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Antimicrobial potentiality of *G. lucidum* isolate against plant pathogens

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Abstract
Ganoderma is a medicinal mushroom with the longest record of medicinal use which offers a wide variety of bioactive compounds that possess significant antimicrobial and antioxidant activities. Currently, it has gained a great attention throughout the world. It is widely distributed and shelf or knob like fungi that feed either as saprobes on dead wood or as parasites on the wood of hardwood trees, conifers or palms and found all around the world. The diverse climatic condition in India made the country a natural habitat for *Ganoderma*. The current studies deal with with microbial activity of *G. lucidum* were tested against different pathogens by dual culture technique and well diffusion method. In dual culture technique maximum percent growth inhibition was recorded against *Fusarium moniliforme* that was 74.99%. In well diffusion method three different solvents like ethanol, di ethyl ether and chloroform were used with different concentrations 250 and 500 ppm. Maximum zone of inhibition (3.2 cm) was recorded in chloroform extract at 500 ppm against *Aspergillus niger*.

Keywords: *G. lucidum*, anti-microbial activity, dual culture, well diffusion

Introduction
Among all the medicinal mushrooms, currently *Ganoderma* is capturing a sound attention in the world market. Also it is a mushroom with the longest record of medicinal use. It is a basidiomycete which has been priced for its therapeutic values for centuries particularly in the Asian countries. The genus comprises of a large and diverse complex of fungi, many of which are wood rotters and others that are pathogenic on economically important trees and perennial crops (Jandaik *et al*., 2010) [4]. The genus *Ganoderma* was established by Finnish mycologist Peter Adolf Karsten in 1881 with *G. lucidum* (Curtis: Fr.) P. Karst from England as the type species. The generic name *Ganoderma* derives from the Greek word ganos means "brightness; sheen", hence "shining" and derma means "skin". Due to its capability of medication among various diverse infections, it is acknowledged as like "Elixir of life", "Food of Gods", "Mushroom of the Universe" and has been revered as "the mushroom of immortality". This mushroom belongs to family Ganodermataceae having double-called basidiospores. Many species of genus *Ganoderma* are reported to possess medicinal properties. They include *G. lucidum*, *G. tropicum*, *G. boninense*, *G. capense*, *G. japonicum* and *G. applanatum*. Amongst all, *G. lucidum* is believed to have maximum number of therapeutic properties (Chen *et al*., 2017) [2]. It grows on hardwood like plum, mulberry, chestnut and Japanese apricot trees and is widely distributed in Asian countries including India.

It is an edible medicinal mushroom that has been used for centuries in Traditional Chinese Medicine (TCM) for its health-promoting properties. Fruiting bodies of *G. lucidum* are quite tough and bitter in taste, for which the special attention was paid for its medicinal properties rather than its value as a source of food. Fruiting bodies, mycelia, and spores of it accumulate a variety of bioactive metabolites with immunomodulatory, cardiovascular, liver protective, antifibrotic, anti-inflammatory, antidiabetic, antiviral, antioxidant, antitumor, and antimicrobial properties. All these pharmacological properties are correlated to a large pool of bioactive compounds produced by fruit bodies, mycelia and spores of medicinal *Ganoderma*. Bioactive compounds mainly include polysaccharides, triterpenoids, fatty acids, nucleotides, protein/ peptides, sterols, vitamins and minerals etc., each having their own outstanding medicinal effects.

Ganoderma-based products have attracted a great deal of attention during the last decade in Europe, Malaysia, North America and Singapore. China, Japan and Korea are the main producers and suppliers of *Ganoderma-based* products. In India, *Ganoderma* based nutraceuticals are growing very rapidly and was estimated to be about US $ 20.00 million in

2000-2001 (Thakur, 2005) [9]. In India, annual market for Ganoderma-based nutraceuticals is estimated to be about Rs. 120 crores. The fruit body of *G. lucidum* is sold in the market @ Rs 600-700/kg (Kaliyaperumal and Kalaichelvan, 2007) [5]. The China Edible Fungi Association recorded that *Ganoderma* production in China was 36700 and 49200 MT in 2012 and 2013, respectively (Hapuarachchi *et al*. 2018) [3].

