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GLYCINE AS GROWTH SUPPLEMENT IN CULTURE MEDIUM OF *CORDYCEPS MILITARIS* ENHANCES ITS GROWTH AND BIOMASS

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Abstract–*Cordyceps militaris* possesses several compounds with medicinal properties, and is commonly used in traditional Chinese functional food and medicine for a variety of health benefits. Because of its rare occurrence in nature, the market demand for artificial *C. militaris* is on the rise. Furthermore, efforts to increase its bioactive ingredients have also been considered in research. In this study, we aimed to investigate the effect of different growth supplements such as potassium fluoride (KF), Glycine (GLY), Adenine sulphate (AS) and Ascorbic acid (AsA) in rice medium on the enhancement of biomass in *C.militaris* fruiting body. A wide range of concentrations of all the growth supplements were added to the culture media during the cultivation of *C. militaris* fruiting bodies. The number, length, fresh weight and dry weight of the fruiting bodies in supplemented media were measured and compared with control (Standard medium). The biomass as fresh weight and dry weight per bottle in *C. militaris* fruiting bodies in the growth medium supplemented with 500 mg/l Glycine was enhanced 51.2% and 77.22% respectively. A remarkable enhancement of dry weight biomass (77.22%) in the fruiting bodies of *C. militaris* was recorded. These results demonstrated that the dose of glycine as amino acid in rice medium could stimulate the growth of *C. militaris* fruiting bodies and may enhance the production of bioactive ingredients that possess useful antioxidant, anticancer and immunomodulatory activities.

INTRODUCTION

Cordyceps militaris is an entomo-pathogenic fungus that belongs to the Cordycipitaceae family classified under the category of medicinal mushrooms by Zhang *et al.* (2012b). This wild macrofungus exists in different forms such as fruiting bodies and mycelial biomass. Wild *Cordyceps militaris* is a rare and endangered species that is found at high altitudes (3000_5000 m) in the Himalayan range (Jang *et al.*, 2015).

This fungus has long been used as an alternative medicine due to its antioxidative, anti-inflammatory, antibacterial, and anticancer activities (Wu *et al.,* 2020; X. Liu *et al.,* 2010; Aida *et al.,* 2009). The beneficial effects of *C. militaris* are attributed to the presence of abundant biologically active substances, including cordycepin, cordyceps acid, cordyceps polysaccharides, ergosterol, (Lin *et al.* 2016; Sharma, 2004; Valverde *et al.,* 2015; Varshney *et al.* 2011; Wang *et al.* 2012; Zhou *et al.* 2019) and mannitol by Ng and Wang (2005) and carotenoids (Lin and Xu, 2020; Nabi *et al.,* 2020; Venil *et al.,* 2020; Bhatt and Patel, 2020; Maoka, 2019). Despite its high value, the production of *C.militaris* faces several challenges, including slow growth rate, variable yield, and in consistent quality under natural or conventional cultivation methods. Therefore, optimizing the culture conditions to enhance the yield and quality of fruiting bodies is crucial for meeting the growing industrial demand.

However, the resource of this wild edible fungus has been decreasing sharply in the recent past and has been reported to be insufficient to meet the huge market demand (Li *et al.,* 2006; Yue-Qin *et al.,* 2002). In view of this, production of *C. militaris* in a controlled environment has been initiated, which involves various technologies including the development of temperature-controlled microfluid devices and the use of biopolymers (Lin *et al.,* 2011;

Zhang *et al.,* 2012). At the same time, approaches to optimize the artificial culture medium (Zhang *et al.,* 2020; Tang *et al.,* 2018; Dong *et al.,* 2012) to promote the production of bioactive compounds in the *C. militaris* fruiting bodies have also been adopted. Growth conditions have been shown to affect the production of metabolites and hence to obtain a uniform and stable content production under controlled conditions is favourably considered. Cultivation in a controlled environment is also important for the protection of *Cordyceps* in the wild. In light of these premises, several studies in the past have focused on optimization of the culture conditions by Shih *et al.* (2007) and media composition by Xie *et al.* (2009) for mycelia liquid culture. However, only a few studies have investigated the conditions for solid-state cultivation of fruiting bodies. Therefore, the present study has attempted to optimize artificial culture conditions to investigate the effect of growth supplements in rice medium on growth and biomass production of *C. militaris* fruiting bodies.

