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FIRST REPORT OF *Rhizoctonia solani* CAUSING RHIZOCTONIA LEAF SPOT OF COTTON (*Gossypium hirsutum* L.) IN INDIA

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ABSTRACT

Cotton is the most important fibre crop of India. Numerous lesions surrounded by prominent dark reddish-brown margins with light greyish centres were observed at two leaf stage and continued up to 30 days after planting in different cultivars of cotton including Jaadoo BG II, RCH 659 BG II, NDLH 1938 and L 1060 at Regional Agricultural Research Station, Lam, Guntur, Andhra Pradesh, India. Isolations from such symptoms resulted in Rhizoctonia and Koch's postulates were proved. Internal transcribed spacer (ITS) region of the isolate was amplified using ITS1 and ITS4 primers and the amplicon was sequenced, deposited in NCBI and accession number MZ350064 was obtained. BLAST search in GenBank revealed 99% homology of *Rhizoctonia solani* Lam isolate (MZ350064) with sequences of *R. solani* (accession numbers, JF 701750 and JF 701751), whereas Thanatephorus sp. LC494248 isolate showed 100% similarity. Phylogenetic analysis revealed that *R. bataticola* out grouped from *R. solani* on comparison with nucleotide sequences. Leaf spot of cotton associated with *R. solani* has not been characterized earlier and thus it becomes first record of confirmation on cotton from India.

Key words : Cotton, *Rhizoctonia solani*, Leaf spot, Characterization.

Introduction

Cotton, the white Gold, is the most important commercial crop of India cultivated in an area of 110.86 l ha with a production of 292.15 l bales and a productivity of 448 kg lint/ha (Waghmare *et al*., 2026). Cotton crop is affected by a number of foliar diseases incited by fungi, bacteria and viruses throughout the season. Fungal leaf spots caused by *Alternaria*, *Corynespora*, *Myrothecium*, *Cercospora* etc. occur during different stages. In view of the regular occurrence and severity, Alternaria and Corynespora leaf spots were investigated to identify the critical weather parameters in disease development and prevent losses by timely interventions. Characterization of Corynespora was reported first time from different parts of India (Salunkhe *et al*., 2019; Bandi *et al*., 2022; Sain *et al*., 2024). In recent years different leaf spot symptoms were observed at two leaf stage which continued up to 30 days after sowing. The present paper reports identification and molecular characterization of causal agent of this new leaf spot in cotton.

Materials and Methods

Unusual leaf spot symptoms were observed at the Regional Agricultural Research Station, Lam, Guntur, Andhra Pradesh, India in upland cotton at two leaf stage which continued up to 30 days after sowing. Numerous lesions surrounded by dark reddish-brown margins with light greyish centres developed on leaves. These symptoms were observed in different cultivars of cotton including Jaadoo BG II, RCH 659 BG II, NDLH 1938 and L1060. Disease incidence of 80-100% was recorded whereas severity of leaf spot ranged from 20-25 per cent disease index during survey in cotton growing mandals in Andhra Pradesh and also at RARS, Lam fields.

Isolation and purification of pathogen

Roving survey was conducted in RARS farm fields (50 acres) to collect samples and cotton leaves with leaf spot symptoms were used for isolating the pathogen. Leaf bits of five mm^2 with healthy and infected leaf portion were surface sterilized using 1% sodium hypo chloride for a min. and rinsed in three changes of sterile distilled

water to remove the disinfectant. Leaf bits were blot dried before transferring aseptically on to potato dextrose agar (PDA) plates and then incubated at 27±1˚C in incubator. Fungal tissue was purified using hyphal tip technique on potato dextrose agar (PDA) at 27±1˚C and observed microscopically to confirm their identity based on morphological characters. Morphological characteristics were consistent with original description of *Rhizoctonia solani* on cotton (Kuhn, 1858; Exner 1953).

Pathogenicity

Prior to characterization, pathogenicity of isolates of *R. solani* was confirmed on cotton hybrid Jaadoo BG II by artificial inoculation under greenhouse conditions. Different doses (10 to 50g) of inoculum multiplied on barley seeds in the laboratory were thoroughly hand mixed with autoclaved soil in pre-sterilized pots in the glass house using 1% formalin; sterile soil without inoculum served as control and pots were maintained under glass house conditions. On third day, cotton seeds of popular hybrid (Jaadoo BG II) were sown in pots (28±2ºC) and observed daily for the development of symptoms (Fig. 1A, B).

