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Original Article

Fluoride-Induced Hormesis Enhances Cordycepin Production and Upregulates the Transcriptional Expression of its Biosynthesis Genes in *Cordyceps militaris*

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Abstract

Cordyceps militaris is a medicinally valuable fungus known for producing cordycepin (3′-deoxyadenosine), a bioactive compound with anticancer, antioxidant, and immunomodulatory properties. However, its low natural yield limits large-scale commercial production. This study investigated the effects of fluoride-induced hormesis and growth supplements on cordycepin biosynthesis and the transcriptional regulation of key biosynthetic genes in *C. militaris* fruiting bodies. *C. militaris* strain 1164 was cultivated on rice-based substrate media supplemented with potassium fluoride (KF, 0.01–1 mM), glycine (500 mg/L), and ascorbic acid (100 mg/L). Cordycepin content was quantified using high-performance liquid chromatography (HPLC), while the expression of nine key genes (RNR, purA, purL, purB, PRPS, NT5E, purC, AMPD, and ADEK) was analyzed using quantitative real-time PCR (qRT-PCR). The results demonstrated that low-dose KF (0.01 mM) significantly enhanced cordycepin production, reaching 65.03 ± 0.73 mg/g dry weight (dwt.), which represents a 69.93% increase compared with the control (38.6 ± 0.15 mg/g dwt.). Glycine and ascorbic acid supplementation also increased cordycepin content to 48.16 ± 0.25 and 61.36 ± 0.41 mg/g dwt., respectively. Gene expression analysis revealed a dose-dependent response, with 0.1 mM KF strongly upregulating purA (6.92-fold), PRPS (4.75-fold), and RNR (3.76-fold). In contrast, 1 mM KF suppressed both gene expression and fungal growth. Glycine and ascorbic acid notably increased RNR expression by 2.5-fold and 2.3-fold, respectively, consistent with the observed enhancement in cordycepin production. This study is the first to demonstrate fluoride-induced hormetic regulation of cordycepin biosynthetic genes. The findings indicate that low-dose KF functions as a metabolic and transcriptional enhancer, providing a novel strategy for optimizing cordycepin production in *C. militaris* through targeted supplementation approaches.

Keywords: *Cordyceps militaris; Cordycepin; Fluoride; Transcriptional Expression*

Introduction

Cordyceps militaris, is a well-known entomopathogenic fungus that is highly valued in traditional medicine for its ability to biosynthesize cordycepin (3′-deoxyadenosine). Cordycepin is a bioactive compound with anticancer, immunomodulatory, antioxidant, and anti-inflammatory properties. Despite its significant pharmacological potential, the natural yield of cordycepin is relatively low, which poses a major limitation for large-scale production and commercial applications (Jędrejko *et al*., 2021; Hu *et al*., 2024; Zhang *et al*., 2020).

The production of *Cordyceps militaris* under controlled environmental conditions has been initiated to ensure stable and enhanced yields of its bioactive compounds. Previous studies have reported that cordycepin production in *C. militaris* can be enhanced through the addition of various growth

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supplements, including glycine, adenosine, inosine, thiamine, glutamine, and other supplements (Kaushik *et al*., 2020; Werapan *et al*., 2022; Wang *et al*., 2022; Kunhorm *et al*., 2022). These approaches also involve advanced technologies such as temperature-controlled cultivation and the use of biopolymers (Jeennor *et al*., 2023; Zhu *et al*., 2025), as well as optimization of artificial culture media—particularly carbon-to-nitrogen ratios and trace elements such as selenium—for cordycepin enhancement (Li *et al*., 2025; Borde & Singh, 2023; Zhao *et al*., 2023). Controlled cultivation of *C. militaris* in modified artificial media not only improves metabolite production but also contributes to the conservation of wild Cordyceps populations (Hou *et al*., 2024). Extensive studies have focused on optimizing culture conditions for the cultivation of *C. militaris* fruiting bodies on solid-state media (Shweta *et al*., 2023; Wang *et al*., 2022).

Low levels of fluoride, a trace element, are well known for their physiological and biochemical effects. At low doses, fluoride can slightly increase Reactive Oxygen Species (ROS) levels and activate antioxidant defense systems (e.g., superoxide dismutase and catalase), leading to the overexpression of stress-response genes such as Heat Shock Proteins (HSPs) and Superoxide Dismutase (SOD). Numerous studies have reported positive effects of fluoride-induced hormesis in Pinus (Doley, 1989), algae (Ismailhodjaev *et al*., 2022), animals (Kulcsár *et al*., 2024), silkworms (Chen, 2003), and Cordyceps (Li *et al*., 2021; Burgstahler, 2002). However, the role of fluoride in modulating gene expression associated with bioactive compound synthesis, particularly in *C. militaris*, remains largely unexplored. Although some studies have reported transcriptomic analyses of Cordyceps species cultivated on germinated soybeans (Yoo *et al*., 2022; Bondy, 2023) and enhancement of cordycepin content in *C. militaris* using corn steep liquor hydrolysate (Chang *et al*., 2024), very few studies have established a comprehensive correlation between the transcriptional expression profiles of genes involved in cordycepin biosynthesis in *C. militaris* using growth supplements and a stressor such as Potassium Fluoride (KF). In this context, the primary aim of the present study is to elucidate how fluoride-induced hormesis modulates the transcriptional machinery responsible for cordycepin biosynthesis in *C. militaris*. To the best of our knowledge, this is the first study to investigate the effects of fluoride-induced transcriptional regulation of cordycepin biosynthetic genes and its impact on cordycepin production in *C. militaris*.

