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Antimicrobial Activity of Biomolecules from Mushroom Fungi against *Colletotrichum capsici* (Syd.) Butler and Bisby, the Fruit Rot Pathogen of Chilli

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A B S T R A C T

Mushroom fungi secrete antifungal, antibacterial and antiviral bioactive compounds of therapeutic and pharmacological value. Very limited work has been done on the exploration of antimicrobial principles from macrobasidiomycetes against plant pathogens. In this view, a study was proposed to screen eight mushroom fungi viz., *Auricularia polytricha*, *Coprinus comatus*, *Ganoderma lucidum*, *Volvariella volvaceae*, *Lentinus edodes*, *Pycnoporus sanguineus*, *Schizophyllum commune*, *Trametes versicolor* against spore germination and mycelial growth of *Colletotrichum capsici* , the fruit rot pathogen of Chilli and to extract antimicrobial molecules from the selected mushroom fungi using different solvents viz., Chloroform, Diethyl ether and Ethyl acetate. Results from dual culture technique revealed that *Ganoderma lucidum*, *Auricularia polytricha* and *Lentinus edodes* showed maximum antifungal activity by inhibiting the mycelial growth of *C. capsici* (54.81%, 53.70 % and 45.55% respectively) with maximum inhibition zone of (4.86 mm, 2.86 mm and 4.86 mm respectively). Though the Chloroform, Diethyl ether and Ethyl acetate fractions of *G. lucidum* cell free extracts inhibited spore germination of *C. capsici* both at 12 and 24 hours, maximum inhibition of spore germination was observed at 24 hours. Among the mushroom fungi, the chloroform extracted fractions followed by Diethyl ether and Ethyl acetate fractions of *G. lucidum* cell free culture filtrates exhibited maximum inhibition of spore germination of *C. capsici* (inhibition of 88 %, 79% and 78 % respectively) at 24 hours. Similarly, maximum inhibition of mycelial growth of *C. capsici* with 40 %, 34.07% and 29.25 % inhibition respectively was recorded in the chloroform extracted fractions followed by Diethyl ether and Ethyl acetate fractions of *G. lucidum* cell free culture filtrates when compared to solvent extracted fractions of *L. edodes* and *A. polytricha* by agar well diffusion technique. The chloroform extracted metabolite of *G. lucidum* followed by Ethyl acetate and Diethyl ether fractions at 2000 ppm concentration inhibited maximum mycelial growth of *C. capsici* (60.55 %, 58.88 % and 55.47 % respectively). It is well proven that chloroform extracted fractions of *G. lucidum* possess antimicrobial activities against the growth of *C. capsici*. Hence, further studies towards the identification of these compounds will pave for development of fungicides against *C .capsici*.

Introduction

Chilli (*Capsicum annum* L.) is an important commercial spice crop grown in almost all the states of India with 1.49 million tonnes of dry chilli produced annually (FAOSTAT, 2013). Such an important crop is affected by many diseases including fungi, bacteria and viruses, the most important being anthracnose (fruit rot) disease caused by a complex of *Colletotrichum* species that cause latent infection and affects ripe fruits (Jeffries *et al.*, 1990) and resulting in both pre and post-harvest fruit decay with yield losses of up to 50% (Liu *et al.*, 2016), about 25% fruit loss at pre-harvest stage and 25-40% loss at post-harvest stage (Sharma and Shenoy, 2014) and severe losses of 10-60% both in yield and quality of the chilli (Bansal and Grover, 1969). Among these, *Colletotrichum capsici* **(**Syd.) Butler and Bisby is reported to survive in plant debris, spreads through seeds from infected fruits and causes both fruit rot and die back in chillies thereby leading to severe yield reduction. Moreover, secondary spread in the field is favoured at a temperature around 27-28°C with relative humidity of 80 per cent through wind borne conidia that aids in fast spread of the disease (Roberts *et al.*, 2001). The intensive use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to humans and the environment, and also in the build-up of resistance of the pathogens.

