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Comparative Analysis Of Cordyceps Militaris Growth On Pearl Millet Substrate: Insights Into Morphology, Wet Weight, Dry Weight, And Cordycepin Level

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Abstract

Cordyceps militaris, a valuable entomopathogenic fungus renowned for its medicinal properties, is gaining attention for its bioactive metabolite, cordycepin. This study investigates the growth performance and bioactive compound yield of C. militaris *(DMRO-1163 strain) cultivated on pearl millet as a solid-state substrate. The cultivation was conducted under controlled conditions using a nutrient-enriched pearl millet medium. Morphological parameters including fruiting body length, wet and dry biomass, and cordycepin concentration were evaluated. The results indicated consistent and robust mycelial colonization within 10 days and the emergence of fruiting bodies between 20–25 days, with full maturity by 50–60 days. The average fruiting body length was 6.94 cm, with mean wet and dry weights of 27.43 g and 4.37 g per jar, respectively. Ultra High-Performance Liquid Chromatography (UHPLC) analysis revealed a cordycepin concentration of 5.965 mg/g in dried fruiting bodies. These findings highlight pearl millet as a cost-effective and efficient substrate for sustainable* Cordyceps militaris *cultivation, offering significant potential for pharmaceutical and nutraceutical applications.*

Keywords: *Cordyceps militaris, Pearl millet substrate, Cordycepin, Medicinal mushroom, Solid-state fermentation, Bioactive compounds, Morphological characterization.*

INTRODUCTION

Cordyceps is a genus of entomopathogenic fungi belonging to the class Ascomycota. It is currently divided into three families: Cordycipitaceae, Ophiocordycipitaceae, and Clavicipitaceae (Li et al., 2021). With over 680 known species, the most notable ones are Cordyceps militaris and Ophiocordyceps sinensis, which are highly esteemed in traditional and oriental medicine across China, Korea, Japan, and other Asian nations (Martinez-Burgos et al., 2024). Cordyceps sinensis is particularly rare and prized, owing to the difficulty of harvesting it from remote mountainous regions such as the Tibetan Plateau and parts of China, Nepal, and Bhutan.

Historically, Cordyceps has been utilized in traditional Chinese and Tibetan medicine for managing fatigue, sexual dysfunction, and chronic illnesses. This has led to its recognition as one of the most valuable medicinal fungi globally. In recent years, scientific interest has surged due to its bioactive constituents, especially cordycepin. This compound is under extensive investigation for its wide array of therapeutic benefits, including immunomodulatory (Liu et al., 2024), anti-inflammatory (Ha et al., 2024), anticancer (Dutta et al., 2024), antioxidant (Guo et al., 2024), antidiabetic (Jee et al., 2024), antihyperlipidemic (Zhang et al., 2024), antithrombotic (Chou et al., 2024), antiviral (Du et al., 2021), antibacterial (Kaewkod et al., 2023), and anti-aging effects (Li et al., 2020).

Recent advancements in artificial cultivation, especially using substrates like pearl millet, have increased accessibility to Cordyceps. Studies suggest that growing Cordyceps militaris on non-insect substrates such as grains (including pearl millet) can significantly enhance the yield of beneficial compounds like cordycepin (Wu et al., 2022). This method offers a sustainable and scalable solution, reducing dependency on wild resources and making Cordyceps available to a broader population. Artificially cultivated Cordyceps often matches or surpasses wild varieties in bioactive content (Ashraf et al., 2020).

The fungus produces fruiting bodies with spore-bearing stalks that contain key bioactive molecules, primarily cordycepin (3′-deoxyadenosine). Although its physical appearance may vary with the substrate, the core morphology remains unchanged. Cordyceps is rich in compounds like polysaccharides, sterols, alkaloids, and especially cordycepin, which holds substantial pharmacological potential (Krishna et al., 2024).

Key therapeutic properties of Cordyceps include (Ashraf et al., 2020):

Antioxidant: Scavenges free radicals and lowers oxidative stress linked to chronic diseases such as cancer and neurodegeneration.

Anti-inflammatory: Suppresses pro-inflammatory cytokine production, useful in treating arthritis and other inflammatory conditions.

Antitumor/Anticancer: Induces apoptosis in cancer cells, suggesting promising applications in oncology.

Immunomodulatory: Enhances both innate and adaptive immunity, boosting resistance to infections and improving vaccine efficacy.

Antidiabetic: Helps regulate blood sugar levels and improve insulin sensitivity.

Energy Boosting: Increases ATP production, reducing fatigue and enhancing physical performance.

