Mycomolecules against *Alternaria solani* causing Early blight of tomato

Ragupathi KP, PR Renganayaki, S Sundareswaran, S Mohan Kumar and A Kamalakannan

DOI: [https://doi.org/10.22271/j.ento.2021.v9.i1b.8125](https://doi.org/10.22271/j.ento.2021.v9.i1b.8125)

**Abstract**

Mycomolecules isolated from mushroom possess antimicrobial properties which forms bioactive compounds of high therapeutic and pharmacological value. Antimicrobial principles from macro basidiomycetes against plant pathogens was not yet well explored. In this view, a study was proposed to screen mushroom fungi *viz.*, *Lentinus edodes, Volvariella volvacea, Ganoderma lucidum* and *Auricularia polytricha* against tomato early blight pathogen *Alternaria solani*. Methanol extracted mycomolecules from the cell free culture of mushroom was used in different experiments of the study. Results from dual culture technique revealed that *Ganoderma lucidum* showed maximum antifungal activity by inhibiting the mycelial growth of *A. solani* (67%). Among the various mushroom fungi, *G. lucidum* cell free culture filtrates exhibited maximum inhibition of spore germination of *A. solani* (53%) at 24 hours. The methanol extracted metabolite fractions of *G. lucidum* at 0.2% concentration inhibited maximum mycelial growth of *A. solani* (69%). Results indicates that methanol extracted cell free culture fractions of *G. lucidum* possess antifungal activities against the growth of *A. solani* and these mycomolecules could be further explored for the development of fungicides against the pathogen.

**Keywords:** Mycomolecules, *Ganoderma lucidum*, basidiomycetes, *Alternaria solani*

**Introduction**

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop grown in varied agro climatic conditions, its production being affected by many fungal, bacterial and viral diseases. Among the fungal diseases of tomato, early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is an important disease-causing production losses varying from 10 - 80 per cent (Datar and Mayee, 1986) [3]. Major insect pests of tomato include Aphids, Tomato Fruit worms & Horn worms, Leaf-footed Bugs & Stink Bugs, Flea Beetles, Whiteflies, Thrips, Spider Mites and Cutworms. (Fouche et al., 2000) [8]. Often tomato is also affected by several nematodes including *Meloidogyne* spp., *Nacobbus aberrans*, *Ditylenchus dipsaci*, *Globoderaro, stochiensis*, *G. pallida*, *Pratylenchus* spp., *Paratrichodorus* spp., *Tylenchorhynchus* spp., *Xiphinema* *fuculum*, *Rotylenchulus reniformis* and *Dolychodorus heterocephalus* (Greco and Vito, 2011) [10]. Disease management in tomato is widely practiced using chemicals (Singh et al., 2001) [22]. Indiscriminate use of chemicals led to development of fungicidal resistance and environmental pollution (Rai et al., 2000) [16]. Extensive use of chemical fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and the environment, and also results in the build-up of resistance of the pathogens. As tomato an important edible crop, large quantity of pesticides was being used, there is a growing demand for chemical pesticide free organic tomato. Current research is focused on search of antimicrobials agents from green channels such as plants, fungi and bacteria in order to identify their biopesticidal compounds. Mushroom fungi are important as natural sources of medicines and possess number of bioactive compounds *viz.*, antibacterial, antifungal, antioxidant, antiviral (Reis et al., 2011; Rhouhana-Toubi et al., 2015) [17, 18]. Ecofriendly approaches for plant disease management includes mushroom fungi as promising source of antimicrobials against plant pathogens as evidenced by the antimicrobial activity of the culture filtrates of *Ophiocordeyceps sinensis* against soil borne pathogens of *Fusarium oxysporum* f. sp. *lycopersici* (Sangeetha et al., 2015) [20]. *Coprinus comatus* against *Fusarium oxysporum* f. sp. *lycopersici* (Jeeva and Krishnamoorthy, 2018) [11], ethanolic extracts of...
Leucopaxillus gignatea against Fusarium solani, Collectotrichum graminicolum and Bacillus subtilis (Feleke and Anila Doshi, 2017) [7]. The present investigation was made with an aim to identify a potential mushroom fungus with antimicrobial activity against Alternaria solani, the tomato early blight pathogen.

