**DOI: https://doi.org/10.36036/MR.32.1.2023.131495**
***Mushroom Research* 32 (1) : 57-66, 2023**

# Morpho-cultural, biochemical and molecular characterization of a pigment producing *Cordyceps militaris* (strain DMRO-1164)

**Satish Kumar, Kirti Kaundal*, Shweta Sharma, Anil Rao and V.P. Sharma**

*Directorate of Mushroom Research, Chambaghat, Solan (HP) - 173213, India*
**Corresponding author;* E-mail: kirtikndl@gmail.com

## ABSTRACT

**The study was conducted to ascertain the cultural growth and nutritional content of the strain DMRO-1164 of *Cordyceps militaris* cultivated in India. Microscopic and molecular studies were undertaken to confirm the identity of the mushroom. Scanning Electron Microscopy was done for micro-morphological characteristics of conidia and conidiophore formation. Molecular identification showed 99.83% similarity with *Cordyceps militaris.* Abundant mycelial density and darkest orange pigment was observed in Potato dextrose agar followed by Oat meal agar and least pigment was observed in Yeast Agar medium. Adenosine and cordycepin content was 1.8 mg/g and 9.8 mg/g, respectively. Proximate analysis of *Cordyceps militaris* showed that 100 g dry mushroom contained: proteins 39.00g, carbohydrate 40g, dietary fibre 1.97g, fat 7.00, sodium 46mg, vitamin C 113 mg, total ash 7.82g and saturated fatty acid 0.12g. Heavy metal analysis showed lesser amount of Pb, Cd (<0.01 mg/kg) and Hg (<0.001 mg/kg). Microbiological evaluation showed least presence of *Staphylococcus* and *Salmonella* on fruiting bodies of *Cordyceps militaris*. The carotenoid and lycopene content were also estimated and found to be 0.461mg/g and 0.298 mg/g. Fruiting bodies was fully developed into orange club shaped structures after 45 days of inoculation.**

**Keywords**: Adenosine, cordycepin, *Cordyceps militaris*, mushroom, medicine, nutrition

*Cordyceps* is an entomopathogenic fungus belonging to the class *Ascomycota*. It is currently divided into three families: *Cordycipitaceae, Ophiocordycipitaceae* and *Clavicipitaceae* (Bhandari *et al.* 2010). Genus *Cordyceps* reported to have more than 680 different species but *Cordyceps militaris* and *Ophiocordyceps sinensis* are being widely used in traditional and oriental system of medicine in China, Korea, Japan, and other Asian countries (Sung *et al*. 2007; Tuli *et al.* 2013; Shrestha *et al*. 2012). *Cordyceps militaris* was approved as the first novel food of the *Cordyceps* species by China's Ministry of Public Health in 2009. In recent years, advanced techniques have revealed that the nutrients and bioactive compounds in the fruiting body of *Cordyceps militaris* are similar to those found in the traditional Chinese invigorator *Ophiocordyceps sinensis* (Yang *et al*. 2019: Chan *et al*. 2015). The main physiologically active ingredients are cordycepin (3'-deoxyadenosine) and adenosine, a nucleoside analogues (Jin *et al*., 2020). The major properties of this fungus are immune modulation, antioxidant, anti-cancer, anti-inflammatory, anti-diabetes, anti-hyperlipidemia, antithrombosis , anti-viral, anti-bacterial, anti-pest and skin whitening (Lee *et al*., 2020; Chin *et al*. 2006; Kim *et al*. 2012; Hong *et al*. 2016; Park *et al*. 2002; Seo *et al*. 2018; Kim *et al*. 2005; Lee *et al*. 2006; Ohta *et al*. 2007; Tran and Tran, 2019; Kim *et al*. 2002). *Cordyceps militaris* is a pathogen that affects the pupae of *lepidopteran*

Recieved on 19-12-2022; Accepted on 31-07-2023
https://epubs.icar.org.in/index.php/MR/article/view/131495

insects (Sung *et al*. 2007) and it has been successfully cultivated on cereal grains and *Bombyx mori* pupae (Stensrud O, 2005). *Cordyceps militaris,* cultivation and utilisation received a lot of attention during COVID-19, and cordycepin content in it has been shown to be therapeutic (Verma, 2020; Jin *et al*, 2018).

The study has been undertaken to confirm the identification of *Cordyceps militaris* isolate, to study optimum culture conditions for large-scale commercialization and its nutritional/nutraceutical composition.

## MATERIALS AND METHODS

### Pure culture

Pure culture of *Cordyceps militaris* (DMRO-1164) was obtained from ICAR-Directorate of Mushroom Research, Chambaghat, Solan, (H.P.) and maintained on PDA (potato dextrose agar) slants according to the procedure of Lin *et al*. (2017).