As chilli is an edible crop and large quantity of pesticides was being used, there is a growing demand for chemical pesticide free organic chilli world over. Current research is focused on search of antimicrobials from green channels such as plants, fungi and bacteria in order to identify biopesticidal compounds. Perusal of literature showed that apart from food, mushroom fungi are important as natural sources of medicines and possess number of bioactive compounds *viz.*, antibacterial, antifungal, antioxidant, antiviral antinemic, anti- tumor, immunosuppressive, antiallergic, anti-inflammatory activities, hypolipidemic, and hepatoprotective activity (Hatvani, 2001; Wasser, 2002; Lindequist *et al.*, 2005; Reis *et al.*, 2011; Rouhana-Toubi *et al.*, 2015). Owing to the current emphasis on the ecofriendly approaches for plant disease management, mushroom fungi can serve 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* and *F. oxysporum* f. sp. *cubense* (Sangeetha and Krishnamooorthy, 2015), *Coprinus comatus* against *F. oxysporum* f. sp. *brachygibbosum*, *Fusarium oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *cubense* (Jeeva and Krishnamoorhy, 2018), ethanolic extracts of *Leucopaxillus gignatea* against *Aspergillus niger, Fusarium solani, Collectotrichum graminicolum and Helminthosporium maydis, Xanthomonas axanopodis pv. punicae, Pseudomonas syringa* and *Bacillus subtilis,* (Feleke and Anila Doshi, 2017). Perusal of literature showed that no work has been attempted on the exploitation of antimicrobials from mushroom fungi against C*olletotrichum capsici* and this kindled interest to undertake the present investigation with an aim to identify a potential mushroom fungus with antimicrobial activity against *C. capsici,* the chilli fruit rot pathogen.

Materials and Methods

The chilli fruit rot pathogen *Colletotrichum capsici* (Acc. No. MK758061) and the mushroom fungal cultures *viz., Ganoderma lucidum, Auricularia polytricha, Lentinus edodes, Coprinus sinensis, Schizophyllum commune, Trametes versicolor, Volvariella volvaceae* and *Pycnoporus sanguineus* obtained from the Department of Plant

Pathology, Tamil Nadu Agricultural University, Coimbatore were used for the studies.

In vitro screening of mushroom fungi against *C. capsici*

Mushroom fungi *viz*. *Ganoderma lucidum, Auricularia polytricha, Lentinus edodes, Coprinus sinensis, Schizophyllum commune, Trametes versicolor, Volvariella volvaceae* and *Pycnoporus sanguineus* were tested for its antagonistic activity against *C. capsici* by following dual culture technique (Dennis and Webster, 1971). 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 *C. capsici* was placed. The dual culture plates were incubated at 28±2ºC for 7 days. Three replications were maintained for each treatment. Plates with *C. capsici* only and respective mushroom fungi served as control. The plates were examined periodically and measurements on the radial mycelial growth of *C. capsici* and mushroom fungi were recorded till the control plate attained full growth (90mm). The percent inhibition of mycelial growth of *C. capsici* was calculated by using the formula proposed by Vincent (1947).

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).

Preparation of solvent extracted metabolites from selected mushroom fungi

Based on the above studies, the mushroom fungi that showed maximum inhibition of mycelial growth of *C. capsici* was selected and used for further studies**.**

Mycelial discs (measuring 9 mm dia.) was cut from margin of a 10 day old culture of *G. lucidum, L. edodes, A. 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. The filtrate was further centrifuged at 10,000 rpm and the Cell Free Culture filtrate (CFC) of *G. lucidum, L. edodes, A. polytricha* was extracted separately with three different solvents *viz*., ethyl acetate, chloroform and diethyl ether. Liquid-liquid extraction was carried out three to four times for each solvent. The ethyl acetate, chloroform and diethyl ether solvent extracts from CFC of *G. lucidum, L. edodes, A. polytricha* was 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 different solvent extracted metabolites from selected mushroom fungi on spore germination and mycelial growth of *C. capsici*

Spore germination test

The different solvent (chloroform, diethyl ether, ethyl acetate) extracted metabolites of *G. lucidum, L. edodes, A. polytricha* were tested separately against spore germination of *C. capsici* using cavity slides (Anonymous, 1943).

A drop of chloroform, diethyl ether and ethyl acetate solvent extracted metabolites of *G. lucidum, L. edodes, A. polytricha* were placed

separately in a cavity slide and a drop of spore suspension (1×10^6 spores/ml) of *C. capsici* prepared in sterile distilled water was added to each of the solvent 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).

$$\text{Inhibition of conidial germination (\%)} = \frac{\text{Total number of conidia} - \text{Number of germinated conidia}}{\text{Total number of conidia}} \times 100$$

Mycelial growth inhibition test

The different solvent (chloroform, diethyl ether, ethyl acetate) extracted metabolites of *G. lucidum, L. edodes, A. polytricha* were tested separately against mycelial growth of *C. capsici* by agar well diffusion method (Stroke and Ridgway, 1980). 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 concentration of solvent extracted (chloroform, diethyl ether, ethyl acetate) metabolites of *G. lucidum, A. polytricha and L. edodes* were made up to 500 ppm and poured into agar wells at the rate of 100µl per well using micro pipette. Then, mycelial disc of *C. capsici* (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 *C. capsici* were recorded (Vincent, 1947).

