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Evaluation of antimycotic value of indigenous wild mushrooms against *Rhizoctonia solani* kuhn causing sheath blight of rice

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Abstract
Mushroom species release various bioactive compounds such as Terpenoids, Flavonoids, Tannins, Alkaloids, and Polysaccharides. They are rich source of natural antibiotics; Basidiomycetes produce a large number of secondary metabolites, which show antibacterial, antifungal, antiviral, cytotoxic and hallucinogenic activity. In this view, the study was proposed to explore the antimicrobial principles from Macrobasidiomycetes against Rice Sheath blight incitant *R. solani*. Eleven species of mushrooms were collected viz., *Ganoderma oregonense, Stereum complicatum, Auricularia auricular-judae, Trametes versicolor, Psathyrella obtusata, Trametes gibbosa, Lepiota brebissonii, Schizophyllum commune, Clitocybe* sp*, Podoscypha* sp*, Coprinus comatus.* The interaction between wild mushroom and plant pathogenic fungi in dual cultures on PDA has been tested, based on the antagonism index (AI) values; six mushrooms were selected for further studies. Results from dual culture technique revealed that *Ganoderma oregonense, Clitocybe* sp and *Lepiota brebissonii* showed maximum antifungal activity by inhibiting the mycelial growth of *R. solani*. (68.50%, 59.25 % and 50.14% respectively) with maximum inhibition zone of (3.20 mm, 2.80 mm and 1.70 mm respectively). With regard to poisoned food technique the culture filtrates of *Ganoderma oregonense* at 15 and 20 per cent conc. completely inhibited the mycelial growth of *R. solani*. Followed by *Clitocybe* sp at 20% Concentration. The least inhibition on the mycelial growth of *R. solani* was observed at 20% concentration by *Lepiota brebissonii*, *Stereum complicatum, Trametes versicolor* and *Schizophyllum commune.* Hence, It is well proven that antimycotic extracts of *Ganoderma oregonense* posses antimicrobial activities against the growth of *R. Solani.*

Keywords: *Rhizoctonia solani,* Mushroom fungi, *Ganoderma oregonense*, Antimycotic activity, Inhibition per cent

Introduction

Rice (*Oryza sativa*) belongs to the genus Oryza (Family: Poaceae) which includes two cultivated and more than 25 wild species that are either perennial or annual, and diploid or tetraploid. The cultivated species are *Oryza sativa* L. and *Oryza glaberrima* L. (Pareja *et al*. 2011) [22]. Rice cultivation started at the 15th century in South East Asia and spread to India, china and Japan. Among the biotic stress factors affecting rice crop, the loss inflicted by pathogen, insect pests and nematodes are considerably more significant. The most important rice diseases like blast, sheath blight, stem rot, grain discoloration and bacterial blight causing more damage to the crop (Sharma *et al*. 2009) [29].

Major constraints in rice production

Among these, Rice sheath blight caused by *Rhizoctonia solani* Kuhn. [Sexual stage: *Thanatephorus cucumeris* (A.B. Frank) Donk)], it is a universal soil saprotrophic and facultative plant parasite (Anees *et al*. 2010) [3] and also found to be known as soil-borne hemibiotrophic pathogen, with approximately 58% yield reduction in test plots of susceptible cultivars (Kouzai *et al*. 2018) [17]. *R. solani* have 14 anastomosis group placed in AG-1 IA (Gonzalez-Vera et. al. 2010). Rice Sheath blight disease occurs near the water level after the infection of sheath turn in to softness leads the infection spreads to healthy plant parts resulting development of water soaked lesions and dormant sclerotia or mycelium presented in surface of the water and soil (Tsiboe *et al*. 2017) [33]. The pathogen survives as sclerotia under unfavourable condition and it may spherical or irregular shaped and measure 4-5 mm in diameter, dark brown to black in colour, basidia and basidiospores are produced and viability for upto 3 years by its saprophytic nature (Kumar *et al*. 2009) [18]. Sclerotia may move from one field to another through irrigation water and during movement they may produce mycelia and secondary or tertiary sclerotia (IRRI 1973) [14]. Depending upon the age of the plant, time of infection and severity, it causes yield loss to the extent of 5.9 to 69 per cent. (Roy, 1993) [26] The maximum disease development was recorded at a temperature level of 25-30ºC and 80-100 per cent RH (Bhunkal *et al*. 2015) also by applying high amount of nitrogenous fertilizer (Akash Datta *et al*. 2017) [2]. Sheath blight of rice has gradually become one of the second most important diseases next to blast disease of rice (Singh *et al*., 2010) [30].

