

Original Research Article

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Harnessing the Antimicrobial Prospects of Mushroom Fungi against *Colletotrichum gloeosporioides* Penz. Causing Post-Harvest Anthracnose Disease of Mango

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ABSTRACT

Mushrooms contain various bio active potential including antifungal, antiviral and antibacterial property. But very limited work has been done on the productive utilization of the antimicrobial properties for plant disease management practices. The current study is formulated to harness the antimicrobial potential of *Ganoderma lucidum*, *Auricularia polytricha*, *Lentinus edodes*, *Coprinus sinensis*, *Schizophyllum commune*, *Pleurotus florida*, *Trichloma mastukae*, *Calocybe indica*, *Volvariella volvacea*, *Fomes* spp and *Pycnoporus sanguineus* against *Colletotrichum gloeosporioides* causing post-harvest anthracnose disease of mango. Results from dual culture test revealed that the *Ganoderma lucidum*, *Auricularia polytricha*, *Pycnoporus sanguineus* and *Pleurotus florida* showed inhibition of radial mycelial growth with inhibition percentage of 56.3 to 70.77 %. Although the mushrooms screened exhibited varied degree of inhibition of mycelial growth of pathogen, *A. polytricha* and *G.lucidum* performed well and the crude cell free culture filtrates from *G. lucidum* and *A. polytricha* tested at 20th day showed maximum inhibition of 36.33% and 47.11% of mycelial growth of *C.gloeosporioides* by *G. lucidum* and *A. polytricha* respectively. These results predicted that the cell free culture filtrate collected contained some antimicrobial compounds that would have been responsible for the antimicrobial effect and offers better scope for development of mycomolecules based fungicide against plant diseases.

Keywords

Mango anthracnose,
Mushroom fungi,
Inhibition percent,
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Introduction

Mango is one the well-known ancient fruit in the world known for its high nutritive value, superb flavour, delicious taste. Though this fruit crop is affected by fungi, bacteria and phytoplasma, Mango anthracnose caused by *Colletotrichum gloeosporioides* Penz being the most important disease leads to severe yield loss due to the pre harvest and post harvest infection even up to 100 per cent (Jha

et al., 2010; Arauz, 2000). Anthracnose disease causes 30-60% yield losses on mango across different countries of the world causing both qualitative and quantitative losses (Shad *et al.*, 2002; Akem, 2006; Chowdhury and Rahim, 2009, Lakshmi *et al.*, 2011). Under the current scenario, continuous and judicious application of fungicide against the pathogen has resulted in loss of effectiveness of fungicide due to buildup of fungicide resistant pathogen apart from creating environmental

pollution and health problem. As an alternative, scientists have started probing for antimicrobial bio active compounds from natural source to overcome the current situation. In that context, mushrooms are in the lime light and evoked interest globally for their bioactive compounds that finds application in pharmaceutical and therapeutic values apart from their nutritive value (Poucheret *et al.*, 2006). Mushroom contain various natural compounds with bio active potentials as antifungal, antibacterial, antiviral, antitumor, antinemic, anti-inflammatory, anti-allergic, anti-antherogenic, antidiabetic properties(Hatvani,2001;Wasser 2002; Lindequist *et al.*, 2005; Quang *et al.*, 2006). Research is focused towards exploitation of antimicrobial compounds from mushrooms against plant pathogens as is evidenced by the effect of culture filtrates of *Lentinula edodes* and *Clitocybe nuda* against *Colletotrichum higginsianum* (Chen and Huang, 2010), *Ganoderma lucidum* against *Colletotrichum capsici* (Priya and Thiribhuvanamala, 2019) and *Coprinus comatus* against *Fusarium oxysporum f.sp. lycopersici* and *F.oxysporum f.sp.cubense* (Jeeva and Krishnamoorthy, 2018). Perusal of literature shows no reports on the antimicrobials from mushrooms against post-harvest pathogens. Currently there is increasing attention to derive safe antimicrobials for post-harvest treatments to mitigate the residual issues in fruits and vegetables. In that context, the present study was attempted to test different mushrooms against mango post-harvest anthracnose pathogen *Colletotrichum gloeosporioides*.