Characterization

Characterization of *R. solani* was performed using universal primers of Internal Transcribed Spacer (ITS) region *viz*., ITS1 and ITS4 following the standard protocol (Williams *et al*., 1990) with slight modifications. Ten-day old culture obtained by inoculating potato dextrose broth (PDB) with mycelial discs (cut from the periphery of actively growing 7 days old culture) followed by incubation for 10 days at 27±1˚C was processed for DNA isolation. The mycelial mat developed on PDB was filtered through sterilized muslin cloth, washed in sterile distilled water and blot dried over filter paper. Mycelial mat of each isolate (0.1 g) was ground to a fine powder using sterilized and pre-chilled mortar and pestle in the presence of liquid nitrogen. Half ml of CTAB isolation buffer was added to the mycelial powder and dispensed into sterilized centrifuge tubes separately, 5 µl of Proteinase-K was added to it and incubated in water bath at 65! for an hour by intermittent vortexing. The samples were cooled down to room temperature and 0.5 ml of Chloroform: Isoamylalcohol (24:1) was added and centrifuged (Centrifuge 5418 R, Eppendorf, Germany) at 12,000 rpm for 15 min at 24^{0}C. Aqueous phase was carefully collected and an equal amount of Chloroform: Isoamylalcohol (24:1) was added, centrifuged at 12,000 rpm (24^{0}C) for 15 min. and aqueous phase was recollected. The process was repeated until a clear supernatant was obtained to which 0.6 volumes of isopropanol, 0.1 volume of 3M sodium acetate were added, mixed gently by tilting the tube and allowed to precipitate by incubating at -20˚C for an hour. Then the samples were spun at 12,000 rpm (4˚C) for 10 min. The supernatant was discarded and the pellet obtained was washed thrice with 70% ethanol by centrifuging at 5000 rpm (4˚C) for 5 min. The pellets were air dried and such ethanol free pellets were dissolved in 20 µl of molecular grade water and preserved at -20˚C for future use. The quantity and concentration of the extracted DNA was verified by measuring the absorbance on Nano-Drop (JENWAY Genova Nano Ver.1.58.3). After calculating the dilution factor the DNA samples were diluted to make 50 ng by adding molecular grade water.

PCR amplification of cotton isolates was based on the amplification of ITS region using universal primers (White *et al*., 1990). Forward and reverse primers, *viz*., ITS 1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4 (5' TCCTCCGCTTATTGATATGC 3'), respectively, synthesized based on the conserved 18S and 28S coding regions of the nuclear rDNA were employed. The PCR amplification was carried out in 0.2 ml PCR tubes with 25µl reactions consisting 2.5µl of 10x buffer, 1.5µl of 25 mM $MgCl_2$, 1.0 µl of dNTPs mix (10 mM each), 0.5µl of 5UTaq polymerase (Thermo Scientific), 1.0µl each of primers (10 µM), 2.0µl of DNA and 15µl of molecular grade water. Amplification was performed (40 times) using a thermocycler (Bio-Rad T100 thermocycler, USA) programmed with initial denaturation at 94˚C for 5 min. followed by denaturation at 94˚C for 1 min., annealing at 55˚C for 30 sec, primer extension at 72˚C for 1 min. and final extension at 72˚C for 10 min. A gradient PCR between 55˚C – 65˚C was maintained to know the annealing temperature at which primers bind to the template DNA and amplify the DNA. At 55˚C, the DNA got amplified and the amplicons were separated by gel electrophoresis (Genaxy, India) at 80V and 80mA for 1 h 45 min.

Sequencing and phylogenetic analysis

Fractionated amplicons were excised from agarose gel, eluted, purified and sequenced by Sanger's dideoxy chain-termination method (ABI 3730 (48 capillaries) electrophoresis). The nucleotide homology search for a partial sequence of ITS1 segment of *R. solani* isolates was performed with the BLASTIN sequence analysis of the NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Accession numbers were obtained for the isolates duly submitting the sequences resulted from Sanger method.

