**Antifungal Efficacy of *Cordyceps militaris*-Mycometabolites against major fungal diseases of *Withania somnifera***

Harshita Gaurav1, Divyanshu Yadav1, Rakesh Pandey2, Pradeep Kumar1, Amritesh Chandra Shukla1\*

1Department of Botany, University of Lucknow, Lucknow 226007, India.

2Department of Microbial Technology and Nematology, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, India.

[gauravh484@gmail.com](mailto:gauravh484@gmail.com) (HG); [divyanshudipps@gmail.com](mailto:divyanshudipps@gmail.com) (DY); [lubiocontrollab@gmail.com](mailto:lubiocontrollab@gmail.com) (RP); [lumushroomlab@gmail.com](mailto:lumushroomlab@gmail.com) (PK) and 1\*Correspondence: [amriteshcshukla@gmail.com](mailto:amriteshcshukla@gmail.com) (ACS)

**Abstract**

*Withania somnifera* (Ashwagandha), a vital medicinal plant, faces significant losses due to fungal diseases such as root rot, wilt, and leaf spot caused by *Fusarium annulatum* and *Alternaria alstroemeriae.* To manage these pathogens, metabolites of *Cordyceps militaris* were extracted following methods from Vinale et al. (2006) and others, with modifications. These metabolites were tested for antifungal efficacy using the poison food technique. Results showed the minimum inhibitory concentrations (MIC) against *F. annulatum* and *A. alstroemeriae* were 15 mg/mL and 20 mg/mL, respectively, with cidal effects observed at 20 mg/mL and 30 mg/mL. In-silico investigations revealed that Cordycepin, a metabolite, exhibited strong binding affinity to the fungal chitin synthetase protein. These findings suggest that *C. militaris* metabolites could serve as a potential alternative to synthetic fungicides, pending further research.

**Keywords**: *Withania somnifera, Cordyceps militaris*, metabolites*,* Antifungal activity, *in-vitro* assay*,* in-silico investigations

**List of Abbreviation:**

*C. militaris*: *Cordyceps militaris*

CSIR: Council of Scientific and Industrial Research

ICAR: Indian Council of Agricultural Research

DMR: Directorate of Mushroom Research

PDA: Potato Dextrose Agar

DMSO: Dimethyl sulfoxide

MGI%: Mycelial Growth Inhibition

MIC: Minimum Inhibitory Concentration

MCC: Minimum Cidal Concentration

BOD: Biological Oxygen Demand

PDB: Protein Data Bank

1. **Introduction**

*Withania somnifera* L. Dunal, commonly known as Ashwagandha, is a prominent medicinal plant belonging to the family Solanaceae. It holds a significant place in traditional medicine, particularly in Ayurveda, where it is revered for its adaptogenic properties, stress-relief potential, and ability to enhance overall vitality. Ashwagandha is often referred to as the "Indian ginseng" due to its wide range of therapeutic applications, including boosting immunity, improving cognitive function, and reducing inflammation. The roots of Ashwagandha are primarily utilized for medicinal purposes, although other parts of the plant also possess therapeutic properties (Paul et al. 2021; Ahmad and Dar 2017).

India stands as the largest producer of Ashwagandha globally, contributing approximately 93% of the total production. This dominance in Ashwagandha production is largely due to India's favorable climatic conditions and the deep-rooted cultural significance of the plant in traditional medicine. The remaining 7% of global production is shared by countries such as the United States (2%), European Union, China, Sri Lanka, and others. The high demand for Ashwagandha in both domestic and international markets underscore its economic importance, making it a vital cash crop for farmers in India (Khabiya et al. 2023).

Despite its significant medicinal and economic value, the cultivation of Ashwagandha is often plagued by various biotic stress factors, among which fungal diseases are particularly detrimental. Among these diseases, leaf spot, root rot and wilt diseases are primarily caused by the fungus *Alternaria* and *Fusarium* sp., poses a serious threat to the yield and quality of Ashwagandha crops. Leaf spot disease is characterized by the appearance of dark, necrotic lesions on the leaves, which can coalesce to form large patches, leading to premature defoliation. This not only reduces the photosynthetic capability of the plant but also directly impacts the overall biomass and root yield, resulting in substantial quantitative and qualitative losses (Mishra 2021; Meena et al. 2019; Pati et al. 2008) while Root rot and wilt diseases are characterized as the fungal disease which affects the roots of ashwagandha plants. It typically arises due to overwatering or poorly drained soil. The symptoms involve leaves turning yellow, plants wilting, and reduced growth (Bharti et al. 2012; Jetawat and Mathur 2016; Jetawat et al. 2016).

The management of fungal disease in Ashwagandha has traditionally relied on the application of synthetic fungicides. Although these chemical agents are effective in managing the disease, their prolonged and uncontrolled use has resulted in several negative consequences, such as the emergence of fungicide-resistant pathogen strains, environmental contamination, and health hazards for humans and animals. These challenges have necessitated the exploration of alternative, eco-friendly approaches to disease management that are both effective and sustainable (Mishra 2021; Chauhan and Ravi 2020; Rawal et al. 2014).