Ganoderma lucidum has shown a high degree of activity against Staphylococcus, Streptococcus and *Bacillus pneumoniae*, perhaps because of increased immune system activity and antiviral effects induced by interferon production (Wasser and Weis, 1999). It also have antifungal activity against some important plant pathogenic fungi such as *Aspergillus niger* Van Tieghem, *Curvularia lunata* Nelson, *Fusarium oxysporum* Schlecht and *Alternaria alternata* Keissl (Singh *et al*. 2014) [8].

Material and Methods

Antimicrobial potential of *G. lucidum* isolate against pathogen

The present investigation was conducted to check the antimicrobial properties of *G. lucidum* extract against the some pathogens *viz*., *Colletotrichum falcatum* Went, *Aspergillus niger* Van Tieghem, *Fusarium moniliforme* (Sacc.) Nirenberg, *Trichoderma viride* Pers. and *Sclerotium rolfsii* Sacc. by dual culture technique and well diffusion method.

Dual culture technique

For interaction of each pathogen against *G. lucidum*, sterilized Petri plates (9cm) were used. An autoclaved melted PDA was poured to the Petri plates. After solidification of PDA in Petri plate, the 5 mm discs of one week old growing colonies were cut from the margin of each pathogen and *G. lucidum* was placed on the opposite sides of the Petri plate 4cm apart on the PDA medium and it was incubated at 25±2ºC. For the control plate, the mycelium bit of the test pathogen was placed in centre of Petri plates and it was incubated at room temperature.

After seven days, the mycelium growth of test pathogens in treated and control plates was recorded. Percent Growth Inhibition (PGI) of pathogens was calculated by using the following formula given by Pau *et al*. (2012) [6].

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where,
PGI = Percent Growth Inhibition
DC = Average diameter of colony in control set
DT = Average diameter of colony in treated set

Well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts against test pathogens. An autoclaved melted PDA was poured to the Petri plates. After solidification of PDA in Petri plate, the 5 mm discs of one week old growing colonies were cut from the margin of each pathogen and placed in the centre of the Petri plates. Then, a hole with a diameter of 5mm was punched aseptically with a sterile cork borer and a volume (20 µL) of the extract solution at desired concentration was introduced into the well. Then, agar plates were incubated under the room condition at room temperature. The antimicrobial agent diffused in the agar medium and inhibited the growth of the test pathogen. For the control plate, the mycelium bit of the test pathogen was placed in centre of Petri plates without any extract addition and it was incubated at room temperature.

After seven days, the mycelium growth of test pathogens in treated and control plates was recorded. Inhibition zone (cm) and Percent Growth Inhibition (PGI) of pathogens was calculated by using the following formula given by Pau *et al*. (2012) [6].

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where,
PGI = Percent Growth Inhibition
DC = Average diameter of colony in control set
DT = Average diameter of colony in treated set

Result & Discussions

Antimicrobial potential of *G. lucidum* isolate against pathogen

Antimicrobial properties of *G. lucidum* were checked against five different pathogens *viz*., *Colletotrichum falcatum* Went, *Aspergillus niger* Van Tieghem, *Fusarium moniliforme* (Sacc.) Nirenberg, *Trichoderma viride* Pers. and *Sclerotium rolfsii* Sacc. by dual culture technique as well as well diffusion method. The experiment was carried out in completely randomized block design. Mean sum of squares was found to be significant for the treatment.

Dual culture technique

In dual culture technique *G. lucidum* showed significant inhibition against all the pathogens. Maximum percent growth inhibition was recorded in *F. moniliforme* (74.99%) which was followed by *A. niger* (72.22%) and least percent growth inhibition was recorded in the case of *T. viride* (46.66%). The data are presented in Table 1, Fig. 1 and Photo 1.

The result of dual culture technique agreed with the result of Badalyan *et al*. (2015) [1]. They tested antifungal activity of *G. lucidum* against five species of phytopathogenic fungi (*Bipolaris sorokiniana* Shoemaker, *Fusarium culmorum* Sacc., *Fusarium oxysporum* Schlecht, *Pestalotiopsis funereal* Henn, *Rhizoctonia cerealis* Bourdot) and four species of their antagonists (*Trichoderma asperellum* Sumuels, *T. harzianum* Rifai, *T. pseudokoningii* Oudem, *T. viride* Pers*)* in dual culture experiment and obsereved that highest antagonistic activity of *G. lucidum* was against *F. oxysporum* as well as *F. culmorum*.