Effect of different concentrations of solvent extracted metabolites of *G. lucidum* on mycelial growth of *C. capsici* by Agar well diffusion assay

Based on the above studies, *Ganoderma lucidum* was identified to possess maximum antimicrobial activity against *C. capsici* and used for the studies. Different solvents *viz.*, chloroform, diethyl ether and ethyl acetate extracted metabolites from culture filtrate of *Ganoderma lucidum* were prepared as mentioned earlier. The solvent extracted metabolites of *G. lucidum* were made up to 1000, 1500 and 2000 ppm and used for testing the Minimum Inhibitory Concentration (MIC) of the metabolites that could inhibit the mycelial growth of *C. capsici* by agar well diffusion assay.

Results and Discussion

Though it is well proven that mushrooms are used as food and in pharmaceuticals since ancient times, the recent research 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 in plant disease management. The best evidence is the fungicide Azoxystrobin derived from mushroom fungi *Strobilurus tenacellus* effective against downy mildew and powdery mildew of grapes. Since the last decade, lot of work has been initiated on the identification of antimicrobial metabolites from mushroom fungi against clinical pathogens especially bacteria. Very limited work has been done on the fungal plant pathogens. The easiest and most reliable way to assess the antagonistic potential of mushroom fungi has to be done by dual culture test (Dennis and Webster, 1971) where the growth nature of the mushroom fungi and test pathogen will give

an indication of the presence or absence of antimicrobial activity of the mushroom fungi.

In vitro screening of mushroom fungi against *C. capsici* by dual culture technique

In our study, among the mushroom fungi tested, *Pycnoporus sanguineus*, followed by *Ganoderma lucidum* and *Auricularia polytricha* showed reduced mycelial growth of *C. capsici* (49 mm, 40.67mm and 41. 67 mm respectively) when compared to control (90 mm) with inhibition per cent of 57.41, 54.81 and 53.70 respectively. Other mushroom fungi, *Lentinus edodes, Trametes versicolor, Volvariella volvaceae and Coprinus sinensis* also showed mycelial growth inhibition of *C. capsici* with 45.55 per cent, 43.63 per cent, 42.92 per cent and 40 per cent respectively. However, inhibition zone was maximum (4.86 mm and 4.86 mm) in *G. lucidum* and *L. edodes* respectively followed by *A. polytricha* (2.76 mm), *V. volavceae* (2.83 mm) and *T. versicolor* (1.1mm) (Table 1; Plate 1). The interactions between respective mushroom fungi and *C.capsici* in dual culture technique is furnished below.

Interactions between mushroom fungi and *C. capsici*

Mushroom fungi and Pathogen	Nature of Interaction
Ganoderma lucidum* and *C. capsici	Clear inhibition zone of 4.86mm; both mushroom fungi and pathogen did not grow over each other even after 10 days
Auricularia polytricha* and *C. capsici	Initially clear inhibition zone of 2.76mm; later mushroom fungi hyperparasitised over the pathogen
Lentinus edodes* and *C. capsici	Pathogen growth was retarded and pushed back with an inhibition zone of 4.86mm
Trametes versicolor* and *C. capsici	Inhibition zone of 1.10 mm; both pathogen and mushroom fungi did not grow over each other
Pycnoporus sanguineus* and *C. capsici	No inhibition zone, but thick mat was formed at the contact of both fungi; later mushroom fungi hyperparasited over the pathogen
Schizophyllum commune* and *C. capsici	No inhibition zone; but hyperparasitization of mushroom fungi over pathogen
Coprinus sinensis* and *C. capsici	No inhibition zone, thick mat formed between both fungi
Volvariella volvaceae* and *C. capsici	No inhibition zone, both fungi did not grow over each other

Similar to our study, Badalyan *et al.*, (2014) reported the antagonistic activity of *Pleurotus ostreatus, Hypholoma fasciculare, Ganoderma lucidum, Lentinus tigrinus* and *Schizophyllum commune Cochliobolus sativus, Fusarium culmorum, Gaeumannomyces graminis* and *Rhizoctonia cerealis* 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) and *Tobacco mosaic virus* infection (Sun *et al.*, 2003). 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).

Based on the inhibition zone and maximum inhibition per cent of mycelial growth of the pathogen, the mushroom fungi *G. lucidum*, *A. polytricha* and *L. edodes* possessing antagonistic activity was assumed to secrete antimicrobial compounds and hence selected for further studies.

