Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2020; 9(1): 05-08
Received: 04-11-2019
Accepted: 08-12-2019

Akila R
Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

Mini ML
Department of Biochemistry, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

Corresponding Author:
Akila R
Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai, Tamil Nadu, India

Solvent extraction and antifungal assay of *Lawsonia inermis* Linn. Against the brown spot fungus *Bipolaris oryzae*

Akila R and Mini ML

Abstract
Rice brown spot caused by *Bipolaris oryzae* is the most devastating pathogen ruining rice production next to sheath rot in Tirunelveli and Thoothukudi districts of Tamil Nadu. This fungus is highly responsible for grain discolouration which ultimately leads to qualitative and quantitative losses in rice grain production. In the current scenario of disease management the hazardous fungicide are being replaced by botanicals. Among the eight botanicals tested against the mycelial growth of *B. oryzae* by poisoned food technique the leaf extract (10%) of Maruthani (*Lawsonia inermis*) and Kodukkapuli (*Pithecolobium dulce*) were highly effective. The antifungal compounds of *L. inermis* were extracted subsequently with the series of solvents. Acetone and ethanol fractions are found effective and having much antifungal activity on the mycelial growth of *B. oryzae.*

Keywords: Rice, brown spot, *Bipolaris oryzae,* partial purification, solvents

Introduction
Rice brown spot (*Bipolaris oryzae*) is reported in all the rice growing countries (Khalili *et al*., 2012) [5]. In India it is reported in every rice growing state and it was first reported at Madras presidency in the year 1919. Bengal famine was an important epiphytotic disease at Bengal during 1942 which caused 90 % losses in grain yield (Ghose *et al*., 1960) [3]. Dry condition coupled with poor nutrition led to the development of brown spot (Baranwal *et al*., 2013) [2]. Now a days due to the development of resistance against a synthetic fungicide in the genome of pathogenic fungi an alternative method of disease management using botanicals is gaining momentum to inhibit the pathogen. As the botanicals are having hundreds of antifungal compounds like phenolics, tannins, steroids, alkaloids and terpenoids the pathogens find it difficult to overcome the resistance. Naik *et al.* 2016 [6] revealed that the leaf extract (10%) of *Lawsonia inermis* resulted in 65% reduction of mycelial growth followed by *Pithecolobium dulce.* Partial purification of antifungal compounds was carried out using a series of solvents one by one. Among the solvents, Acetone extract of *L. inermis*. Exhibited the highest antifungal activity against many fungi like *Alternaria solani, Drechslera halodes, D. graminea, Fusarium solani and Curvularia lunata* when tested under poisoned food technique (Ankita Sharma and Kanika Sharma, 2011) [1].

Materials and Methods
Screening of botanicals for antifungal activity
Eight botanicals namely Eucalyptus, Arjuna, Jamoon Turmeric Notchi, Vasambu Maruthani and Kodukaipuli (Manila tamarind) were tested for their antifungal activity against the pathogen *B. oryzae*

Botanical extraction
Twenty five gram of fresh leaves were collected manually and extracted with 25 ml of sterile water (1 g/ml, W/V) using pestle and mortar. The extract was filtered through muslin cloth and finally through Whatman no 1 filter paper and filter sterilized using Seitz filter (45 µm). This formed the standard plant extract (100 %) (Shekhawat and Prasada, 1971) [8]. Ten ml. of the standard plant extract solution (100%) was mixed with 90 ml. of the sterilized PDA medium to get the required concentration (10 %) of the plant extract.

Poisoned food technique
Twenty ml. of this mixture was poured into sterilized Petri dishes and allowed to set. A 9 mm. actively growing PDA culture disc of *Bipolaris oryzae* was placed at the centre of the medium.