Materials and Methods

The mango anthracnose pathogen *Colletotrichum gloeosporioides* and the pure cultures of mushrooms viz *Ganoderma lucidum*, *Auricularia polytricha*, *Lentinus edodes*, *Coprinus sinensis*, *Schizophyllum*

commune, *Fomes spp*, *Trichloma mastukae*, *Calocybe indica*, *Volvariella volvacea*, *Pleurotus florida* and *Pycnoporus sanguineus* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore.

Screening of antagonistic potential of mushroom fungi under *in vitro*

Mycelial cultures of mushrooms viz., *Volvariella volvacea*, *Pleurotus florida*, *Ganoderma lucidum*, *Auricularia polytricha*, *Lentinus edodes*, *Coprinus sinensis*, *Schizophyllum commune*, *Calocybe indica*, *Trichloma mastukae*, *Fomes spp* and *Pycnoporus sanguineus* were screened for their antagonistic potential against *Colletotrichum gloeosporioides* causing post harvest anthracnose disease of mango by dual culture technique (Dennis and Webster, 1971).

The PDA medium was prepared and poured into Petri dish. A 9 mm mycelial disc of the mushroom fungi were placed at one side of Petri dish and similarly test pathogen was placed at opposite side of the same Petri dish. The pathogen were inoculated separately which served as control. Three replications were maintained for each treatment. The Petri dishes were incubated at $28 \pm 2^{\circ}\text{C}$. Periodical observations on the mycelial growth, pattern and their antagonistic behaviour were done. The percentage inhibition of mycelial growth in pathogen over control was calculated by using the formula (Vincent, 1947).

$$\text{Per cent inhibition over control (\%)} = \frac{C - T}{C} \times 100$$

Where, C is the radial mycelial growth of the pathogen (mm) in control

T is the radial mycelial growth of the pathogen (mm) in dual culture plate.

Testing the efficacy of crude culture filtrates of mushroom fungi against *Colletotrichum gloeosporioides*

Extraction of crude culture filtrates from mycelium of mushroom fungi

Based on results obtained from the preliminary antagonistic screening of mushroom fungi against *C.gloeosporioides*, the mushrooms *Auricularia polytricha* and *Ganoderma lucidum* were shown to be effective against *Colletotrichum gloeosporioides* as shown by the inhibition of mycelial growth of pathogen. The mycelial disc (9mm) from 10 days old culture of *Auricularia polytricha* and *Ganoderma lucidum* were placed into 250 ml conical flask containing sterilized Potato Dextrose (PD) broth. The flasks were kept in incubator cum shaker at 25°C with agitation at 150 rpm. After incubation, the mycelial mat was separated from broth using Whatman filter paper No 1. Then the culture filtrate was centrifuged at 10,000 rpm for 10 mins. The supernatant was then filtered through the membrane filter (0.2 µm) to avoid bacterial contamination. This extract is used as cell free crude culture filtrates. The crude culture filtrate were collected from *Auricularia polytricha* and *Ganoderma lucidum* at various periodical intervals viz., 10th, 15th, 20th and 25th days of inoculation and tested for mycelial inhibition studies.

Mycelial inhibition test

The crude culture filtrate of *G. lucidum* and *A. polytricha* extracted at various periodical intervals (10th, 15th, 20th, 25 days) were tested against the mycelial growth of *C.gloeosporioides* by agar well diffusion technique (Stokes and Ridgway, 1980). The PDA medium was poured into the sterilized plates and allowed to solidify. After solidification, four wells were made at equal

distance leaving 1cm space from edge of the plate using sterilized corkborer. A 100 µl of crude culture filtrate of *G.lucidum* and *A.polytricha* (separately) was pipetted and poured into the well. Using sterilized cork borer, a 9 mm mycelial disc of *C. gloeosporioides* from 10 days old culture was placed at the centre of the plates and incubated at room temperature (28 ± 2°C). Three replications maintained for each treatment. Sterile water served as control instead of using culture filtrate. The percentage inhibition was calculated by using the formula (Vincent 1947).