In recent years, the use of biocontrol agents, particularly those derived from fungi, has gained considerable attention as a viable alternative to synthetic fungicides. One such biocontrol agent that has shown promise in the management of plant diseases is *C. militaris*, an entomopathogenic fungus known for its production of bioactive secondary metabolites (Ashraf et al. 2020; Chen et al. 2020). *C. militaris* has been extensively studied for its medicinal properties, including immunomodulatory, antitumor, and antimicrobial activities (Qu et al. 2020; Park et al. 2009). However, its potential as a biocontrol agent in agriculture is only beginning to be explored.

Moreover, In the current investigation *C.* *militaris* was used for the extraction of metabolites(s). Different researchers have proved that secondary metabolites have good antifungal activity under In-vitro analysis.

Thus, the present study aims to determine the in vitro and in silico antifungal activity of metabolites of *C.* *militaris* against the major deteriorating fungal pathogens of Ashwagandha.

1. **Materials and method**

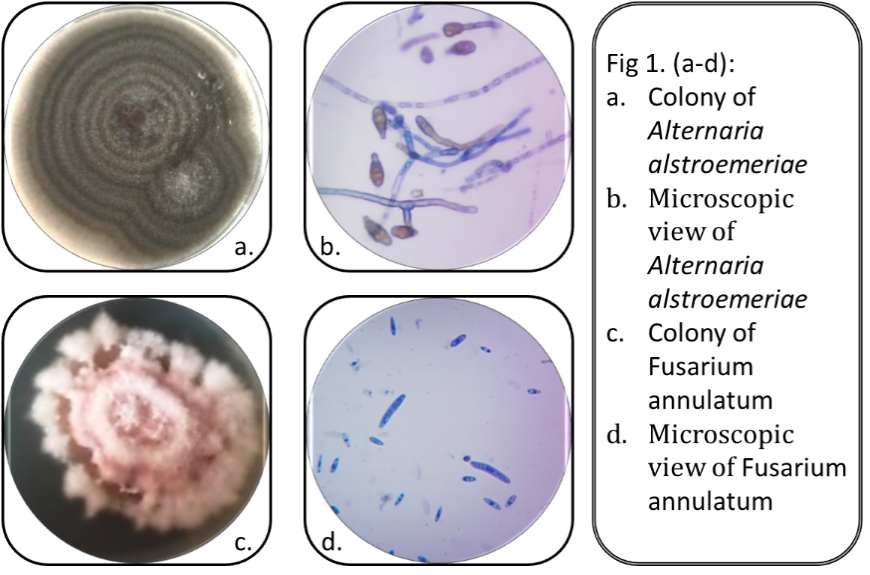
During frequent field visits across various districts of the Lucknow division, it was observed that several farmers were experiencing reduced root yields. A survey was subsequently conducted, revealing that the decline in crop yield was primarily attributed to major fungal diseases. Leaf spot, root rot, and wilt diseases affecting *Withania somnifera* were caused by *Alternaria alstroemeriae* and *Fusarium annulatum*, respectively. These diseases typically emerge during the monsoon season and significantly impact both the quantity and quality of the herbage and yield.

* 1. **Fungal strains, culture conditions**
     1. **Pathogenic fungi**

In this study, two significant fungal pathogens known to deteriorate *Withania somnifera*, namely *Alternaria alstroemeriae* and *Fusarium annulatum*, were isolated from diseased Ashwagandha plant samples. These pathogens were cultured on potato dextrose agar medium and subsequently purified and identified through both morphological and molecular analyses at the CSIR-National Chemical Laboratory in Pune, Maharashtra as well as the pathogenicity test was confirmed using Koch postulates (Koch, 1988). For further detailed in-vitro analysis, the isolated fungal pathogens were re-cultured on potato dextrose agar and preserved at 4°C for comprehensive investigation.

***Alternaria alstroemeriae*** is a fungal pathogen known for causing leaf spot diseases. Morphologically, it produces dark brown to black conidia, which are typically obclavate or ellipsoid in shape and possess transverse and longitudinal septa. The conidia are formed in chains and exhibit a beak-like extension. Under the microscope, conidiophores are septate, simple, or branched, and arise singly or in small groups. The conidia often have a smooth or slightly roughened surface, contributing to their distinctive appearance. This fungus thrives in humid conditions, leading to significant agricultural losses in affected crops (Simmons 2007; Thomma 2003).