### *In Vitro* cultivation of *C. militaris*

*C. militaris* strain was cultivated in a brown rice medium. Substrate was prepared using 12g of brown rice supplemented with 35ml nutritional broth (glucose 20g, peptone 5g, yeast extract 3g, $KH_2PO_4$ 0.5g, Tri Ammonium citrate 1g, $MgSO_4$ 0.5g, Vitamin $B_{12}$ 50mg and water 1000 ml) was taken into 500ml beaker (Fig. 5), autoclaved for 20 minutes at 121°C according to the method of Shrestha *et al*., (2012). The substrate was inoculated with 3 ml liquid spawn of *C. militaris* and covered with polypropylene sheet. The beakers were incubated at 21°C for spawn run, with relative humidity 60–70% under dark conditions for 15 days. For fruiting body formation and development, the beakers were exposed to light conditions at 21°C and relative humidity 85–90%. Data pertaining to days taken for mycelial colonization and days taken for ascocarp formation were recorded. Average biological efficiency (BE) was determined according to the calculations by Nguyen *et al*. (2019).

$$BE\,(\%) = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

### Taxonomy

For the confirmation of identity of the fungus, taxonomic and molecular studies were undertaken. Standard protocols were followed for description of *C. militaris* (Atri *et al*., 2005). A colour notation was recorded according to Kornerup and Wanscher (1978) for morphological descriptions. Fresh specimens were examined macroscopically in the laboratory using a free-hand sections mounted in 5% KOH and stained with 1% Congo red. The spore shape quotient (Q = L/W) was calculated considering the mean value of the length and width of 20 ascospores. The specimen was studied using light microscopy (Motic Inc., Hong Kong) and scanning electron microscopy (SEM; Emcraft, Gyeonggi-do, Korea). For SEM examination, the conidia were obtained from the dried sample and were directly mounted on double-sided adhesive tape pasted on a metallic specimen-stub and sputtered with gold coating. These gold-plated spores were observed at different magnifications in high vacuum mode to observe the pattern of spore structure.

### DNA extraction, amplification and sequencing

Identity of the culture was confirmed using molecular biology approaches through homology searches for ITS 5.8S rDNA sequences on NCBI. The DNA was extracted by CTAB method as described by Stajich et al (2010). PCR was performed on DNA extracted to amplify 5.8S rRNA genes region using universal primers ITS-1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-4(5'-TCCTCCGCTTATTGATATGC-3'). PCR conditions were set at 97°C for 1 min, 97°C for 1 min and 48°C for 1 minute, 72°C for 1 minute (30 cycles), and a final extension at 72°C for 7 minutes. The amplicons were sequenced using Sanger sequencing method (Sanger *et al.,* 1977). The obtained sequences were compared on NCBI using BLAST searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Tamaru *et al*., 2011).

**Growth and pigment production**

To optimize growth and pigment development conditions, *C. militaris* strain was grown on different solid media, Potato dextrose agar (PDA), Yeast extract agar (YEA), Modified nutrient agar (MNA), Dextrose tryptone agar modified (DTAM), Dextrose starch agar (DSA) and Oatmeal agar (OMA) at 25°C. Vegetative growth of mycelium in the solid media was measured by taking the diameter of colony on X and Y axis for which 10 mm discs of *C. militaris* were inoculated in each Petri dish. The diameter of the mycelium extension (mm) was measured at 5 to 25 days at each 5 day interval after inoculation on different culture medium. The morphological characteristics of mycelia such as texture, density, and colour were assessed by visual observations.

**Proximate composition**

To determine the proximate composition, the fruit body was dried at 45°C till constant weight was obtained. The nutritional attributes of each sample were analysed using standard protocols. Total nitrogen was estimated by the Kjeldahl method and crude protein was calculated using a factor of 4.38 (Kalaè, 2013). The crude fat was analysed by following method (James, 1995). Total ash was assayed following Manzi *et al*. (2001). Moisture content was estimated according to the method suggested by AOAC (AOAC, 2003). Miner-als including heavy metals were determined by the method of Jackson using an atomic absorption spectrophotometer (Jackson, 1967). Total carbohydrate content was calculated by employing the following formula: carbohydrates (%) = 100 – (moisture content + protein content + crude fat + ash content + crude fiber).

**Microbiological Analysis**

*C. militaris* was microbiologically investigated for total microbial count (TMC), yeast & mould, Salmonella and Staphylococcus after 3 months of preservation followed by (Ratajczak *et al*, 2015).

**Heavy Metal Analysis**

To determine the effect of Cd, Pb, and Hg on fruit body was done by methods described by Zheng *et al*. (2016) and Zhou *et al*. (2018).

**Bioactive compounds**

Determination of major bioactive components adenosine and cordycepin was done using high-performance liquid chromatography (HPLC) (Singpoonga *et al*, 2020).