MATERIALS AND METHODS

Cordyceps militaris Cultivation

The stock culture of *C. militaris* strain 1164 was collected from ICAR DMR Solan, Himachal Pradesh and maintained on agar slants containing 2% glucose, 2% peptone, 0.2% KH_2PO_4, and 0.3% $MgSO_4$. The inoculated plants were incubated at 21–23 _C in the dark for 10 days and then stored at 4°C for mycelial growth. The normal solid medium comprised 36 g of rice with 63 ml of the nutrient solution (2% glucose, 2% peptone, 0.2% KH_2PO_4, and 0.3% $MgSO_4$) in a 300 ml cylindrical glass bottle. The different concentrations of Potassium Floride, Adenine sulphate, Glycine and Ascorbic acid were added to the solid media. All media were then sterilized for the cultivation of *C. militaris* on a solid medium. Each solid medium was prepared for 5 parallel bottles. After the completion of mycelial formation, they were transferred to normal and growth supplements solid culture media and incubated in the cultivation shed, where the temperature was maintained at 21°C to 23°C with the air humidity above 70% and in the dark for 20 days for base cultivation. After the complete spread of *C.militaris* mycelia on the medium surface, they were subjected to an alternating light–dark cycle of 21–23°C in 12 h of light and 16–20 °C in 12 h of darkness for 30 days to stimulate fruiting body growth. The cultivation environment and growth status were examined regularly. After 30 days, *C. militaris* fruiting bodies from normal (control) and growth supplements media were harvested and number, length, fresh and dry weight of fruiting bodies were measured.

Statistical Analysis

All the assays were performed in triplicate, and the results were expressed as means standard deviation (SD). The degree of statistical significance between the control and sample groups was analysed using an unpaired t-test (GraphPad Prism 10 software, San Diego, CA, USA). Significant values are represented as * $p < 0.05$, **$p < 0.01$, and *** $p < 0.001$.

RESULTS AND DISCUSSION

Number, length, fresh weight and dry weight per culture bottle of fruiting bodies of *C. militaris* raised in the Normal (control) and growth supplement, Potassium fluoride (KF), Adenine sulphate (AS), Glycine (GLY) and Ascorbic acid (AsA) added medium. In the present study, we successfully obtained *C. militaris* fruiting bodies from normal (Control) and KF (0.001,0.01, 0.1 and 1.0mM), AS (50,100 and 150 mg/l.), GLY (0.5,1.0 and 1.5 g/l.) and AsA (50,100 and 100 mg/l) supplemented media.

Biomass production in *C. militaris* fruiting bodies raised in KF supplemented medium

As shown in Figure 1 and 6A and 6B, we found that in all the media normal and supplemented with the 0.001, 0.01, and 0.1 mM KF, the growth of fruiting bodies was examined. This was not in line with earlier studies that have reported the inhibitory effect of high doses of fluoride on the growth of *Cordyceps militaris* (Chae *et al.,* 2018; Li *et al.,* 2021).

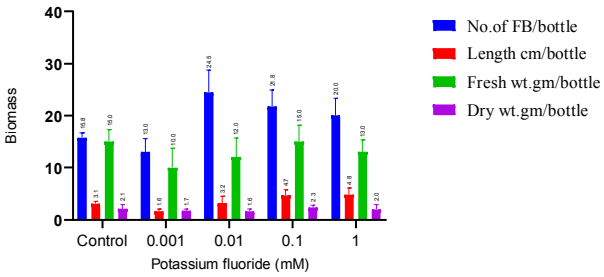

Fig. 1. Number, length, fresh weight and dry weight of fruiting bodies of *C.militaris* onControl(normal), 0.001 mM, 0.01 mM,0.1mM and 1.0 mM potassium fluoride supplemented medium. (mean ±SD, n = 3), *** $p < 0.0001$