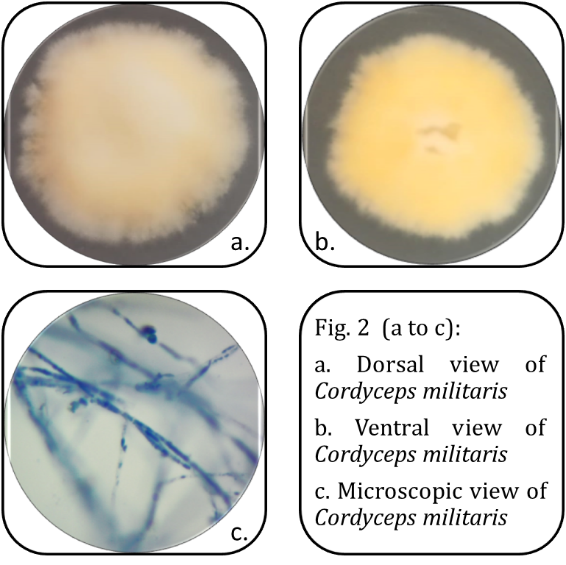
***Fusarium annulatum*** is a filamentous fungus known for causing wilt and rot diseases in various plants. Morphologically, it produces white pinkish to violet mycelium with septate hyphae. The fungus forms crescent-shaped macroconidia, which are 3 to 5 septate. The conidia have a distinct foot-shaped basal cell. Microconidia, when present, are oval to ellipsoid, typically single-celled, and smaller. Chlamydospores may also be observed in older cultures (Leslie and Summerell 2006).



* + 1. **Fungi used as a bioagent**

The fungal culture of *Cordyceps militaris* was obtained from the Indian Council of Agricultural Research (ICAR) – Directorate of Mushroom Research (DMR), Solan (Himachal Pradesh) (DMRO 1164).

*Cordyceps militaris* is an entomopathogenic fungus with a distinct morphology characterized by its elongated, slender, and bright orange to yellowish stromata, typically 3–5 cm in length. The stromata emerge from the host insect's body, usually caterpillars. Microscopically, it exhibits septate hyphae and produces cylindrical to fusiform, multi-septate ascospores within long, thread-like asci. The ascospores often fragment into part-spores. The fungus is renowned for its bioactive compounds, particularly cordycepin, which is studied for its pharmacological properties.



* 1. **Metabolite extraction from *Cordyceps militaris***

The potential fungi i.e. *Cordyceps militaris* were used for extraction of mycometabolites, which was done by following the method of Vinale et al. 2006; Stracquadanio et al. 2020; Khan et al. 2020; Xiao et al. 2022 with slight modifications:

