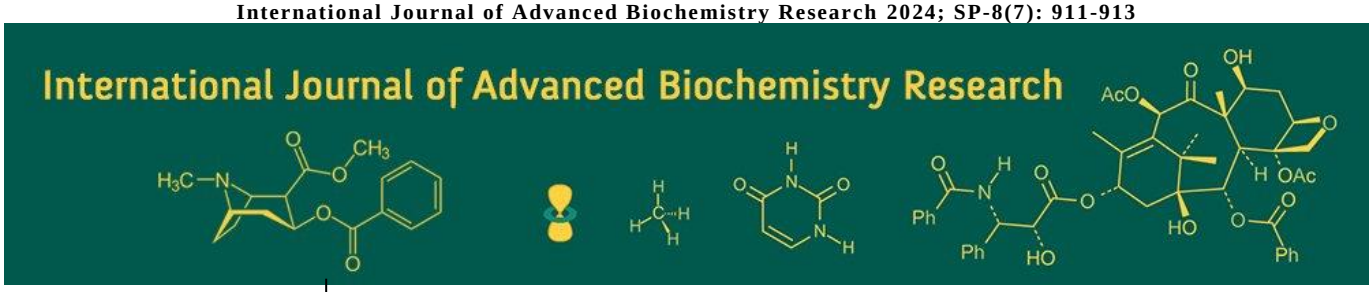

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Paramasivan M
Associate Professor, Department of Plant Pathology, Regional Research Station, TNAU, Virudhachalam, Tamil Nadu, India

I Johnson
Associate Professor, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

A Thangam
Guest Lectures, Government Arts and Science College for Women, Alangulam, Tamil Nadu, India

R Ramjegathesh
Assistant Professor, Department of Plant Pathology, National Research Centre for Pulses, Vamban, Tamil Nadu, India

S Thangeswari
Assistant Professor, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Navarasu
Ph.D. Scholar, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Corresponding Author:
I Johnson
Associate Professor, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Curvularia lunata causing leaf blight barnyard millet (*Echinochloa frumentacea* (Roxb.) link in India and its management by botanicals

Paramasivan M, I Johnson, A Thangam, R Ramjegathesh, S Thangeswari and Navarasu

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Abstract
The management of leaf blight pathogen with nineteen plant extracts tested under *in vitro* conditions. Among twenty plant extracts the 5v (Vilvum, Vembu, Vanni, Vagai and Vengai) plants extracts (10%) effectively inhibited mycelium growth (84.81,64.77,63. 26,61.36 and 48.56 percent reduction over control) of Leaf blight pathogens. The fungus was further identified by amplifying internal transcribed spacer region sequence DNA extraction was carried out by scraping the mycelium and reproductive structures formed in monocultures of the *Curvularia* sp. DNA extraction was carried out according to the CTAB-based protocol, To amplify the DNA from the isolates, primers ITS1 (forward: 5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (reverse: 5'TCCTCCGCTTATTGATATGC-3'). The resulting 550-bp sequence was submitted to GenBank with Accession No. OP604146.

Keywords: Barnyard millet, leaf blight, molecular characterization and *Curvularia*

Introduction
Barnyard millet is a highly nutritious crop affected by several pathogens, of which leaf spot is an important disease hindering productivity. Indian barnyard millet, *Echinochloa frumentacea* has 10.5% protein, 3.6% fat, 68.8% carbohydrate and 398 kcal/100 g energy. The millet relieved people from starvation especially in Tamil Nadu during aberrant weather conditions. The dehulled and heat treated barnyard millet is beneficial for type-II diabetics. This crop is shorter duration it can be easily grown under rainfed or with minimum water supply. Larger cultivation of kuthiraivali in climate changes the Helminthosporium leaf blight incidence was high (47.80%), Grain smut (5%) and *Curvularia* leaf blight (45.50%) for the three years (2017-2020).

