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Effect of *Metarhizium anisopliae* on *Rhizoctonia solani* and *Meloidogyne graminicola* in Rice Seedlings

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A B S T R A C T

An experiment was conducted in the laboratory of Department of Plant Pathology, SHUATS, Prayagraj, U.P. during 2019-2020 to observe the effect of 15 botanical extracts against *Rhizoctonia solani* by Poison Food Technique. Among the treatments *Piper nigrum*, *Centella asiatica* and *Lawsonia inermis* were found to be effective at different concentration (5%, 10%, 15%) with an inhibition percentage of 85.5%, 80% and 75.5% respectively as compared to control. Mycelial growth of *Rhizoctonia solani* was not inhibited by *Aerva lanata*, *Ocimum sanctum* and *Biophytum sensitivium*. Antagonistic effect of *Metarhizium anisopliae* against *Rhizoctonia solani* was evaluated in *in-vitro* condition by dual culture method and an inhibition of 60% was recorded against *Rhizoctonia solani*. A pot experiment was conducted in the shade net to evaluate the activity of *Metarhizium anisopliae* against *Meloidogyne graminicola* in rice seedlings. Root gall population was found to be reduced in treatment containing *Metarhizium anisopliae* + *Meloidogyne graminicola* and *Metarhizium anisopliae* + *Rhizoctonia solani* + *Meloidogyne graminicola* as compared to control pots with an inhibition percentage of 54.4% and 74.2% respectively.

Introduction

Rice is one of the important food crops and provides an essential part of the daily dietary intake for nearly half of the world's population. Diseases are considered as major constraints for rice production as 10 to 30 per cent of the annual rice harvest is lost due to infection by many diseases.

Sheath blight is a fungal disease of rice caused by a necrotrophic soil-borne fungus *Rhizoctonia solani* with teleomorpic stage *Thanatephorus cucumeris* (Turaidar *et al.*, 2018). *Rhizoctonia solani* is having a wide range of hosts like maize, wheat, barley, oat, soybean, peanut, alfalfa, chickpea, field pea, potato, sugar beet. Sheath blight is a devastating disease in all rice growing regions of the world. The yield loss ranging from 4-50% have been reported depending on the crop stage at the time of infection, severity of the diseases and environmental conditions (Singh *et al.*, 2016).

Meloidogyne graminicola is an obligate sedentary endoparasitic nematode causing root knot in rice seedlings adapted to flooded conditions. Under upland or intermittently flooded conditions, yield losses caused by *M. graminicola* range from 20% to 80% and 11% to 73%, respectively (Mantelin *et al.*, 2017). Entomopathogenic fungi, *Metarhizium anisopliae* has well established action against insects. But certain studies have reported its antagonistic activity against plant pathogenic fungus such as *Alternaria porri* (Gothandapani *et al.*, 2015), *Fusarium oxysporum* (Picardal *et al.*, 2019) and *Meloidogyne* sp. (Devi, 2018; Abdollahi, 2018).

Botanicals are now emerging as safer and more compatible approach to control phytopathogens. By considering the importance of the botanicals, 15 widely available indigenous plants of Kerala have been considered in this study based on their therapeutic uses against *Rhizoctonia solani*. Castellanos *et al.*, (2020) evaluated pepper essential oil against *Fusarium oxysporum* and *Aspergillus niger*. Rajnikant *et al.*, (2015) reported the antifungal potential of *Murraya koenigii* leaf extract against *Fusarium oxysporum*. Keeping the above in view, present research was undertaken to evaluate the effect of *Metarhizium anisopliae* against *Meloidogyne graminicola* in rice seedlings and *in-vitro* experiment was conducted to evaluate the effect of *Metarhizium anisopliae* on radial growth of *Rhizoctonia solani* and *in-vitro* evaluation of botanical extracts on the radial growth of *Rhizoctonia solani.*

Materials and Methods

Isolation of the pathogen

The entomopathogenic fungus culture *Metarhizium anisopliae* was provided by the Central Plantation Crops Research Institute (CPCRI), Kayamkulam, Kerala. Sub-culturing was done and PDA slants were allowed to grow at 27 ± 1℃ for 15 days. *Rhizoctonia solani* was isolated from the disease infected plants. The infected part was cut into small pieces (0.5cm), surface sterilized with mercuric chloride (0.1%) for 15-30 seconds, rinsed with distilled water to remove the disinfectant and blotted dry. The sterilized pieces were plated (4 pieces/dish) on potato dextrose agar (PDA) medium in petri dishes under aseptic conditions and incubated at 27℃ for 1 week and then sub cultured.