Testing the solvent extracted metabolites of selected mushroom fungi (*G. lucidum, A. polytricha and L. edodes*) against spore germination and mycelial growth of *C. capsici*

The use of extraction solvents is important to extract antimicrobial components of interest as many of the macro fungi extracted with polar and non polar solvents contained bioactive compounds with antifungal, antibacterial and antiviral activities against human pathogens (Wasser, 2002). In our study different solvents *viz.,* Chloroform, Diethyl ether and Ethyl acetate were used to extract the antimicrobial compounds from 20 day old crude cell free culture filtrates of *G. lucidum, L. edodes and A. polytricha.* All three extracts used in the study had antimicrobial activity of *C. capsici*. The results obtained in the present study showed that the Chloroform, Diethyl ether and Ethyl acetate fractions of *G. lucidum* cell free extracts inhibited spore germination of *C. capsici* both at 12 and 24 hours; with maximum inhibition of spore germination at 24 hours. The Chloroform, Diethyl ether and Ethyl acetate extracted constituents of *G. lucidum* showed spore germination inhibition of 85 %, 73.75% and 70.25 % respectively at 12 hours and inhibition of 88 %, 79% and 78 % respectively at 24 hours. In the case of *A. polytricha*, ethyl acetate fractions of metabolites exhibited inhibition of spore germination of *C. capsici* with inhibition of 55.75 % followed by Chloroform and Diethyl ether fractions at 52.75 and 52.5 per cent inhibition respectively at 12 hours. The spore germination inhibition at 24 hours was observed to be 66.25% in Ethyl acetate fraction followed by Chloroform and Diethyl ether (59.5 % and 59% respectively). The chloroform fraction of *L. edodes* inhibited spore germination of *C. capsici* with inhibition of 57.75 % and 67.25% at 12 and 24 hours respectively. Similarly, the Ethyl acetate fractions and Diethyl ether fractions recorded inhibition of spore germination (56.5% and 65.25% respectively) at 12 hours and (53.25 % and 64.25% respectively) at 24 hours (Table 2a). Chen and Hyuang (2010) reported that the culture filtrates of *Lentinula edodes* and *Clitocybe nuda* completely inhibited the spore germination of *Colletotrichum higginsianum.* Also, culture filtrates of *Ganoderma lucidum* inhibited spore germination of *Alternaria brassicicola* and culture filtrates of *Coprinus comatus, L. edodes*, *Tremella aurantialba* and *C. nuda* suppressed the germination of *Phytophthora capsici.*

The agar well diffusion of solvent extracted constituents of cell free culture filtrates of *G. lucidum*, *L. edodes* and *A. polytricha* (Table 2b) showed that all the metabolites extracted from all the three solvents (Chloroform, Diethyl ether and Ethyl acetate) at 500 ppm exhibited inhibition of mycelial growth of *C. capsici.* Variations in inhibition of mycelial growth were observed among different solvent extracted metabolites against *C. capsici.* In the present study, among the different solvents, Chloroform extracted metabolites of *G. lucidum* recorded 40 per cent inhibition of mycelial growth of *C. capsici* followed by Diethyl ether (34.07 % inhibition) and Ethyl acetate fractions (29.25% inhibition). In the case of *L. edodes*, Chloroform fractions exhibited 36 per cent inhibition of mycelial growth followed by Ethyl acetate (32.96%) and Diethyl ether fractions (31 per cent). The Ethyl acetate fractions followed by Diethyl ether fractions

of *A. polytricha* recorded 32.96 and 30 per cent inhibition of mycelial growth respectively followed by Chloroform fractions (22 per cent inhibition). From the study, it is observed that chloroform extracted fractions followed by Diethyl ether and Ethyl acetate fractions of *G. lucidum* showed maximum inhibition of mycelial growth of *C. capsici* with 40 %, 34.07% and 29.25 % respectively when compared to other solvent extracted fractions of *L. edodes* and *A. polytricha* which clearly shows the antimicrobial nature of metabolites from *G. lucidum*. Widest inhibitory zone (33mm) was obtained with acetone extract from mycelium of *Ganoderma lucidum* against *Pseudomonas aeruginosa* (Sheetal Mehta and Savitha Jandaik, 2012).