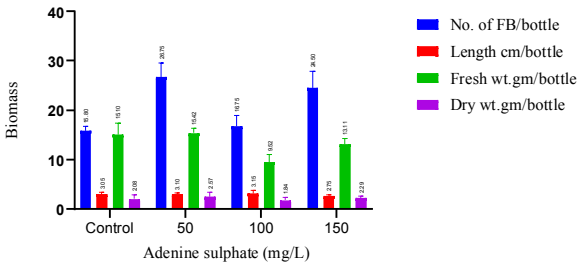

Fig. 2. Number, length, fresh weight and dry weight of fruiting bodies of *C. militaris* on Control (normal), 50 mg/l., 100 mg/l., and 150 mg/l. adenine sulphate supplemented medium. (mean ±SD, n = 3), *** p < 0.0001

Notably, as shown in Fig. 12. 25±0.51 g/bottle dry weight of fruiting bodies was obtained from 0.1mM KF. The mass of freeze-dried fruiting bodies from normal Medium (Control), 0.001mM KF, 0.01KF, 0.1 mMKF and 1.0mM KF was 2.08±0.82, 1.71±0.34, 1.6±0.44, 2.25±0.51 and 1.97±0.93 g/bottle, respectively. We found that the biomass of *C. militaris* fruiting bodies was promoted in the solid-state medium containing a dose of 0.1mM KF Fig.6. The optimization of fluoride addition (0.1 mM) resulted in an increase in biomass of fruiting bodies to 9.5±0.45 percent.

Biomass production in *C. militaris* fruiting bodies raised in Adenine sulphate (AS) supplemented medium

Adenine sulphate provides adenine, a purine base essential for synthesizing nucleotides, such as ATP, DNA, and RNA. During the active growth of the fruiting body, rapid cell division and metabolic activity require large amounts of nucleotides for DNA replication.RNA synthesis for protein production.

Studies suggest that adding adenine sulphate to the cultivation medium stimulates the growth of mycelium and enhances the yield and quality of the fruiting body. Adenine sulphate can regulate key pathways involved in cell differentiation and induction of fruiting body development (Masuda *et al.*, 2007; Liu *et al.*, 2018). We next analysed the biomass production of fruiting bodies in *C. militaris* cultured in Adenine sulphate (AS) supplemented medium (Fig. 6C). As shown in Fig. 2, the mass of freeze-dried fruiting bodies from normal Medium (Control), 50 mg/l,100 mg/l and 150 mg/l. AS was 2.08±0.82,2.575±0.82 g/bottle, 2.575±0.91 g/bottle, 1.842±0.58 and 2.295±0.31 g/bottle, respectively. Notably, the highest 2.575±0.91 g/bottle dry weight biomass of fruiting body was achieved from 50 mg/l. adenine sulphate supplemented medium which is 14% higher than the dry weight biomass produced from KF supplemented medium.

Biomass production in *C. militaris* fruiting bodies raised in Ascorbic acid (AsA) supplemented medium

Ascorbic acid, commonly known as vitamin C, plays a significant role in various biological processes. By acting as an antioxidant, a growth regulator, and a metabolic enhancer, it significantly influences the yield, bioactive compound production, and nutritional value of the fungus. The role of vitamin C (ascorbic acid) in the growth of *Cordyceps* fungi has not been extensively studied. However, some researches indicate that *Cordyceps* species, such as *Cordyceps militaris*, contain various bioactive compounds, including vitamins like B1, B2, B12, E, and K, but not specifically vitamin C (Ko *et al.* 2017; Li *et al.*, 2004; Kim and Yun, 2005; Leung and Wu, 2007; Li *et al.*, 2010; Kang *et al.*, 2014) also reported vitamin B1 as an active growth supplement in *C. militaris* for cordycepin production.

In the present study we observed that (Fig. 3 and Fig. 6D) the mass of freeze-dried fruiting bodies from normal Medium (Control), 50 mg/l,100 mg/l and 150 mg/l. As A was 2.02±0.63,3.225±0.66 g/bottle, 3.212±0.26 g/bottle and 3.177±0.43g/bottle, respectively. Notably, the highest 3.225±0.66 g/bottle dry weight biomass of fruiting body was achieved from 50 mg/l. ascorbic acid supplemented medium which is 59% higher than the dry weight biomass produced from normal control medium.