Preparation of leaf extracts

The leaves of medicinal plants *viz., Justicia adhatoda, Chrysopogon zizanioides, Centella asiatica, Lawsonia inermis, Tubernaemontana coronaria, Cardiospermum halicacabum, Phyllanthus niruri, Clitoria ternatea, Aerva lanata, Vitex negundo, Piper nigrum, Murraya koenigii, Biophytum sensitivium, Ocimum sanctum, Manihot esculenta* were collected from different localities of Alappuzha district (Kerala state). These were washed 2-3 times in tap water and air dried at room temperature (25o-30℃)**.** The dried leaves were ground into powder form, sieved and packed. 10gm sample of powdered dried leaves mixed in 50 ml of water. The extract was filtered primarily through double layered muslin cloth then through Whattman no.1 filter paper followed by centrifugation at 3000 rpm for 10 min and made to the required concentration by adding distilled water. In PDA media each plant extract was mixed at 5%, 10%, 15% concentration and then autoclaved at 121 at 15psi.

Poison food technique

Five-millimetre diameter disc of *Rhizoctonia solani* was kept with the help of cork borer at the centre of each petriplate containing the 5,

10, 15% of medicinal plant powder extract in PDA. Three replications were maintained. The plates were incubated at 27±1℃ for three days and colony diameter was recorded. Percent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{C}{T}$$

where, C = colony diameter in control

T = colony diameter in treatment

Dual culture method

In this method, an agar disc of the antagonist, *Metarihizium anisopliae* was placed opposite to the *Rhizoctonia solani* in same petriplate. A control plate of *Rhizoctonia solani* was maintained. Antagonistic activity was tested 4 days after incubation by measuring the radius of the *Rhizoctonia solani* and the radius of control. Percentage inhibition of radial growth (PIRG) was calculated using the formula developed by Dickinson (1976).

where, R1= radius of *Rhizoctonia solani* colony in control plate; R2= radius of *Rhizoctonia solani* colony in dual culture plate

Application of *Metarhizium anisopliae* in the pots

10 days old seedlings of rice were used. The conidia were collected from *Metarhizium anisopliae* culture surfaces by flooding with sterile distilled water (Al-Hazmi *et al.,* 2016). Serial dilutions were prepared and the number of conidia was measured by a haemocytometer to achieve the concentrations of 1× 105 cfu ml-1. Then 10 ml of *M. anisopliae* conidial solution were added in each pot infested by *Meloidogyne graminicola* at 2J/gm of soil and to the pots contained both *Meloidogyne graminicola* and 10ml of *Rhizoctonia solani*. One treatment of infested pots of *Meloidogyne graminicola* was maintained as control. Combination treatment of *Meloidogyne graminicola* and *Rhizoctonia solani* were also maintained (Niveditha *et al.,* 2019).

Results and Discussion

In-vitro effect of botanical extracts against *Rhizoctonia solani* (Table-1)

In- vitro effect of botanical extracts on the radial growth of *Rhizoctonia solani* at different concentrations (5%, 10%,15%) shows that.

At 5% concentration the treatment of botanical *Centella asiatica* significantly reduced the radial growth of *Rhizoctonia solani* as compared to other botanical extracts including the control. Maximum percentage of inhibition was observed in *Centella asiatica* (61.6%) followed by *Piper nigrum* (47.3%), *Lawsonia inermis* (38%), *Vitex negundo* (22.2%), *Chrysopogon zizaniodes* (22.2%), *Manihot esculenta* (11%), *Phyllanthus niruri* (8%), *Murraya koenigii* (6.6%).