Variations in antimicrobial activity of *G. lucidum* extracts was observed in different solvent fractions and it is explained that the reason for the differences in their antimicrobial effectiveness may be due to the differences in the molecular weight of the compounds or due to the genetic makeup of the test organisms (Uma Gowrie *et al*.,2014; Gebreyohannes *et.al*., 2019). Moreover the *Ganoderma* compounds identified are mostly Triterpenes (lanostanoid-type triterpene and polyketides (Farnesyl quinone), small peptides (ganodermin) and polysaccharides with antimicrobial properties (Wang and Ng, 2006; Zhang *et al.,* 2015; Basnet *et al.,* 2017). The antifungal agent phellinsin A from *Phellinus* sp. inhibited the growth of *Colletotrichum lagenarium, Pyricularia grisea, Rhizoctonia solani, Aspergillus fumigatus* and *Trichophyton mentagrophytes* (Hwang *et al.,* 2000; Chowdhary *et al.,* 2015). Antibacterial activity of *L. edodes* against Gram negative and Gram positive bacteria *viz., Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Salmonella typhi* has been reported (Ishikawa *et al.,* 2001; Komemushi *et al.,* 1996; Quereshi *et al.,* 2010). In some other studies, crude methanolic extract (100 ppm) from *Clitocybe* sp, *Boletus affinis* var. *maculosus* would exhibited maximum inhibition against *Colletotrichum coffaenum* (89.08 per cent and 76.69 per cent) followed by *Leucocoprinus fragilissimus, Collybia strictipes* and *Lactarius* sp(Shahid *et al.,* 2016)

Based on the above studies, *G. lucidum* possessing maximum antimicrobial activity with respect of inhibition of spore germination and mycelial growth inhibition was taken for further studies.

Testing different concentrations of solvent extracted metabolites of *G. lucidum* against *C. capsici*

The antimicrobial metabolites of *G. lucidum* were extracted using different solvents (chloroform, diethyl ether, ethyl acetate) were made up to different concentrations of 1000,1500 and 2000 ppm to test the desired concentration that could inhibit maximum mycelial growth of *C. capsici*. From the results (Table 3; Plate 2) it is observed that all the solvent extracted antimicrobial metabolites of *G. lucidum* at 1000 ppm, 1500 ppm and 2000 ppm exhibited mycelial growth inhibition of *C. capsici* ranging (43.75 per cent to 60.55 per cent). The Chloroform extracted metabolite of *G. lucidum* (60.55 %) followed by Ethyl acetate and Diethyl ether (58.88 % and 55.47 % respectively) fractions at 2000 ppm inhibited maximum mycelial growth of *C. capsici*. The chloroform extract of *Hygrophorus agathosmus* and the dichloromethane extract of *Suillus collitinus* were the most active extracts against both yeast and bacteria (Yamac and Fatma Bilgili, 2006). The chloroform extract (100 µl concentration) of *G. lucidum* basidiocarp showed antibacterial activity against *S. typhi* with inhibition zone of 18mm and antifungal

activity against *C. albicans* with inhibition zone of 17mm (Uma Gowrie *et al*., 2014). Ethyl acetate was found to be the best solvent for extracting antimicrobial substances from *L. edodes*, *A. polytricha* and *V. volvaceae* which showed inhibition of mycelial growth of *Alternaria solani*, *Colletotrichum capsici*, *Phytopthora* and *Rhizoctonia solani* (Radhajeyalakshmi *et al.*, 2011). Also ethyl acetate fractions from fruiting body and CFC filtrate condensate of *Pisolithus albus* exhibited antifungal activities against *Fusarium oxysporum.* f. sp. *lycopersici*, *Macrophomina phaseolina* and *Rhizoconia solani* (Ganeshkumar and Krishnamoorthy, 2014). The fruiting body, mycelia and spores of *G. lucidum* contain ganoderic acid, polysaccharides, triterpenoids, fatty acids, nucleotides, protein, peptides, sterols (Yoon *et al.*, 1994; Mizuno *et al.*, 1995; Kim *et al.*, 1999; Uma Gowrie *et al.*, 2014) which account for more than 400 bioactive compounds.

Table.1 Screening the antagonistic activity of mushroom fungi against *C. capsici* by dual culture technique