Carbendazim and Validamycin are still widely used for the management of sheath blight of rice (Peng *et al*., 2014) [24]. Indiscriminate use of chemical fungicides is causing a rigorous intimidation to the environment and community health and they are dangerous to other beneficial rhizosphere micro flora existing in an agricultural environment (Shanmugaiah *et al*., 2010) [28]. Presently Exploration of bioactive compounds effective in treating plant pathogenic microorganisms is with the perception of environment friendly and sustainable agriculture. Currently, there is a growing interest in searching for new

antimicrobial agents from natural sources such as bacteria, fungi, and plants. Natural products, especially microbial and plant products constitute the major sources of new bio-molecule. (Shanmugaiah *et al*., 2010, Harikrishnan *et al*., 2016) [28, 12].

Mushroom species release various bioactive compounds such as terpenoids, flavonoids, tannins, alkaloids, and polysaccharides. They are widely distributed in nature and predominantly occur in climates ranging from tropics to the tundra. They have wide habitat range. They grow on damp places having lignocellulolytic material of coniferous and deciduous forests, on wood, desert sands, lake dunes, gardens, open fields, marshy places, on the heaps of stored straw, farmyard manure etc. From a taxonomic point of view, mainly basidiomycetes (the spore droppers) with a distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand" (Chang and Mile (1992) [9]. Wild mushrooms have been collected and consumed by people for thousands of years. Basidiomycetes produce a large number of secondary metabolites (Anke 1989) [4] which show antibacterial, antifungal, antiviral, cytotoxic and hallucinogenic activity or which can be source of plant growth regulator or flavours (Janssens *et al*. 1992; Breheret *et al*. 1997; Marumoto *et al*. 1997) [14, 7, 19]. Mushrooms are rich source of natural antibiotics. Among them, the cell wall glucans are well known for their immunomodulatory properties and many of the externalized secondary metabolites to combat pathogenic microorganisms. Additionally, the exudates from mushroom mycelia are active against many plants' pathogenic fungi and other microorganism. Several compounds extracted from *Lentinus edodes* revealed antifungal and antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*. The chloroform and ethyl acetate extracts of the dried mushroom have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Barros *et al*. 2007) [5].

Owing to the current emphasis on the eco-friendly approaches for plant disease management, mushroom extracts can serve as promising source of antimycotic activity against plant pathogens as evidenced by the antimicrobial activity of the culture filtrates of Ganoderma which was identified to have antifungal potential against sheath blight pathogen of rice was developed as an emulsifiable concentrate. (Sajeena and Marimuthu, 2013) [26], 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) [9]. So, keeping in view the importance of higher fungi and wild mushrooms in having potential antifungal products the present research work has been conducted.

Materials & Methods

1. Isolation, maintenance and identification of the test pathogen

Diseased rice plants with characteristic symptoms of sheath blight disease were collected from traditional rice growing area of Mayiladuthurai district, Tamil Nadu. The infected portion of the sheath was cut into small bits; surface sterilized with 1% sodium hypochlorite solution for 50 seconds and washed thrice with sterile distilled water. Further, a piece of specimen was transferred to sterile Petri dishes containing PDA medium. The plates were incubated at room temperature (28±2°C) for 5 days and the isolates were purified by single hyphal tip method. Molecular Characterization and identification of virulent isolate of *R. solani* were done with Accession number. OL077520 and the culture is maintained on PDA slant for further studies

2. Collection and identification of wild mushroom

Various wild mushroom samples were collected from the Eastern ghats (yercaud hills) and kodaikanal hills. During collection, sporocarps were removed from ground and wooden timbers with a great care to avoid damage to the base of the stipe to reveal any volva, rotting base, and bulb or buried substrata included cones, fruits and other fungi. Soil was removed using a soft brush (Stojchev *et al*. 1998) [30]. The habitat and morphological characteristics of the macro fungi were noted (Peksen and Karaca 2003) and photographed for diagnosis during the collection if specimen was on the wood or litter, including substratum it was collected to facilitate identification. The specimens were collected in the plastic bags or boxes for further identification in the laboratory (Afyon *et al*. 2005) [1]. The specimens were wrapped in the aluminum foil, which offers a good protection. Care was taken to avoid distortion of fleshy fungi and labeled the specimen. The collection was brought to the lab and soon preserved in a preservative liquid or as dried specimens. The collected sporocarps were described for the morphological characters like colour, size, shape, odour and texture. Accurate and consistent notation of sporocarps colour, including colour changes of mature sporocarps and colours of different development stages, presence or absence of texture, lamellae that are important for describing macrofungi were noted.