At 10% concentration C*entella asiatica* significantly reduced the radial growth of *Rhizoctonia solani* from other treatments including control. The results showed that the maximum percentage of inhibition is observed in *Centella asiatica* (74.4%) followed by *Piper nigrum* (53.3%), *Phyllanthus niruri* (51.1%), *Lawsonia inermis* (50%), *Murraya koenigii* (46.6%), *Chrysopogon zizaniodes* (41.1%), *Manihot esculenta* (32.6%), *Vitex negundo* (33.3%), *Tubernaemontana coronaria* (21.1%), *Justicia adhatoda* (10%), *Clitoria ternatea* (3%). Whereas treatments *Cardiospermum halicacabum*, *Aerva lanata*, *Biophytum*

sensitivium, *Ocimum sanctum* not inhibited the growth of *Rhizoctonia solani*.

At 15% concentration *Centella asiatica* significantly reduced the radial growth of *Rhizoctonia solani* as compared with other treatments, including control. The results showed that the maximum mycelial growth inhibition percentage was observed in *Piper nigrum* (85.5%) followed by *Centella asiatica* (80%), *Manihot esculenta* (77%), *Lawsonia inermis* (75.5%), *Chrysopogon zizaniodes* (66%), *Phyllanthus niruri (*63.3%), *Murraya koenigii* (61.1%), *Tubernaemontana coronaria* (60%). Treatments *Aerva lanata*, *Biophytum sensitivium* and *Ocimum sanctum* not inhibited the growth of *Rhizoctonia solani*.

Among the botanicals, *Piper nigrum*, *Centella asiatica*, *Manihot esculenta*, *Lawsonia inermis*, *Chrysopogon zizaniodes* recorded significant inhibition over the radial growth of *Rhizoctonia solani.* From the mean value of treatments at 5%, 10% and 15% concentration the inhibition rate were recorded high in *Centella asiatica* and *Piper nigrum* which can be used as soil amendments with the organic matter or as combination with fungicides for spraying and seed treatment.

The results showed that the antifungal activity increases with the increase in concentration of the extract. The present investigations are in line with the investigations carried out by other workers such as Castellanos *et al.*, (2020), Singh *et al.*, (2000), Sharma *et al*., (2009), Seema *et al.*, (2011).

In-vitro effect of *Metarhizium anisopliae* against *Rhizoctonia solani*

In-vitro effect of *Metarhizium anisopliae* against *Rhizoctonia solani* by dual culture method (Table - 2) revealed that the *Metarhizium anisopliae* was effective against *Rhizoctonia solani* and recorded significant inhibition of 60% against the test pathogen over control.

The result revealed that the entomopathogenic fungi - *Metarhizium anisopliae* inhibited the mycelial growth and spore germination of *Rhizoctonia solani*. Similarly Dara *et al.*, (2018) reported the fungitoxicity of *Metarhizium anisopliae* against *Macrophomina phaseolina* in strawberry.

Effect of *Metarhizium anisopliae* against *Meloidogyne graminicola*

The results of pot experiment (Table-3) in shade house indicated that the number of galls of *Meloidogyne graminicola* were significantly reduced in T3 (*Rhizoctonia solani* + *Meloidogyne graminicola* + *Metarhizium anisopliae)* by 74.2% and T1 *(Metarhizium anisopliae* + *Meloidogyne graminicola)* by 54.4% as compared to the control T0 (*Meloidogyne graminicola*) and T2 (*Rhizoctonia solani* + *Meloidogyne graminicola).*

The present study revealed that *Metarhizium anisopliae* can be adopted as a method for suppressing *Meloidogyne graminicola* rootgall populations in rice seedlings. Similar findings were reported by against nematodes, *Meloidogyne* sp. by Devi (2018) and Abdollahi (2018) against *Meloidogyne incognita.*

Biological control agents are perceived to have specific advantages over synthetic fungicides, including fewer non-target and environmental effects, efficacy against fungicide- resistant pathogens and reduced probability of resistance development. *Metarhizium anisopliae* can have different fungitoxic effect on various developmental stages of the plant pathogenic fungus and nematodes.

