spices as well as for the prevention[8]. The World Health Organisation defines herbal medicines as treatments that consist of herbs, raw herbs, formation of medicine and completed herbal goods. These remedies contain active substances derived from portions of plants, other plant materials, or combinations thereof. Traditional herbal medicines (THMs) are specific therapeutic methods observed in various cultures such as traditional Chinese medicine (TCM) in China, Ayurvedic therapy in India, Kampo treatment in Japan, Aboriginal healthcare in Australia and TeRongoā in New Zealand[13].

Medicinal mushrooms have consistently maintained their significance throughout human civilization. The genus Cordyceps is highly diversified within the family Clavicipitaceae, with over 750 species exhibiting a wide range of species, morphological variations and ability to adapt to different hosts. The majority of these varied species are primarily found in Asian nations including Korea, Japan, Nepal and China, as well as in other regions of the world with damp temperate and tropical environments. The global spread of various species under diverse environmental situations worldwide is indicated by their occurrence[10].

The link among the Cordyceps species as well as related hosts involves intricate and well-coordinated systems. These organisms adapt their life cycles to match the traits of their hosts in order to grow and survive. They do this by evading the hosts immune systems and producing defensive secondary metabolites[14]. These metabolites have the potential to be used as novel medications. These species have become highly significant due to their property of producing a wide range of natural compounds with various biological functions.

In recent times, artificially cultivated Cordyceps species have outperformed their wild counterparts due to the rarity and high cost involved in their harvesting and processing[19]. It is widely utilised in Asia both as a raw material for drugs and as a functional. *Cordyceps militaris* is the second most widely studied and researched species within its domain. Various pharmacological properties of this species have been recorded such as regulation of blood glucose, reduction of lipids in the blood, inhibition of tumour growth, antimicrobial effects, antiviral effects, antiprotozoal effects, anti-inflammatory effects, protection of neurons, antioxidant effects and immune protection. Hence, *C. militaris* might be regarded as a significant contender for the management of many illnesses[11].

The principle of "prevention is better than cure" is widely recognised in modern society, leading to the development of safe and beneficial characteristics of food products. Functional foods are foods that are marketed as healthy and have physiological qualities in addition to their nutritional benefits. Furthermore, substances derived from nature that have the ability to regulate or impact the body's systems

when consumed regularly, are also referred to as functional foods. The European Consensus defines a food as functional if it goes beyond providing basic nourishment and instead improves specific bodily functions in a way that promotes better health and reduces the risk of disease[9].

Functional foods are naturally occurring compounds that can undergo biotechnological operations to incorporate or remove specific elements. These treatments aim to enhance health and prevent diseases in specific individuals or specified groups depending on factors such as gender and age[15]. The presence or intentional inclusion of specific elements such as flavonoids, anthocyanins, polyphenols fatty acids, minerals, carotenoids and fibres, among others, improves the nutritive quality of the food. In the past, functional foods used to include vitamins, minerals and other nutritious elements in their production[2].

Prebiotics and probiotics are well studied functional nutrients that specifically address metabolic syndrome, high blood pressure, cellular damage and oxidation[1]. These possess distinctive capabilities to modify and boost the immune system and they can strengthen immunity by forming a mutually beneficial relationship with people. In addition to its therapeutic properties, *C. militaris* could be a promising option to be considered as a functional food because it contains metabolites with therapeutic or protective abilities.

This species has been found to contain a diverse array of bioactive substances including cordycepin, D-mannitol, ergosterol, carotenoids, peptides, vital amino acids, aromatic oils, minerals, vitamins, nucleosides, sterols and different forms of carbohydrates including mono, oligo and polysaccharides[6]. These findings highlight the species pharmacological and palliative importance[20]. In Asia, the industrial sector engages in extensive fermentation and industrial harvesting of stromata. This is done to address the decrease in the natural population and meet the growing requirement for medicinal, culinary and nutritive purposes.

Moreover, the industrial production of fermented culture is mostly driven by the presence of bioactive substances. Presently, Government has granted approval for many health foods, derived from this fungus. In addition, health food products containing *C. militaris* mycelia in the form of medicinal powder and capsules have been officially approved for commercial use. These products are claimed to have positive effects on the kidneys and lungs and are said to be effective towards coughing, asthma, mucus, cold limbs, exhaustion, giddiness, tinnitus and other health conditions[4].