Materials and Methods

C. militaris strain 1164 stock culture was obtained from ICAR–DMR, Solan, Himachal Pradesh. The master culture was propagated on Potato Dextrose Agar (PDA) slants prepared in 50 mL test tubes, following the method described by Li *et al*. (2021). The slants were incubated in the dark at 21–23 °C for 10 days and subsequently stored at 4^0C for the preservation of the mycelial culture. Spawn was prepared in a liquid medium containing potato dextrose broth (24 g/L), peptone (5 g/L), yeast extract (3 g/L), KH_2PO_4 (1 g/L), $MgSO_4$ (0.5 g/L), and vitamin B_1 (50 mg/L). The cultures were incubated in an incubator shaker at 120 rpm for 10–15 days at 22 °C. After sufficient mycelial multiplication, the cultures were inoculated into a rice-based substrate medium consisting of 25 g of rice supplemented with 50 mL of nutrient medium in 300 mL cylindrical glass bottles. The nutrient medium consisted of glucose (30 g/L), peptone (5 g/L), yeast extract (3 g/L), KH_2PO_4 (1 g/L), tri-ammonium citrate (1 g/L), $MgSO_4$ (0.5 g/L), vitamin B_1 (50 mg/L), and vitamin B_{12} (10 mg/L). Different concentrations of potassium fluoride (1 mM, 0.1 mM, and 0.01 mM), glycine (500 mg/L), and ascorbic acid (100 mg/L) were added to the rice substrate media. The inoculated substrates were incubated in the dark for 15 days to allow mycelial growth initiation. After the mycelium fully colonized the substrate surface in the culture bottles, the cultures were transferred to a cultivation chamber maintained at 21–23 °C with a 12 h light/12 h dark photoperiod to induce fruiting body formation. After 60 days, the fruiting bodies were harvested for subsequent analyses.

Quantification of Cordycepin

Cordycepin content in the dry biomass of *C. militaris* was analyzed using High-Performance Liquid Chromatography (HPLC), following the method described by Yu *et al*. (2006). The effects of different

growth supplement media on cordycepin enhancement were evaluated. For each treatment group, 1 g of dried fruiting body was extracted with 20 mL of 70% HPLC-grade methanol prepared in distilled water. The samples were sonicated for 1 hour at ambient temperature to ensure thorough mixing and extraction. The resulting extracts were centrifuged at 4,000 × g (5,000 rpm) for 30 minutes. The supernatant was carefully transferred to a new vial and subsequently filtered through a 0.22 µm syringe filter. The filtered extracts were stored at −20 °C until further HPLC analysis.

Cordycepin in *C. militaris* samples was quantified using a Waters HPLC system (USA) equipped with a 1525 binary pump, a Waters 2998 photodiode array detector, and an Avantor ACE Generix 5 C18(2) column (250 × 4.6 mm). The mobile phase consisted of methanol and ultrapure water (10:90, v/v) containing 10 mmol/L KH_2PO_4. The column temperature was maintained at 40 °C, and the flow rate was set at 1.0 mL/min for elution. Chromatograms were analyzed at an absorbance wavelength of 261 nm. A standard calibration curve was prepared using cordycepin standard solution (10 mg dissolved in 10 mL of 70% HPLC-grade methanol) and was used to evaluate cordycepin content in samples from the different treatment groups. Subsequently, cordycepin concentration was calculated from the HPLC chromatograms using the following formula:

$$\frac{Sample\ Area}{Standard\ Area} \times \frac{Standard\ weight}{Dilution\ of\ standard} \times \frac{Dilution\ of\ sample}{Sample\ weight} \times Standard\ Potency \times 100$$

Sample Area = Peak area of the sample from HPLC chromatogram.
Standard Area = Peak area of the standard solution.
Standard Weight = Weight of standard taken (mg).
Dilution of Standard = Dilution factor applied to standard solution.
Dilution of Sample = Dilution factor applied to sample solution.
Sample Weight = Weight of test sample (mg).
Standard Potency = Assay purity of the reference standard (%).

RNA Isolation

RNA isolation was performed using the RNeasy Plant Mini Kit (QIAGEN). Briefly, 100 mg of dried fruiting body was homogenized in liquid nitrogen, followed by the addition of 450 µL of cell lysis and RNase inactivation buffer. The lysate was vortexed vigorously and then transferred to a QIA shredder spin column, followed by centrifugation at 8,000 × g for 2 minutes. Half the volume of absolute ethanol was added to the lysate to facilitate RNA binding. The mixture was then transferred to a RNeasy Mini spin column and washed sequentially with 500–700 µL of wash buffers to remove proteins, polysaccharides, and other contaminants. After the final drying step, RNA was eluted in 50–100 µL of RNase-free water. RNA concentration and purity were assessed using a NanoDrop spectrophotometer by measuring absorbance ratios at 230/260 nm and 260/280 nm. The purified RNA was stored at −20 °C for further analysis.

Quantitative mRNA expression analysis of genes involved in cordycepin biosynthesis

The mRNA expression of cordycepin biosynthetic genes were analysed using dx/dt VEGA master mix (Rubizon Pvt. Ltd. Bangalore, India) for real time (2X) with SYBR green for qRT PCR analysis by adding 200ng purified RNA followed by 10µL VEGA Master mix (2X) and 0.8µl (100pmole) of each forward and reverse primer for 20µl PCR reaction each specific genes ; NT5E (5´- Nucleosidase), ADEK (Adenosine kinase), AMPD (AMP deaminase), RNR (Ribonucleoside-diphosphate reductase), purl (Phosphoribosyl-formyl-glycinamide synthase), pur-A (Adenyl succinate synthase), pur-B (Adenyl succinate lyase), pur-C (Phosphoribosyl-aminoimidazole-succinocarboxamide synthase), PRPS (Ribose-phosphate pyrophosphokinase). The primer sequence information of all the genes along with accession number is provided in Table 1 (Kaushik *et al*., 2020). RNA sample of fruiting bodies grown on each combination of medium was screened for mRNA expression using qRT-PCR (QIAquant96, Qiagen, Germany). The Ct (Threshold cycle) values of gene expression were normalized to the eukaryotic housekeeping gene, 18S ribosomal RNA, and values represented in the graph are the relative fold change of untreated sample as a control which was calculated using a standard delta-delta Ct method:

Relative fold change = 2ˆ(-ΔΔct)

Where ΔΔct = ΔΔct of treated – ΔΔct of Control

ΔΔct treated = Ct of target gene in treated group – Ct of 18srNA gene in treated group) and ΔΔct control = Ct of target gene in control group – Ct of 18srNA gene in control group (Xie *et al*.,2023).