Table 1: Antimicrobial activity of *G. lucidum* GJN – 2 by dual culture method

Sr. No.	Test Organism	PGI (%)*
1.	*Colletotrichum falcatum* Went	66.10
2.	*Aspergillus niger* Van Tieghem	72.22
3.	*Fusarium moniliforme* (Sacc.) Nirenberg	74.99
4.	*Trichoderma viride* Pers.	46.66
5.	*Sclerotium rolfsii* Sacc.	60.27
S.Em. ±		0.71
CD at 0.05		2.15
CV%		2.23

*****Percent Growth Inhibition

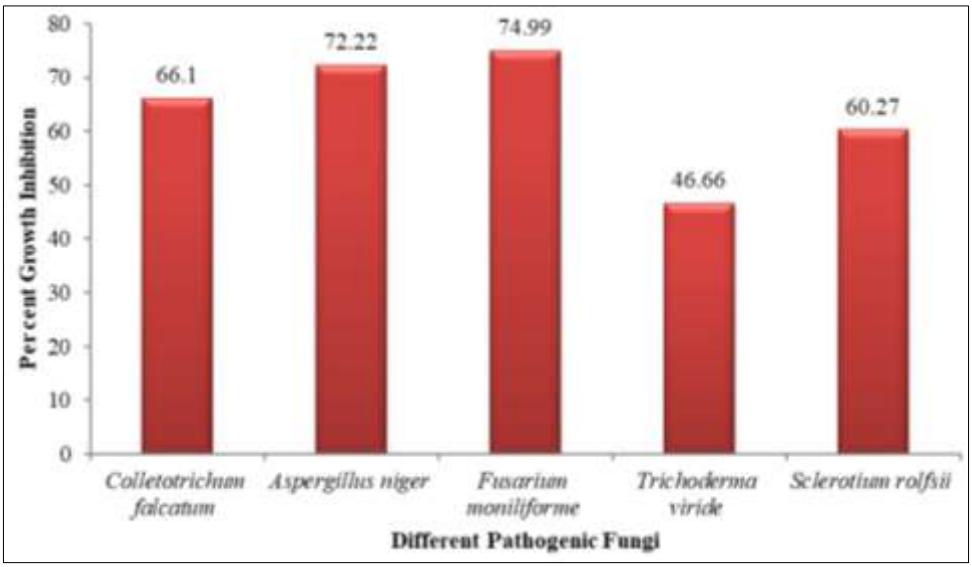

Fig 1: Antimicrobial activity of *G. lucidum* GJN -2 by dual culture method

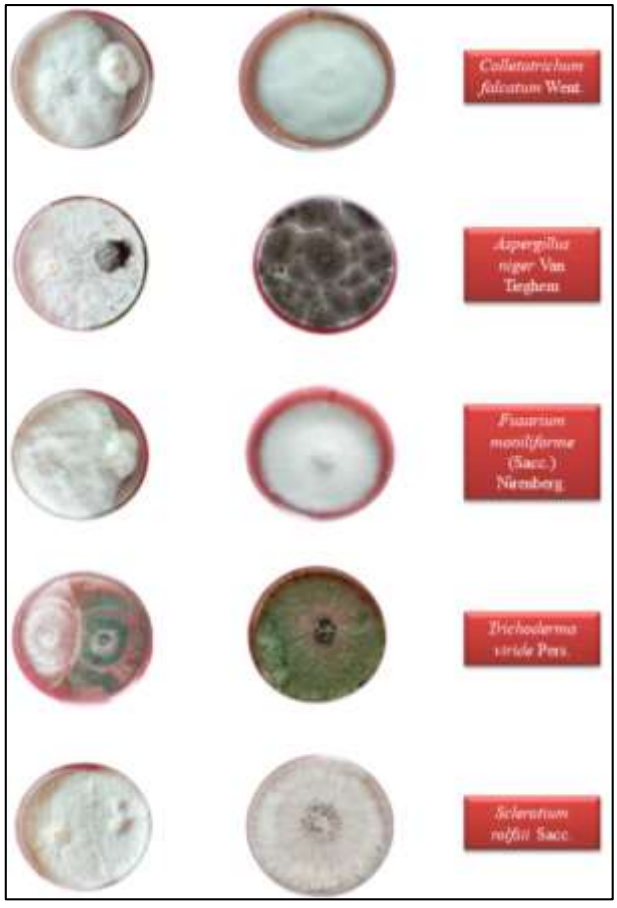

Photo 1: Antagonistic effect of *G. lucidum* against different pathogens by dual culture method