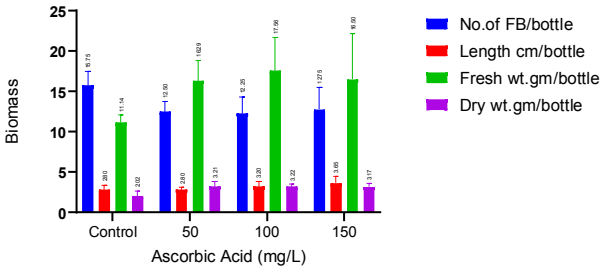

Fig. 3. Number, length, fresh weight and dry weight of fruiting bodies of *C. militaris* on Control (normal), 50 mg/l, 100 mg/l, and 150 mg/l. Ascorbic acid supplemented medium. (mean ±SD, n = 3), *** p < 0.0001

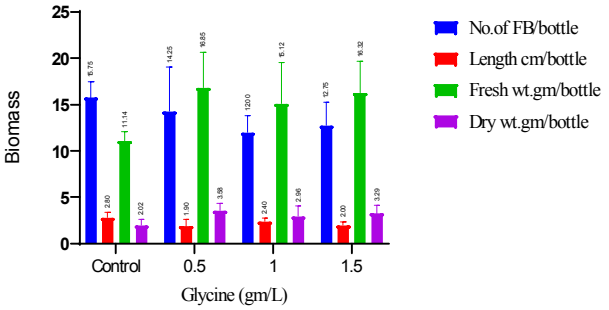

Fig. 4. Number, length, fresh weight and dry weight of fruiting bodies of *C. militaris* on Control (normal), 500mg/lit., 1.0 g/l., and 1.5 g/l. Glycine supplemented medium. (mean ±SD, n = 3), *** p < 0.0001

Biomass production in *C. militaris* fruiting bodies raised in Glycine (GLY) supplemented medium.

Glycine serves as a building block for protein synthesis in fungal cells, contributing to the structural and enzymatic components necessary for fruiting body formation. It can also act as a nitrogen source, which is vital for the growth and differentiation of fungal tissues, including the development of fruiting bodies by Masuda *et al.* (2007).

Though specific studies on role of glycine in the growth of *Cordyceps* fruiting bodies are not reported. However, research has shown that various amino acids can influence fungal development and metabolite production by Kredich and Guarino (1961) which incited us to utilize glycine as amino acids as growth supplement in medium for the enhancement of biomass of fruiting bodies in *Cordyceps militaris.* As depicted in Fig. 4 and 6E biomass of freeze-dried fruiting bodies from normal Medium (Control), 0.5 g/l,1.0 g/l and 1.5 g/l glycine

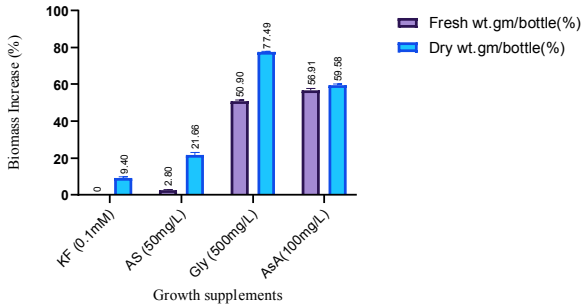

Fig. 5. Fresh weight and dry weight of fruiting bodies of *C.militaris* on KF (0.1mM), AS (50 mg/l), GLY (500 mg/l.) and AsA (100 mg/l.) supplemented medium. (mean ±SD, n = 3), *** p < 0.0001

(GLY) was 2.02±0.63,3.525±0.766 g/bottle, 2.962±1.11 g/bottle and 3.29±0.83 g/bottle, respectively. The amount of dry weight biomass of fruiting body of *Cordyceps militaris* was significantly increased to 3.525±0.766 g/bottle when glycine was added 0.5 g/l in medium.

We next compared the percentage increase of freeze-dried biomass of fruiting bodies produced in optimum concentrations of all the growth supplements KF, AS, AsA, and GLY in the medium shown in Fig. 5 and 6E. We found that the increase of freeze-dried fruiting body biomass in KF (0.1mM), AS (50 mg/l), AsA (100 mg/l.) and GLY (500 mg/lit.) was 9.4±0.45%, 21.66±1.34%, 59.58±0.49% and 77.49±0.44% respectively.