**Table 1**: Mycomolecules and their mushroom source against major pathogens

| Mycomolecule       | Mushroom Source          | Target pathogen          |
|--------------------|--------------------------|--------------------------|
| Ganodermin        | Ganoderma lucidum        | Botrytis cinerea         |
| Pleurostrin       | Pleurotus ostreatus      | Botryosphaeria berengeriana |
| Eryngin           | Pleurotus eryngii        | Mycosphaerella arachidicola |
| Lyophyllin        | Lyophyllium shimeiji     | Physalospora piricola    |
| Grifoline         | Albatrellus dispansus    | Alternaria alternate     |
| Hypsin            | Hypsizigus marmoreus     | Botrytis cinerea         |
| Ratusilactone     | Lactarius rafus          | Alternaria brassicaceae  |
| Cordymin          | Cordyceps militaris      | Rizoctonia solani        |
| Cinnamic acids    | Ganoderma lucidum        | Pencillium ochrochloron  |
| Chrysotrione      | Hygrophorus chrysodon    | Fusarium verticillioides |
| Agrocybin         | Agrocybe cylindracea     | Mycosphaerella arachidicola |
| Lentin            | Lentinus edodes          | Mycosphaerella arachidicola |
| Hydroxypyrrene    | Cordyceps militaris      | Fusarium oxysporum       |
| Phellinsin        | Phellinus sp             | Pyricularia grisea       |

*(Sivanandhan et al., 2017) [23]*

**Materials and Methods**

The tomato early blight pathogen *Alternaria solani* and the mushroom fungal cultures *viz.*, *Lentinus edodes*, *Volvariella volvaceae*, *Ganoderma lucidum* and *Auricularia polytricha* obtained from the Department of Pathology, Tamil Nadu Agricultural University, Coimbatore were used for the studies.

**In vitro screening of mushroom fungi against A. solani**

Mushroom fungi *viz.*, *Lentinus edodes*, *Volvariella volvaceae*, *Ganoderma lucidum* and *Auricularia polytricha* were tested for its antagonistic activity against *A. solani* by following dual culture technique (Dennis and Webster, 1971) [6]. A 9 mm mycelial disc of mushroom fungi was placed at the edge of the Petri plates containing PDA medium on one side. Similarly, on the opposite side a 9 mm mycelial disc of *A. solani* was placed. The dual culture plates were incubated at 28±2ºC for 7 days. Three replications were maintained for each treatment. Plates with *A. solani* only and respective mushroom fungi served as control. The plates were examined periodically and measurements on the radial mycelial growth of *A. solani* and mushroom fungi were recorded till the control plate attained full growth (90mm). The percent inhibition of mycelial growth of *A. solani* was calculated by using the formula proposed by Vincent (1947) [27].

**Percent inhibition of growth (PI) = C-T/C×100**

Where, C is the growth of pathogen in control (mm) and T is the growth of pathogen in treatment (mm).

**Solvent extraction of metabolites from mushroom fungi**

Mycelial discs (measuring 9 mm dia.) was cut from margin of a 10 day old culture of *Lentinus edodes*, *Volvariella volvaceae*, *Ganoderma lucidum* and *Auricularia polytricha* grown in PDA medium in petridishes and inoculated in 250 ml conical flasks containing 100 ml of sterilized PD broth. The flasks were placed on a rotary shaker maintained at 120 rpm and incubated at 25ºC for 20 days. After incubation, the culture filtrate and the mycelial mat were separated by filtration through Whatman No. 40 filter paper and the filtrate was centrifuged at 10,000 rpm and the Cell Free Culture filtrate (CFC) was extracted separately with methanol as solvent. Liquid-liquid extraction was carried out three to four times with methanol. The extracts from CFC of mushrooms were evaporated separately under reduced pressure using a rotary evaporator to obtain the residues. The condensate or residue so obtained from solvent was dried and dissolved in methanol (1mg/ml) and filtered with membrane filter (0.48 μm), stored at 4ºC used for further studies.

**Effect of methanol extracted metabolites on A. solani spore germination test**

The methanol extracted metabolites of various mushrooms were tested separately against spore germination of *A. solani* using cavity slides. A drop of extracted metabolites of mushrooms were placed separately in a cavity slide and a drop of spore suspension (1x10⁶ spores/ml) of *A. solani* prepared in sterile distilled water was added to extracted metabolite and thoroughly mixed. The cavity slide was placed in the Petri dish moistened with cotton and incubated at room temperature (28 ± 2ºC). Three replications were maintained for each treatment. The spore suspension in sterile water alone served as control. The spore germination was observed and recorded after 6, 12 and 24 hours under phase contrast microscope and the percent inhibition of spore germination was calculated using the formula (Akhter et al., 2006) [1].