Results and Discussion

The results obtained from this study indicated that mushrooms screened showed various degrees of antagonistic activity against *C.gloeosporioides* and it proved the earlier success of derivation of Azoxystrobin from *Strobilurus tenacellus* against downy mildew and powdery mildew diseases of grapes. In our study, among the mushrooms screened, the mycelium of *Auricularia polytricha*, *Ganoderma lucidum*, *Pycnoporus sanguineus* and *Pleurotus florida* inhibited the mycelial growth of *Colletotrichum gloeosporioides* (26.3mm, 36.6mm, 39.6mm and 39.3mm respectively) as observed by inhibition per cent of 70.77, 59.33, 56.00 and 56.33 respectively. Other mushrooms, *Fomes spp*, *Volvariella volvacea*, *Schizophyllum commune*, *Coprinus sinensis*, *Calocybe indica* and *Tricholoma matsukae* also showed mycelial growth inhibition of 50.44%, 41.11%, 51.88%, 50.00%, 22.11%, 22.66% respectively against the test pathogen *Colletotrichum gloeosporioides* (Fig.1).

As in our study, Priya *et al.*, (2018) reported that the antagonistic activity of *Ganoderma lucidum* followed by *Auricularia polytricha* against *Colletotrichum capsici*. Similarly, Badalyan *et al.*, (2014) reported that the

mushrooms *Ganoderma lucidum*, *Pleurotus ostreatus*, *Hypholoma fasciculare*, *Lentinus tigrinus* exhibited various antimicrobial activity against *Cochliobolus sativus*, *Fusarium culmorum*, *Gaeumannomyces graminis* and *Rhizoctonia cerealis*. The interactions between the pathogen *Colletotrichum gloeosporioides* and the mushroom were documented from the dual culture assay. Plates are given in Table-1. Antagonistic interactions between different mushroom fungi and plant pathogenic fungi in dual cultures and various types of

competitive interactions viz., over growth of pathogen, inhibition at mycelial contact, inhibition of mycelia at distance and partial or complete replacement of plant pathogenic fungi were reported by Badalyan, 2002; Jeeva and Krishnamoorthy, 2018; Priya *et.al.*, 2019). This clearly shows that the interactions could be due to certain metabolites that would be responsible for the retardation of *C. gloeosporioides*. These results paved way to exploit antimicrobial metabolites from potential mushrooms *G.lucidum* and *A.polytricha* against *C. gloeosporioides*.

Table.1 Interaction between mushroom fungi and *C.gloeosporioides*

Mushroom fungi with <i>C.gloeosporioides</i>	Nature of interaction
<i>Ganoderma lucidum</i>	Pathogen growth was retarded and pushed back.
<i>Auricularia polytricha</i>	Formation of light yellowish green pigment at the interaction zone
<i>Lentinus edodes</i>	Clear zone of inhibition were formed ;both the mushroom and pathogen did not grow each other
<i>Fomes</i> spp	Thick mat of hyphae were formed at zonal point and later pathogen gets hyper parasitized by the mushroom fungi
<i>Volvariella volvacea</i>	The mushroom fungi hyperparasitised over the pathogen but no inhibition zone
<i>Pycnoporus sanguineus</i>	No inhibition zone. A very thin zone were formed at contact point
<i>Pleurotus florida</i>	No inhibition zone ;no hyperparasitization;both the mushroom and the pathogen growth ceases at the point of interaction
<i>Schizophyllum commune</i>	Thick mat of mycelial hyphae were formed at the zonal point and further growth of pathogen gets retarded
<i>Coprinus sinensis</i>	No inhibition zone. Both fungi did not grow each other
<i>Calocybe indica</i>	Linear mycelial growth of the pathogen near the mushroom fungi
<i>Tricholoma matsukae</i>	Pathogen grow over the mushroom fungi