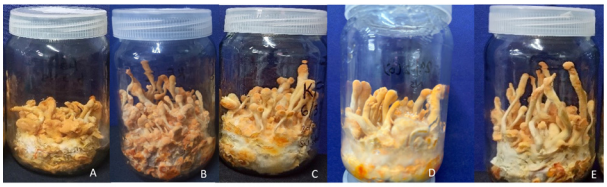

Fig. 6. Fruiting bodies of C. militaris in A. normal (control) medium and B. KF 0.1mM, C. AS (50 mg/l.), D. AsA (100 mg/l.), E. GLY (500 mg/l.) supplemented medium.

CONCLUSION

Cultivation technologies to support and empower the production of *C. militaris* are valuable for meeting increasing market demands, but very little attention is paid to enhance the biomass production of *Cordyceps militaris* using different growth supplements in rice media. This is the first study, to the best of our knowledge, that utilizes the different growth supplements for promoting biomass production of *C. militaris*. Results depicted in our study concluded that a significant increase of 77.49% dry weight biomass of fruiting body of cordyceps militaris was obtained in the glycine supplemented medium may be critical in artificial cultivation systems of *Cordyceps militaris* for the nutrition, metabolic pathways, secondary metabolite and cordycepin synthesis.

Conflict of Interest – None

REFERENCES

Aida, F.M.N.A., Shuhaimi, M., Yazid, M. and Maaruf, A.G. 2009. Mushroom as a potential source of prebiotics: a review. *Trends in Food Science & Technology.* 20(11-12): 567-575.

Bhatt, T. and Patel, K. 2020. Carotenoids: potent to prevent diseases review. *Natural Products and Bioprospecting.* 10: 109-117.

Chae, Y., Kim, D. and An, Y.J. 2018. Effects of fluorine on crops, soil exoenzyme activities, and earthworms in terrestrial ecosystems. *Ecotoxicology and Environmental Safety.* 151: 21-27.

Dong, J.Z., Lei, C., Ai, X.R. and Wang, Y., 2012. Selenium enrichment on *Cordyceps militaris* link and analysis on its main active components. *Applied Biochemistry and Biotechnology.* 166: 1215-1224.

Elkhateeb, W.A., Daba, G.M., Thomas, P.W. and Wen, T.C. 2019. Medicinal mushrooms as a new source of natural therapeutic bioactive compounds. *Egypt. Pharm. J.* 18: 88-101.

Hur, H. 2008. Chemical Ingredients of *Cordyceps militaris. Mycobiology.* 36: 233-235.

Jang, S.H., Kim, S.H., Lee, H.Y., Jang, S.H., Jang, H., Chae, S.W., Jung, S.J., So, B.O., Ha, K.C., Sin, H.S. and Jang, Y.S. 2015. Immune-modulating activity of extract prepared from mycelial culture of Chinese caterpillar mushroom, *Ophiocordyceps sinensis* (Ascomycetes). *Int J Med Mushrooms*. 17(12): 1189-99.

Kang, C., Wen, T.C., Kang, J.C., Meng, Z.B., Li, G.R. and Hyde, K.D. 2014. Optimization of large scale culture conditions for the production of cordycepin with *Cordyceps militaris* by liquid static culture. *The Scientific World Journal*. 2014(1): 510627.

Kim, H.O. and Yun, J.W. 2005. A comparative study on the production of exopolysaccharides between two entomopathogenic fungi *Cordyceps militaris* and Cordyceps sinensis in submerged mycelial cultures. *Journal of Applied Microbiology*. 99(4): 728-738.

Ko, Y.F., Liau, J.C., Lee, C.S., Chiu, C.Y., Martel, J., Lin, C.S., Tseng, S.F., Ojcius, D.M., Lu, C.C., Lai, H.C. and Young, J.D. 2017. Isolation, culture and characterization of *Hirsutella sinensis* mycelium from caterpillar fungus fruiting body. *PLoS One.* 12(1): e0168734.

Kredich, N.M. and Guarino, A.J. 1961. Studies on the biosynthesis of cordycepin. *Biochimica et Biophysica Acta*. 47(3): 29-534.