Well diffusion method

In well diffusion method, three different solvents such as ethanol, di ethyl ether and chloroform with 250 ppm and 500 ppm concentration were used to evaluate the bioefficacy of *G. lucidum* against different pathogens. The data are presented in Table 2, Fig. 2 and Photo 2, 3 and 4.

In the case of *C. falcatum*, the maximum inhibition zone was noted in ethanol extract at 500 ppm concentration which was 2.3 cm while the least inhibition zone was noted in chloroform extract at 250 ppm concentration (1.6 cm). In *A. niger*, maximum zone of inhibition was recorded in chloroform at 500 ppm which was 3.2 cm and minimum zone of inhibition recorded in di ethyl ether extract at 250 ppm (1.8 cm).

Ethanol extract at 500 ppm showed maximum inhibition zone (2.6 cm) against *F. moniliforme* and chloroform extract at 250 ppm showed least inhibition zone (1.8 cm). In *T. viride*, maximum inhibition zone was recorded in ethanol extract which was same as chloroform extract at 500 ppm (2.1 cm) and least inhibition zone was recorded in di ethyl ether (1.2 cm). Chloroform extract at 500 ppm showed maximum inhibition zone (2.7 cm) against *S. rolfsii* and di ethyl ether extract showed least inhibition zone (1.8 cm).

In general, maximum zone of inhibition was recorded in chloroform extract at 500 ppm (3.2 cm) against *A. niger* and least zone inhibition (1.2 cm) was recorded in case of di ethyl ether extract at 250 ppm against *T. viride*.

The results obtained in the present study were in harmony with the findings of Radhika and Rajan (2021) [7] who assessed antifungal activity of four extract of *G. lucidum viz*., water, chloroform, methanol and ethanol against five fungal pathogens namely, *A. nige*, *A. terreus*, *A. dowii*, *Fusarium* spp. and *Penicillium* spp. by well diffusion test and observed that maximum growth inhibition was observed in the case of chloroform extract against *A. niger*.

Table 2: Antimicrobial activity of *G. lucidum* GJN – 2 by well diffusion method

Sr. No.	Test organism	Ethanol extract				Di ethyl ether extract				Chloroform extract			
		250 ppm		500 ppm		250 ppm		500 ppm		250 ppm		500 ppm	
		ZI* (cm)	PGI** (%)	ZI (cm)	PGI (%)	ZI (cm)	PGI (%)	ZI (cm)	PGI (%)	ZI (cm)	PGI (%)	ZI (cm)	PGI (%)
1.	*Colletotrichum falcatum* Went	1.9	78.88	2.3	74.44	1.7	81.11	2.2	75.55	1.6	82.22	1.9	78.88
2.	*Aspergillus niger* Van Tieghem	2.4	73.33	2.9	66.66	1.8	80.00	2.3	74.44	2.8	68.88	3.2	64.44
3.	*Fusarium moniliforme* (Sacc.) Nirenberg	2.0	77.77	2.6	71.11	1.9	78.88	2.5	72.22	1.8	79.99	2.4	73.33
4.	*Trichoderma viride* Pers.	1.7	81.11	2.1	76.66	1.2	86.66	1.9	78.88	1.5	83.33	2.1	76.66
5.	*Sclerotium rolfsii* Sacc.	2.1	76.66	2.3	74.44	1.8	80.00	2.1	76.66	2.1	76.66	2.7	69.99
S.Em. ±		0.041	0.454	0.048	0.453	0.032	0.406	0.045	0.497	0.041	0.454	0.052	0.574
CD at 0.05		0.124	1.380	0.147	1.378	0.096	1.236	0.136	1.511	0.124	1.381	0.157	1.745
CV%		4.04	1.17	3.96	1.24	3.81	1.00	4.06	1.31	4.17	1.16	4.19	1.57