Table.2 Testing the efficacy of crude culture filtrate of *G.lucidum* and *A.polytricha* against *C.gloeosporioides*

Days interval	<i>G.lucidum</i>		<i>A.polytricha</i>	
	Mean mycelial of the pathogen Growth	% inhibition over control	Mean mycelial of the pathogen Growth	% inhibition over control
10 th day	90.00 ^b (71.61)	0.00	90.00 ^c (71.61)	0.00
15 th day	58.66 ^a (49.96)	34.88 ^{ab} (36.18)	52.33 ^b (46.31)	41.88 ^b (40.31)
20 th day	57.33 ^a (49.19)	36.33 ^a (37.05)	47.60 ^a (43.60)	47.11 ^a (43.32)
25 th day	59.33 ^a (50.35)	34.11 ^b (35.71)	51.33 ^b (4.74)	43.00 ^b (40.95)
Control	90.00 (71.61)	00	90.00 (71.61)	0.00 ^d
SEd	1.265	-	1.235	-
CD (p=0.05)	2.854	-	2.787	-

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.

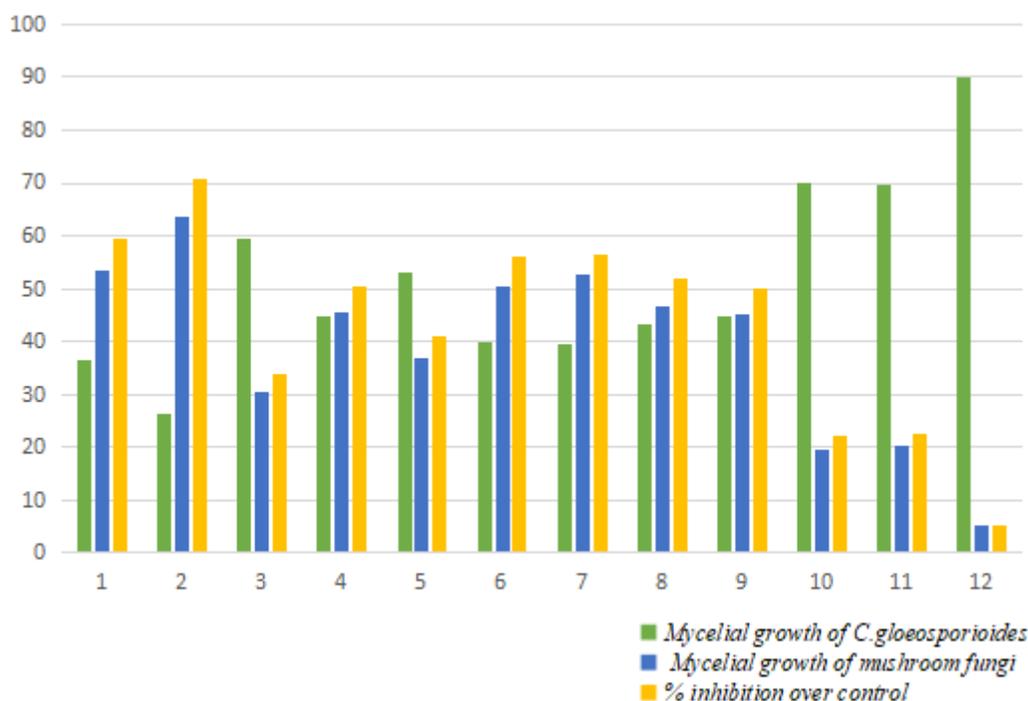


Fig.1 Screening the antagonistic activity of mushroom fungi against *C.gloeosporioides* by dual culture assay