Materials and Methods
In 2021-22, Small Yellowish brown spot on leaves to increase in oblong shaped lesions, the center of spot brown coloured around with yellow margins. Lesions are most occurring in leaf margin of Indian barnyard millet of TamilNadu in India. Early symptoms appeared as brown circular spots on the leaves. Pathogen isolations were made on Potato dextrose Agar (PDA) media. Symptomatic leaf samples were cut into 4 to 6 mm 2 pieces, surface sterilized (10% bleach for 1 min, 90% ethanol for 30 sec) and rinsed in sterilized water three times, followed by air drying. These samples were plated onto PDA media and incubated at 25°C for 6 days in the dark.
The isolates were grown in Petri dishes in a potato-dextrose-agar medium (PDA). Subsequently, the characteristics of the colonies and the conidia were observed. The analyzed colonies morphological characteristics were as follows: colony coloration black thin mycelium, border shape irregular, colony reverse side with dark pigmentation and fungus of dark brown colony, bearing large stroma, appeared on the media. Conidiophores were brown, septate, geniculate, simple or unbranched, with dark brown scar. PDA after ten days of incubation in a chamber at a temperature of 25 °C ± 2 °C and a 12-hour photoperiod. The conidia were collected with a platinum loop and transferred to slides, where conidia were using an optical microscope (40X) coupled to a camera, conidia measuring Conidia

were brown, straight to pyriform, with 3 to 4 cells, with large and curved central cells, smooth walled, ranging in size from 5.3 to 19.56 µm, and produced apically in a sympodial manner. Based on morphological characteristics, the pathogen was identified as *Curvularia lunata* (Wakk.)

Results and Discussions

The conidia were collected with a platinum loop and transferred to slides, where conidia were using an optical microscope (40X) coupled to a camera, conidia measuring Conidia were brown, straight to pyriform, with 3 to 4 cells, with large and curved central cells, smooth walled, ranging in size from 5.3 to 19.56 µm, and produced apically in a sympodial manner. Based on morphological characteristics, the pathogen was identified as *Curvularia lunata* (Wakk.), The fungus was further identified by amplifying internal transcribed spacer region sequence DNA extraction was carried out by scraping the mycelium and reproductive structures formed in monocultures of the *Curvularia* sp. DNA extraction was carried out according to the CTAB-based protocol, To amplify the DNA from the isolates, primers ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'TCCTCCGCTTATTGATATGC-3'). The total volume of the amplification reactions was 50 µL, comprising 25 µL of Taq DNA Polymerase 2x Master Mix (Enzymes & Reagents), 5 µL of each 10 µM primer oligonucleotide, 10 µL of ultrapure water and 5 µL DNA of the samples. PCR reactions were run on a thermal cycler (TC-5000) programmed for an initial cycle of 5 min at 94 °C (initial denaturation), followed by 35 cycles of 30 s at 94 °C (denaturation), 30 s at 59 °C (annealing), 30min 70°C (Extension) 7mins 72°C (final extension) The resulting 550-bp sequence was submitted to GenBank with Accession No. OP604146**.** For pathogenicity testing, a conidial suspension (10^6conidia ml−1) from a 7-day-old culture of C.lunata was used to inoculate ten leaves of Barnyard millet (CO (Kv2) variety, followed by incubation in a controlled environment chamber at 25 °C with 70–80% humidity.

As a control, ten leaves of Barnyard millet (CO (Kv2) variety were inoculated with sterile distilled water. Two weeks after inoculation, symptoms were observed only on the leaves inoculated with conidia and the fungus was consistently re-isolated.*C.lunata* was observed on Barnyardmillet in *Curvularia* leaf spot diseases, caused by different *Curvularia* species (iftikhar *et al*., 2016) [1], Silva *et al*. (2014) [3] presented evidence regarding the transmission of *Curvularia* sp. from seeds of other species of grass, Brachiaria, rice, sorghum and millet. To our knowledge, this is the first report of *C. lunata* leaf blight on *Echinochloa frumentacea* India.

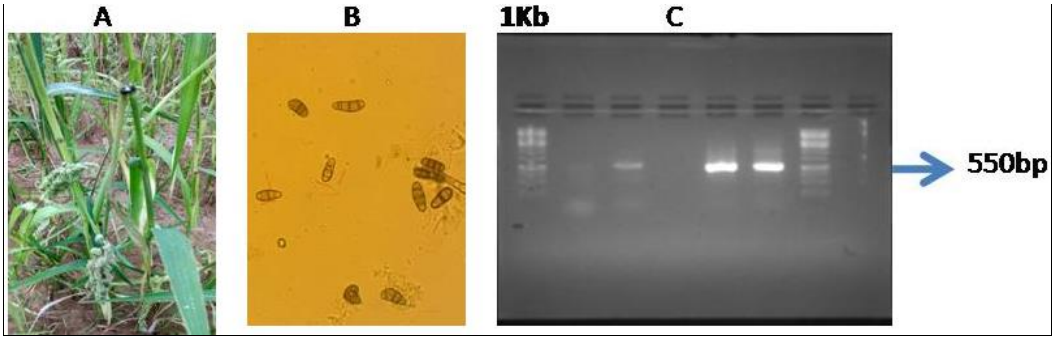

Fig 1: A) Symptoms of Leaf spot of Barnyard millet, B) conidia for *Curvularia lunata*, C) Molecular characterization of *C.lunata* PCR amplification 550 bp