**Effect of methanol extracted metabolites on A. solani mycelial growth inhibition test**

The methanol extracted metabolites of various mushrooms were tested separately against mycelial growth of *A. solani* by agar well diffusion method (Stroke and Ridgway, 1980) [54]. After solidification of PDA medium in Petri dishes, four wells (5mm in diameter) were made on the plate using sterile cork borer on all four sides, giving equal distance and also by leaving one cm space from the periphery. The different concentrations *viz.*, 0.05%, 0.1% and 0.2% of various mushrooms metabolites were poured into agar wells at the rate of 100μl per well using micro pipette. Then, mycelial disc of *A. solani* (5mm diameter) taken from ten days old culture was placed at the centre of each Petri dish and incubated at 28±2ºC for seven days. Observations on the per cent inhibition of mycelial growth of *A. solani* were recorded (Vincent, 1947) [27].

**Statistical analysis**

The data obtained from various experiments were analysed...
Results and Discussion
Mushrooms are used as food and in pharmaceuticals since ancient times, the recent findings has proved that the mushroom fungi possess secondary metabolites of antimicrobial nature to be effective against many plant pathogens. There is great scope for developing biopesticidal molecules from mushroom fungi that can be used for development of fungicides like Azoxy strebin in plant disease management.

Table 2: Antagonistic activity of mushroom fungi against A. solani by dual culture technique

| Treatment       | A. solani mycelial growth (mm) | Mushroom fungal growth (mm) | Inhibition zone (mm) | % inhibition over control |
|-----------------|--------------------------------|-----------------------------|----------------------|--------------------------|
| Lentinus edodes | 38.29                          | 47.17                       | 4.58                 | 57.46                    |
| Volvariella volvacea | 53.52                        | 34.05                       | 2.40                 | 40.53                    |
| Ganoderma lucidum | 29.45                         | 53.62                       | 6.93                 | 67.28                    |
| Auricularia polytricha | 43.54                 | 42.75                       | 3.71                 | 51.62                    |
| Control         | 90.00                          | 90.00                       | -                    | -                        |
| SEd             | 1.48                           | 0.86                        | 0.14                 | 1.26                     |
| CD (P=0.05)     | 3.30                           | 1.99                        | 0.32                 | 2.90                     |

Table 3: Effect of methanolic mushroom extracts on A. solani spore germination

| Treatment       | 6h | 12h | 24h |
|-----------------|----|-----|-----|
| Lentinus edodes |    |     |     |
| Volvariella volvacea |    |     |     |
| Ganoderma lucidum |    |     |     |
| Auricularia polytricha |    |     |     |
| Control         |    |     |     |
| SEd             |    |     |     |
| CD (P=0.05)     |    |     |     |

Table 4: Antimicrobial activity of methanolic mushroom extracts against A. solani by agar well diffusion technique

| Treatment       | 0.05% | 0.10% | 0.20% |
|-----------------|-------|-------|-------|
| Lentinus edodes |       |       |       |
| Volvariella volvacea |       |       |       |
| Ganoderma lucidum |       |       |       |
| Auricularia polytricha |       |       |       |
| Control         |       |       |       |
| SEd             |       |       |       |
| CD (P=0.05)     |       |       |       |

In vitro screening of mushroom fungi against A. solani by dual culture technique

Among the mushroom fungi tested, Ganoderma lucidum followed by Lentinus edodes and Auricularia polytricha showed reduced mycelial growth of A. solani (29 mm, 38 mm and 44 mm respectively) when compared to control (90 mm) with inhibition per cent of 67, 57 and 52 respectively. However, inhibition zone was maximum (6.93 mm and 4.58 mm) in G. lucidum and L. edodes respectively followed by A. polytricha (3.71 mm) and V. volvacea (2.40 mm) (Table 2). Badalyan et al., (2014) [35] reported the antagonistic activity of Pleurotus ostreatus and Ganoderma lucidum by dual culture technique. Constituents of Ganoderma and Agrocybe aegerita was found to reducing local lesions of Ground nut bud necrosis virus in cowpea (Sajeena and Marimuthu, 2013) [39] and Tobacco mosaic virus infection (Sun et al., 2003) [30]. This could be due to the effect of Ganoderma constituents in inhibiting the viral replication by interfering with their adsorption, viral integration, assembly and release (Gao et al., 2003) [39].