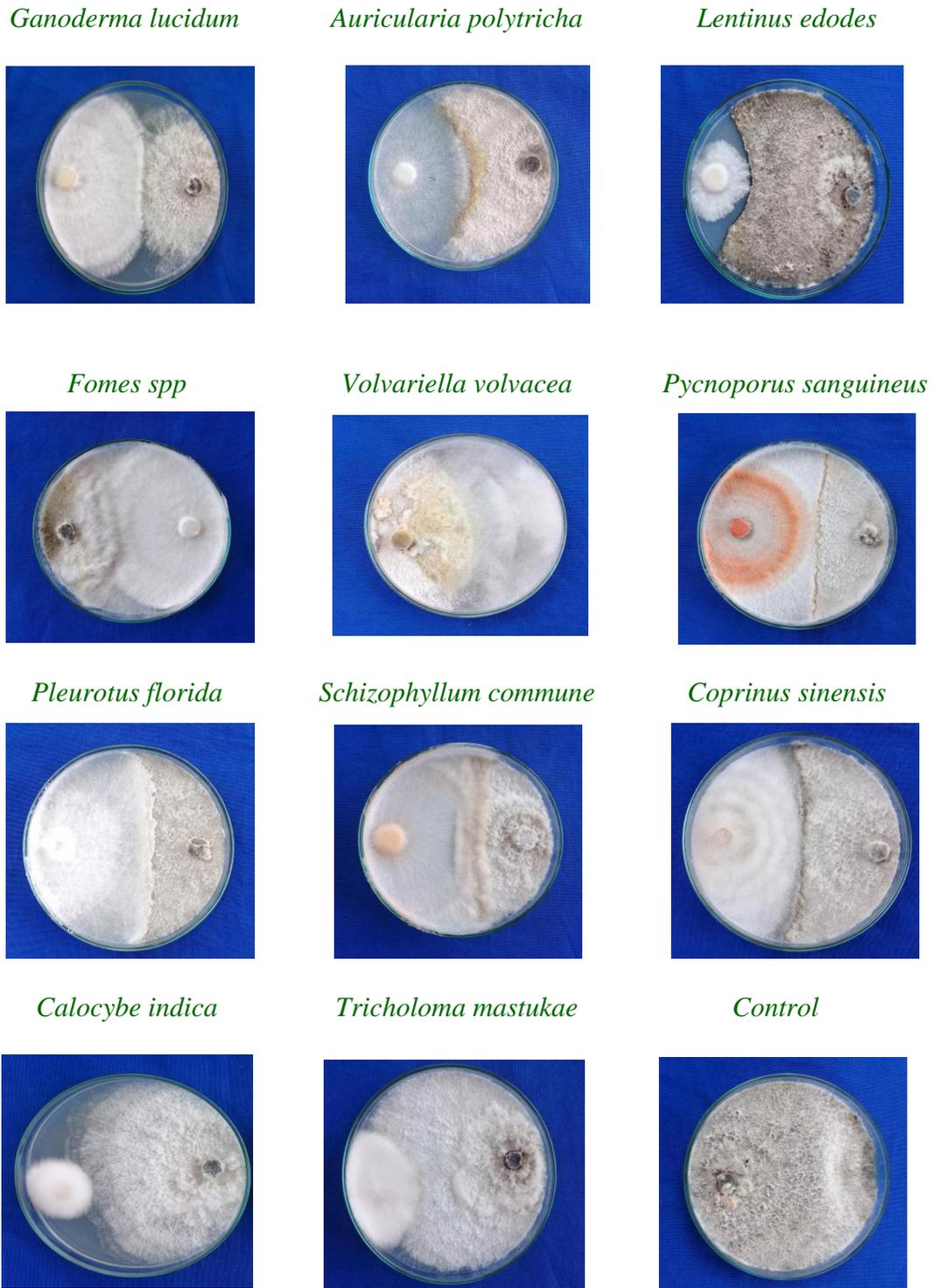


Plate.1 *In vitro* effect of antagonistic activity of mushroom fungi against *Colletotrichum gloesporioides* by dual culture