Identification was also made based on various specific characters such as the diameter of fruiting bodies, cap, odor, flesh, gills, stem and veil (Watling 1971) [34]. Furthermore, monographs, identification keys and field guide of mushroom were referred for further species identification (Ostry and O'Brien 2010; Kaul 2001) [20, 15]. Apart from that mushrooms were identified and confirmed by their morphological characters using the software identification of mushrooms (Discover mushroom, 1997 – Version: 5.1.2600.5512.); (Match maker, 2003, Version 1.10).

3. Isolation and maintenance of pure culture of wild mushrooms

Isolations from the fresh specimens collected from hills were made following standard tissue culture technique. Each specimen after washing with the jet of sterile water was cut across the pileus region with the help of sterilized sharp blade to obtain bits (1-2 mm) of tissue, which were dipped in 1% sodium hypochlorite solution for 50 seconds using sterile forceps for 10 to 20 seconds. These were repeatedly washed with sterile water (5 washings) and placed on sterilized filter paper to remove excess moisture, the bits were then transferred on PDA slants (Sud 1995) [31], aseptically with the help of sterilized inoculating needle and incubated 20±1^0 C. Stock cultures were maintained in the refrigerator at 40C. Cultures were revived after a period of 7-10 days on fresh slants.

4. Evaluation of anti plant pathogenic activity of wild mushrooms

The anti-plant pathogenic activity of higher basidiomycetes was done *in vitro* by adopting following standard methodology.

4.1 Testing antagonistic activity

Competitive interaction between wild mushrooms and plant pathogenic microorganisms was evaluated by dual culture experiments on Petri dishes (90 mm diameter containing 20 ml PDA. In each Petri dish two 2 mm diameter mycelial disks, one each from mushroom and fungal colonies was placed on the agar surface 30 mm apart. Immediately after inoculation, the plates were sealed with plastic film and incubated in darkness at 24 ^{0} C for 10 days. Colony growth and type of interaction was examined regularly. The presence of dense zones of mycelium, aggregated structures such as mycelial cords, pigmented hyphae, exudate droplets, dark pseudo sclerotial lines, and fruiting body primordia in the interaction zones was also noted. The antagonistic activity of the various7 mushroom cultures was tested by the method described by Huang and Hoes (1976) [12].

4.2. Evaluation of culture filtrate extracts of mushroom against *R.solani*

Mycelial culture and culture filtrates extract method as described by Anke *et al*. (1977) was used. A small square of 5x5 mm of mycelial culture of each of the mushroom was maintained on Potato Dextrose Agar slant and used to inoculate each of the 250 ml Erlenmeyer flasks containing 100 ml of potato dextrose broth and sterilized. The flasks were incubated for 5 days on a rotary shaker at 100 rpm for 48 h at ambient temperature of 25±1^{0}C. 10-days old culture filtrate is filtered through Whatman filter paper no 1. Different concentration (5%, 10%, 15% and 20%) of culture filtrate is prepared by dissolving 5ml, 10ml, 15ml and 20ml of mushroom filtrate in 100ml of PDA medium to make the given concentrations respectively. The culture filtrate was tested by poisoned food technique (Vincent 1947) [33]. Desired quantity of culture filtrate at different concentration was aseptically mixed into double strength sterilized PDA medium. This medium was poured in sterilized Petriplates and allowed to solidify. Medium without any filtrate treatment served as control.

After solidification a 5 mm diameter mycelial disk of pathogenic fungus was placed in the middle of plates. Each treatment was replicated thrice, with three plates in each replication. Immediately after inoculation the plates were incubated in incubator at 25±1^{0}C. Data on mycelia growth were recorded when control Petriplate was fully covered. Per cent inhibition was calculated by formula (Vincent 1947) [33].