**ß-Carotene and lycopene**

ß-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of ß -carotene and lycopene were calculated according to the following equations

Lycopene (mg/ 100 ml) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$

ß-carotene (mg/100 ml) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$

**RESULTS**

**In vitro cultivation of** ***C. militaris***

*C. militaris* was cultivated in brown rice medium (12g of brown rice and 35ml nutrient broth) in 500ml beakers, liquid spawn was inoculated and incubated at 21°C with relative humidity 60-70%in the dark, until complete spawn run (Fig. 1). After 15 days, the flasks were exposed to light at 21°C and RH 85–90% for fruit body initiation. Primordia formation starts after 20-25 days and fully developed into mature vertical stromata within 50 to 60 days. The biological efficiency was 40-50% when monochromatic LED lights were used to induce the fruiting body of *C. militaris*.

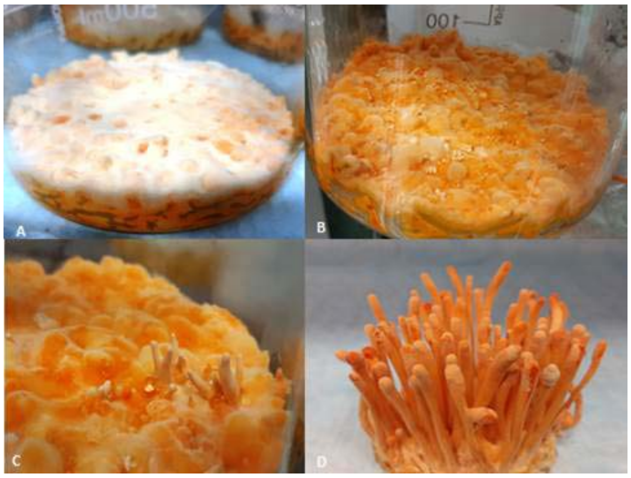

**Fig. 1.** Cultivation of strain DMRO-1164 on brown rice substrate

### Morphological, microscopic and scanning electron microscopy (SEM) studies

The fruiting body up to 7-8 cm long, 0.2-0.5 cm in dia, erect, often clavate shaped, fertile head, orange to bright orange, covered with tiny granules giving powdery appearance, stipe orange and stuffed (Fig. 2). Conidia were generally globose with 1.5~2 µm in diameter. The oval or ellipsoidal conidia varied from 2~4 × 1.5~2 µm in size, cylindrical conidia were as large as 7 × 3 µm. Ascospores discharged from fresh stroma of *C. militaris* specimen were observed for germination and conidiation periodically through SEM. Part-spores of *C. militaris* started to swell and germinate unidirectionally when observed through SEM. Oval part-spores, relatively smaller than cylindrical ones, were usually observed as jointed, mostly in pairs and did not germinate, whereas cylindrical part-spores, which were usually detached from adjacent part-spores, swelled and mostly germinated (Fig. 3). Elongated pyriform, cylindrical and globose conidia were produced on tips of simple and undifferentiated hyphae. First-formed conidia were somewhat pyriform or cylindrical (Fig. 2). Conidiogenous hyphae, i.e., phialides, were very similar to vegetative hyphae, but more slender. Slimy conidial heads were frequently observed, instead of chains. Even thin slimy film could be observed between an immature globose conidium and a mature broadly oval conidium.

**Fig 2.** Fruiting bodies of *C. militaris*

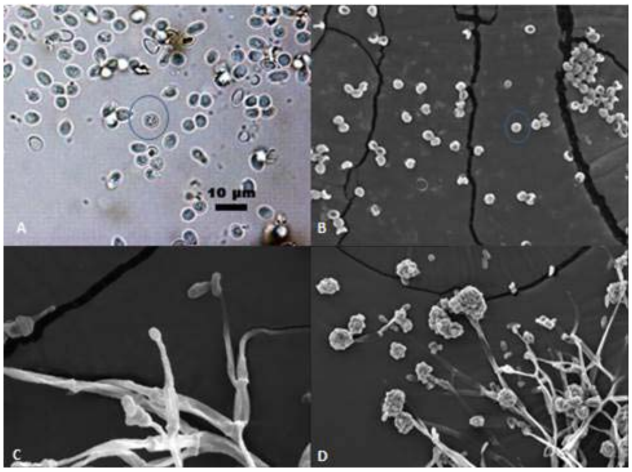


**Fig 3.** Scanning electron micrographs of profuse vegetative and conidial hyphae of *C. militaris*. A – Ellipsoidal conidia produced singly; B- Globose conidia; C- Conidia adhered in slimy heads; D-Oval spore under SEM

### Confirmation of identification and phylogenetic analysis of the *Cordyceps militaris* specimen

The dry mycelial powder of *Cordyceps* sample was used to isolate the genomic DNA. The ITS sequence was amplified using the universal flanking primers ITS1 and ITS4. The sequencing was outsourced through Eurofin ltd. The Sequence obtained was compared on NCBI using BLAST-n and phylogenetic relations with closest sequences were established using fast minimum evolution method (Fig 4). According to the nucleotide sequence analysis, ITS sequences of DMRO-1164 showed up to 99% homology with *Cordyceps militaris* specimen (KACC43321) on NCBI.