Material and Methods

Preparation of inoculum culture: The inoculum culture of *C. militaris* was acquired from Veer Enterprises Ltd. located in Surat. The culture was preserved on potato dextrose agar slants (PDA, HiMedia Laboratories Pvt. Ltd., Mumbai, India) at a temperature of 23°C for a period of 14 days and then stored at a temperature of 4°C. Prior to the experiments, the strain was cultivated on potato dextrose agar plates for a duration of 14 days[7].

Mycelium preparation: From the PDA plate culture, samples was taken, each measuring 1cm^2 using a sterilised cutter. These samples were then transferred to 100 ml inoculum medium containing potato dextrose broth (PDB), yeast extract, peptone, magnesium sulphate, potassium diphosphate and 20gm of brown rice at the bottom. Further the medium was stored at dark condition till 11 days for mycelium growth at 20- 22°C and at medium humidity.

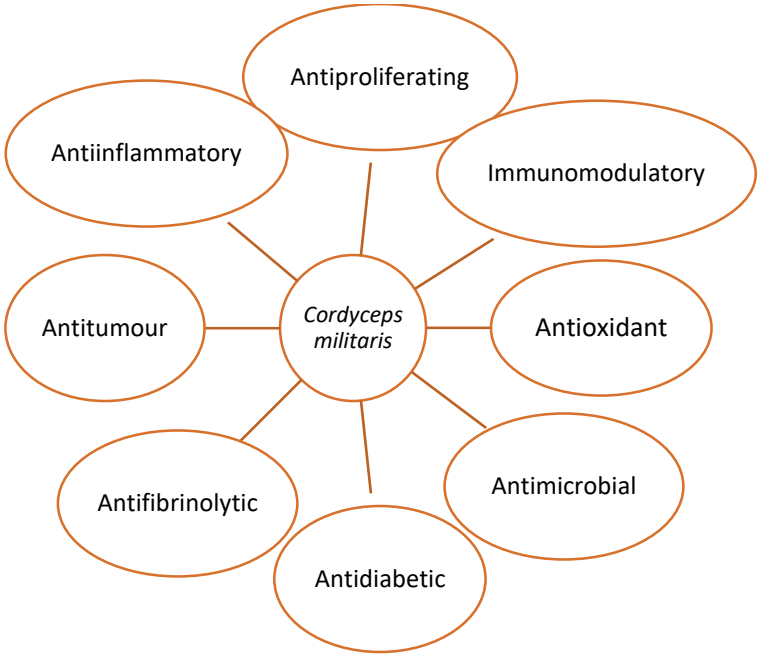

Figure 1: Therapeutic applications of *Cordyceps militaris*

Fruiting body yield of *C. militaris:* After the mycelium growth, the samples were stored in light condition for approximately 55-60 days for the growth of fruiting body. All the samples were stored at aseptic condition at 20°C and 60% humidity. Samples were regularly maintained to optimize the fruiting body yield.

Results and Discussion

The choice of substrate can significantly impact the yield of fruiting bodies in *C. militaris* cultivation[16]. Brown rice is a commonly used substrate in the cultivation of *Cordyceps militaris* and its use can influence the yield in several ways.

Nutrient Content: Brown rice provides a suitable nutrient profile for the growth of *C. militaris.* It contains carbohydrates, proteins and other essential nutrients that support fungal growth. The fungus utilizes these nutrients during the different stages of its life cycle including mycelium colonization and fruiting body development.

Texture and Structure: The physical characteristics of the substrate, such as particle size and structure, can impact the growth and development of the fungus. Brown rice, when properly prepared, provides a favorable texture and structure for the mycelium to colonize and form fruiting bodies.

Moisture Retention: Brown rice can retain moisture well, creating a suitable environment for fungal growth. Adequate moisture is crucial for the development of fruiting bodies and the substrate's ability to retain water can influence the overall yield.

Presence of Bioactive Compounds: Brown rice contains various bioactive compounds that may contribute to the medicinal properties of *Cordyceps militaris*. These compounds could influence the synthesis of bioactive metabolites in the fungus, potentially impacting the quality of the harvested fruiting bodies.