* Zone of Inhibition

** Percent Growth Inhibition

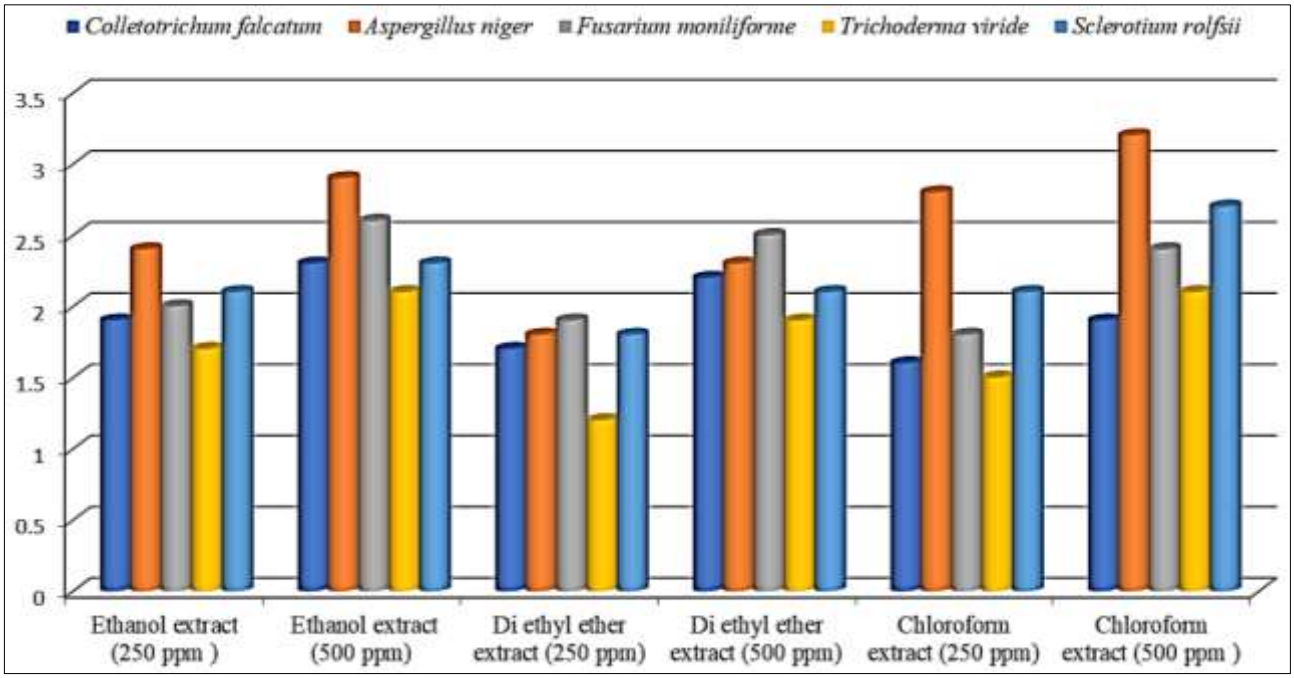

Fig 2: Antimicrobial activity of *G. lucidum* GJN – 2 by