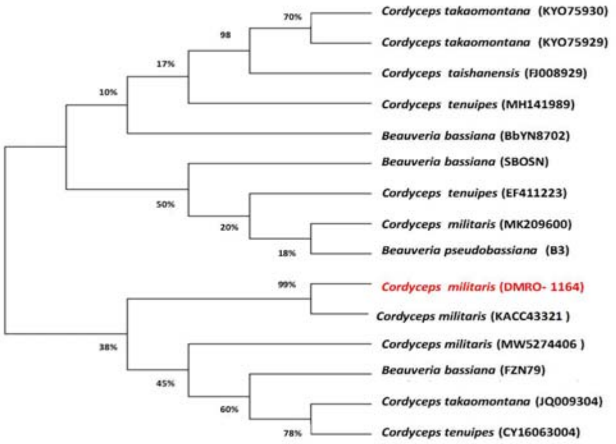


**Fig 4.** Phylogenetic tree was derived from ITS 5.8S rDNA gene sequences of *C. militaris*

### Physiological and pigmentation studies

Mycelial growth of *C. militaris* was longitudinally radial, aerial initially creamish white, becoming densely matted and woolly in appearance. Potato dextrose agar (PDA), Yeast extract agar (YEA), Modified nutrient agar (MNA), Dextrose Tryptone agar modified (DTAM), Dextrose starch agar (DSA), and Oat meal agar (OMA) were evaluated for mycelial growth observation and pigment production. Mycelial discs (10 mm) were cut from the colonies of *Cordyceps militaris* with the help of the sterilized cork borer and inoculated in each of six different agar media plates and incubated at 25$^{o}$C. Colony growth and diameter was recorded on x and y axis every week for three subsequent weeks. After three weeks of incubation, most of the colonies completely covered the plates. Colony diameter was measured in triplicate in each medium. The average colony diameter was calculated. PDA, OMA and DTAM showed abundant mycelial density and radial growth (Fig. 5).

With respect to growth and pigmentation of the colonies on different media, PDA supported the maximum radial growth of 85.2mm in three weeks and showed orange pigment with white margins, but the density of mycelia was low. Mycelia with orange pigments were found in high density on OMA with radial growth of 80.0 mm while DTAM supported at growth of 79.6 mm with very high mycelial and orange pigment was visible. There was moderate mycelial growth and very little pigment production on MNA (72.1 mm) and DSA (72.6 mm). On YEA there is little radial growth and no pigment could be seen (Fig 6).