Contamination Resistance: Brown rice, when properly prepared and sterilized, can help resist contamination from unwanted microorganisms. Contamination can negatively affect the yield of *Cordyceps militaris* by competing with the fungus for nutrients and space. The radial growth of *C. militaris* was assessed by measuring the diameter of the colonies every two days for a total of 14 days, until the hyphae covered the entire 90 mm Petri plate. Linear model with a positive correlation was expressed in mycelia growth curve ($y = 10.133x + 1.222$ and $R^2 = 0.9945$ where x is the culture day after inoculation and y is the colony diameter at the corresponding culture day)[12].

Factor effecting the mycelial growth: The mycelial growth curve of *Cordyceps militaris* is intricately linked to the formation of fruiting bodies in a meticulously synchronised life cycle. The mycelial growth phase correlates to the vegetative stage of the fungus, wherein it undergoes proliferation and establishes itself in the chosen substrate.

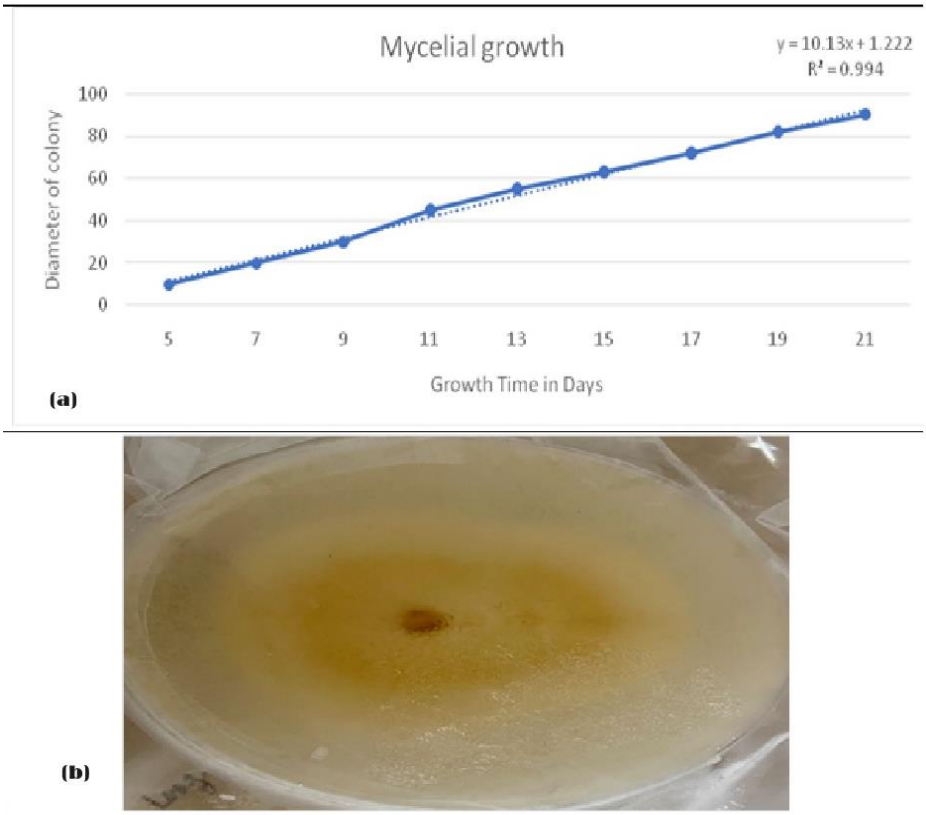

Figure 2: (a) Linear regression analysis of radial growth and culturing days. (b) Mycelial growth of *Cordyceps militaris*