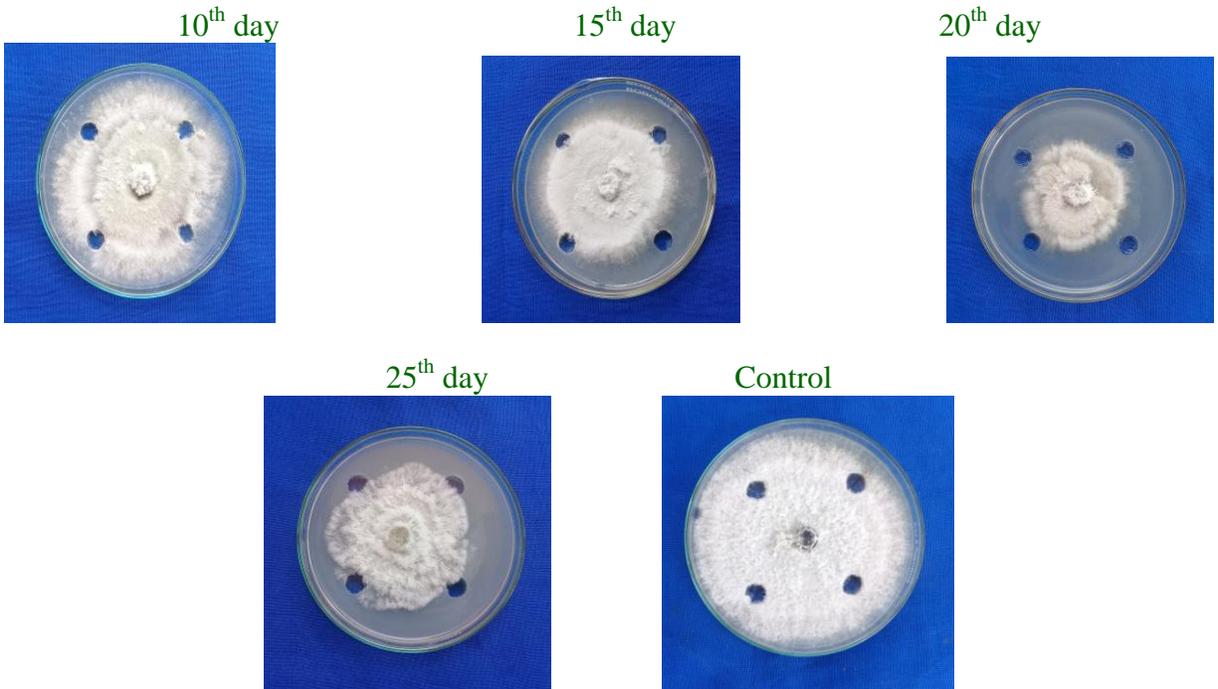


Plate.2a Antimicrobial activity crude culture filtrate of *Ganoderma lucidum* and against *Colletotrichum gloeosporioides*