Cordycepin and other Cordyceps-derived compounds are being explored for use in drug development targeting cancer, diabetes, inflammation, and immune disorders. Cordyceps is also gaining popularity in dietary supplements due to its energizing, anti-aging, and immune-boosting effects (Das et al., 2021). The goal is to make Cordyceps more accessible through scalable cultivation practices, especially for inclusion in functional foods and nutraceuticals.

Recent research supports the use of pearl millet as an effective substrate for Cordyceps cultivation. Pearl millet offers a favorable nutritional profile that supports mycelial growth and enhances cordycepin synthesis. Studies have demonstrated that cultivation on pearl millet can yield cordycepin concentrations comparable to or even exceeding those in wild fungi. Under optimal conditions, cordycepin content ranges from 5 to 10 mg/g of dry weight.

C. militaris is now widely cultivated in both liquid and solid-state systems, making it the most extensively studied and commercially viable species of the genus (Krishna et al., 2024). Optimizing growth conditions—such as glucose supplementation, pH, temperature, and light intensity—can significantly influence biomass yield and cordycepin production. For instance, a temperature of 30°C has been associated with higher cordycepin levels despite slower growth rates (Turk et al., 2021). The incorporation of by-products like spent brewery grain can also enhance yields, with some studies reporting cordycepin levels up to 10.58 mg/g in fresh stromata (Thy, 2019).

These findings highlight pearl millet as a promising and sustainable substrate for Cordyceps cultivation. It supports large-scale production while minimizing environmental impact and reducing the need for wild harvesting.

The present study focuses on optimizing the cultivation of Cordyceps militaris (DMRO-1163 strain) using pearl millet as the primary substrate. The objective is to develop an eco-friendly, scalable method for producing Cordyceps with a high cordycepin yield. This approach not only addresses the overexploitation of wild Cordyceps but also aligns traditional medicinal knowledge with modern scientific advancements. It enables the reliable production of Cordyceps-based pharmaceutical and nutraceutical products while Contributing To The Conservation Of Natural Ecosystems.

MATERIALS AND METHODS

Pure Culture:

The pure culture of Cordyceps militaris (DMRO-1163) was obtained from Kehloor Biosciences and Research Centre (KBRC), Bilaspur, Himachal Pradesh. The culture was maintained on Sabouraud Dextrose Agar (SDA) slants under standard laboratory conditions.

In Vitro Cultivation of Cordyceps militaris
The Cordyceps militaris strain was cultivated using a pearl millet-based solid-state medium. For spawn preparation, a loopful of culture from SDA slants was inoculated into Sabouraud Dextrose Broth (SDB) and incubated at 20 °C for 3–5 days to develop liquid spawn.

The cultivation substrate consisted of 30 g of pearl millet supplemented with 60 mL of a nutritional solution (comprising glucose 20 g, sucrose 10 g, peptone 5 g, yeast extract 3 g, KH_2PO_4 2 g, $MgSO_4$ 0.5 g, vitamin B_{12} 20 mg, vitamin B_1 50 mg, and distilled water up to 1000 mL). The mixture was dispensed into 250 mL glass jars and sterilized by autoclaving at 121 °C for 20 minutes, following the method of Shrestha et al. (2012) with modifications.

After cooling to room temperature, 5 mL of the Cordyceps militaris liquid spawn was inoculated into each jar. Incubation was carried out at 20 °C for 10 days in complete darkness, followed by 40–50 days under alternating light and dark conditions. During the cultivation period, the incubation environment was maintained at 20 °C with 80–90% relative humidity and 500 lux white light for 14 hours per day. Observations were recorded on the duration required for mycelial colonization and ascocarp (fruiting body) formation.

Morphological Characterization:
Morphological traits of the Cordyceps militaris fruiting bodies were assessed based on parameters such as length, color, and weight. The length was measured in centimeters, and both wet and dry weights were calculated in grams per jar. Color observations were documented to describe visual differences. These evaluations contributed to a comprehensive morphological profile and provided insight into the impact of using pearl millet as a substrate on the development of Cordyceps militaris.

Bioactive Compound Analysis:
Quantification of the key bioactive compound cordycepin was performed using Ultra High-Performance Liquid Chromatography (UHPLC). Cordycepin analysis was carried out at CSIR-IHBT (Institute of Himalayan Bioresource Technology), Palampur. The analytical procedure involved the following steps:

Sample Preparation: Extraction of cordycepin from the harvested Cordyceps militaris samples.