well diffusion method

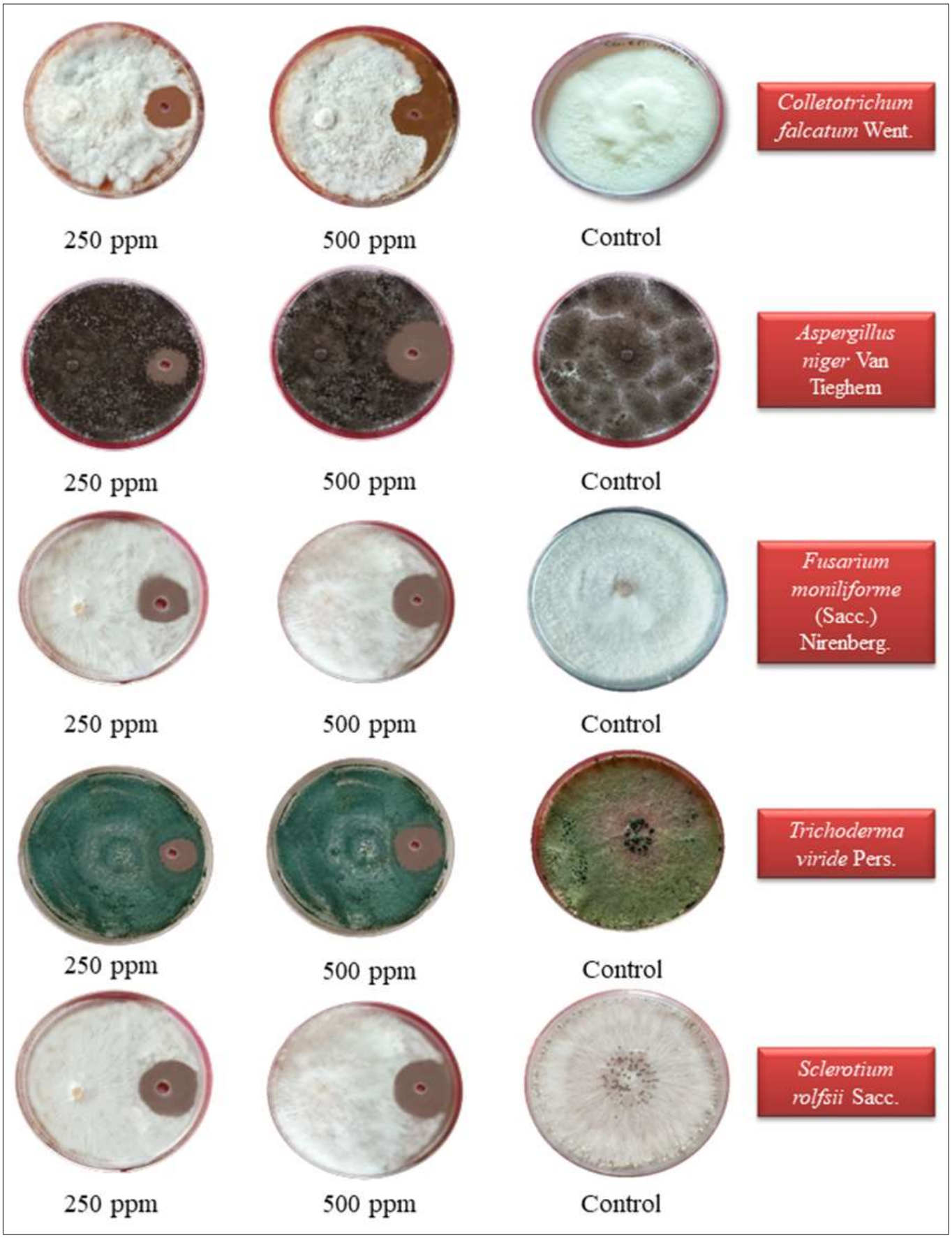

Photo 2: Growth inhibition of different pathogens by ethanol extract of *G. lucidum*