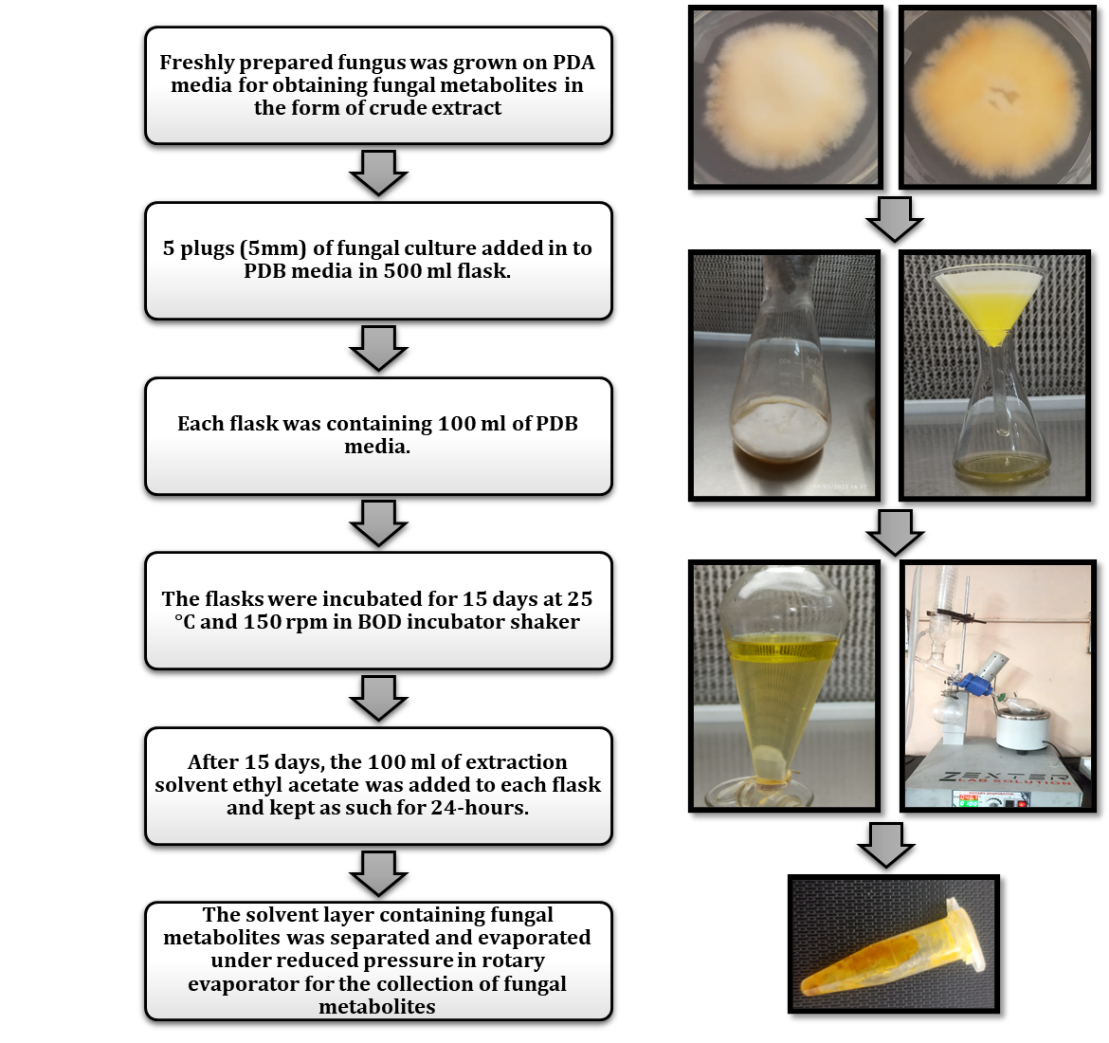


Fig. 3: Flow chart showing the steps for extracting the metabolites from *C. militaris*

* 1. **Antifungal efficacy**

**2.3.1 Poison food technique**

The antifungal activity of secondary metabolites from *C. militaris* was assessed using the poison food technique originally developed by Grover and Moore 1962, with slight modifications as per Shukla and Dikshit 2016 and Shukla et al. 2021. The experiment utilized Potato Dextrose Agar (PDA) (Hi-media) medium combined with various concentrations of *C. militaris* metabolites dissolved in dimethyl sulfoxide (DMSO), a universal solvent. The mixture was poured into sterilized petri dishes (90 mm diameter) to maintain a uniform thickness. A control was prepared with PDA medium containing only DMSO. Mycelial discs (5 mm in diameter) from 5–7-day-old fungal cultures were then placed at the centre of the petri dishes, which were sealed with parafilm. All experiments were conducted in triplicate, with the inoculated plates incubated at 25±2˚C for seven days. The colony diameter was measured at regular intervals of 24 hours for both treated and control plates (Shahi et al. 1999; Shukla & Dikshit 2016; Shukla et al. 2021). The percentage of mycelial growth inhibition (MGI%) was calculated using the corresponding formula.

**Mycelial Growth Inhibition% (MGI) = [(Dc - Dt) / Dc] x 100**

Dc indicates Mean colony diameter in control set

Dt indicates Mean colony diameter in treatment set

**2.3.2 Nature of toxicity of the secondary metabolites of C. militaris**

The toxicity of *Cordyceps* *militaris* metabolites was assessed as either fungistatic or fungicidal at their respective minimum inhibitory concentrations (MIC) against the test fungi *Alternaria* *alstroemeriae* and *Fusarium* *annulatum*. This evaluation followed the methods outlined by Shahi et al. 1999; Shukla and Dikshit 2016; and Shukla et al. 2021. To determine antifungal activity, fungal discs from the MIC experimental setup were re-inoculated upside down on fresh plain PDA medium. The inoculated petri dishes were incubated in a BOD incubator at 25 ± 2°C for seven days, after which the results were recorded. If fungal growth was observed on the 7th day, it indicated that the metabolites had a fungistatic effect. Conversely, the absence of fungal growth signified a fungicidal effect. All experiments were conducted in triplicate. The lowest concentration of metabolites that resulted in no fungal growth after the 7-day incubation period was considered the Minimum Cidal Concentration (MCC).

* 1. **In-silico assays**

Molecular docking was conducted to predict the potential antifungal mechanisms of metabolites from *C. militaris*, as reported in various studies. The 3D structures of the fungal protein, chitin synthetase (PDB ID: 7STM), were obtained from the Protein Data Bank (PDB). The structures of three ligands—Cordycepin, Ergothioneine, and D-mannitol—were sourced from PubChem with CIDs 6303, 5351619, and 6251, respectively. These ligands were selected based on literature, as studies indicate that they are compounds found in *C. militaris* (Xiao et al., 2022; Ashraf et al., 2020; Reisa et al., 2013; Tuli et al., 2013). Using Open Babel software, ligands and heteroatoms were removed, and the proteins were optimized and minimized. Docking was then performed with the PyRx virtual screening tool, and binding affinities were saved as CSV files. The final protein-ligand interactions were visualized using Discovery Studio (Khan et al. 2022; Khanzada et al. 2021).

1. **Results**

**3.1 Antifungal Activity**

The in-vitro antifungal properties of C. militaris metabolites were tested against the plant fungal pathogens *Alternaria alstroemeriae* and *Fusarium annulatum* using the poison food technique. The findings revealed that the minimum inhibitory concentration (MIC) of the metabolites was 20 mg/ml for *A. alstroemeriae* and 15 mg/ml for *F. annulatum*. Additionally, the minimum fungicidal concentration (MCC) was determined to be 30 mg/ml for *A. alstroemeriae*, while for *F. annulatum* it was recorded as 20 mg/ml.