Leung, P.H. and Wu, J.Y. 2007. Effects of ammonium feeding on the production of bioactive metabolites (cordycepin and exopolysaccharides) in mycelial culture of a *Cordyceps sinensis* fungus. *Journal of Applied Microbiology*.103(5): 1942-1949.

Li, R., Jiang, X.L. and Guan, H.S. 2010. Optimization of mycelium biomass and exopolysaccharides production by *Hirsutella* sp. in submerged fermentation and evaluation of exopolysaccharides antibacterial activity. *African Journal of Biotechnology.* 9(2).

Li, S., Yang, F. and Tsim, K.W. 2006. Quality control of Cordyceps sinensis, a valued traditional chinese medicine. *Journal of Pharmaceutical and Biomedical Analysis*. 41(5): 1571-1584.

Li, S., Li, P. and Ji, H. 2011. RP-HPLC determination of ergosterol in natural and cultured Cordyceps. *Chin. J. Mod. Appl. Pharm*. 1: 297-299.

Li, S.P., Li, P., Lai, C.M., Gong, Y.X., Kan, K.K., Dong, T.T., Tsim, K.W. and Wang, Y.T. 2004. Simultaneous determination of ergosterol, nucleosides and their bases from natural and cultured Cordyceps by pressurised liquid extraction and high-performance liquid chromatography. *Journal of Chromatography A.* 1036(2): 239-243.

Li, Y., Ban, L., Meng, S., Huang, L., Sun, N., Yang, H., Wang, Y. and Wang, L. 2021. Bioactivities of crude polysaccharide extracted from fermented soybean curd residue by *Cordyceps militaris*. *Biotechnology & Biotechnological Equipment*. 35(1): 334-345.

Lin, L. and Xu, J. 2020. Fungal pigments and their roles associated with human health. *Journal of Fungi*. 6(4): 280.

Lin, S., Liu, Z.Q., Xue, Y.P., Baker, P.J., Wu, H., Xu, F., Teng, Y., Brathwaite, M.E. and Zheng, Y.G. 2016. Biosynthetic pathway analysis for improving the cordycepin and cordycepic acid production in Hirsutella sinensis. *Applied Biochemistry and Biotechnology*. 179: 633-649.

Lin, Y.S., Yang, C.H., Lu, K., Huang, K.S. and Zheng, Y.Z. 2011. Synthesis of agar microparticles using temperature controlled microfluidic devices of Cordyceps militaris cultivation. *Electrophoresis*. 32(22): 3157-3163.

Liu, T., Liu, Z., Yao, X., Huang, Y., Qu, Q., Shi, X., Zhang, H. and Shi, X. 2018. Identification of cordycepin biosynthesis-related genes through de novo transcriptome assembly and analysis in *Cordyceps cicadae*. *Royal Society Open Science*. 5(12): 181247.

Liu, X., Zhou, B., Lin, R., Jia, L., Deng, P., Fan, K., Wang, G., Wang, L. and Zhang, J. 2010. Extraction and antioxidant activities of intracellular polysaccharide from *Pleurotus* sp. mycelium. *International Journal of Biological Macromolecules.* 47(2): 116-119.

Maoka, T. 2020. Carotenoids as natural functional pigments. *Journal of Natural Medicines*. 74(1): 1-16.

Masuda, M., Urabe, E., Honda, H., Sakurai, A. and Sakakibara, M. 2007. Enhanced production of cordycepin by surface culture using the medicinal mushroom *Cordyceps militaris*. *Enzyme and Microbial Technology*. 40(5): 1199-1205.

Nabi, F., Arain, M.A., Rajput, N., Alagawany, M., Soomro, J., Umer, M., Soomro, F., Wang, Z., Ye, R. and Liu, J. 2020. Health benefits of carotenoids and potential application in poultry industry: A review. *Journal of Animal Physiology and Animal Nutrition*. 104(6): 1809-1818.

Ng, T.B. and Wang, H.X. 2005. Pharmacological actions of Cordyceps, a prized folk medicine. *Journal of Pharmacy and Pharmacology.* 57(12): 1509-1519.

Sharma, S. 2004. Trade of Cordyceps sinensis from high altitudes of the Indian Himalaya: conservation and biotechnological priorities. *Current Science-Bangalore.* 86: 1614-1618.