UHPLC Analysis:
The prepared extract was injected into the UHPLC system and passed through a high-pressure chromatographic column.

Detection was performed using a UV or Photodiode Array (PDA) detector, targeting the specific absorption spectrum of cordycepin.

Data Interpretation: Cordycepin peaks were identified on chromatograms and quantified using calibration curves developed with known cordycepin standards.

RESULTS

Pure Culture:
C. militaris was cultivated on a pearl millet medium (30 g of pearl millet and 60 mL of nutritional solution) in 250 mL glass jars. Liquid spawn (5 mL) was inoculated and incubated at 20 °C with a relative humidity of 80–90% in dark conditions until a white mycelial mat formed over the substrate. After 10 days, a dense mycelial mat was observed, after which the jars were exposed to a photoperiod of 14 hours light and 10 hours dark to initiate fruiting body development. Primordia formation began within 20–25 days, and fully mature vertical stromata developed by 50–60 days.

Figure 1 shows the pure culture of Cordyceps militaris (DMRO-1163) growing in a test tube on SDA slants. The culture displays a distinctive orange to yellowish mycelial mass, with even, smooth texture and clearly defined edges, indicating healthy growth and successful isolation on the solid medium.

Figure 1: Pure culture of Cordyceps militaris (DMRO-1163) on SDA slants.

Figure 2 illustrates the preparation of Cordyceps militaris spawn in Sabouraud Dextrose Broth (SDB). A test tube containing the pure culture is shown centrally, flanked by two conical flasks containing liquid medium. Inoculation appears recent, as the broth is still clear. The setup also includes a Bunsen burner in the background, suggesting aseptic techniques were followed. These flasks were incubated at 20 °C on a shaker for 3–4 days for culture propagation.

Figure 2: Spawn preparation from pure culture.

Figure 3 shows the flasks after 4 days of incubation. The broth has a light yellow tint and appears slightly turbid, indicating initial mycelial growth. The flasks are sealed with cotton plugs to maintain sterility while allowing gas exchange. At this stage, the culture remains in the liquid phase without aggregation at the surface.

Figure 3: Prepared spawn after 4th day of incubation.

Figure 4 displays the preparation of the solid substrate using pearl millet and the nutritional solution for the cultivation of Cordyceps militaris.

Figure 4: Preparation of solid substrate (pearl millet and nutritional solution) for cultivation.

Figure 5 illustrates a mature cluster of Cordyceps militaris fruiting bodies at around 60 days, showing bright orange color and elongated structure. These fruiting bodies, resembling slender tendrils, indicate a successful and contamination-free harvest, optimal for pharmacological and nutraceutical studies.

Figure 5: 60th day growth of fully matured Cordyceps militaris fruiting bodies ready for harvest.

Figure 6 shows the measurement of fruiting body length using a ruler. The longer fruiting body extends beyond 7 cm, while the shorter one reaches just past 3 cm. These measurements are vital for standardization and commercial evaluation.

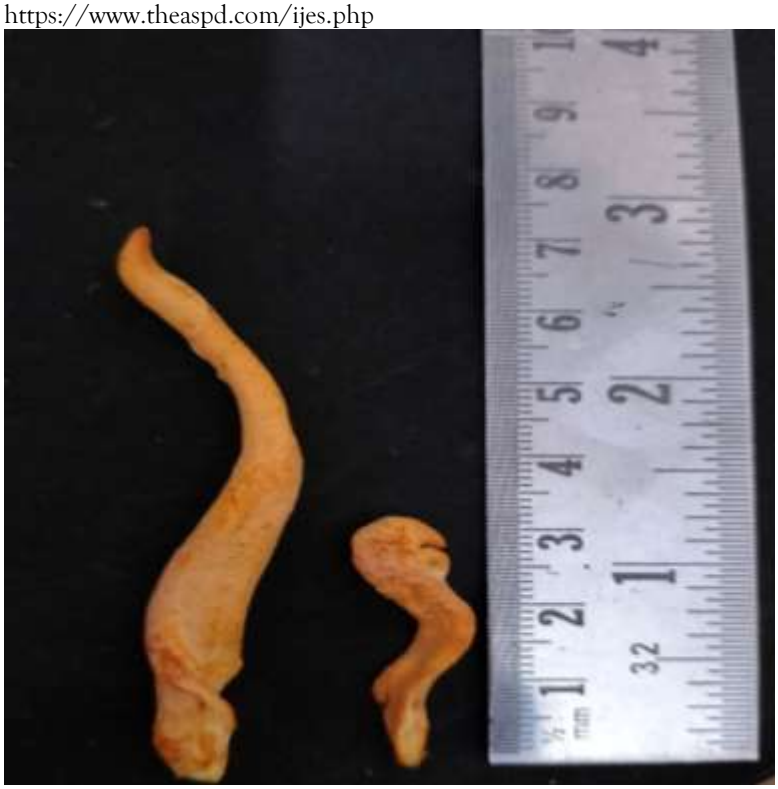

Figure 6: Measurement of Cordyceps militaris fruiting body length.