Mushroom fungi metabolites against A. solani spore germination and mycelial growth Many of the macro fungi extracted with polar and non polar solvents contained bioactive compounds with antifungal, antibacterial and antiviral activities (Wasser, 2002) [29]. Antimicrobial compounds from 20 day old crude cell free culture filtrates of G. lucidum, L. edodes, V. volvacea and A. polytricha. Irrespective of mushroom species spore germination was higher with increase in duration. Among the treatments G. lucidum recorded higher percentage of spore germination inhibition (53.07%) followed by L. edode (37.93%) at 24 h. Control showed highest (90.67%) spore germination. Chen and Hyuang (2010) [40] reported that the culture filtrates of Lentinula edodes completely inhibited the spore germination of Colletotrichum higginsianum. Also, culture filtrates of Ganoderma lucidum inhibited spore germination of Alternaria brassicicola and culture filtrates of L. edodes suppressed the germination of Phytophthora capsici.(Table 3)

The agar well diffusion of methanol extracted constituents of cell free culture filtrates of G. lucidum, L. edodes, V. volvacea and A. polytricha (Table 3) showed that all the metabolites extracted exhibited significantly varied inhibition of mycelial growth of A. solani. Ganoderma compounds identified are mostly Triterpenes (lanostanoid-type triterpene and polyketides (Farnesyl quinone), small peptides (ganodermin) and polysaccharides with antimicrobial properties (Basnet et al., 2017) [3]. Antibacterial activity of L. edodes against bacteria has been reported (Qureshi et al., 2010) [41]. In some other studies, crude methanolic extract of Clitocybe sp exhibited maximum inhibition against Colletotrichum coffeaeum (Shahid et al., 2016) [22].

Testing different concentrations of methanol extracted metabolites against A. solani

The antimicrobial metabolites extracted using methanol was made up to different concentrations of 0.05%, 0.1% and 0.2% to test the desired concentration that could inhibit mycelial growth of A. solani. From the results (Table 4) it is observed that all the extracted antimicrobial metabolites of all mushroom exhibited significantly varied mycelial growth inhibition of A. solani. G. lucidum inhibited maximum (69.41%) mycelial growth of A. solani at 0.2%. G. lucidum basidiocarp showed antibacterial activity against S. typhi and...
antifungal activity against *C. albicans* (Uma Gowrie et al., 2014) [20]. Antimicrobial substances from *L. edodes*, *A. polytricha* and *V. volvacea* showed inhibition of mycelial growth of *Alternaria solani* (Radhajeyalakshmi et al., 2011) [15]. The fruiting body, mycelia and spores of *G. lucidum* contain ganoderic acid, polysaccharides, triterpenoids, fatty acids, nucleotides, proteins, peptides, sterols (Kim et al., 1999) [12] which account for more than 400 bioactive compounds.

**Conclusion**

Antimicrobial molecules from fungi, bacteria and plants to manage plant diseases can mitigate the environmental hazards and pollution by indiscriminate use of chemical fungicides. Among the mushroom fungi screened against *A. solani*, the macrofungi *Ganoderma lucidum* found to be with several high value bioactive mycomolecules needs to be identified and it has great scope for developing effective bio-fungicides against plant pathogens.