Table 1: *Primer Sequences of the Genes Involved in Cordycepin Biosynthesis*

Gene Symbol	Accession no.	Forward Primer	Reverse Primer
NT5E	KP090958	GCTCGTGTTCCTGCCATAGT	GCTCTACTATCGCCCGAGTG
ADEK	KP090962	CCATTGGGCTCACGAGTCTT	CGTGACGAAAAGACAGTGCC
AMPD	KP090965	CCGTGAGCATGTCGAGGAC	GCAGCAAAACACCATCCGTC
RNR	KY435930	CAGTCCCAGTCGCTCAACAT	TCCTTCACATACGTGGCTCG
purl	KP090952	AGACCTTTGCGCTCGAGAAA	GGTACTGCATCTCGGACTCG
pur-A	KP090957	CCGTACCCGAGACTTGACAC	AGGCAGTTGGGATTGAACGAG
pur-B	KP090955	CCTTTCCAAGTTTGCCTGCC	CGGCCCTAACGTATTCGACA
pur-C	KP090954	ACATACATCCCGACGATGCC	TTGTCGAAGCTGTCCTGGTC
PRPS	KP090946	CACGACTACGAGAACCCCAG	TCGGGAATGTGCTTCACGAT
18sr RNA	MG642880	GACGCGTTCGGCACCTTA	TTCAGCCTTGCGACCATAC

Statistical Analysis

All the experiments were performed in triplicate, and the results were expressed as (MEAN ± SEM). The statistical significance was analysed using ANOVA to assess the difference between control and the different groups from GraphPad Prism 10 Software, USA.

Results

Impact of growth supplements and potassium fluoride on cordycepin production

Different categories of growth supplements such as Amino acids (Glycine, Glutamine and Aspartic acid), Nucleosides (Hypoxanthine, Adenosine, Adenine sulphate, Thymidine and Inosine) and Vitamins (Ascorbic acid, Thiamine, Pyridoxine and biotin) and stressor KF were used in rice substrate medium to enhance the growth of fruiting bodies of *C. militaris* (Figure 1a-f).

Figure 1: *Fruiting bodies of cordyceps militaris cultivated on rice substrate medium on control (a) supplemented with potassium fluoride 1mM(b), 0.1mM(c) and 0.01mM(d) supplemented with glycine 500mg/L(e) and supplemented with Ascorbic acid 100mg/L(f).*

As represented by RP-HPLC dendrograms in Figure 2 the cordycepin was enhanced in the fruiting body of *C. militaris* grown on medium supplemented with ascorbic acid (Vitamin), glycine (Amino acid) and KF as compared to normal medium. As shown in Figure 3 ascorbic acid depicted maximum amount of cordycepin production in fruiting bodies (61.36c.) followed by glycine (48.16±0.25mg/gm dwt) as compared to control (38.6±0.15mg/gm dwt). However, the Ascorbic acid showed an increase of 60.47±0.26 percent cordycepin production followed by glycine 25.81±0.1percent as compared to control (Figure 4).

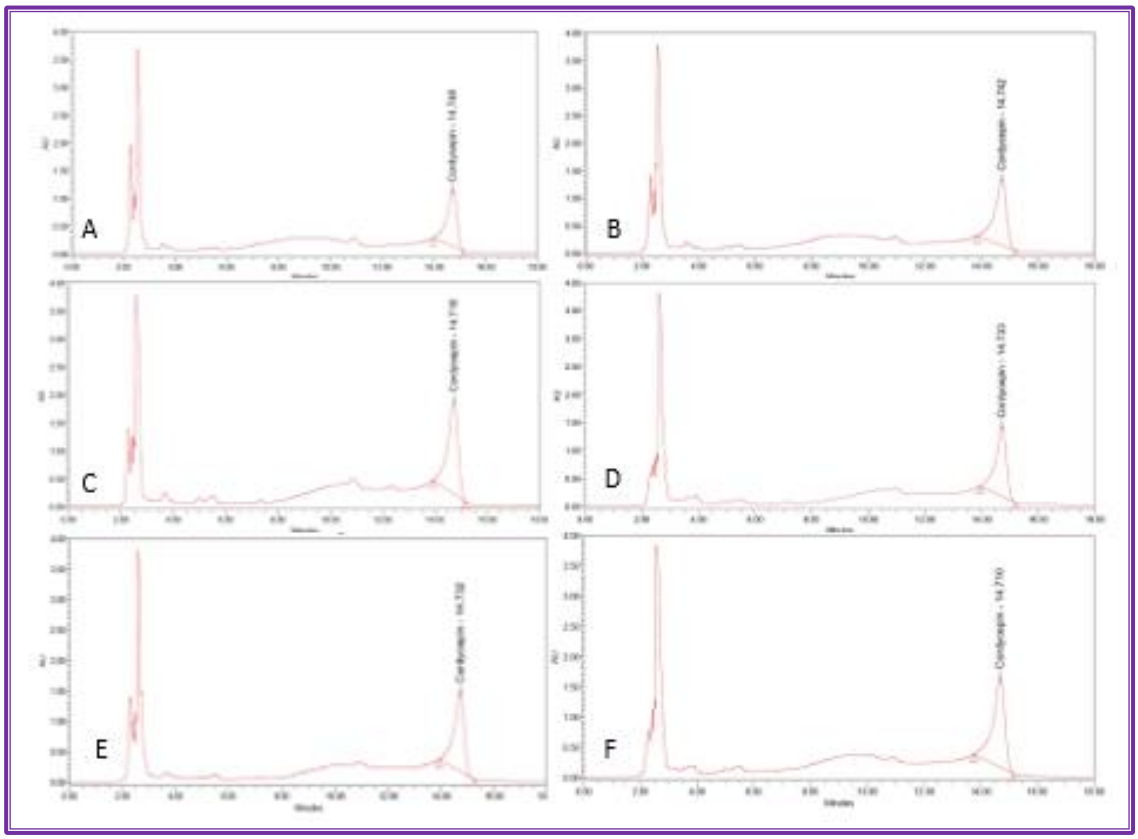

Figure 2: *HPLC chromatogram of cordycepin in fruiting bodies of C. militaris cultivated on basal rice medium without growth supplements showed in Control (A) HPLC chromatogram of cordycepin in fruiting bodies of C. militaris cultivated on basal rice medium supplemented with 1mM potassium fluoride showed in (B) HPLC chromatogram of cordycepin in fruiting bodies of C. militaris cultivated on basal rice medium supplemented with 0.1mM potassium fluoride showed in (C) HPLC chromatogram of cordycepin in fruiting bodies of C. militaris cultivated on basal rice medium supplemented with 0.01mM potassium fluoride showed in (D) HPLC chromatogram of cordycepin in fruiting bodies of C. militaris cultivated on basal rice medium supplemented with 500mg/L Glycine showed in (E)) HPLC chromatogram of cordycepin in fruiting bodies of C. militaris cultivated on basal rice medium supplemented with 100mg/L Ascorbic Acid showed in (F).*