The plates were incubated at room temperature (28±2ºC) for seven days. PDA without plant extract was served as control. Three replications were maintained for each treatment. The mean diameter of the mycelial growth of the pathogen was recorded and the results were expressed as per cent reduction of mycelium over control (Schmitz, 1930) [7]

Extraction and fractionation of antifungal compounds from the leaves of *L. inermis*

Extraction was carried out by following the procedure prescribed by Ankita Sharma and Kanika Sharma, 2011 [1]. Dried leaf powder (40 g) was successively extracted with 240 ml of each solvent in the following series viz., petroleum ether-chloroform-acetone-ethanol and water using shaker and flash evaporator. Each time before extracting with next solvent the residue leaf powder was dried in an oven at 50 ºC and used for extraction with next solvent. After complete extraction respective solvents were evaporated under reduced pressure and dried. The residues of each fractions were separately dissolved in acetone. All the extracts obtained were stored at 4 ºC in airtight bottles for further studies to get a concentration of 0.1 g/ml. Per cent extractive values were calculated by following formula

$$\text{Per cent extractive} = \frac{\text{Wt. of dried extract}}{\text{Wt. of dried leaf material (40 g)}} \text{ X } 100$$

Antifungal screening of the fraction

The antifungal activity of prepared fractions were tested using poisoned food technique and agar well method. Accurately 200 µl of different fractions were mixed with 20 ml of molten PDA medium and culture disc of brown spot pathogen *Bipolaris oryzae* was inoculated in the centre of the Petri plate and observed for the growth of mycelium for seven days. The same stock solutions of various solvents were tested against the pathogen using agar well method also. The culture disc was placed on the centre of the Petri plate and the pathogen was allowed to grow for 3 days. On 3rd day after inoculation the fractions of respective solvents was poured in to the four agar wells which were made on the four corners of the Petri plate

Results and Discussion

Screening of botanicals against *B. oryzae*

Among the 8 botanicals tested against the mycelial growth of *Bipolaris oryzae* by Poisoned food technique the leaf extract (10 %) of Maruthani (*L. inermis*) and Kodukaipulli (*P. dulce*) were on par with each other, performed well and recorded 76.40 per cent reduction over control (Table 1; Fig.1). This result was in confirmation with the finding of Ramachandra Naik *et al*., 2016 [6] who reported that the leaf extracts (10%) of Maruthani (*L. inermis*) and Manila tamarind (*Pithecolobium dulce*) were highly effective on the mycelial growth of *B. oryzae* which in turn recording 65 and 60.56 percent reduction of mycelial growth.

According to Harish *et al.*, 2008 [4] about fifty plant extracts were tested against *B. oryzae* by poisoned food technique. Two leaf extracts (10 %) *Nerium oleander* and *Pithecolobium dulce* exerted the higher percentage inhibition of mycelial growth (77.4 and 75.1 respectively) and spore germination (80.3 and 80 respectively).

Fractionation of *L. inermis* and antifungal assay

The percent extractive values of partially purified leaf extract of *L. inermis* was obtained with different organic solvents. The results were given in Table 2, 3 and 4. Petroleum ether fraction gave the maximum percent extractive (2.25 %). Next to this chloroform and ethanol fractions are having the same extractive value of 1.25. Water extract is having the highest percent extractive value but when sterile water is used for the preparation of aqueous extract, the aqueous extract amended plate was contaminated with *Aspergillus* spp. and *Penicillium* spp. Endophytic fungal spores proliferated well as contaminants in this aqueous extract. Ethanol amended plate showed 6.4 cm mycelial growth compared to control (9 cm) in turn recording 29 per cent reduction which is followed by acetone fraction.

Various fractions were tested by inhibition zone method also. Of all the extracts tested acetone and ethanol extract showed maximum inhibition activity on the mycelial growth of *B. oryzae* (Fig. 2 & 3). This finding corroborates with the finding of Ankita Sharma and Kanika Sharma, 2011 [1] who reported that acetone extract fraction was very much effective and inhibiting the maximum mycelial growth of fungi like *Alternaria solani*, *Drechslera graminae*, *D. halodes*, *Fusarium solani*, *F. moniliforme*, *Curvularia lunata* and *Rhizoctonia solani* compared to other solvents like Petroleum ether, benzene, chloroform and methanol. They also added that petroleum ether fraction of *Eucalyptus citriodora* was also effective and inhibiting maximum mycelial growth of the fungi mentioned above. Alkaloids, steroids, quinines and tannins are highly soluble in these fractions.