Shih, L., Tsai, K.L. and Hsieh, C. 2007. Effects of culture conditions on the mycelial growth and bioactive

metabolite production in submerged culture of *Cordyceps militaris*. *Biochemical Engineering Journal*. 33(3): 193-201.

Tang, J., Qian, Z. and Wu, H. 2018. Enhancing cordycepin production in liquid static cultivation of Cordyceps militaris by adding vegetable oils as the secondary carbon source. *Bioresource Technology*. 268: 60-67.

Valverde, M.E., Hernández-Pérez, T. and Paredes-López, O. 2015. Edible mushrooms: improving human health and promoting quality life. *International Journal of Microbiology*. 2015(1): 376387.

Varshney, V.K., Pandey, A., Kumar, A., Rathod, D. and Kannojia, P. 2011. Chemical screening and identification of high cordycepin containing cultured isolate (s) of medicinal Chinese caterpillar mushroom, *Ophiocordyceps sinensis* (Berk.) GH Sung *et al*. *International Journal of Medicinal Mushrooms*. 13(4).

Venil, C.K., Velmurugan, P., Dufossé, L., Renuka Devi, P. and Veera Ravi, A. 2020. Fungal pigments: Potential coloring compounds for wide ranging applications in textile dyeing. *Journal of Fungi*. 6(2): 68.

Wang, J., Liu, Y.M., Cao, W., Yao, K.W., Liu, Z.Q. and Guo, J.Y. 2012. Anti-inflammation and antioxidant effect of Cordymin, a peptide purified from the medicinal mushroom *Cordyceps sinensis*, in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. *Metabolic Brain Disease*. 27: 159-165.

Wu, L., Sun, H., Hao, Y., Zheng, X., Song, Q., Dai, S. and Zhu, Z. 2020. Chemical structure and inhibition on á-glucosidase of the polysaccharides from *Cordyceps militaris* with different developmental stages. *International Journal of Biological Macromolecules*. 148: 722-736.

Xie, C.Y., Gu, Z.X., Fan, G.J., Gu, F.R., Han, Y.B. and Chen, Z.G. 2009. Production of cordycepin and mycelia by submerged fermentation of *Cordyceps militaris* in mixture natural culture. *Applied Biochemistry and Biotechnology*. 158: 483-492.

Yang, F.Q., Feng, K., Zhao, J. and Li, S.P. 2009. Analysis of sterols and fatty acids in natural and cultured Cordyceps by one-step derivatization followed with gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* 49: 1172-1178.

Yue-Qin, C., Ning, W., Hui, Z. and Liang-Hu, Q., 2002. Differentiation of medicinal *Cordyceps* species by rDNA ITS sequence analysis. *Planta Medica*. 68(07): 635-639.

Zhang, L., Dai, X., Xu, F., Wang, F., Gong, B. and Wei, Y., 2012. Preparation of imidazole-functionalized silica by surface-initiated atom transfer radical polymerization and its application for hydrophilic interaction chromatography. *Analytical and Bioanalytical Chemistry*. 404: 1477-1484.

Zhang, Y., Li, E., Wang, C., Li, Y. and Liu, X., 2012. *Ophiocordyceps sinensis*, the flagship fungus of China: terminology, life strategy and ecology. *Mycology*. 3(1): 2-10.

Zhang, Y., Zeng, Y., Cui, Y., Liu, H., Dong, C. and Sun, Y. 2020. Structural characterization, antioxidant and immunomodulatory activities of a neutral polysaccharide from *Cordyceps militaris* cultivated on hull-less barley. *Carbohydrate Polymers*. 235: 115969.

Zhou, Y., Wang, M., Zhang, H., Huang, Z. and Ma, J. 2019. Comparative study of the composition of cultivated, naturally grown *Cordyceps sinensis*, and stiff worms across different sampling years. *PLoS One*. 14(12): e0225750.

Zhu, J.S., Halpern, G.M. and Jones, K. 1998. The scientific rediscovery of a precious ancient Chinese herbal regimen: *Cordyceps sinensis*: Part II. *J. Altern. Complement. Med*. 4: 429-457.