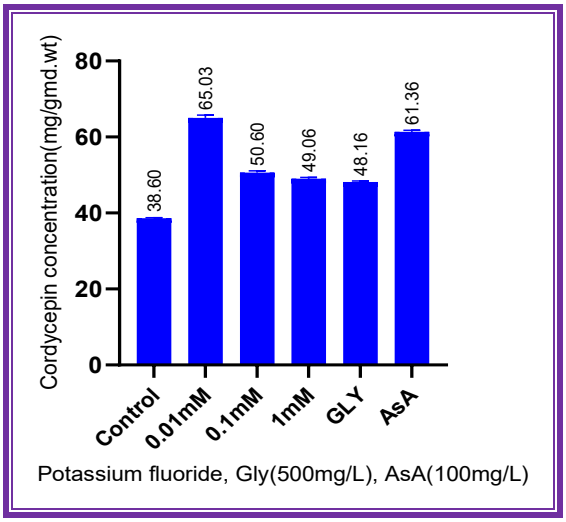

Figure 3: *Effect of growth supplements (Potassium fluoride, Glycine (Gly) and Ascorbic acid (AsA) on cordycepin production in in fruiting bodies of C militaris. Cordycepin production was found to be significantly higher (65.03mg/g dwt.) in 0.01mM potassium fluoride as compared to Gly (48.16mg/gdwt) and AsA (61.36mg/gdwt.) as compared to control. Data was presented as the mean _ SEM of 3 independent experiments. The significance level was accepted at p<0.05 as compared to control*

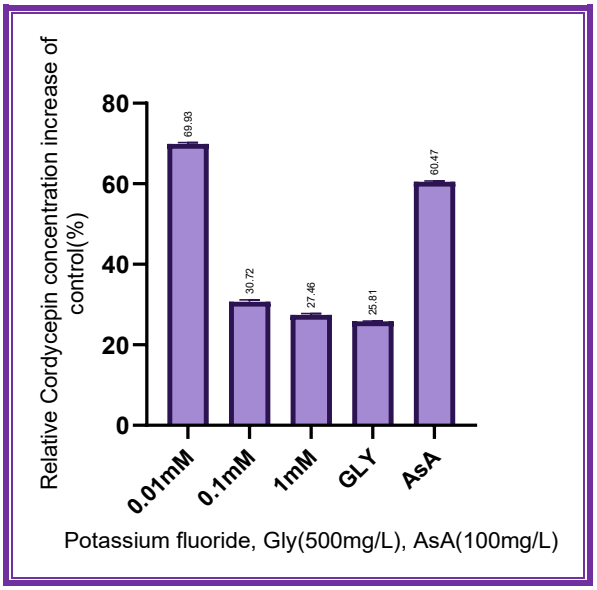

Figure 4: *The graph representing the percentage increase of cordycepin as compared to control, Gly and AsA in C. militaris fruiting bodies. We found that a significant increase in the cordycepin concentration up to 69.93% in the medium supplemented with 0.01 mM potassium fluoride as compare to control, Gly and AsA*

Potassium fluoride (KF) was added as a fluoride source to the culture media. We successfully obtained fruiting bodies in normal as well as fluoride supplemented medium (0.01mM, 0.1mM and 1mM). The cordycepin concentration was significantly increased to 65.03±0.73 mg/g dwt. in the medium supplemented with KF 0.01mM (Figure 3). A reduction in cordycepin was observed in 0.1mM (56.6±0.52mg/g dwt.)) and 1.0mM (49.06±0.3mg/g dwt.) We observed an increase of 69.93±015 percent cordycepin production in 1gm dry weight of fruiting body on medium supplemented with KF 0.01mM as compared to control (Figure 4).

Impact of Modified media on Transcriptional Regulation of Cordycepin Pathway Genes:

In the present study, an amino acid (glycine), a vitamin (ascorbic acid), and a low dose of potassium fluoride (KF) were evaluated as supplements in the culture medium for their potential to induce transcriptional activation of cordycepin biosynthetic genes in the fruiting bodies of *C. militaris*. Gene expression profiles associated with cordycepin biosynthesis were analyzed using glycine and ascorbic acid as growth supplements and KF as a stressor, each applied individually in the cultivation medium. Quantitative real-time PCR (qRT-PCR) analysis showed that glycine supplementation upregulated the expression of RNR (2.5-fold), NT5E (0.04-fold), purA (1.5-fold), and ADEK (0.01-fold) genes. Ascorbic acid treatment resulted in increased expression of RNR (2.25-fold), NT5E (0.05-fold), purA (0.95-fold), and ADEK (0.01-fold) compared with the control (Figure 5).