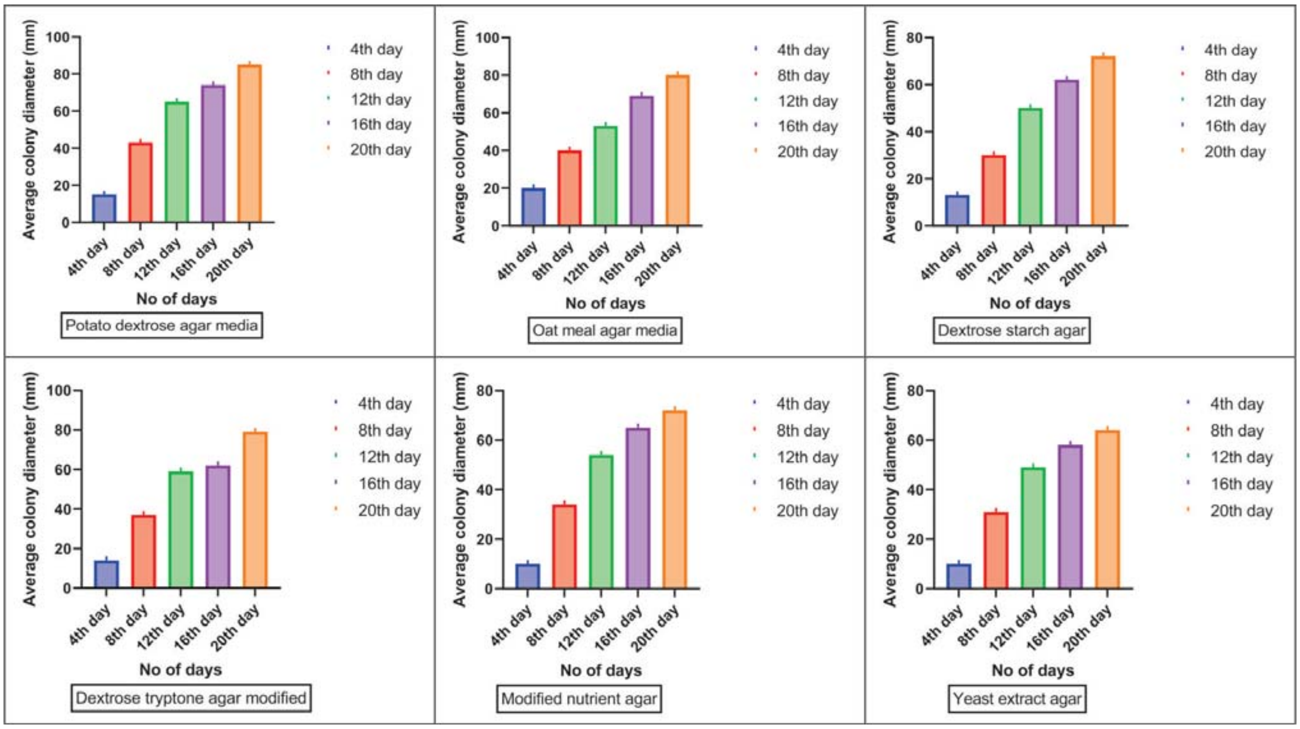


**Fig. 5.** Growth of *C. militaris* mycelium on different media

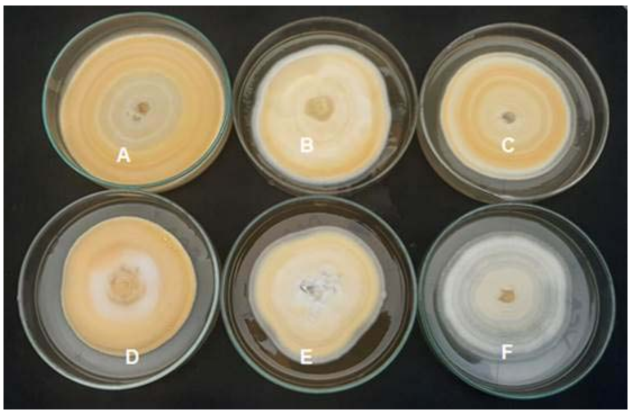


**Fig 6.** Pigmentations in *C. militaris* colony after three weeks of light exposure on different agar media [A- Potato dextrose agar; B - Oat meal agar; C - Dextrose tryptone agar modified; D - Dextrose starch agar; E -Modified nutrient Agar; F- Yeast extract agar]

### Biochemical, bioactive, heavy metal and microbiological analysis of *C. militaris* fruit bodies

The fruit body analysis of *Cordyceps militaris* (Table 1) demonstrated that the dried fruit bodies were particularly good sources of proteins (39 g/100 g) and carbohydrate (40 g/100 g). Moreover, fruit bodies had significant amounts of total ash (7.82 g/100 g), sodium (46 mg/100 g), and dietary fibre (1.97 g/100 g). ß-carotene and lycopene content in the extract of fruiting body was 0.461mg/g and 0.298 mg/g. Cordycepin and adenosine were the key bioactive components of the *C. militaris*. The concentration of adenosine was 1.8 mg/g, while cordycepin was 9.8 mg/g. Lead, Cadmium, and Mercury were discovered to be within permissible limits, according to the results of the heavy metal contamination. Besides these, pathogenic bacteria such as *Salmonella* and *Staphylococcus* were found absent. Considerably low total bacterial count (1570 cfu) and yeast & and mould (220 cfu) were obtained.

## DISCUSSION

In the study, DMRO-1164 was identified as *Cordyceps militaris* based on taxonomic and molecular studies of the fruiting body, conidia, and

**Table 1.** Biochemical, key bioactive components, heavy metal and microbial analysis of *Cordyceps militaris*

| Parameter (dry weight basis) | Unit | Results |
|---|---|---|
| Total Energy | Kcal/100g | 288 |
| Fat | g/100g | 7.0 |
| Protein | g/100g | 39.0 |
| Dietary Fibre | g/100g | 1.97 |
| Sodium | mg/100g | 46 |
| Total Carbohydrate | g/100g | 40.0 |
| Sugar | g/100g | 2.15 |
| Vitamin C | mg/100g | 113 |
| Saturated Fatty Acid | g/100g | 0.12 |
| Total Ash | g/100g | 7.82 |
| Total Moisture Content (fruit body) | % | 4.62 |
| Cordycepin | mg/g | 9.50 |
| Adenosine | mg/g | 1.80 |
| ß-carotene | mg/g | 37.42 |
| Lycopene | mg/g | 1.359 |
| **Microbiological Analysis** | | |
| Total Bacterial count | cfu/g | 1570 |
| Yeast & Moulds | cfu/ g | 220 |
| Salmonella | cfu/25 g | Absent |
| Staphylococcus | cfu/25 g | Absent |
| **Heavy Metal Analysis** | **Max permissible limit** | **Results** |
| Lead as Pb | Max 0.20 mg/kg | <0.01 mg/kg |
| Cadmium as Cd | Max 0.10 mg/kg | <0.01 mg/kg |
| Mercury as Hg | Max 0.20 mg/kg | <0.001 mg/kg |