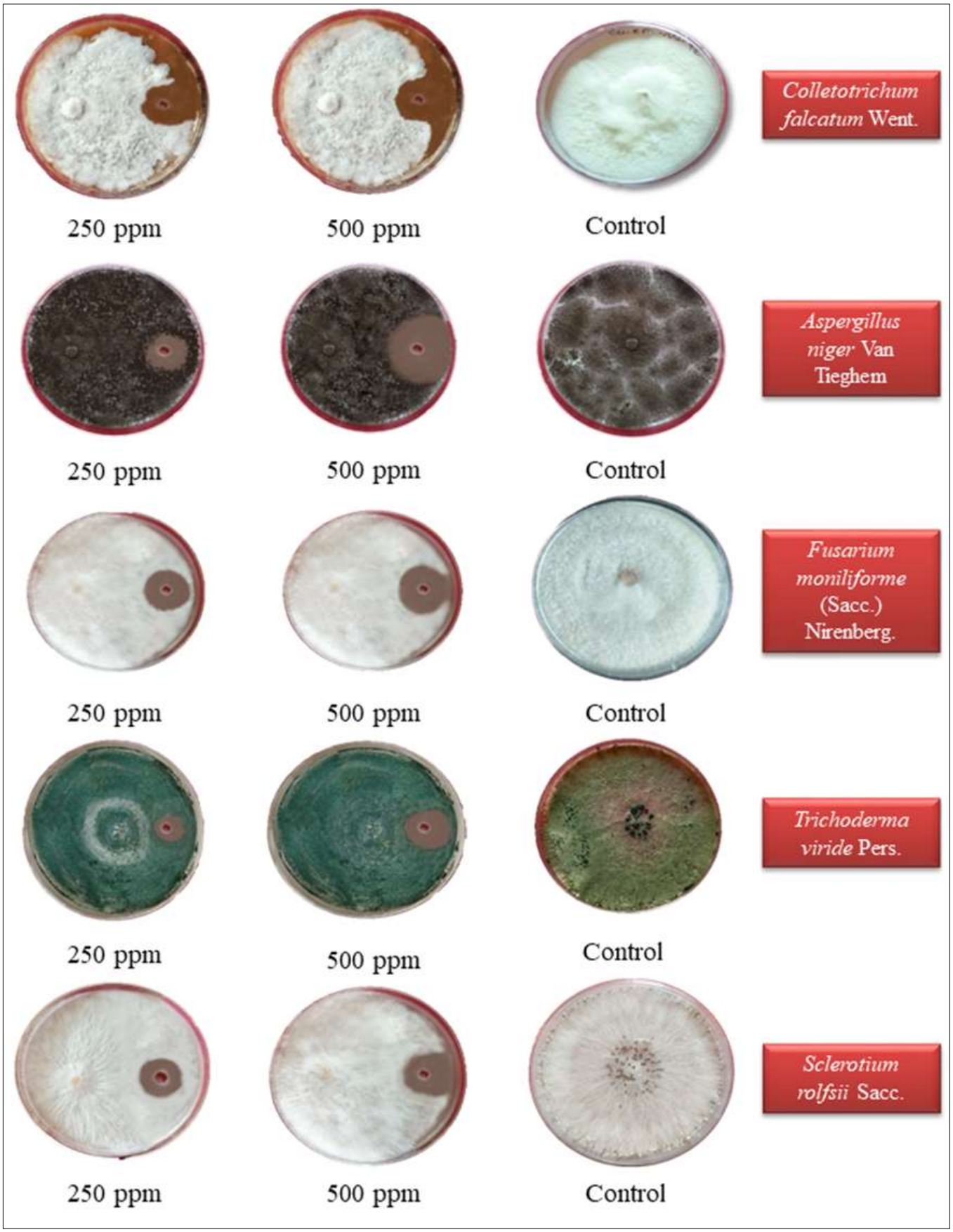

Photo 3: Growth inhibition of different pathogens by di ethyl ether extract of *G. lucidum*