Treatment	*Colletotrichum capsici* Average radial mycelial growth (mm)	Mushroom fungal growth (mm)	Inhibition zone (mm)	%inhibition over control
Ganoderma lucidum	40.67^{ab} (39.58)	44.40^{c} (41.78)	4.86	54.81^{ab} (47.76)
Auricularia polytricha	41.67^{b} (40.16)	45.33^{c} (42.32)	2.76	53.70^{b} (47.12)
Lentinus edodes	49.00^{cd} (44.43)	36.00^{a} (36.87)	4.86	45.55^{cd} (42.45)
Pycnoporus sanguineus	38.33^{a} (38.23)	51.70^{d} (45.97)	0	57.41^{a} (49.26)
Coprinus sinensis	54.00^{e} (47.29)	36.00^{a} (36.87)	0	40.00^{e} (39.23)
Schizophyllum commune	47.00^{c} (43.28)	39.00^{b} (38.65)	0	47.78^{c} (43.73)
Volvariella volvaceae	51.37^{de} (45.74)	35.70^{a} (36.69)	2.83	42.92^{de} (40.93)
Trametes versicolor	50.73^{de} (45.40)	38.30^{b} (38.23)	1.10	43.63^{de} (41.34)
Control	90.00^{f} (71.57)	-	-	0.00^{f} (0.57)
SEd	2.9511	0.5156		
CD (p=0.05)	1.4047	1.0930		

Values are the mean of three replications. Means followed by a common letter are not significantly different at 5%level by DMRT.Values in parenthesis are arcsine transformed values

Table.2a Effect of solvent fractions of culture filtrates of selected mushroom fungi on spore germination of *C. capsici*

Mushroom fungi	Solvent extracted metabolites																	
	Ethyl acetate						Chloroform						Diethyl ether					
	6 hours		12 hours		24 hours		6 hours		12 hours		24 hours		6hours		12 hours		24 hours	
	SG	PI	SG	PI	SG	PI	SG	PI	SG	PI	SG	PI	SG	PI	SG	PI	SG	PI
Ganoderma lucidum	-	100[a] (89.19)	29.75[a] (33.05)	70.25[a] (56.95)	22.00[a] (27.97)	78.0[a] (62.03)	-	100[a] (89.19)	15.00[a] (22.79)	85.0[a] (67.21)	12.0[a] (20.27)	88.0[a] (69.73)	-	100[a] (89.19)	26.25[a] (30.82)	73.75[a] (59.18)	21.25[a] (27.45)	79.0[a] (62.73)
Auricularia polytricha	-	100[a] (89.19)	44.25[b] (41.70)	55.75[b] (48.30)	33.75[b] (35.52)	66.25[b] (54.48)	-	100[a] (89.19)	47.25[c] (43.42)	52.75[c] (46.58)	40.5[c] (39.52)	59.5[c] (50.48)	-	100[a] (89.19)	47.5[b] (43.57)	52.5[b] (46.43)	41.00[c] (39.82)	59.0[c] (50.18)
Lentinus edodes	-	100[a] (89.19)	43.5[b] (41.27)	56.5[b] (48.73)	34.75[b] (36.12)	65.25[b] (53.88)	-	100[a] (89.19)	42.25[b] (40.54)	57.75[b] (49.46)	32.75[b] (34.91)	67.25[b] (55.09)	-	100[a] (89.19)	46.75[b] (43.14)	53.25[b] (46.86)	35.75[b] (36.72)	64.25[b] (53.28)
Control	-	100[a] (89.19)	54.0[c] (47.29)	46.0[c] (42.71)	83.0[d] (65.65)	17.0[d] (24.35)	-	100[a] (89.19)	54.0[d] (47.29)	46.0[d] (42.71)	83.0[e] (65.65)	17.0[e] (24.35)	-	100[a] (89.19)	54.0[c] (47.29)	46.0[c] (42.71)	83.0[e] (65.65)	17.0[e] (24.35)
Control (without metabolite)	-	100[a] (89.19)	47.0[b] (43.28)	53.0[b] (46.72)	77.0[c] (61.34)	23.0[c] (28.66)	-	100[a] (89.19)	47.0[c] (43.28)	53.0[c] (46.72)	77.0[d] (61.34)	23.0[d] (28.66)	-	100[a] (89.19)	47.0[b] (43.28)	53.0[b] (46.72)	77.0[d] (61.34)	23.0[d] (28.66)
SEd	-		1.7369		1.4720		-		6.5256		1.0288		-		1.2179		1.0878	
CD (p=0.05)	-		3.7021		3.1374		-		13.9091		2.1928		-		2.5960		2.3186	

Where, SG- No. of spores germinated, PI- Percent inhibition of spore germination. Values are the mean of four replications. Means followed by a common letter are not significantly different at 5%level by DMRT. Values in parenthesis are arcsine transformed values

Table.2b Antimicrobial activity of solvent extracted metabolites of selected mushroom fungi against *C. capsici* by agar well diffusion technique