ascospores (Lincoff ,1981). Sehgal and Sagar (2006) have also conducted study on morphology and anatomy of *Cordyceps militaris* and our results pertaining to measurement with slight variations are in agreement with them. The contents of adenosine and cordycepin were found quite good in the strain. Wang *et al*. (2016) also studied cordycepin content of *cordyceps militaris*. ß-carotene and lycopene are the pigments responsible for orange colour in C. militaris and are antioxidant in nature. Our results for the concentration of the ß-carotene and lycopene are in agreement to Choi *et al*., (2020) and Joshi *et al*, (2019). Shrestha *et al*. (2012) showed that the biological efficiency was 14 –27% when brown rice was used as the culture medium for cultivation. Similar studies were done by Chao *et al*. (2019). Many researchers have focused on the production of polysaccharides from *Cordyceps militaris* due to their biological activities and health benefits (Hung *et al*., 2020). Major compounds found in *Cordyceps militaris* were studied by Chan *et al*. (2015). These findings support previous studies in which *Cordyceps militaris* was rich in adenosine, cordycepin, and various amino acid, which have been generally used as an active ingredient in anti-ageing and rejuvenating skin care products due to their hygroscopic properties (Ferreira *et al*., 2020). COVID-19, a new pandemic disease caused single stranded RNA virus was first identified in November-December 2019 in Wuhan, China (Phelan *et al*., 2020 ). *Cordyceps militaris* has been proved to have antiviral activities against several human viruses including influenza, human immunodeficiency, murine leukemia and epstein-barr virus (Chanda *et al*., 2015; Ohta *et al*., 2007; Ryu *et al*., 2014). The researchers are also considering evaluating inhibitory potential of this fungus against SARS-CoV-2. This study could provide useful information for the development of economic and efficient cultivation of strain DMRO-1164 with good concentration of cordycepin and can be commercialized in the bio-industry where fermentation processes such as functional foods, pharmaceuticals and cosmetics are used.

## REFERENCES

1. AOAC. 2003. *Official Methods of Analysis. Vol. I 17th ed.* Association of Analytical Washington, DC, USA

2. Atri, N.S., A. Kaur and H. Kour. 2005. Wild Mushrooms - Collection and Identification: In Frontiers in Mushroom Biotechnology, Rai, R.D., Upadhyay, R.C. and Sharma, S.R.(eds). pp. 10-26. National Research Centre for Mushrooms (ICAR), Chambaghat, Solan.

3. Bhandari, A.K., J.S. Negi, V.K. Bisht, C.S. Rana, M.K. Bharti and N. Singh. 2010. Chemical constituent, inorganic elements and properties of *Cordyceps sinensis* " a review. *Nat Sci* **8**: 253-256.

4. Chan J.S.L., G.S. Barseghyan, M.D. Asatiani, S.P. Wasser. 2015. Chemical composition and medicinal value of fruiting bodies and submerged cultured mycelia of caterpillar medicinal fungus *Cordyceps militaris* CBS-132098 (Ascomycetes) *Int J Med Mushrooms* **17**: 649-659.

5. Chanda, S., A. Banerjee, S. Nandi, S. Chakrabarti, and M. Sarkar. 2015. Cordycepin: an adenosine analogue executes anti rotaviral effect by stimulating induction of type I interferon. *Journal of Virology and Antiviral Research* **4(2):** 1-12.

6. Chao S.C., S.L. Chang, H.C. Lo, W.K. Hsu, Y.T. Lin, and T.H. Hsu. 2019. Enhanced production of fruiting body and bioactive ingredients of *Cordyceps militaris* with LED light illumination optimization. *J Agr Sci Tech* **21**(2): 451-462.

7. Zeng, X., X. Xu, H.M. Boezen and X. Huo. 2016. Children with health impairments by heavy metals in an e-waste recycling area. *Chemosphere* **148**: 408-15. doi: 10.1016/j.chemosphere.2015.10.078

8. Chin, J.E., H.S. Lee and K.C. Kim. 2006. Effect of mushroom extracts on tyrosinase promoter. *Korean J Sanitation* **21**(3): 1-8.

9. Choi, J.E., B. Park, J. Lee and J. Kim. 2020. Anti-atopic dermatitis properties of *Cordyceps militaris* on TNFá/IFNã-stimulated HaCaT cells and experimentally induced atopic dermatitis in mice. *Phys Act Nutr* **24** (4): 7-14.

10. Ferreira M.S., M.C. Magalhães, J.M. Sousa-Lobo and I.F. Almeida. 2020. Trending anti-aging peptides. *Cosmetics* **7**(4): 91. doi: 10.3390/cosmetics7040091.

11. Hong, S.M., H.D. Cho, J.H. Kim, J.Y. Lee, J.M. Park and K.I. Seo. 2016. Anti-oxidant and anti-proliferative effects of water extract mixture of *Cordyceps militaris* and *Allium tuberosum*. *J Life Sci* **26**: 805-811.

12. Hung, D.N., L.C. Wang, L.H. Lay and V.T. Phuong. 2020. Impact of different fermentation characteristics on the production of mycelial biomass, extra-cellular polysaccharides, intra-cellular polysaccharides, and on the antioxidant activities of *Cordyceps militaris* (L.) Fr. (strains AG-1, PSJ-1). *Acta Agric Slov* **116**: 337-350.