Table.1 *In- vitro* effect of botanical extracts on the radial growth (mm) of *Rhizoctonia solani*

Treatment no.	Treatment name	Radial growth of *Rhizoctonia solani* (mm) after 3 days					
		Mean of 3 replicates of 5%	Inhibition %	Mean of 3 replicates of 10%	Inhibition %	Mean of 3 replicates of 15%	**Inhibition %**
T0	Control	90	0	90	0	90	**0**
T1	*Justicia*	90	0	81.33	10	76.33	**15.5**
T2	*Chrysopogon zizanioides*	71.66	22.2	53.33	41.1	30.33	**66**
T3	*Centella asiatica*	35	61.1	23.33	74.4	18	**80**
T4	*Lawsonia inermis*	55	38	45	50	22.33	**75.5**
T5	*Tubernaemonta na coronaria*	89	0	74.33	21.1	36.33	**60**
T6	*Cardiospermum halicacabum*	90	0	90	0	85	**5**
T7	*Phyllanthus niruri*	82.33	8	44	51.1	33.66	**63.3**
T8	*Clitoria ternatea*	90	0	87.66	3	71	**21.1**
T9	*Aerva lanata*	90	0	90	0	90	**0**
T10	*Vitex negundo*	70.33	22.2	60.66	33.3	55.33	**38.8**
T11	*Piper nigrum*	45	47.3	42	53.3	13.33	**85.5**
T12	*Murraya koenigii*	84	6.6	48.33	46.6	35	**61.1**
T13	*Biophytum sensitivium*	90	0	90	0	90	**0**
T14	*Ocimum sanctum*	90	0	90	0	90	**0**
T15	*Manihot esculenta*	80	11	59	32.6	20.66	**77**
S.Ed(±)		1.616		1.944		**1.997**	
CD (5%)		**3.306**		**3.977**		**4.085**	

Table.2 *In vitro* effect of *Metarhizium anisopliae* on the radial growth (mm) of *Rhizoctonia solani*

Treatment no	Treatment name	Mean of six replicates	
		3 days	Inhibition %
T0	Control (*Rhizoctonia solani alone)*	90	**0**
T1	*Rhizoctonia solani* + *Metarhizium anisopliae* (1:1)	36	**60**
T2	*Rhizoctonia solani* + *Metarhizium anisopliae* (1:2)	34	**62.2**
S.Ed(±)		**1.587**	
C.D (5%)		**3.959**	

Table.3 Effect of *Metarhizium anisopliae* on root galls population of *Meloidogyne graminicola* in rice seedlings at 60 days after germination

SL NO.	TREATMENTS	NO. OF ROOT GALLS/PLANT	
		Mean of the six replicate	% reduction over control
T0	*Meloidogyne graminicola*	32	**0**
T1	*Metarhizium anisopliae* + *Rhizoctonia solani* + *Meloidogyne graminicola*	15	**54.4**
T2		34	**0**
T3	*Rhizoctonia solani* + *Meloidogyne graminicola*+*Metarhizium anisopliae*	9	**74.2**
S.Ed		**3.681**	
C.D (5%)		**7.73**	

Fig.1 Comparative radial growth (mm) of *Rhizoctonia solani* at 5%, 10%, 15% concentration as affected by plant extracts