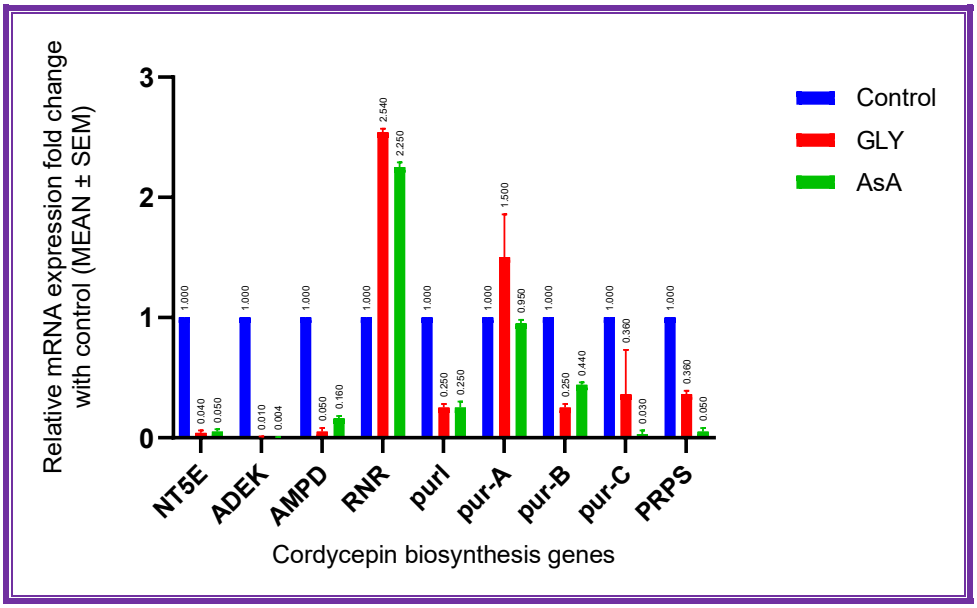

Figure 5: *Glycine and ascorbic acid relative fold change analysis with control*

Relative transcriptional levels of nine key cordycepin biosynthetic genes were also analysed in the fruiting body samples supplemented with 0.01mM,0.1mM and 1.0mM KF. Significant upregulation was observed for RNR (5.26-fold), purA (1.52-fold), purL (1.46-fold), and purB (1and .05-fold) Moderate increases were also noted for PRPS (0.95-fold), NT5E (0.83-fold), purC (0.64-fold), AMPD (0.43-fold), and ADEK (0.30-fold) KF 0.01mM. Further enhancement in gene expression was recorded at 0.1 mM KF, where purA exhibited a dramatic increase (6.92-fold), followed by PRPS (4.75-fold), RNR (3.76-fold), purB (2.48-fold), and purC (2.25-fold). Upregulation was also seen in AMPD (1.34-fold), purL (1.25-fold), NT5E (0.94-fold), and ADEK (0.50-fold). However, at 1 mM KF, a general decline in transcriptional activation was observed, although some genes still showed moderate upregulation. The expression of purA (3.83-fold) and purL (3.15-fold) remained elevated, whereas purB (1.49-fold), RNR (1.45-fold), NT5E (0.63-fold), purC (0.55-fold), ADEK (0.13-fold), AMPD (0.12-fold), and PRPS (0.06-fold) showed reduced fold changes at 1.0mM KF compared to lower concentrations (Figure 6).

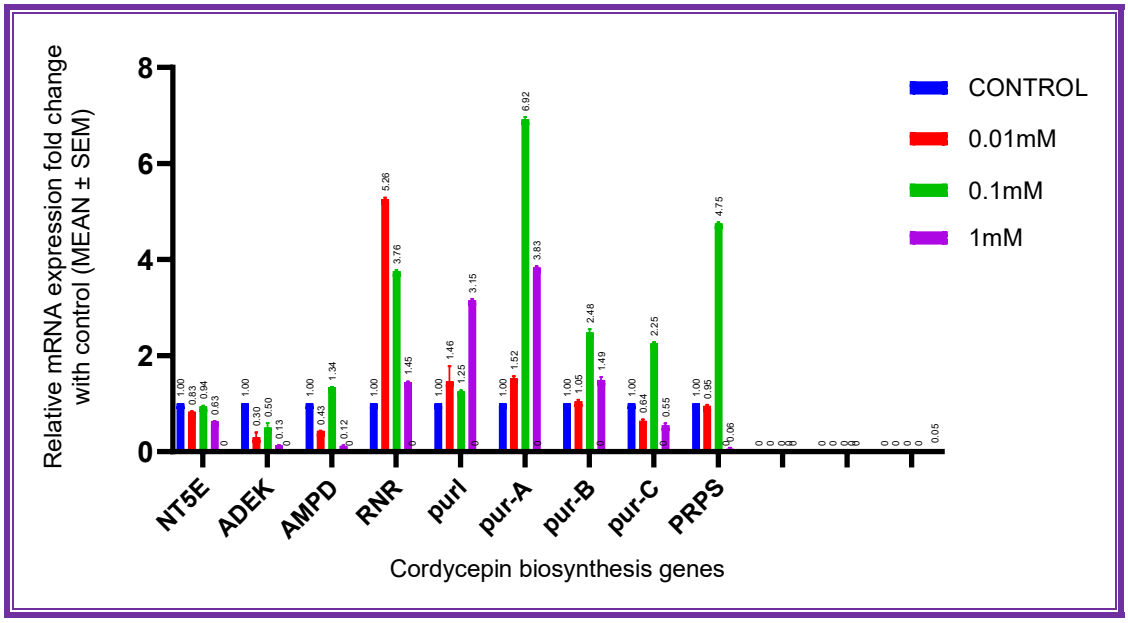

Figure 6: *Potassium fluoride relative fold change analysis with control*

For further evaluation, a heatmap was generated depicting the differential regulation of genes playing role in cordycepin biosynthesis upon the addition of different growth supplements and KF(Figure 7).

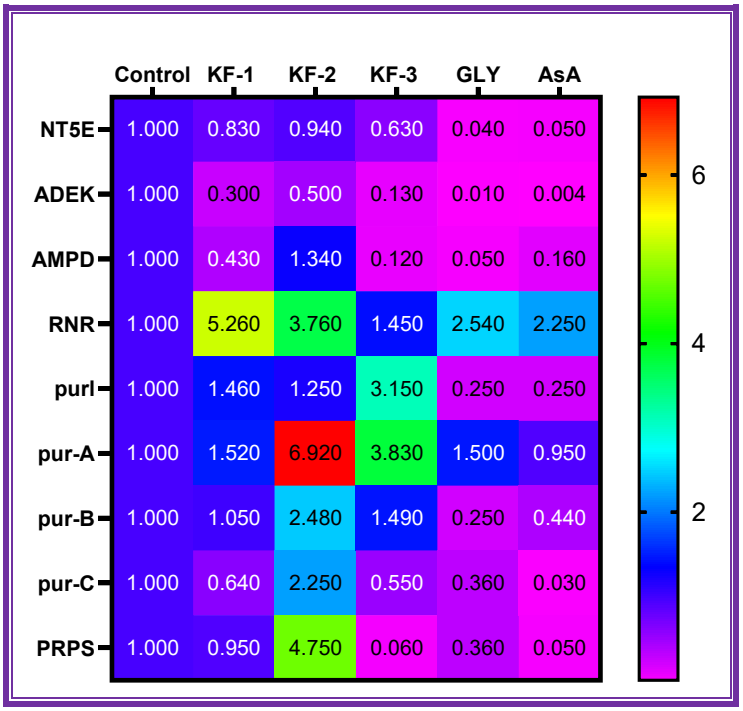

Figure 7: *Heatmap representing the differential expression of genes involved in cordycepin biosynthesis: Growth supplements Glycine (GLY) and ascorbic acid (AsA) and Potassium fluoride (KF-1 0.01mM), (KF-2 0.1mM), (KF-3 1mM) were tested for inducing upregulation of genes involved in cordycepin biosynthesis but KF-1 was observed to best for enhancement of expression of cordycepin biosynthesis.*