Table 1. Antifungal activity of the metabolites of C. militaris against test pathogens.

|  |  |  |
| --- | --- | --- |
| Concentrations | Mycelial growth inhibition (MGI) (%) | |
| *Alternaria* *alstroemeriae* | *Fusarium annulatum* |
| 2 mg/ml | 32.25 | 42.46 |
| 4 mg/ml | 45.16 | 67.12 |
| 8 mg/ml | 61.29 | 76.71 |
| 10 mg/ml | 75.80 | 90.41 |
| 15 mg/ml | 90.32 | 100% (Static) |
| 20 mg/ml | 100% (Static) | 100% (Cidal) |
| 25 mg/ml | 100% (Static) | 100% (Cidal) |
| 30 mg/ml | 100% (Cidal) | 100% (Cidal) |

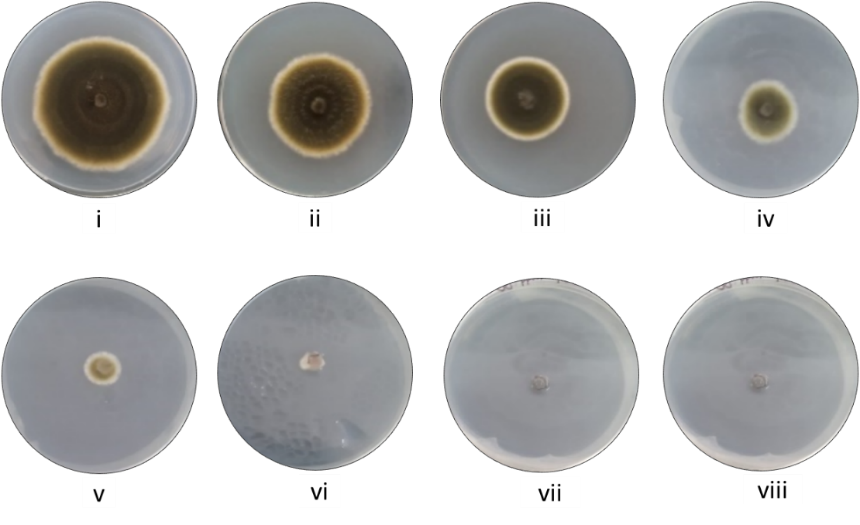


Fig. 4(A). Growth inhibition of *Alternaria* *alstroemeriae* by using metabolites of C. militaris at dose of (i) 2 mg/ml (ii) 4 mg/ml, (iii) 8 mg/ml, (iv) 10 mg/ml, (v) 15 mg/ml (vi) 20 mg/ml (vii) 25 mg/ml and (viii) 30 mg/ml.

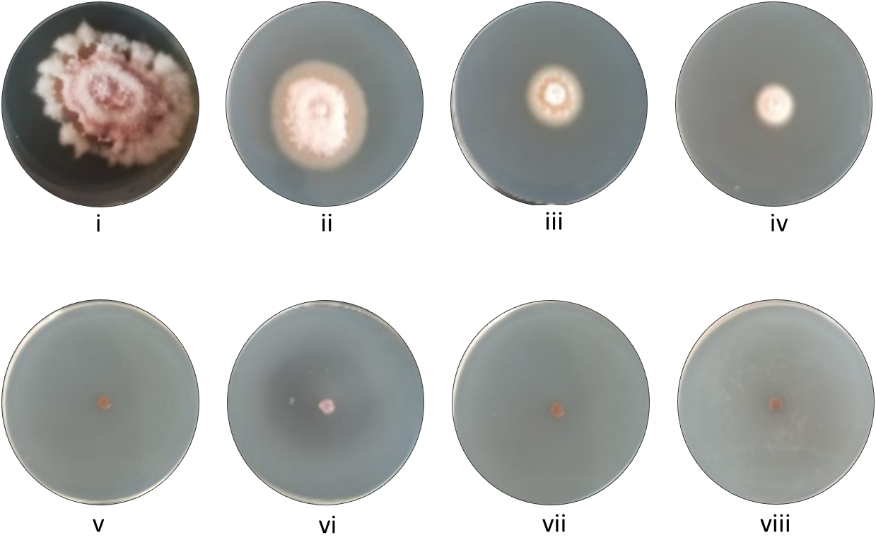


Fig. 4(B). Growth inhibition of *Fusarium annulatum* by using metabolites of C. militaris at dose of (i) 2 mg/ml (ii) 4 mg/ml, (iii) 8 mg/ml, (iv) 10 mg/ml, (v) 15 mg/ml (vi) 20 mg/ml (vii) 25 mg/ml and (viii) 30 mg/ml.

Fig. 5. Antifungal activity of metabolites of *C. militaris* on *Fusarium annulatum* and *Alternaria* *alstroemeriae* at different concentrations showing percent inhibition of mycelia growth.