Table 1: Efficacy of Plant extracts against leaf blight of Kuthiraivali

Plant extracts (10%)	Scientific Name	Parts used	Colony diameter (cm)	Percent inhibition over control (%)
Vanni	*Prosopis cineraria*	Leaf	3.2	63.63
Vengai	*Pterocarpus marsupium*	leaf	4.5	48.56
Kodukapuli	*Pithecellobium dulce*	Leaf	6.2	29.54
Vilvum	*Aegle marmelos*	Leaf	2.8	84.81
poovarasu	*Thespesia populnea*	Leaf	7.3	17.04
Duranta	*Duranta erecta*	Leaf	3.9	55.68
Manjanathi	*Morinda tinctoria*	Leaf	6.7	23.86
Notchi	*Vitex negundo*	Leaf	4.1	53.40
Redsandal	*Pterocarpus santalinus*	Leaf	6.5	26.13
MalaiVembu	*Melia dubia*	Leaf	7.3	17.04
Vagai	*Albixia lebbeck*	Leaf	3.4	61.36
Neem	*Azadirachta indica*	Leaf	3.1	64.77
Sarakontrai	*Cassia fistula*	Leaf	8.4	4.50
Pavala malli	*Nyctanthes arbor-tristis*	Leaf	7.5	14.72
Hardwickia	*Hardwickia binata*	Leaf	7.2	18.18
Arali (nerium)	*Nerium oleander*	Leaf	6.8	22.72
Pungam	*Pongamia pinnata*	Leaf	4.5	48.86
Guava	*Psidium guajava*	Leaf	6.8	22.72
Eucalyptus	*Eucalyptus globulus*	leaf	5.1	42.04
Control	-		8.8	-
C.D.			0.93	
SE(d)			0.46	
C.V.			9.93	

Nineteen plant extracts tested the *Aegle marmelos* (10%) 84.17 percent reduction and *Azadirachta indica* (10%) 64.77 percent reduction under *In Vitro* Iwuagwu *et al*., (2020) found that Alcohol extract of *Piper guineensis* had the highest radial growth inhibition of *Azardiractha indica*, which had an inhibition value of 81.02%. The biocontrol and plant extracts easily available farmers' use of organic and rainfed conditions.

Conclusion:

This study assessed the effectiveness of nineteen plant extracts against *Curvularia lunata*, the pathogen causing leaf blight in barnyard millet. Among the tested extracts, *Aegle marmelos* (Vilvum) showed the highest efficacy, with an 84.81% reduction in mycelial growth. *Azadirachta indica* (Neem) also demonstrated significant effectiveness with a 64.77% reduction. These findings suggest that plant extracts offer a promising, eco-friendly approach to managing leaf blight. The results highlight the potential of using locally available plants for disease control, particularly in organic and rainfed farming. Further research is needed to validate these results in field conditions and integrate them into comprehensive pest management strategies.

Reference

1. Iftikhar S, Shahid AA, Nawaz K, Ali SW. First report of *Curvularia lunata* var. *aerial* causing leaf blight on tomato in Pakistan. J Plant Pathol. 2016;98:171-185.
2. Iwuagwu CC, Ononuju CC, Umechuruba CI, Nwogbaga AC, Obidiebube AE, Okolie H, *et al*. Effect of Plant extracts on radial growth of *Helminthosporium oryzae* causative of brown spot disease of rice under *in-vitro* African Crop Science Journal. 2020;28(3):473-480.
3. Silva MSBS, Rodrigues AAC, Oliveira LJMG, Silva EKC, Pereira TS, *et al*. Sanidade de sementes de arroz, biocontrole, caracterização e transmissão de *Curvularia lunata* em semente-plântula de arroz. Revista Ceres. 2014;61:511-517.
4. Das IK, Padmaja PG, editors. Biotic stress resistance in millets. Academic Press; c2016 Aug 3.
5. Lodama KE. Fumonisin production by and biological control of Fusarium species associated with cowpea seed. University of Pretoria (South Africa); c2010.