13. Jackson, M.L. 1967. *Soil chemical analysis*. New Delhi: Pren-tice Hall of India Ltd.

14. James, C.S. 1995. *Analytical Chemistry of Foods 1 edition*, Chapman and Hall New York.

15. Jin, Y., X. Meng, Z.D. Qiu, Y.P. Su, P. Yu and P. Qu. 2018. Anti-tumor and anti-metastatic roles of cordycepin, one bioactive compound of *Cordyceps Militaris*. *Saudi Journal of Biological Sciences* **25**(5): 991-995.

16. Jin Feng Li, Van Ahn Hoang, Jong Chan Ahn, Dong Uk Yang, Dong Wook Lee and Deok Chun Yang. 2020. Isolation of new strain of *Cordyceps militaris* HB8 and optimal condition for production of adenosine and cordycepin in fruit body. *Korean J Plant Res* **33**(6): 696-706.

17. Joshi, M., A. Sagar, S.S. Kanwar and S. Singh. 2019. Anticancer, antibacterial and antioxidant activities of *Cordyceps militaris*. *Indian J Exp Biol* **57**: 15-20.

18. Kalaè, P. 2013. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. *J Sci Food Agric* **93**(2): 209-18. doi: 10.1002/jsfa.5960.

19. Kim, H.J., T.H. Lee, Y.S. Kwon, M.W. Son and C.K. Kim. 2012. Immunomodulatory activities of ethanol extract of *Cordyceps militaris* in immune-compromised mice. *J Korean Soc Food Sci Nutr* **41**(4): 494-500.

20. Kim, H.S., Y.J. Rho and M. Choe. 2005. *Cordyceps militaris* increases hepatic glucokinase activities. *J Korean Soc Food Sci Nutr* **34**(2): 158-161.

21. Kim JR, Yeon SH, Kim HS and Ahn YJ (2002): Larvicidal activity against Plutella xylostella of cordycepin from the fruiting body of *Cordyceps militaris*. Pest Manag. Sci. 58(7): 713-717.

22. Kornerup, A., and J.H. Wanscher. 1978. *Methuen handbook of color*, 3rd ed. Methuen, London.

23. Lee, C.T., K.S. Huang, J.F. Shaw, J.R. Chen, W.S. Kuo, G.X. Shen, A.M. Grumezescu, A.M. Holban, Y.T. Wang, J.S. Wang, Y.P. Hsiang, Y.M. Lin, H.H. Hsu and C.H. Yang. 2020. Trends in the immunomodulatory effects of *Cordyceps militaris*: total extracts, polysaccharides and cordycepin. *Frontiers in Pharmacology* **11**: 1-20.

24. Lee, S.M., N.S. Park and E.J. Park. 2006. Effects of *Cordyceps militaris* cultivated on rice on lipid metabolism in rats fed high fat-cholesterol diet. *J Food Sci Nutr* **11**: 36-41.

25. Lin, J., X. Zhang, B. Song, W. Xue, X. Su, X. Chen and Z. Dong. 2017. Improving cellulase production in submerged fermentation by the expression of a Vitreoscilla hemoglobin in *Trichoderma reesei*. *AMB Express* **7**: 203. doi: 10.1186/s13568-017-0507-x.

26. Lincoff, G. 1981. *Guide to Mushrooms*. A Fireside Book, Published by Simon and Schuster, New York, 512p.

27. Manzi, P., A. Aguzzi and L. Pizzoferrato. 2001. Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry* **73:** 321-325. Doi: 10.1016/S0308-8146(00)00304-6.

28. Nagata, M. and I. Yamashita. 1992. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *Nippon Shokuhin Kogyo Gakkaish* **39(**10): 925-928.

29. Nguyen, B.T.T., N.X. Ngo, V.V. Le, L.T. Nguyen, R. Kana and H.D. Nguyen. 2019. Optimal culture conditions for mycelial growth and fruiting body formation of Ling Zhi mushroom *Ganoderma lucidum* strain GA3. *Vietnam J Sci Technol* **61**(1): 62-67.

30. Ohta, Y., J.B. Lee, K.A. Hayashi, D.K. Fujita, D.K. Park and T. Hayashi. 2007. In vivo anti-influenza virus activity of animmunomodulatory acidic polysaccharide isolated from *Cordyceps militaris* grown on germinated soybeans. *J Agric Food Chem* **55**: 10194-10199.

31. Park, C.S., C.J. Kwon, M.A. Choi, G.S. Park and K.H. Choi. 2002. Antioxidative and nitrite scavenging activities of *Cordyceps militaris* extracts. *Korean J Food Preserv* **9**: 109-113.