Extracts used in this study was very small quantity so the presence of active molecules will be lesser. We could formulate the botanical fungicide from the leaf extract of *L. inermis* using the solvent either acetone or ethanol. This extract was eco-friendly, biodegradable and will not cause any phytotoxicity on plants and safe to human and animals. In future these fractions have to be analysed by LC-MS and the structure of individual active principle can be unveiled.

Table 1: Effect of botanicals (10 %) on the mycelial growth of *Bipolaris oryzae* (Poisoned Food Technique)

S. No	Common name	Scientific name	*Mycelial growth (cm)	Per cent reduction over control
1	Eucalyptus	*Eucalyptus globulus*	5.20	41.60
2	Arjuna	*Terminalia arjuna*	7.50	15.73
3	Jamoon	Syzygium cumini	4.63	47.98
4	Turmeric	*Curcuma longa*	5.80	34.83
5	Notchi	*Vitex negundo*	7.63	14.27
6	Vasambu	*Acorus calamus*	4.76	46.52
7	Maruthani	*Lawsonia inermis*	2.10	76.40
8	Kodukaipuli (Manila tamarind)	*Pithecolobium dulce*	2.10	76.40
9	Control		8.9	-
	CD(P=0.05)		0.83	

Table 2: Percent extractive value of various solvents

	Fractions	Percent extractive value
1	Petroleum ether fraction	2.25
2	Chloroform	1.25
3	Acetone	1.0
4	Ethanol	1.25
5	Water	15

Table 3: Effect of various solvent extracts on the mycelial growth of *Bipolaris oryzae* (Poisoned food technique)

S. No	Solvent extracts	*Mycelial growth (cm.)	Per cent reduction over control
1	Petroleum ether fraction	6.7[a]	26
2	Chloroform	6.9[a]	23
3	Acetone	7.6[b]	15
4	Ethanol	6.4[a]	29
5	Aqueous	Contaminated with *Aspergillus* spp., *Penicillium* sp.	-
6	Acetone -control	9.0 [C]	-
	CD (0.05)	0.82	

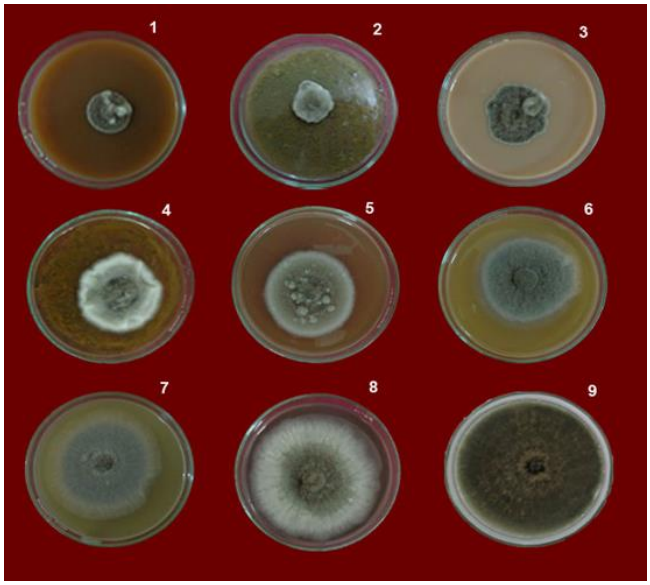
*Mean of three replications

The treatment means are compared using Duncan Multiple Range Test (DMRT)
In a column, means followed by a common letter are not significantly different (P=0.05)

Table 4: Effect of solvent extracts on the mycelial growth of *B. oryzae* (Inhibition zone technique-30 µl/well)

S. No	Solvent extracts	*Inhibition zone (cm.)
1	Petroleum ether fraction	Nil
2	Chloroform	Nil
3	Acetone	0.54
4	Ethanol	0.49
5	Aqueous	Nil
6	Acetone -control	Nil

*Mean of three replications



1. *Lawsonia inermis*
2. *Pithecolobium dulce*
3. Syzygium cumini
4. *Acorus calamus*
5. *Eucalyptus globules*
6. *Curcuma longa*
7. *Terminalia arjuna*
8. *Vitex negundo*
9. *Control*