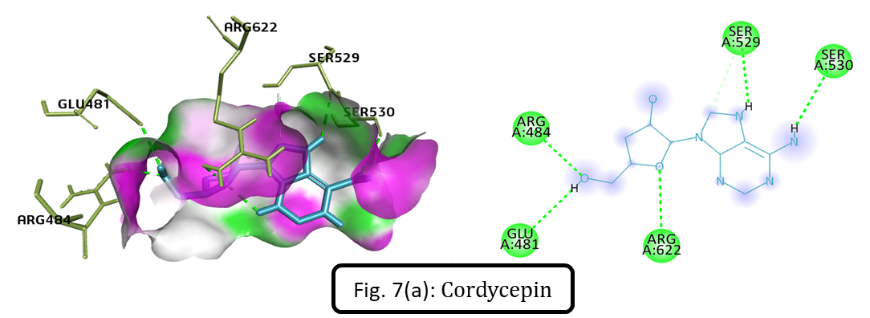
Table 2. Nature of toxicity of the metabolites of *C. militaris* against test pathogens.

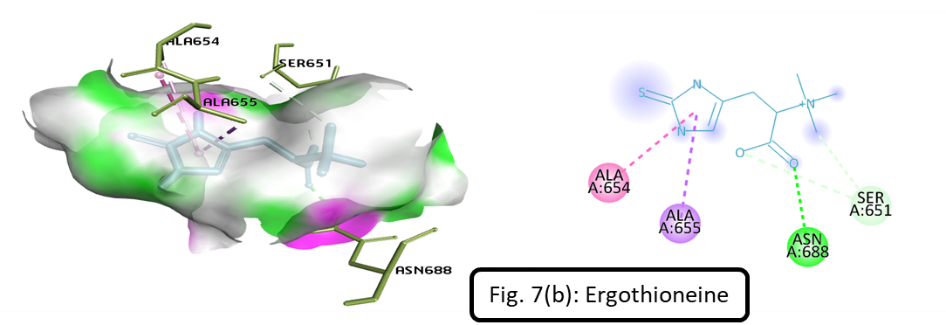
|  |  |  |
| --- | --- | --- |
| Pathogen | Minimum Inhibitory Concentration | Minimum Cidal Concentration |
| *Fusarium annulatum* | 15 mg/ml | 20 mg/ml |
| *Alternaria* *alstroemeriae* | 20 mg/ml | 30 mg/ml |

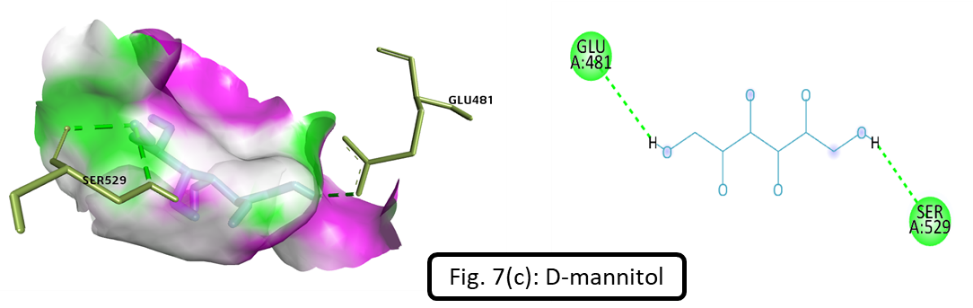
Fig. 6. Minimum Inhibitory Concentration (MIC) and Minimum Cidal Concentration (MCC) of metabolites against both the test pathogens.

**3.2 Molecular Docking Analysis**

To investigate the mechanism underlying the antifungal activity, docking interactions between the ligands and the fungal chitin synthetase receptor were analysed. Among the tested ligands, cordycepin exhibited the highest binding affinity, with values ranging from -6.7 to -5.9. Lower Kd values indicate stronger binding affinity between a ligand and its target. The results revealed that cordycepin formed multiple bond interactions with fungal chitin synthetase (Fig. 7a). In contrast, Ergothioneine formed various bonds (Fig. 7b) with binding affinity values between -5.7 and -4.6, while D-mannitol showed binding affinity values between -4.9 and -4.4 (Fig. 7c). The binding affinities were ranked in the following order: D-mannitol > Ergothioneine > cordycepin (Table 5).







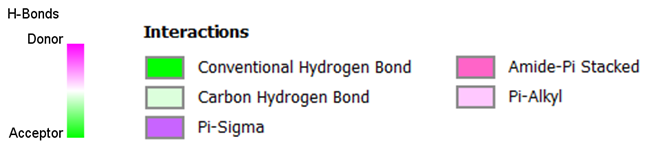


Fig. 7: Docking interactions of ligands binding with fungal protein. (a) Cordycepin (b) Ergothioneine and (c) D-mannitol.