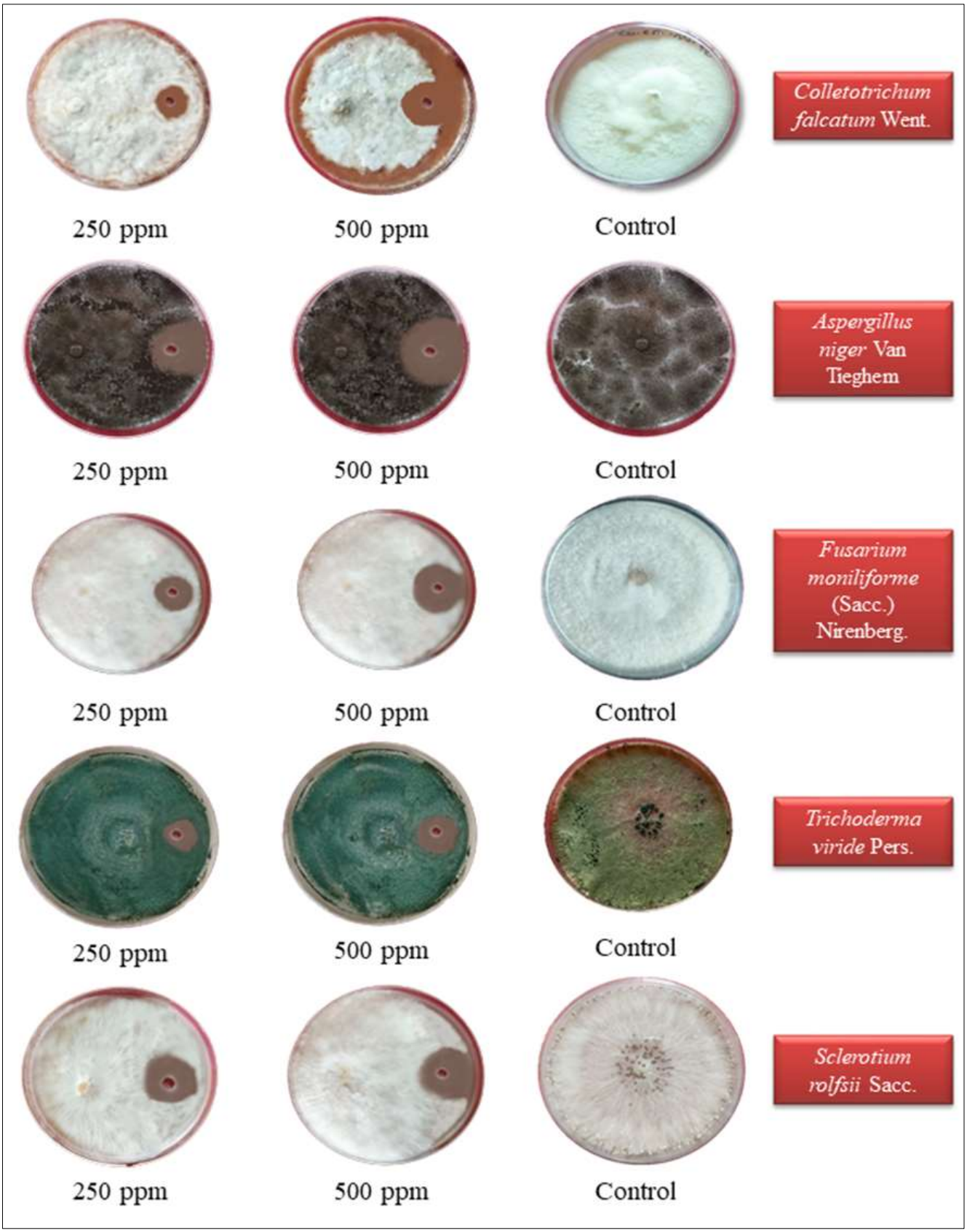

Photo 4: Growth inhibition of different pathogens by chloroform extract of *G. lucidum*

References

1. Badalyan SM, Shnyreva AV, Iotti M, Zambonelli A. Genetic resources and mycelial characteristics of several medicinal polypore mushrooms (Polyporales, Basidiomycetes). International journal of medicinal mushrooms, 2015, 17(4).
2. Chen B, Ke B, Ye L, Jin S, Jie F, Zhao L, *et al*. Isolation and varietal characterization of *Ganoderma resinaceum* from areas of *Ganoderma lucidum* production in China. Scientia Horticulturae. 2017 Oct 20;224:109-14.
3. Hapuarachchi KK, Elkhateeb WA, Karunarathna SC, Cheng CR, Bandara AR, Kakumyan P, *et al*. Current

status of global *Ganoderma* cultivation, products, industry and market. Mycosphere. 2018;9:1025-1052.
4. Jandaik S, Singh R, Sharma M. Comparative growth characteristics and yield attributes of lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (higher Basidiomycetes) on different substrates in India. International journal of medicinal mushrooms. 2013;15(5):75-78.
5. Kaliyaperumal M, Kalaichelvan PT. Cultural diagnosis of Ganoderma lucidum complex from southern India. MYCOLOGIA. 2007;4:15-9.
6. Pau CG, Leong S, Teck C, Wong SK, Eng L, Jiwan M, *et al*. Isolation of indigenous strains of *Paecilomyces lilacinus* with antagonistic activity against *Meloidogyne incognita*. International Journal of Agriculture & Biology. 2012 Apr 1;14(2).
7. Radhika R, Rajan S. Antifungal potentials of *Ganoderma lucidum* extracts. Plant Cell Biotechnol. Mol. Biol. 2021 May 15;22:22-7.
8. Singh J, Gupta S, Malviya S, Ahrwar B. *In-vitro* evaluation of antimicrobial activity of *Ganoderma lucidum*. International Journal of Advanced Research. 2014;2(6):460-6.
9. Thakur MP. Biology of edible mushrooms. Fungal Divers. Biotech. 2005;6:305-348.