Discussion

Our RP-HPLC results demonstrate a classic hormetic, dose-dependent response to potassium fluoride (KF), in which a low concentration (0.01 mM) markedly enhances cordycepin production, whereas a higher dose (1 mM) attenuates this effect. This pattern of low-dose stimulation coupled with high-dose inhibition is a well-documented characteristic of hormesis. In fungi and plants, subtoxic levels of fluoride can transiently stimulate growth and secondary metabolite production, while higher concentrations inhibit key metabolic enzymes and impose physiological stress (Kaewkod *et al*., 2024; Rajak *et al*., 2023). Specifically in *C. militaris*, Li *et al*. (2021) demonstrated that low fluoride levels enhance fruiting body development and bioactive metabolite content, which closely aligns with our observed effects at 0.01 mM KF.

The qRT-PCR expression profiles support a model in which low-dose Potassium Fluoride (KF) reprograms purine metabolic flux and the terminal branch of cordycepin biosynthesis. At 0.01 mM KF, strong upregulation of RNR (5.26-fold) coincided with moderate increases in purA, purL, and purB expression (Yang *et al*., 2025; Wang *et al*., 2022). Elevated RNR expression therefore provides a direct route for increasing cordycepin production. Consistently, overexpression of the small RNR subunit has been shown to enhance cordycepin production in Cordyceps/Ophiocordyceps systems (Zhang *et al*., 2020).

At 0.1 mM potassium fluoride (KF), we observed coordinated upregulation of purA (6.92-fold), PRPS (4.75-fold), RNR (3.76-fold), purB (2.48-fold), and purC (2.25-fold), along with smaller increases in AMPD and purL. This transcriptional profile suggests two coupled processes: (i) PRPS-driven elevation of phosphoribosyl pyrophosphate (PRPP) to support de novo purine synthesis (Wang *et al*., 2022), and (ii) reinforcement of the IMP→AMP node (purA, purB, purC, and purL), which supplies AMP substrate to the RNR-dependent terminal pathway (Yang *et al*., 2025; Wang *et al*., 2022; Peng *et al*., 2024).

Although the cns1–cns4 genes were not quantified in this experiment, contemporary models indicate

that Cns1 and Cns2 form an obligate complex that governs cordycepin biosynthesis in parallel with pentostatin (PTN) formation, while Cns3 and Cns4 modulate related biosynthetic steps and self-protection mechanisms (Peng *et al*., 2024; Zeng *et al*., 2024; Yang *et al*., 2025). Thus, the RNR-centered transcriptional surge observed under low-dose KF likely operates in concert with, rather than independently of, the cns gene machinery to enhance 3′-deoxyadenosine production.

By contrast, at 1 mM potassium fluoride (KF), transcriptional levels were reduced across most gene loci (PRPS, 0.06-fold; AMPD, 0.12-fold; ADEK, 0.13-fold; purC, 0.55-fold; RNR, 1.45-fold), despite purA and purL remaining above 3-fold. This partial collapse of the transcriptional network is consistent with the inhibitory effects of fluoride at higher doses on central metabolic enzymes, cellular energy homeostasis, and redox balance, which can uncouple upstream nucleotide anabolism from the terminal deoxygenation–dephosphorylation pathway (Jarosz & Pitura, 2021). The sustained upregulation of purA and purL likely reflects a stress-responsive attempt to maintain IMP and AMP pools. However, with PRPS and AMPD suppressed and NT5E and ADEK remaining muted, the system may become substrate-limited and less capable of efficiently channeling AMP to 3′-dADP and ultimately to cordycepin.

Our findings support a bidirectional mechanism of fluoride-induced hormesis in cordycepin biosynthesis: (1) low-dose potassium fluoride (KF) transiently enhances purine biogenesis through activation of PRPS and purA/purB/purC/purL, and (2) high-dose KF imposes metabolic and redox stress that downregulates PRPS, AMPD, NT5E, and ADEK, thereby suppressing cordycepin accumulation. This interpretation aligns with broader evidence that modifications in culture media can reprogram transcriptional networks and metabolite flux to favor cordycepin biosynthesis under specific conditions (In-on *et al*., 2022; Wang *et al*., 2023; Lusakunwiwat *et al*., 2024; Buradam *et al*., 2024). Data from the present investigation further confirm that low-dose KF supplementation (0.01–0.1 mM) supports gene upregulation accompanied by increased cordycepin synthesis without inducing toxicity (Wang *et al*., 2023; Li *et al*., 2024). The present study further suggests that maintaining high RNR expression, preserving PRPS and AMPD activity, and stabilizing cns1–cns2 function may yield additional improvements in cordycepin production (Kaushik *et al*., 2020; Peng *et al*., 2024; Yang *et al*., 2025).

Limitations and Future Scope

The present study provides novel insights into the hormetic role of fluoride in enhancing cordycepin production and the transcriptional upregulation of cordycepin biosynthetic genes in *Cordyceps militaris*. However, protein abundance, enzyme activity, and post-transcriptional regulation were not evaluated, leaving functional confirmation incomplete. Large-scale industrial trials are required to establish reproducibility, scalability, and cost-effectiveness. In addition, large-scale cultivation trials using bioreactors or substrate-based systems will be necessary to confirm industrial feasibility and yield stability.

Future research should address these gaps by integrating multi-omics approaches, including proteomics and metabolomics, to validate functional enzyme activities and metabolic fluxes associated with cordycepin biosynthesis. Further exploration of signaling pathways and regulatory networks underlying fluoride-induced hormesis would help elucidate its molecular basis. Additionally, integrating genetic and metabolic engineering tools, such as CRISPR–Cas systems, could provide sustainable strategies to stabilize and maximize cordycepin production, thereby expanding the industrial and therapeutic potential of *C. militaris*.