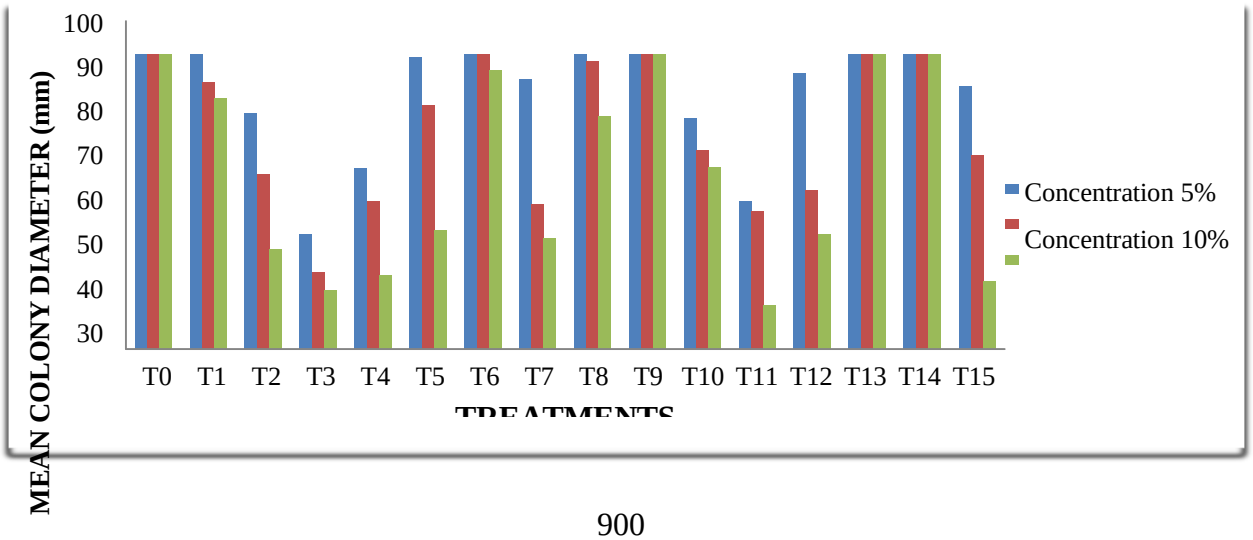


Plate.1 Evaluation of antagonistic activity of medicinal plant powder extract against *Rhizoctonia solani* using Poison Food Technique