32. Phelan, A.L., R. Katz and L.O. Gostin. 2020. The novel coronavirus originating in Wuhan, China: challenges for global health governance. *JAMA* **323**(8):709-710. doi: 10.1001/jama.2020.1097. PMID: 31999307

33. Ratajczak, M., M.M. Kubicka, D. Kamiñska, P. Sawicka and J. D³ugaszewska. 2015. Microbiological quality of non-sterile pharmaceutical products. *Saudi Pharm J* **23**(3):303-7. doi: 10.1016/j.jsps.2014.11.015.

34. Ryu, E., M. Son, M. Lee, K. Lee, J.Y. Cho, S. Cho, S.K. Lee, Y.M. Lee, H. Cho, G.H. Sung, and H. Kang. 2014. Cordycepin is a novel chemical suppressor of Epstein-Barr virus replication. *Oncoscience* **1**(12), 866-881.

35. Sanger, F., S. Nicklen and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *PNAS* **74** (12): 5463-5467.

36. Sehgal, A.K. and A. Sagar. 2006. In vitro isolation and influence of nutritional conditions on the mycelia growth of the entomopathogenic and medicinal fungus *Cordyceps militaris*. *J Plant Path* **3**: 315-321.

37. Seo, K.H., J.Y. Park, H.J. Noh, J.Y. Lee, E.Y. Young, J.G. Han, J.H. Kim and M.S. Cheong. 2018. Anti-inflammatory effects of various mushrooms in LPS-stimulated RAW264.7 cells. *Korean J Plant Res* **31**(5): 478-488.

38. Shrestha, B., S.K. Han, J.M. Sung and G.H. Sung. 2012. Fruiting body formation of *Cordyceps militaris* from multi-ascospore isolates and their single ascospore progeny strains. *Mycobiology* **40**(2): 100-106.

39. Singpoonga, N., R. Rittiron, B. Seang-on and Peerasak Chaiprasart and Yuranan Bantadjan. 2020. Determination of adenosine and cordycepin concentrations in *Cordyceps militaris* fruiting bodies using near-infrared spectroscopy. *ACS Omega* **5**(42): 27235-27244.

40. Stajich, J.E., S.K. Wilke, D. Ahrén, C.H. Au, B.W. Birren. et al. 2010. Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). *PNAS* **107**: 11889-11894.

41. Stensrud, O., N.L. Hywel-Jones and T. Schumacher. 2005. Towards a phylogenetic classification of *Cordyceps*: ITS nrDNA sequence data confirm divergent lineages and paraphyly. *Mycological Research* **109**: 41-56.

42. Sung, G.H., N.L. Hywel-Jones, J.M. Sung, J.J. Luangsa-Ard, B. Shrestha and J.W. Spatafora. 2007. Phylogenetic classification of *Cordyceps* and the clavicipitaceous fungi. *Stud Mycol* **57**: 5-59.

43. Tamaru, Y., H. Miyake, K. Kuroda, A. Nakanishi, C. Matsushima, R.H. Doi amd M. Ueda. 2011. Comparison of the mesophilic cellulosome-producing *Clostridium cellulovorans* genome with other cellulosome-related clostridial genomes. *Microb Biotechnol* **4**(1): 64-73. doi: 10.1111/j.1751-7915.2010.00210.x.

44. Tran, N.Q. and D.X. Tran. 2019. Xanthine oxidase inhibitory potential, antioxidant and antibacterial activities of *Cordyceps militaris* (L.) link fruiting body. *Medicines* **6**(1): 1-13.

45. Tuli, H.S., A.K. Sharma, S.S. Sandhu and D. Kashyap. 2013. Cordycepin: a bioactive metabolite with therapeutic potential. *Life Sci* **93**(23): 863-9. doi: 10.1016/j.lfs.2013.09.030.

46. Verma, A.K. 2020. Cordycepin: a bioactive metabolite of *Cordyceps militaris* and polyadenylation inhibitor with therapeutic potential against COVID-19. *Journal of Biomolecular Structure & Dynamics* **3**: 1-8.

47. Wang, X., L. Fang, Li. Fang, C. Hongyan, W. Sun, X. Chen, H. Gao and W. Shen. 2016. Determination of cordycepin content of *Cordyceps militaris* recombinant rice by high performance liquid chromatography. *Tropical Journal of Pharmaceutical Research* **15** (10): 2235-2239.

48. Yang, N.N., N. Jiang, Q.Y. Ma, F.D. Kong, Q.Y. Xie, L.M. Zhou, Z.F. Yu and Y.X. Zhao. 2019. Chemical study of the strain *Cordyceps* spp. from cell fusion between *Cordyceps militaris* and *Cordyceps cicadae*. *Journal of Asian Natural Products Research* **21**(5): 449-455. doi: 10.1080/10286020.2018.1451518

49. Zhou, L., S. Wang, Q. Hao, L. Kang, C. Kang, J. Yang, W. Yang, J. Jiang, L.Q. Huang and L. Guo. 2018. Bioaccessibility and risk assessment of heavy metals, and analysis of arsenic speciation in *Cordyceps sinensis*. *Chin Med* **13**: 40.