Mushroom fungi	Solvents used	Average mycelial growth (mm)	%inhibition over control
Ganoderma lucidum	Ethyl acetate	63.67^{ef} (52.93)	29.25^{ef} (32.74)
	Chloroform	54.00^{a} (47.29)	40.00^{a} (39.23)
	Diethyl ether	59.33^{c} (50.38)	34.07^{c} (35.71)
Lentinus edodes	Ethyl acetate	61.00^{cde} (51.35)	32.00^{cde} (34.45)
	Chloroform	57.00^{b} (49.02)	36.00^{b} (36.87)
	Diethyl ether	62.00^{def} (51.94)	31.00^{def} (33.83)
Auricularia polytricha	Ethyl acetate	60.33^{cd} (50.96)	32.96^{cd} (35.04)
	Chloroform	70.00^{g} (56.79)	22.00^{g} (27.97)
	Diethyl ether	63.00^{ef} (52.54)	30.00^{ef} (33.21)
Control		90.00^{h} (71.57)	0.00^{h} (0.51)
SEd		0.9661	
CD (p=0.05)		2.0152	

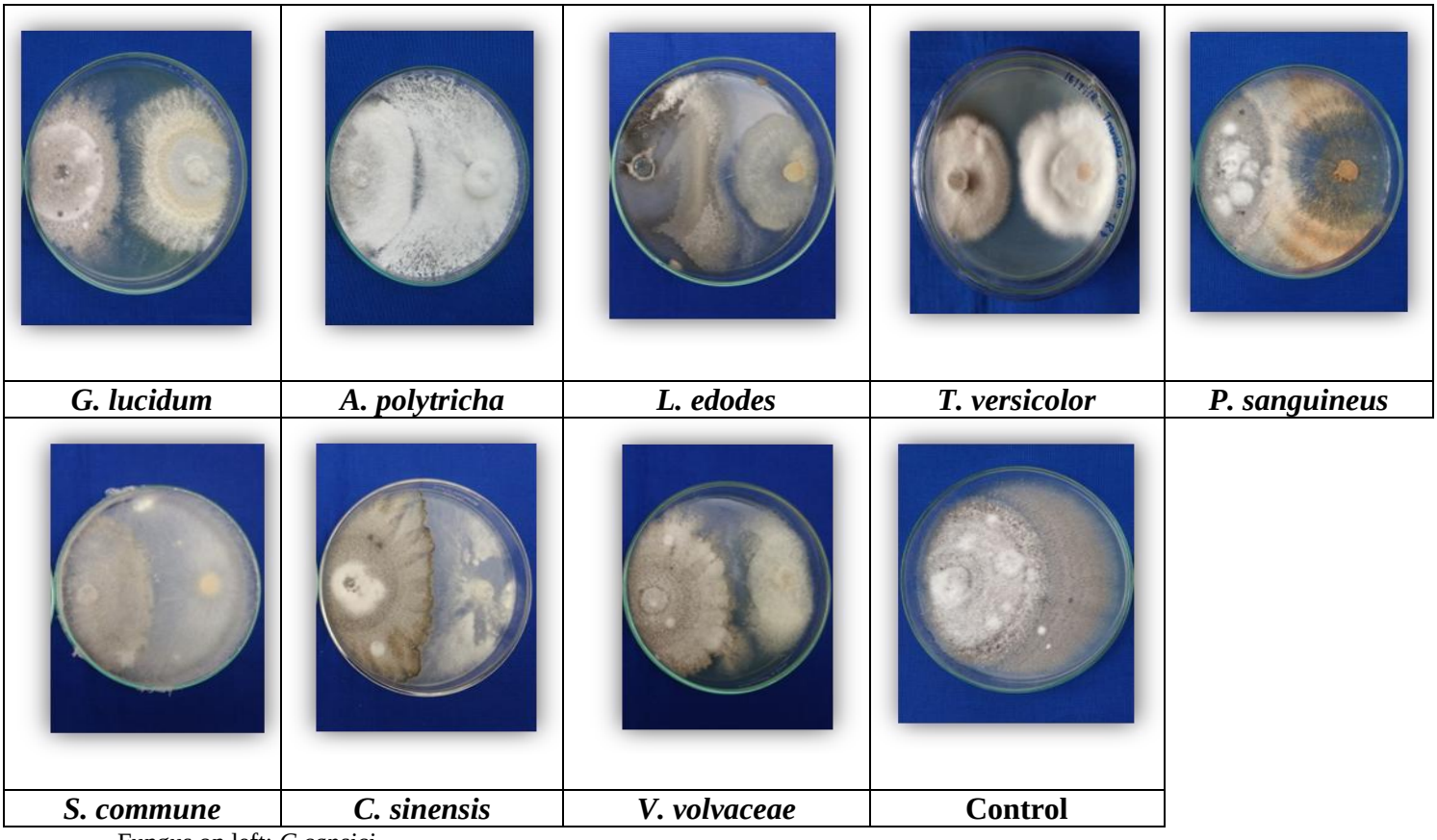
Values are the mean of three replications
Means followed by a common letter are not significantly different at 5%level by DMRT
Values in parenthesis are arcsine transformed values