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

∴ C- Mycelial growth of pathogen in control; T- Mycelial growth of pathogen in dual plate.

Results

1. Myco-ecological characteristics

Naturally growing mushroom fungi flora was collected out during Dec – Jan in the Eastern Ghats (yercaud hills). The representative sample of various mushrooms were collected and brought to the Department of Plant Pathology, Annamalai University. These were characterized morphologically and preserved for further study. A total of 11 wild mushroom has been collected which belong to basidiomycetes, The collected mushrooms were identified following standard descriptive keys (Phillips 1991; Lincoff 1981) [24, 18] and have been listed in Table.1.

2. *In vitro* antagonistic activity of certain wild mushrooms

The interaction between wild mushroom and plant pathogenic fungi in dual cultures on PDA has been presented in (Table 2.). Five type of competitive interaction were observed: Over growth of pathogen over mushroom, inhibition at mycelial contact, inhibition at mycelia distance, overgrowth, partial or complete replacement of plant pathogenic fungi.

In this study not only different type of interaction between mushroom culture and plant pathogenic fungi but also differences in strength of interaction were observed in table **3.** On the basis of the antagonism index (AI) values, mushrooms were divided into four groups: which has also been presented in (Table 2)

a. Strongly active = (+ + +)
b. Active = (+ +)
c. Moderately active = (+)
d. Weakly active = (-)

2.1 Efficacy of wild mushrooms against *R. solani* (Dual culture)

The result of the experiments (Table 3.) showed that *Ganoderma oregonense* was found to be more antagonistic to *R. solani.* Statistically, *G. oregonense* recorded 68.50 per cent growth inhibition with a colony diameter of 28.35 mm. followed by *Clitocybe* sp (59.25%), *Lepiota brebissonii* (50.14%), *Stereum complicatum* (46.00%), *Trametes versicolor* (36.00%) and least inhibition was recorded by *Schizophyllum commune* (33.50%). Hence *G. oregonense* was proved to be best against *R. solani.*

2.2 Effect of certain wild mushrooms culture filtrate extract against *R. solani* by poison food technique.

The result on the effect of different concentration of culture filtrate of wild mushroom on the mycelia growth of *R.solani* under *in vitro* condition revealed in (table 4.) that the culture filtrate of *G. oregonense* completely inhibited the mycelial growth at 15 and 20 % concentration under *in vitro* condition, followed by *Clitocybe* sp at 20% Concentration. The least inhibition on the mycelia growth of *R. solani* was observed at 20% concentration by *Lepiota brebissonii*, *Stereum complicatum, Trametes versicolor* and *Schizophyllum commune.*

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 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.

Table 1: Diversity of Collected and identified wild mushroom

S. No	Scientific name	Phylum (Basidiomycota) & class	order	family	Habitat	Nature	Period of collection
1.	*Ganoderma oregonense*	Agaricomycetes	Polyporales	Ganodermataceae	On the tree	Non-edible	Throughout the year
2.	*Stereum complicatum*	Agaricomycetes	Russulales	Stereaceae	On the wood, and wooden debris	Non-edible	Aug- Nov
3.	*Auricularia auricular-judae*	Agaricomycetes	Auriculariales	Auriculariaceae	On the wood	Edible	Sep- Oct
4.	*Trametes versicolor*	Agaricomycetes	Polyporales	Polyporaceae	On dead and decaying stumps of trees	Non-edible	Throughout the year
5.	*Psathyrella obtusata*	Agaricomycetes	Agaricales	Psathyrellaceae	On the ground	Non-edible	Sep-Oct
6.	*Trametes gibbosa*	Agaricomycetes	Polyporales	Polyporaceae	On the wood log	Non-edible	Aug-Dec
7.	*Lepiota brebissonii*	Agaricomycetes	Agaricales	Agaricaceae	On the ground	Non-edible	Sep- Oct
8.	*Schizophyllum commune*	Hymenomycetes	Schizophyllales	Schizophyllaceae	On the wood	Non-edible	July to September
9.	*Clitocybe sp*	Agaricomycetes	Agaricales	Tricholomataceae	On broadleaf wood	Non-edible	On ground amongst grasses
10.	*Podoscypha sp*	Agaricomycetes	Polyporales	Meruiaceae	On the dead wood	Non-edible	June to September
11.	*Coprinus comatus*	Agaricomycetes	Agaricales	Agaricacea	On the ground	Non-edible	Sep- Oct