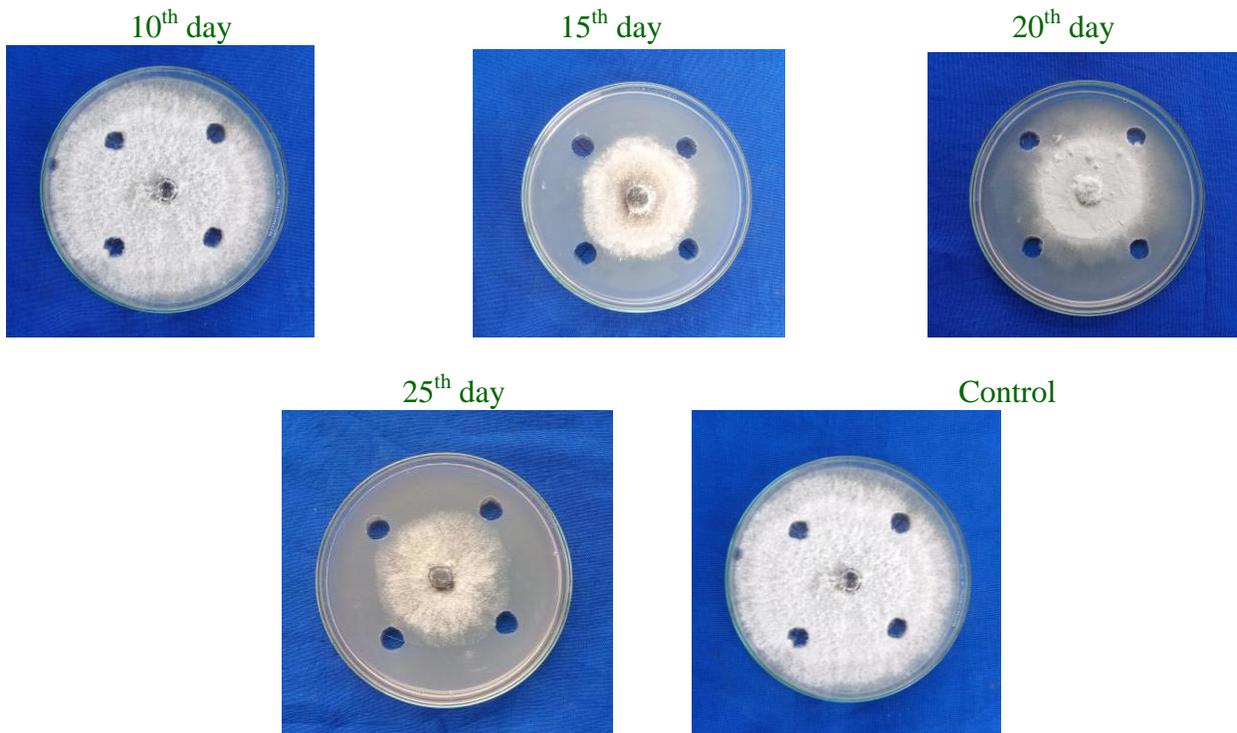


Plate.2b Antimicrobial activity crude culture filtrate of *Auricularia polytricha* against *Colletotrichum gloeosporioides*

Testing the efficacy of crude culture filtrate from potential mushroom fungi *Ganoderma lucidum* and *Auricularia polytricha* against *C.gloeosporioides*

Based on the maximum inhibition per cent and interaction studies the culture filtrates of *G.lucidum* and *A. polytricha* collected at various periodical interval 10th, 15th, 20th and 25th days were tested to exploit the antimicrobial metabolites against *C.gloeosporioides*. Results indicated that the crude culture filtrate collected on 10th day did not exhibit any antifungal effect against any the pathogen tested. The culture filtrate collected from 20th day showed maximum mycelial inhibition of 36.33 % and 47.11% of *C.gloeosporioides* with respect to *G. lucidum* and *A. polytricha* respectively (Table 2; Plate 2 a and b).

From the findings, it is concluded that antagonistic effect of mushroom fungi by dual culture assay may offer an indication for the presence of antimicrobial metabolites and such tests will be useful to screen large number of samples. In our study, the mushroom *G. lucidum* and *A.polytricha* secreted maximal production of antifungal metabolites on 20th day and contributed for maximum inhibition of mycelial growth of *C.gloeosporioides*.

Globally research is focused on the development of newer fungicide through approaches to manage the plant diseases to mitigate the pesticide residues in food channel. This study further implies that the mushroom fungi screened against *C.gloeosporioides* possess antagonistic activity with various interactions such as formation of inhibition zone, thick mat of mycelium and hyper parasitic activity. Among them the need for identification of bio active compounds from the *G. lucidum* and *A. polytricha* is required for developing

fungicide against broad spectrum activity. The study paves way and kindles interest for identification of the antimicrobial compound in *A. polytricha* and *G. lucidum* responsible for inhibition of pathogen growth.

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