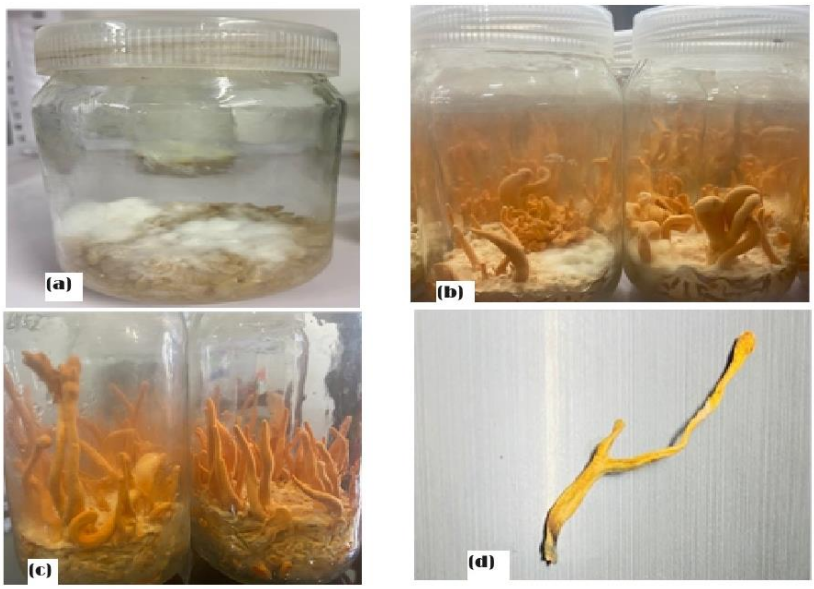

Figure 3: (a) White colour Mycelium growth after 10 days in dark condition. (b and c) The top panel shows the formation of a fruiting body or stroma. In the centre, the perithecia are poorly developed and have low density while at the bottom, perithecia are well-developed. (d) Dry mushroom of *Cordyceps militaris*

During this phase, the mycelium assimilates nutrients from the substrate, undergoes replication and establishes an interconnected network of hyphae. The mycelial network undergoes a transition from the vegetative phase to the reproductive phase in response to various environmental cues including changes in temperature, humidity and light. This change is marked by the initiation of fruiting body formation.

The mycelium undergoes a morphological metamorphosis, leading to the production of specialised structures that give rise to the fruiting bodies. Optimal aeration and efficient gas exchange are important for the successful development of C. *militaris*. Proper ventilation facilitates the elimination of metabolic waste and maintains adequate levels of oxygen, thereby producing an advantageous setting for the growth of fruiting bodies. Modulating the speeds and patterns of airflow can enhance crop productivity. The brown rice substrate's physical features are crucial and can be modified by altering the particle size and structure. Optimising the texture and structure of the substrate enhances the ability of the mycelium to colonise it, creating an ideal base for the development of fruiting bodies[18].

The substrate might be pulverised or formed into pellets to optimise the conditions for mycelial growth. Regulating moisture levels is another crucial element. Brown rice exhibits favourable moisture retention characteristics, while an excessive amount of water might give rise to complications such as contamination. Hence, it is essential to control the moisture content by accurately managing hydration levels and ensuring adequate drainage or aeration. This is critical for creating a favourable environment that promotes the healthy growth of mycelium and the creation of fruiting bodies[5]. The correlation between the mycelium's growth curve and the emergence of fruiting bodies is contingent upon the timing and sequencing of these developmental stages[17]. The growth pattern of mycelium typically exhibits exponential growth during the vegetative phase and the transition to the reproductive phase occurs once the mycelium reaches a certain level of maturity and optimal environmental conditions are available[3]. The commencement of fruiting body development is a pivotal milestone in the life cycle of *Cordyceps militaris*. The mycelial network channels its energy and resources towards the formation of primordia, which later mature into fully fledged fruiting bodies. The velocity and efficacy of this shift can be influenced by variables such as the composition of the substrate, environmental parameters and culture methods.

The figure 3a illustrates the white colour mycelium growth at the 9th day of the growth period, which is the starting time of the fruiting to emerge whereas figure 3b and c displayed the variations in the yield of fruiting bodies and the biological efficiency of *C. militaris* cultivated on brown rice under the control pattern cultivation circumstances. At about 60 days, the fruiting was harvested from the bottle, the approximate weight of mushroom was 7.27gm/bottle and the dry weight of the mushroom was 2.32gm/bottle as in figure 3d. The percentage yield of the mushroom was 313% [18].

Conclusion

Cordyceps militaris is a fungus which is both edibly and medicinally used, containing a wide range of bioactive compounds. It has been utilised in the traditional Chinese medical system for an extensive period of time. It is regarded as a great source of many bioactive constituents, with cordycepin being widely recognised as having the highest nutraceutical potential. Due to its high cost in its natural

form, measures have been made to cultivate the fruiting body using artificial mediums. The choice of substrate can significantly impact the yield of fruiting bodies in *C. militaris* cultivation[16].

Brown rice is a commonly used substrate in the cultivation of *Cordyceps militaris* and its use can influence the yield in several ways to enhance the nutrient quantity, texture, moisuture retention in mushroom and contamination resistance. This study reported that the brown rice and potato dextrose media favoured the fruiting body yield of *C. militaris* respectively.

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