Conclusion

This study demonstrates that low-dose potassium fluoride (0.01 mM) supplementation in the culture medium significantly enhances cordycepin production in *Cordyceps militaris* fruiting bodies. In contrast, higher concentrations (0.1 mM and 1 mM) negatively affected both fungal growth and cordycepin content. This is the first report to highlight the potential of low-dose fluoride as a metabolic enhancer in *C. militaris*. The study further revealed a dose-dependent transcriptional response, with peak

expression of key biosynthetic genes, including purA, RNR, and PRPS, observed at 0.01 mM KF. Conversely, higher fluoride levels (1 mM) resulted in reduced gene expression, indicating a hormetic effect. Collectively, these findings suggest that low-dose KF functions as a transcriptional enhancer of cordycepin biosynthesis, whereas excessive fluoride suppresses this pathway.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

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References

Bondy, S. C. (2023). The hormesis concept: strengths and shortcomings. *Biomolecules*, *13*(10), 1512. https://doi.org/10.3390/biom13101512

Borde, M., & Singh, S. K. (2023). Enhanced production of cordycepin under solid-state fermentation of Cordyceps militaris by using combinations of grains/substrates. *Brazilian Journal of Microbiology*, *54*(4), 2765-2772. https://doi.org/10.1007/s42770-023-01169-x

Buradam, P., Thananusak, R., Koffas, M., Chumnanpuen, P., & Vongsangnak, W. (2024). Expanded Gene Regulatory Network Reveals Potential Light-Responsive Transcription Factors and Target Genes in Cordyceps militaris. *International Journal of Molecular Sciences*, *25*(19), 10516. https://doi.org/10.3390/ijms251910516

Burgstahler, A. W. (2002). Paradoxical dose-response effects of fluoride. *Fluoride*, *35*(143), e147. https://hero.epa.gov/reference/2054892/

Chang, Y., Liu, X., Jiao, Y., & Zheng, X. (2024). Improved cordycepin production by Cordyceps militaris using corn steep liquor hydrolysate as an alternative protein nitrogen source. *Foods*, *13*(5), 813. https://doi.org/10.3390/foods13050813

Chen, Y. (2003). Differences in fluoride effects on fecundity among varieties of the silkworm Bombyx mori. *Fluoride*, *36*(3), 163-169. https://www.fluorideresearch.org/363/files/FJ2003_v36_n3_p163-169.pdf

Doley, D. (1989). Fluoride-induced enhancement and inhibition of shoot growth in four taxa of Pinus. *New Phytologist*, *112*(4), 543-552. https://doi.org/10.1111/j.1469-8137.1989.tb00349.x

Hou, S. J., Cheng, K. C., Lin, S. H., Hsiao, I. L., Santoso, S. P., Singajaya, S., ... & Lin, S. P. (2024). Improvement of extracellular polysaccharides production from Cordyceps militaris immobilized alginate beads in repeated-batch fermentation. *Lwt*, *193*, 115752. https://doi.org/10.1016/j.lwt.2024.115752

Hu, Y., Wu, Y., Song, J., Ma, M., Xiao, Y., & Zeng, B. (2024). Advancing Cordyceps militaris Industry: Gene Manipulation and Sustainable Biotechnological Strategies. *Bioengineering*, *11*(8), 783. https://doi.org/10.3390/bioengineering11080783

In-On, A., Thananusak, R., Ruengjitchatchawalya, M., Vongsangnak, W., & Laomettachit, T. (2022). Construction of light-responsive gene regulatory network for growth, development and secondary metabolite production in Cordyceps militaris. *Biology*, *11*(1), 71. https://doi.org/10.3390/biology11010071

Ismailhodjaev, B., Mirzaqobulov, J., Kholmirzaeva, B., Kuatbekova, K., Eskaraev, N., & Abduraimova, N. (2022, June). Comparative study of the activity and localization of carbonic anhydrase in some microalgae and their dependence on the concentration of carbon dioxide and nitrogen supply. In *AIP Conference Proceedings* (Vol. 2432, No. 1, p. 050066). AIP Publishing LLC. https://doi.org/10.1063/12.0015245

Jarosz, Z., & Pitura, K. (2021). Fluoride toxicity limit—can the element exert a positive effect on plants?. *Sustainability*, *13*(21), 12065. https://doi.org/10.3390/su132112065

Jędrejko, K. J., Lazur, J., & Muszyńska, B. (2021). Cordyceps militaris: An overview of its chemical constituents in relation to biological activity. *Foods*, *10*(11), 2634. https://doi.org/10.3390/foods10112634

Jeennor, S., Anantayanon, J., Panchanawaporn, S., Chutrakul, C., Vongsangnak, W., & Laoteng, K. (2023). Efficient de novo production of bioactive cordycepin by Aspergillus oryzae using a food-grade expression platform. *Microbial Cell Factories*, *22*(1), 253. https://doi.org/10.1186/s12934-023-02261-5

Kaewkod, T., Ngamsaoad, P., Mayer, K. O., Cheepchirasuk, N., Promputtha, I., & Tragoolpua, Y. (2024). Antioxidant, Antibacteria, and Anti-Inflammatory Effects of Cordyceps militaris Extracts and Their Bioactive Compounds. *Journal of Food Biochemistry*, *2024*(1), 1862818. https://doi.org/10.1155/jfbc/1862818

Kaushik, V., Singh, A., Arya, A., Sindhu, S. C., Sindhu, A., & Singh, A. (2020). Enhanced production of cordycepin in Ophiocordyceps sinensis using growth supplements under submerged conditions. *Biotechnology Reports*, *28*, e00557. https://doi.org/10.1016/j.btre.2020.e00557

Kulcsár, S., Turbók, J., Kövér, G., Balogh, K., Zándoki, E., Ali, O., ... & Mézes, M. (2024). Exposure to a combination of fusarium mycotoxins leads to lipid peroxidation and influences antioxidant defenses, fatty acid composition of phospholipids, and renal histology in laying hens. *Toxins*, *16*(5), 226. https://doi.org/10.3390/toxins16050226

Kunhorm, P., Chueaphromsri, P., Chaicharoenaudomrung, N., & Noisa, P. (2022). Enhancement of cordycepin production from Cordyceps militaris culture by epigenetic modification. *Biotechnology letters*, *44*(4), 581-593. https://doi.org/10.1007/s10529-022-03241-2