Table 3. Binding affinities of ligands interaction with fungal protein in terms of Kd values using molecular docking analysis.

|  |  |  |
| --- | --- | --- |
| **Protein** | **Ligand** | **Binding affinity** |
| Chitin synthetase | Cordycepin | -6.7 to -5.9 |
| Ergothioneine | -5.7 to -4.6 |
| D-mannitol | -4.9 to -4.4 |

1. **Discussion**

The findings of the current study highlight the potential of *Cordyceps militaris* metabolites as natural antifungal agents against *Fusarium annulatum* and *Alternaria alstroemeriae*, significant pathogens of *Withania somnifera*. The minimum inhibitory and fungicidal concentrations of these metabolites, determined as 15 mg/mL and 20 mg/mL for *F. annulatum* and 20 mg/mL and 30 mg/mL for *A. alstroemeriae*, respectively, demonstrate substantial efficacy. These results align with prior studies where fungal metabolites, particularly from entomopathogenic fungi, have shown promising antimicrobial properties (Vinale et al., 2006; Khan et al., 2020).

The in-silico analysis revealed that cordycepin, a prominent metabolite of *C. militaris*, interacts with specific amino acids of fungal chitin synthetase, forming strong bonds. This mechanism underpins its antifungal activity and is consistent with the reported molecular interactions of fungal bioactive compounds targeting structural proteins of pathogens (Xiao et al., 2022). Such insights not only confirm the bio efficacy of these metabolites but also provide a molecular basis for their mode of action.

These findings support further exploration of *C. militaris* metabolites for pot and field-level applications as sustainable alternatives to synthetic fungicides. Their development as bioagents could address rising concerns about chemical residues and environmental impact, fostering sustainable agriculture practices.

1. **Acknowledgment**

The authors are thankful to the Head, Department of Botany, University of Lucknow for providing the research facilities. Besides the authors are also thankful to Indian Council of Agricultural Research (ICAR) – Directorate of Mushroom Research (DMR), Solan (Himachal Pradesh) for providing the fungal strain and CSIR- National Chemical Laboratory for confirming the pathogen. Further, one of the authors, is thankful to the Council of Science and Technology, Uttar Pradesh project No. CST/AAS/D- 2198; for providing the financial support.

1. **Conflict of interest statement**

The authors have declared no conflict of interest.

1. **References**

M. Ahmad, and N.J. Dar. 2017 “*Withania somnifera*: ethnobotany, pharmacology, and therapeutic functions, in: D. Bagchi (Ed.), Ethnobotany, Pharmacology, and Therapeutic Functions.” Sustained Energy for Enhanced Human Functions and Activity, *Elsevier* Inc 137–154.

Ashraf, S.A., A. E. Elkhalifa, A. J. Siddiqui, M. Patel, A. M. Awadelkareem, M. Snoussi, M. S. Ashraf, Adnan, S. Hadi. 2020 “Cordycepin for Health and Wellbeing: A Potent Bioactive Metabolite of an Entomopathogenic Medicinal Fungus *Cordyceps* with Its Nutraceutical and Therapeutic Potential.” *Molecules* 2020, 25, 2735.

Bharti, N., P. Agrawal, B. Misra, A. Tripathi, R. Singh, D. Maji, H. Pratap Singh, and A. Kalra. 2013. “Efficacy of combined applications of antagonist bacteria and chemical resistance inducers for the management of Fusarium solani causing root rot in *Withania somnifera.*” *Biocontrol Science and Technology.* 23 (2), 239-244.

Chauhan P., S. Ravi. 2020. “Optimization of media and appraisement of fungicides and bioagents on leaf spot of ashwagandha, *Withania somnifera* (L.) Dunal caused by Alternaria *alternata*”. *Journal of Pharmacognosy and Phytochemistry*. 9 (5), 1049-1052.

Chen, B., Y. Sun, F. Luo, and C. Wang. 2020. “Bioactive Metabolites and Potential Mycotoxins Produced by Cordyceps Fungi: A Review of Safety.” *Toxins*. 12, 410.

Grover, R.K., and J. D. Moore. 1962. “Toximetric studies of fungicides against brown spot organism *Sclerotia fructicola* and *S. laxa*.” *Phytopathology* 52 876-880.

Jetawat, R. P. S., and K. Mathur. 2016. “Management of Ashwagandha root rot disease with fungicides, biocontrol agents and botanicals.” *Journal of Applied and Natural Science.* 8 (1), 305 – 309.

Jetawat, R. P. S., K. Singh, R. S. Ratnoo, and B. D. S. Nathawat. 2015. “Physiological studies for the rot root pathogen of ashwagandha (*Withania somnifera*).”  *Annals of Plant and Soil Research.* 17 583-585.

Khabiya, R., G.P. Choudhary, A.C. Jnanesha, A. Kumar, R.K. Lal. 2023. “An insight into the potential varieties of Ashwagandha (Indian ginseng) for better therapeutic efficacy.” *Acta Ecologica Sinica.*

Khan, A., A. A. M. Khan, A. M. U. B. Mahfuz, J. M. Sanjana, A. Ahsan, D. R. Gupta, M. N. Hoque, and T. Islam. 2022. “Highly potent natural fungicides identified in silico against the cereal killer fungus Magnaporthe oryzae.” *Scientific reports* 12 20232.