Table.3 Testing the different concentrations of solvent extracted metabolites of *G. lucidum* against *C. capsici*

Treatment	Solvent	Concentation (ppm)	Average mycelial growth(mm)	%inhibition over control
Ganoderma lucidum	Ethyl acetate	1000	50.62^{f}(45.36)	43.75^{f} (41.41)
		1500	48.07^{e} (43.89)	46.58^{e} (43.04)
		2000	37.00^{b} (37.46)	58.88^{b} (50.11)
	Chloroform	1000	45.10^{d} (42.19)	49.88^{d} (44.93)
		1500	40.17^{c} (39.33)	55.36^{c} (48.08)
		2000	35.52^{a} (36.58)	60.55^{a} (51.09)
	Diethyl ether	1000	50.15^{f} (45.09)	44.27^{f} (41.71)
		1500	44.50^{d} (41.84)	50.55^{d} (45.32)
		2000	40.07^{c} (39.27)	55.47^{c} (48.14)
Control			90.00^{g} (71.57)	0.00^{g} (0.44)
SEd			0.5104	
CD (p=0.05)			1.0425	

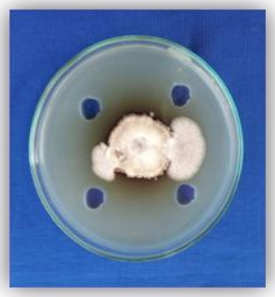
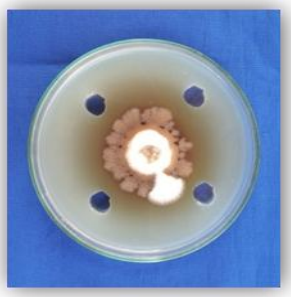
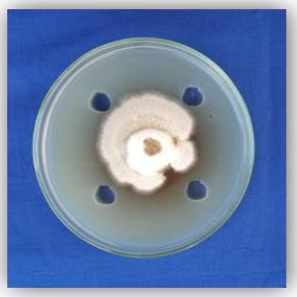
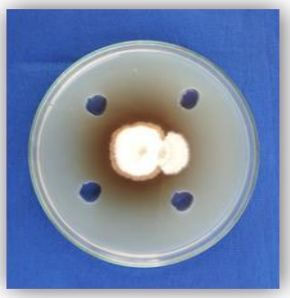
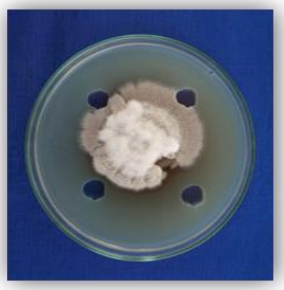
Values are the mean of four replications. Means followed by a common letter are not significantly different at 5%level by DMRT. Values in parenthesis are arcsine transformed values

Plate.1 *Invitro* effect of antagonistic activity of mushroom fungi against *C. capsici* by dual culture technique



Fungus on left: *C.capsici*
Fungus on right: Mushroom fungus

Plate.2 Antimicrobial activity of different solvent extracted fractions of Cell free culture filtrates of *G. lucidum* against *C. capsici*

Ethyl acetate			
	1000 ppm	**1500 ppm**	**2000 ppm**
Chloroform			
	1000 ppm	**1500 ppm**	**2000 ppm**
Diethyl ether			
	1000 ppm	**1500 ppm**	**2000 ppm**
			
		Control	

Since, the chloroform extracted antimicrobial metabolites of *G. lucidum* at 2000 ppm showed maximum antimicrobial activity as evidenced by the above results, further work proceeded with higher concentrations using chloroform as solvent that could show maximum mycelial growth inhibition.

Globally research is focused on the identification of antimicrobial molecules from fungi, bacteria and plants to manage plant diseases in a green channel system to mitigate the environmental hazards and pollution by indiscriminate use of fungicides. This study implies that all the mushroom fungi screened against *C. capsici* possessed antagonistic activity either with formation of inhibition zone, thick mat of mycelium and hyperparasitisation. Among these, the macrofungi *Ganoderma lucidum* with maximum secretion of bioactive compounds belonging to several chemical groups needs to be identified and has great scope for developing fungicides against broad group of plant pathogens.

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