Fig 1: Effect of botanicals (10 %) on the mycelial growth of *B. oryzae*

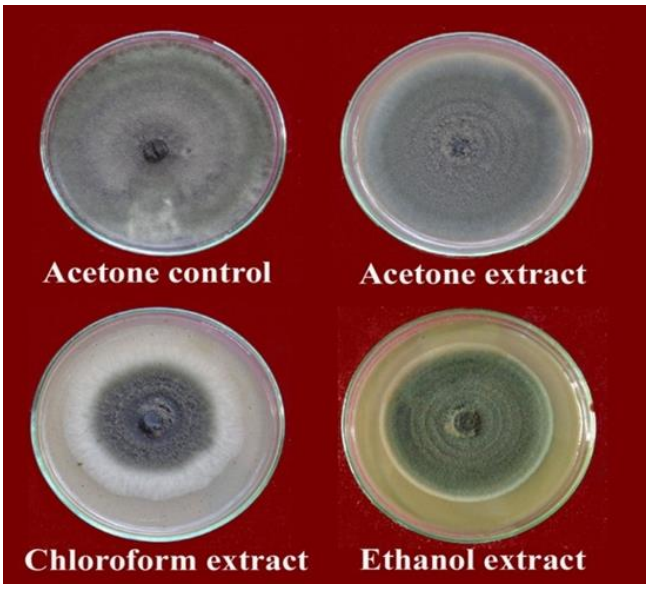


Fig 2: Effect of various solvent extracts on the mycelial growth of *Bipolaris oryzae*- Poisoned food technique (200 µl/20 ml medium)

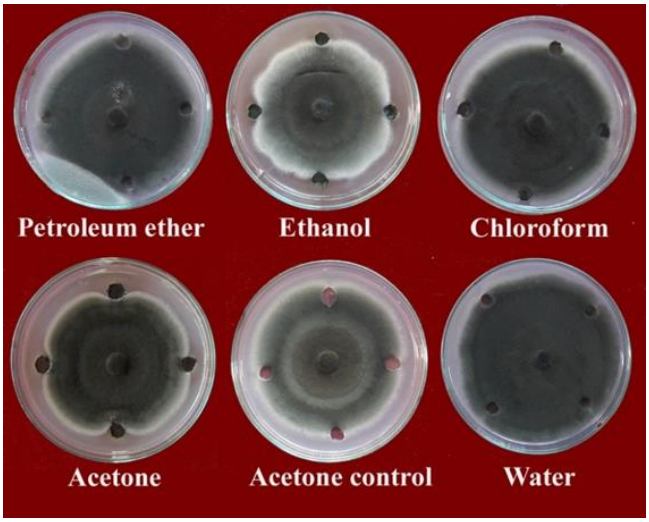


Fig 3: Effect of various solvent extracts on the mycelial growth of *Bipolaris oryzae* – Inhibition zone method (30 µl/well)

References

1. Ankita Sharma, Kanika Sharma. Assay of antifungal activity of *Lawsonia inermis* Linn. And *Eucalyptus citriodora*. J of Pharmacy research. 2011; 4(5):1313-14.
2. Baranwal MK, Kotasthane A, Magculia N, Mukherjee PK, Savary S, Sharma AK *et al*. A review on crop losses, epidemiology and disease management of rice brown spot to identify research priorities and knowledge gaps, 2013.
3. Ghose RLM, Ghatge MB, Subramanian V. Rice in India, New Delhi, ICAR, 1960, pp. 474.
4. Harish S, Duraiswamy S, Ramalingam R, Ebenezar EG, Seetharaman K. Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. Biocontrol. 2008; 53:555-567.
5. Khalili E, Sadravi M, Naeimi S, Khosravi V. Biological control of rice brown spot with native isolates of three Trichoderma species. Braz. J Microbiol. 2012; 43:297-305.
6. Ramchandra Naik M, Akila R, Thiruvudai Nambi. Management approaches for brown spot of rice caused by *Bipolaris oryzae*. J Farm Sci. 2016; 29(3):370-376.

7. Schmitz H. Poisoned food technique. Industrial and Engineering chemistry. Analyst. 1930; 2:361.
8. Shekawat PS, Prasadha R. Antifungal properties of plant extracts. Inhibition of spore germination. Phytopathol. 1971; 24:800-802l.