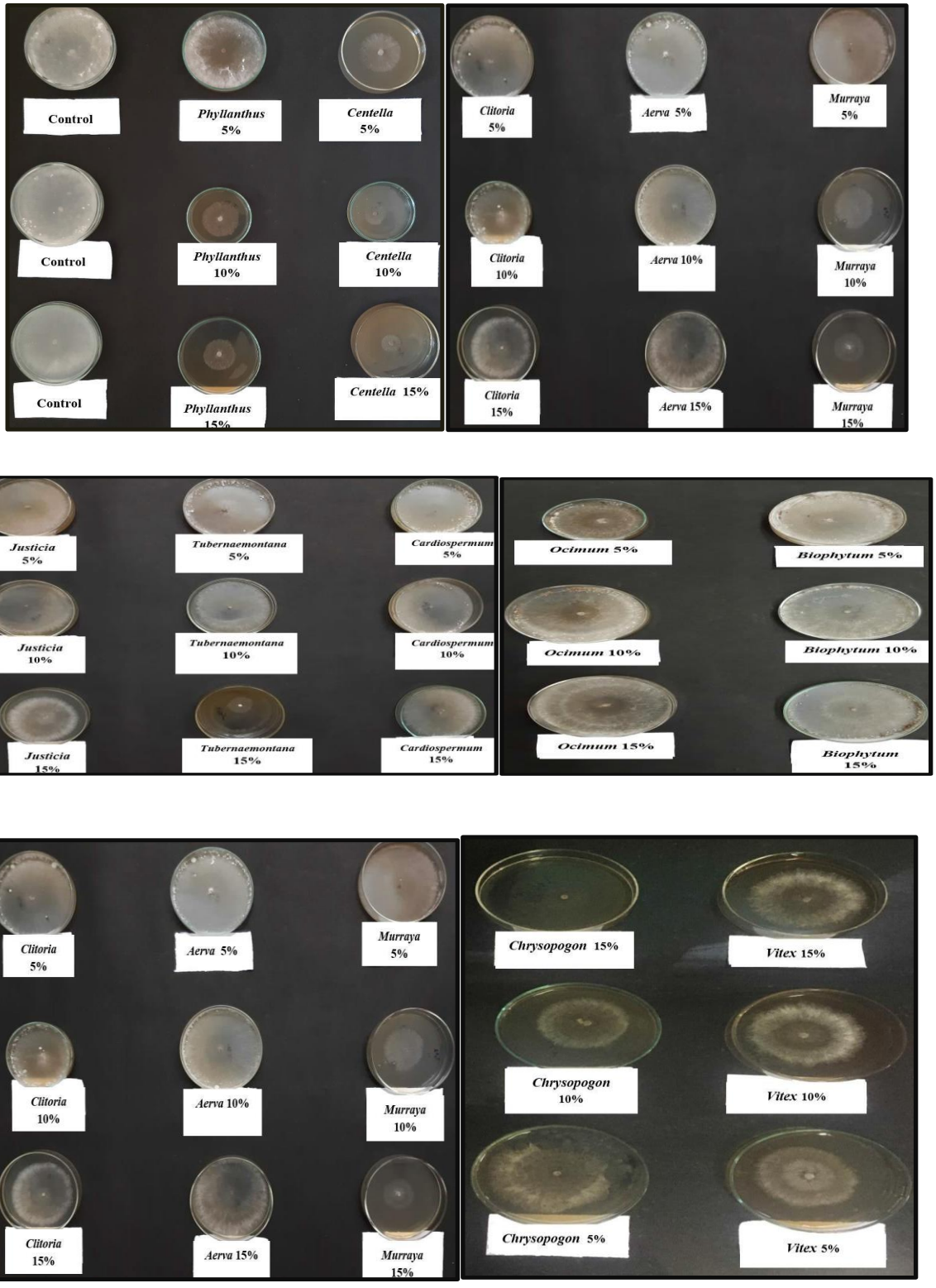
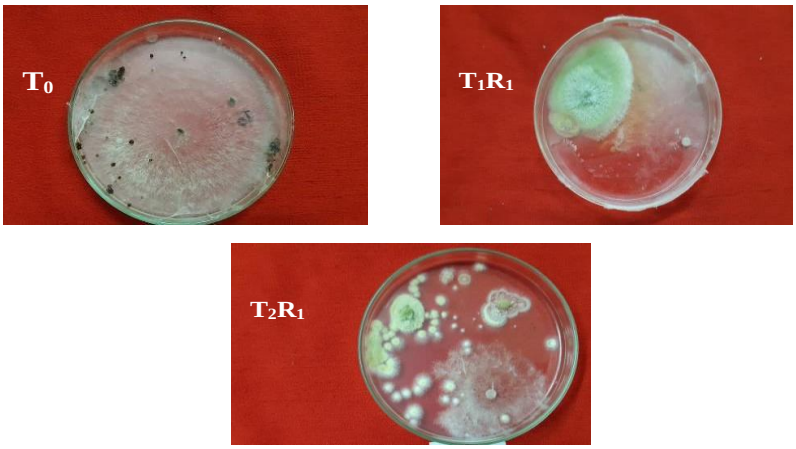


Plate.2 Evaluation of antagonistic activity of *Metarhizium anisopliae* against *Rhizoctonia solani* (T1R1, T2R1) as comparing with control (T0) using Dual Culture Technique



Our results suggested that mycelial growth and spore formation of *Rhizoctonia solani* is affected by the entomopathogenic fungus *Metarhizium anisopliae* and the culture filtrates of *Metarhizium anisopliae* showed nematicidal activity against *Meloidogyne graminicola* by reducing the root gall population in rice seedlings.

The results of poison food technique showed that the leaf extracts of *Piper nigrum*, *Centella asiatica*, *Lawsonia inermis* exhibit antifungal properties against *Rhizoctonia solani*. The inhibition of the growth of the pathogenic fungi is due to the active ingredients predominantly found in the plants which infers that leaf extracts in general have great potentiality in the control of fungal diseases in commercially important crop plants. It can be concluded that keeping aside the environmentally hazardous commercial fungicides, these leaf extracts could be suitable substitute for controlling fungal pathogens

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