Khan, A., S. S. Alhewairini, and A. Mohammad. 2020. “Antifungal activity of Cordyceps militaris extracts against some phytopathogenic fungi.” *Journal of Fungi* 6 (3), 120.

Khanzada, B.; N. Akhtar, M. K. Okla, S. A. Alamri, A. Al-Hashimi, M. W. Baig, S. Rubnawaz, H. AbdElgawad, A.H. Hirad, I. U. Haq, et al. 2021. “Profiling of Antifungal Activities and In Silico Studies of Natural Polyphenols from Some Plants.” *Molecules*, 26, 7164.

Leslie, J. F., and B. A. Summerell. 2006. “The *Fusarium* Laboratory Manual.” *Blackwell Publishing.*

Meena, R. P., K. A. Kalariya, P. L. Saran, and P. Manivel. 2019. “Evaluation of ashwagandha (Withania somnifera L.) Dunal accessions and breeding lines against leaf spot disease caused by Alternaria alternata under subtropical condition of India.” *Journal of Applied Research on Medicinal and Aromatic Plants*. 2214-7861.

Mishra, R. S. 2021. “Management of Alternaria leaf spot of Ashwagandha (Withania somnifera L.) by organic products.” *The Pharma Innovation Journal.* 10 (10) 1270-1272.

Park, B. T., K. H. Na, E. C. Jung, J. W. Park, and H. H. Kim. 2009. “Antifungal and Anticancer Activities of a Protein from the Mushroom Cordyceps militaris.” *Korean J Physiol Pharmacol.* 13 49－54.

Pati P.K., M. Sharma, R.K. Salar, A. Sharma, A.P. Gupta, and B. Singh. 2008. “Studies on leaf spot disease of *Withania somnifera* and its impact on secondary metabolites.” *Indian J. Microbiol*. 48 432–437.

Paul, S., S. Chakraborty, U. Anand, S. Dey, S. Nandy, M. Ghorai, S.C. Saha, M. T. Patil, R. Kandimalla, J. Pro´ ck´ow and A. Dey. 2021 “Withania somnifera (L.) Dunal (Ashwagandha): a comprehensive review on ethnopharmacology, pharmacotherapeutics, biomedicinal and toxicological aspects, Biomed.” *Pharmacother* 143 112175,

Qu, S.-L., S.-S. Li, D. Li, and P. J. Zhao. 2022. “Metabolites and Their Bioactivities from the Genus *Cordyceps*.” *Microorganisms*, 10, 1489.

Rawal, P., R. P. Singh, Lekha. 2014.” Integrated Root Rot Management of Ashwagandha (*Withania* *somnifera* (L) Dunal).” *Asian Resonance.* 3 (3), 108-114.

Reis, F.S., L. Barros, R. C. Calhelha, A. Ciric, L. Griensven, M. Sokovic´, and I. Ferreira. 2013. “The methanolic extract of *Cordyceps militaris* (L.) Link fruiting body shows antioxidant, antibacterial, antifungal and antihuman tumor cell lines properties.” *Food and Chemical* *Toxicology*. 62, 91–98.

Shahi S.K., A. C. Shukla, A. K. Bajaj, G. Medgely, and A. Dikshit. 1999. “Broad spectrum antimycotic drug for the control of fungal infection in human beings.” *Current Science*. 836-839.

Shukla A.C., N. Agrawal, S. Singh, and Lalhlenmawia, 2021. “Fundamentals of Pharmacognosy and Phytochemistry.” Authored Book, Publisher- *Today and Tomorrow's Printers* and Publisher, India, [ISBN 13: 978817019] 399.

Shukla, A.C. and A. Dikshit, 2016. “Protocols in medicinal and aromatic plants,” Eds. ed. *Today & Tomorrow’s Printers and Publishers*, New Delhi. India.

Simmons, E.G. 2007. “Alternaria: An Identification Manual.” CBS-KNAW Fungal Biodiversity Centre.

Stracquadanio, C., J. M. Quiles, and F. J. Muñoz-Ledesma. 2020. “Efficacy of fungal secondary metabolites for the control of plant pathogenic fungi”. *Agriculture* 10 (5), 155.

Thomma, B. P. H. J. 2003. “Alternaria spp.: from general saprophyte to specific parasite.” *Molecular Plant Pathology* 4 (4), 225-236.

Tuli, H. S., and S. S. Sandhu. 2013. “Therapeutic potentials of Cordyceps with special reference to Cordyceps militaris.” *Biotech*, 3 (6), 595–602.

Vinale, F., K. Sivasithamparam, E. L. Ghisalberti, R. Marra, S. L. Woo, and M. Lorito. 2006. “Trichoderma–plant–pathogen interactions”. *Soil Biology and Biochemistry* 38 (3): 409-419.

Xiao, Y., H. Liu, and Y. Zhao. 2022. “Recent advances in the antifungal effects of *Cordyceps* *militaris* and its bioactive components.” *Frontiers in Microbiology* 13, 842416.