**References**

1. Akhter N, Begum MF, Alam MS. Inhibitory effect of different plant extracts, cow dung and cow urine on conidial germination of *Bipolaris sorokiniana*. J Bio-sci 2006;14:87-92.
2. Badalyan SM, Gharibyan NG, Innocenti G. Antifungal/Antagonistic Activity of Different *Ganoderma* Collections against Plant Pathogenic Fungi and Their Antagonists. 8th international conference on mushroom biology and mushroom 2014, 4.
3. Basnet BB, Li Liua, Li Bao, Hongwei Liu. Current and future perspective on antimicrobial and anti-parasitic activities of *Ganoderma* sp.: an update. Mycology 2017;8(2):111-124.
4. Chen JT, Huang JW. Antimicrobial Activity of Edible Mushroom Culture Filtrates on Plant Pathogens Plant Pathology Bulletin 2010;19:261-270.
5. Datar VV, Mayee CD. Technical bulletin-1. Marathwad Agricultural University, Parbhani. Phytopathometry, 46.
6. Dennis, C and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma*: II. Production of volatile antibiotics. Transactions of the British Mycological Society 1986;57(1):41-IN44.
7. Feleke HT, Anila Doshi. Bioactive compounds of antimicrobial nature from Indian wild mushrooms. Indian J. of Natural Products and Resources 2017;8(3):254-262.
8. Fouche C, Gaskell M, Koike S, Mitchell J, Smith R. Insect Pest Management for Organic Crops: UC Vegetable Research and Information Center Publication 2000, 7251.
9. Gao Y, Zhou S, Huang M, Xu A. Antibacterial and Antiviral value of the Genus *Ganoderma* P. Karst. Species (*Aphyllophoromyctidaeae*): A Review. International Journal of Medicinal Mushrooms 2003;5(3):235-246.
10. Greco N, Di Vito M. Main Nematode Problems of Tomato. Acta Hortic 2011;914:243-249.
11. Jeeva S, Krishnamoorthy AS. Antifungal potential of Myco-molecules of *Coprinopsis cinerea* (Schaeff) S Gray s.lat against *Fusarium* spp, Madras Agric J 2018;105(1-3):56-60.
12. Kim HW, Kim BK. Biomedical triterpenoids of *Ganoderma lucidum* (Curt: Fr.) P. Karst. (*Aphyllophoromyctidaeae*). *Int. J. Med.Mushrooms*, 1999;1:121-138.
13. Panse VG, Sukatme PV. Statistical methods for agricultural workers. ICAR publication, New Delhi 1985, 359.
14. Quereshi S, Pandey AK, Sandhu SS. Evaluation of antibacterial activity of different *Ganoderma lucidum* extracts. People’s Journal of Scientific Research 2010;3(1):9-13.
15. Radhajeyalakshmi R, Velzhahan R, Prakasham V. *In vitro* evaluation of solvent extracted compounds from edible macromycetes against phytopathogenic fungi. Archives of Phytopathology and Plant Protection 2011;1:1-8.
16. Rai NK, Leepika Tuli BK Sarma, Singh UP. Effect of plant extracts on spore germination of some fungi. Indian J. Plant Pathol 2000;18:44-47.
17. Reis FS, Pereira E, Barros L, Sousa MJ, Martins A, Ferreira IC. Biomolecule profiles in inedible wild mushrooms with antioxidant value. Molecules 2011;16(6):4328-4338.
18. Rouhana-Toubi A, Wasser SP, Fares F. The shaggy ink cap medicinal mushroom, *Coprinus comatus* (higher Basidiomycetes) extract induces apoptosis in ovarian cancer cells via extrinsic and intrinsic apoptotic pathways. Int J Med Mushrooms 2015;17(12):1127-1136.
19. Sajeeva A, Marimuthu T. Efficacy, stability and persistence of Ganosol, a *Ganoderma* based fungicide against plant pathogens. The Journal of Plant Protection Sciences 2013;5(1):17-25.
20. Sangeetha C, Krishnamoorthy A, Nakkeeran S, Ramakrishnan S, Amirtham D. Evaluation of bioactive compounds of *Ophiocordyceps sinensis* [Berk.] Sacc. against *Fusarium* spp. Biochem. Cell. Arch 2015;15(1):431-435.
21. Shahid AA, Asif M, Shahbaz M, Ali M. Antifungal Potential of *Ganoderma lucidum* Extract against Plant Pathogenic Fungi of *Calendula officinalis* L. Paper presented at the 5th International Conference on Biological, Chemical and Environmental Sciences (BCES-2016) March 2016.
22. Singh J, Majumdar VL. Efficacy of plant extracts against *Alternaria alternata*, the incitant of fruit rot of pomegranate (*Punica granatum*). J Mycol Pl Pathol 2001;31:346-349.
23. Sivanandhan S, Ameer Khusro, Michael Gabriel Paulraj, Savarimuthu Ignacimuthu, Naif Abdullah AL-Dhabi. Biocontrol Properties of Basidiomycetes: An Overview. J Fungi 2017;3:2. doi:10.3390/jof3010002.
24. Stroke JE, Ridgway GL. Clinical bacteriology, Edward Arnold Ltd. London 1980, p. 143.
25. Sun H, Zhao CG, Tong X, Qi YP. A lectin with mycelia differentiation and anti phytovirus activities from the edible Mushroom *Agrocybe aegerita*. Journal of Biochemical Molecular Biology 2003;36:214-22.
26. Uma Gowrie S, Chathurdevi G, Rani K. Evaluation of Bioactive Potential of Basidiocarp Extracts of *Ganoderma lucidum* International Journal of Pharmaceutical Research & Allied Sciences 2014;3(1):36-46.
27. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature 1947;159:850-850.
28. Wang HX, Ng TB. A laccase from the medicinal mushroom *Ganoderma lucidum*. Applied Microbiol. Biotechnol 2006;72:508-513.
29. Wasser SP. Review of Medicinal Mushrooms Advances: Good news from old Allies. Herbal Gram 2002;56:28-33.