Fruiting Body Yield

Table 1 presents the wet and dry weight data of fruiting bodies from 30 jars:

Fruiting Body Weight	Sum of 30 jars (g)	Mean (g/jar)	Standard Deviation (g)
Wet Weight	823.00	27.433	3.33
Dry Weight	131.15	4.372	0.67

Table 1: Wet and dry weight of Cordyceps militaris fruiting bodies cultivated on pearl millet substrate.

Fruiting Body Length

Table 2 summarizes the average length of fruiting bodies from the 30 jars:

Substrate (PM01)	Mean Length (cm)	Standard Deviation (cm)
Pearl Millet	6.94	0.270185

Table 2: Length of Cordyceps militaris fruiting bodies cultivated on pearl millet substrate.

Cordycepin Content

Figure 7 Displays the UHPLC chromatogram showing the cordycepin peak, while **Table 3** details the concentration of cordycepin extracted from fruiting bodies grown on pearl millet substrate:

Substrate	Cordycepin Level (mg/g)
Pearl Millet	5.965

Table 3: Cordycepin concentration in Cordyceps militaris cultivated on pearl millet substrate.

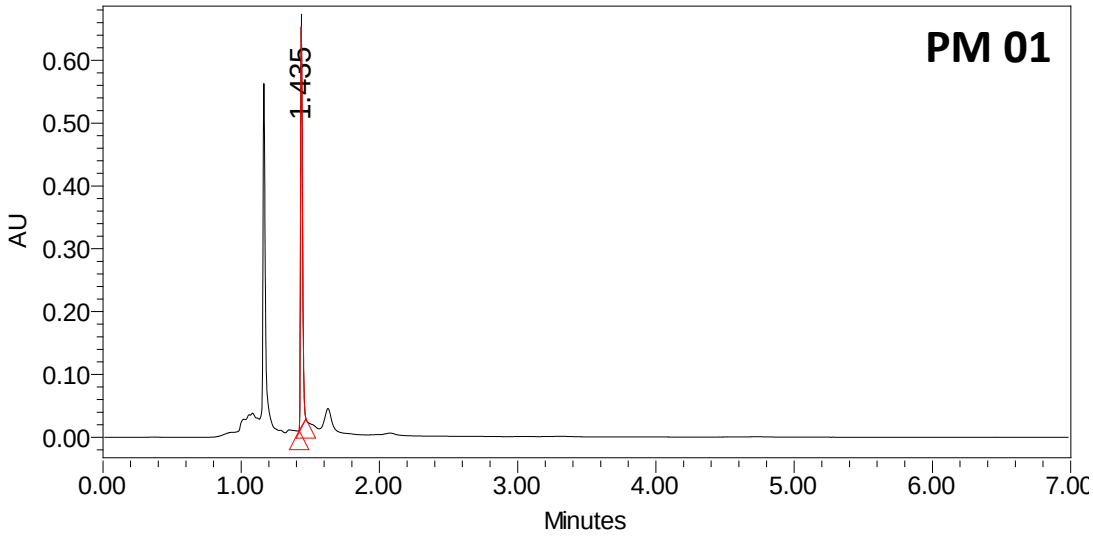

Figure 8: UHPLC chromatogram showing cordycepin peak.

CONCLUSION

This study successfully demonstrates that pearl millet is a promising substrate for the artificial cultivation of Cordyceps militaris. The fungus showed excellent growth characteristics, including high biomass yield and notable cordycepin production, under controlled environmental conditions. The observed cordycepin content of 5.965 mg/g supports the use of pearl millet as a viable alternative to more conventional substrates for functional mushroom production. Moreover, the morphological consistency and quality of the fruiting bodies make pearl millet a suitable candidate for commercial-scale cultivation. These results contribute to the ongoing efforts in developing scalable, eco-friendly methods for producing bioactive compounds from Cordyceps militaris, reducing reliance on wild harvesting, and aligning traditional medicinal uses with modern biotechnological practices.

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