Li, W., Zhao, B., Liu, X., He, Z., Xie, L., & Qian, Z. (2025). Purification, structural characterization, and in vitro immunomodulatory activity of a low-molecular-weight polysaccharide from cultivated Chinese cordyceps. *International Journal of Biological Macromolecules*, *301*, 140394. https://doi.org/10.1016/j.ijbiomac.2025.140394

Li, X., Jiang, R., Wang, S., Li, C., Xu, Y., Li, S., ... & Wang, L. (2024). Prospects for cordycepin biosynthesis in microbial cell factories. *Frontiers in Chemical Engineering*, *6*, 1446454. https://doi.org/10.3389/fceng.2024.1446454

Li, X., Wang, J., Zhang, H., Xiao, L., Lei, Z., Kaul, S. C., ... & Zhang, Z. (2021). Low dose of fluoride in the culture medium of Cordyceps militaris promotes its growth and enhances bioactives with antioxidant and anticancer properties. *Journal of fungi*, *7*(5), 342. https://doi.org/10.3390/jof7050342

Lusakunwiwat, P., Thananusak, R., Nopgason, R., Laoteng, K., & Vongsangnak, W. (2024). Holistic transcriptional responses of Cordyceps militaris to different culture temperatures. *Gene*, *923*, 148574. https://doi.org/10.1016/j.gene.2024.148574

Peng, T., Guo, J., & Tong, X. (2024). Advances in biosynthesis and metabolic engineering strategies of cordycepin. *Frontiers in Microbiology*, *15*, 1386855. https://doi.org/10.3389/fmicb.2024.1386855

Rajak, P., Roy, S., Khatun, S., Mandi, M., Ganguly, A., Das, K., ... & Biswas, G. (2023). Fluoride contamination, toxicity and its potential therapeutic agents. *Toxicology International,* *29*(4), 553-565. https://doi:10.18311/ti/2022/v29i4/30844

Shweta., Abdullah, S., Komal., & Kumar, A. (2023). A brief review on the medicinal uses of Cordyceps militaris. *Pharmacological Research - Modern Chinese Medicine*, *7*, 100228. https://doi.org/10.1016/j.prmcm.2023.100228

Wang, L., Yan, H., Zeng, B., & Hu, Z. (2022). Research progress on cordycepin synthesis and methods for enhancement of cordycepin production in Cordyceps militaris. *Bioengineering*, *9*(2), 69. https://doi.org/10.3390/bioengineering9020069

Wang, X., Li, Y., Li, X., Sun, L., Feng, Y., Sa, F., ... & Cheng, X. (2023). Transcriptome and metabolome profiling unveils the mechanisms of naphthalene acetic acid in promoting cordycepin synthesis in Cordyceps militaris. *Frontiers in Nutrition*, *10*, 1104446. https://doi.org/10.3389/fnut.2023.1104446

Werapan, B., Nutaratat, P., Ariyaphuttarat, S., & Prathumpai, W. (2022). Cordycepin production by the potential fungal strains Cordyceps militaris BCC 2819 and Cordyceps cicadae BCC 19788 in submerged culture during batch and fed-batch fermentation. *African Journal of Biotechnology*, *21*(10), 483-503. https://doi.org/10.5897/AJB2022.17514

Xie, J., Wang, J., Wang, X., Chen, M., Yao, B., Dong, Y., ... & Wu, Y. (2023). An engineered dermal substitute with mesenchymal stem cells enhances cutaneous wound healing. *Tissue Engineering Part A*, *29*(17-18), 491-505. https://doi.org/10.1089/ten.tea.2023.0071

Yang, Y., Huang, J., Dong, G., & Hu, X. (2025). Telomere-to-Telomere Assembly of the Cordyceps militaris CH1 Genome and Integrated Transcriptomic and Metabolomic Analyses Provide New Insights into Cordycepin Biosynthesis Under Light Stress. *Journal of Fungi*, *11*(6), 461. https://doi.org/10.3390/jof11060461

Yoo, C. H., Sadat, M. A., Kim, W., Park, T. S., Park, D. K., & Choi, J. (2022). Comprehensive transcriptomic analysis of Cordyceps militaris cultivated on germinated soybeans. *Mycobiology*, *50*(1), 1-11. https://doi.org/10.1080/12298093.2022.2035906

Yu, L., Zhao, J., Li, S. P., Fan, H., Hong, M., Wang, Y. T., & Zhu, Q. (2006). Quality evaluation of Cordyceps through simultaneous determination of eleven nucleosides and bases by RP-HPLC. *Journal of Separation science*, *29*(7), 953-958. https://doi.org/10.1002/jssc.200600007

Zeng, J., Zhou, Y., Lyu, M., Huang, X., Xie, M., Huang, M., ... & Wei, T. (2024). Cordyceps militaris: A novel mushroom platform for metabolic engineering. *Biotechnology Advances*, *74*, 108396. https://doi.org/10.1016/j.biotechadv.2024.108396

Zhang, F. L., Yang, X. F., Wang, D., Lei, S. R., Guo, L. A., Liu, W. J., & Song, J. (2020). A simple and effective method to discern the true commercial Chinese cordyceps from counterfeits. *Scientific Reports*, *10*(1), 2974. https://doi:10.1038/s41598-020-59900-9

Zhang, H., Yu-Xian, Wang, Y. X., Tong, X. X. Yokoyama, W., Cao, J., Wang, F., Peng, C., & Guo, J. L. (2020). Overexpression of ribonucleotide reductase small subunit, RNRM, increases cordycepin biosynthesis in transformed Cordyceps militaris. *Chinese Journal of Natural Medicines*, *18*(5), 393-400. https://doi.org/10.1016/S1875-5364(20)30046-7

Zhao, B., Ding, H., Hu, T., & Guo, Y. (2023). Synergistic effects of the Se and Zn supplemental combination on the nutrient improvement of mannitol and adenosine and the multi-element bioaccessibility in Cordyceps cicadae. *LWT*, *173*, 114354. https://doi.org/10.1016/j.lwt.2022.114354

Zhu, Z., Xiang, Y., Yan, X., Shi, C., Chen, C., Liu, H., ... & Wu, D. (2025). Stable cross-linked polyacrylamide stationary phase based on atom transfer radical polymerization for hydrophilic interaction liquid chromatography. *Microchemical Journal*, 213, 113818. https://doi.org/10.1016/j.microc.2025.113818