Table 2: Interaction between certain wild mushroom fungi and *R. solani* by Dual culture technique

Scientific name	Interactions	Antagonism index (AI)
Ganoderma oregonense	Clear inhibition zone of 3.20mm, both mushroom fungi and pathogen did not grow each other even after 10 days.	+++
Stereum complicatum	Initially clear inhibition zone of 1.30 mm formed, later mushroom fungi hyperprasitised over the pathogen	++
Auricularia auricular-judae	No, inhibition zone, but hyperparitization of mushroom fungi over pathogen	-
Trametes versicolor	inhibition zone of 1.15 mm; both pathogen and mushroom fungi did not grow over each other	++
Psathyrella obtusata	No inhibition zone; but hyperparasitization of mushroom fungi over pathogen	-
Trametes gibbosa	No inhibition zone, both fungi did no grow each other	-
Lepiota brebissonii	inhibition zone of 1.70 mm; both pathogen and mushroom fungi did not grow over each other	++
Schizophyllum commune	inhibition zone of 1.10 mm; both pathogen and mushroom fungi did not grow over each other	+
Clitocybe sp	Clear inhibition zone of 2.80 mm formed later mushroom fungi hyperparsitied over the pathogen.	+++
Podoscypha sp	Pathogen growth was retarded and pushed back with an inhibition zone of 4.86mm	+
Coprinus comatus	No inhibition zone; but hyperparasitization of mushroom fungi over pathogen	-

Table 3: Screening of antagonistic activity of mushroom fungi against *R. solani* by Dual culture.

S. No	Mushroom species	*R. solani* Average mycelial growth (mm)	Inhibition zone (mm)	% inhibition over control
1.	*Trametes versicolor*	57.60	1.15	36.00^{e} (36.87)
2.	*Stereum complicatum*	48.60	1.30	46.00^{d} (42.71)
3.	*Schizophyllum commune*	59.85	1.10	33.50^{f} (35.37)
4.	*Ganoderma oregonense*	28.35	3.20	68.50^{a} (55.86)
5.	*Lepiota brebissonii*	44.87	1.70	50.14^{c} (45.08)
6.	*Clitocybe* sp	36.67	2.80	59.25^{b} (50.33)
7.	Control	90.00 (71.57)	0.00	100
	CD (p=0.05)	1.807		3.234

*Values in the column followed by common letters do not differ significantly by DMRT (P = 0.05)

Table 4: Screening of antagonistic activity of certain mushroom fungi against *R. solani* by posion food technique

S. No	Mushroom species	Mycelial growth (mm)							
		5 %	% inhibition over control	10 %	% inhibition over control	15 %	% inhibition over control	20 %	% inhibition over control
1.	*Trametes versicolor*	80.00	11.11 [e] (19.47)	71.21	20.80 [e] (27.13)	56.30	37.44 [e] (37.73)	29.80	66.89 [e] (54.87)
2.	*Stereum complicatum*	75.10	16.55 [d] (24.01)	51.73	42.52 [d] (40.69)	49.00	45.60 [d] (42.48)	28.35	68.50 [d] (55.86)
3.	*Schizophyllum commune*	84.00	6.75 [f] (15.06)	76.90	14.60 [f] (22.46)	51.85	42.45 [f] (40.66)	33.74	62.51 [f] (52.24)
4.	*Ganoderma oregonense*	32.00	64.44 [a] (53.39)	12.10	86.60 [a] (68.52)	0.00	100 [a] (90.00)	0.00	100 [a] (90.00)
5.	*Lepiota brebissonii*	57.17	36.50 [c] (37.16)	32.62	63.80 [c] (53.01)	25.00	72.25 [c] (58.21)	11.89	86.80 [c] (68.70)
6.	*Clitocybe* sp	48.72	45.90 [b] (42.64)	28.36	68.50 [b] (55.86)	15.72	82.53 [b] (65.29)	0.00	100 [b] (90.00)
7.	Control	90.00 (71.57)	0.00 (0.00)	90.00 (71.57)	0.00 (0.00)	90.00 (71.57)	0.00 (0.00)	90.00 (71.57)	0.00 (0.00)
	CD(=0.05)		2.413		3.475		4.405		5.234

*Values in the column followed by common letters do not differ significantly by DMRT (P = 0.05)

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