Results and Discussion

All the six samples collected from fields yielded

Rhizoctonia. Purified colonies of *Rhizoctonia* were light yellowish brown to white colour with profusely growing cottony fluffy aerial mycelium in concentric rings which later produced round brown sclerotia. Mycelia width measured 8.13±1.43 µm (Fig. 1D). Morphological characteristics were consistent with original description of *Rhizoctonia solani* on cotton (Kuhn, 1858; Exner, 1953; Jeeva *et al*., 2006; Abd-Elsalam *et al*., 2010; Olutoyosi *et al*., 2017).

Pathogenicity

Immediately after two leaf stage, seedlings of inoculated Jaadoo BG II showed characteristic reddish-brown coloured spots with light grey centre (Fig. 1C). Repeated pathogenicity test two times resulted in same symptoms and microscopic examination of leaf spot isolate was done to identify the pathogen morphologically.

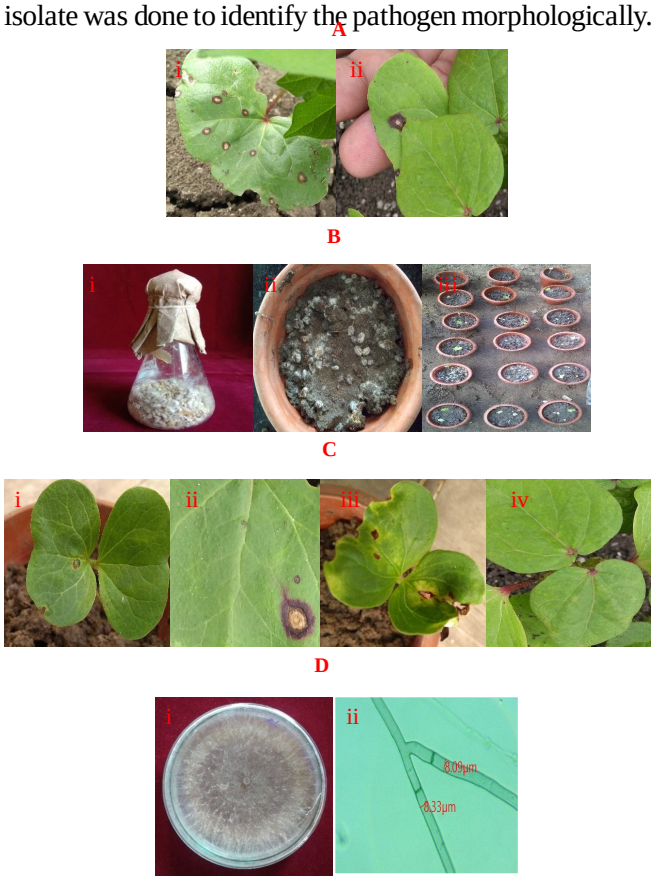


Fig. 1 : Field symptoms of Rhizoctonia leaf spot on cotton, A: i and ii, Artificial inoculaion of *R. solani* Lam isolate for pahogenicity test (B): pahogen multiplied on barley seeds (i), multiplied pathogen mixed with sterilized soil (ii) and seedling emergence from inoculaed soil (iii), inoculated plants showing symptoms on leaves (C): initial symptoms on leaves (i and ii), advanced symptoms on matured leaves (iii) and healthy seedlings (iv), cultural and morphological characters of *R. solani* (D): Colony appearance of pathogen at 4 DAI (i), right angle branching mycelium (ii) at 400X magnification.



Fig. 2 : Phylogenetic analysis of nucleotide sequence of the ITS region of the *Rhizoctonia solani* isolate of Lam (MZ350064), Andhra Pradesh along with available corresponding ITS region sequences.

Koch's postulates were proved by re-isolation and comparing with the original isolate in morphological and cultural characteristics (Fig. 1D).

To confirm the identity of the pathogen, DNA was extracted and internal transcribed spacer (ITS) region of isolate was amplified using ITS1 and ITS4 primers and the amplicon was sequenced (White *et al*., 1990). The sequenced data was deposited in NCBI for which accession number MZ350064 was allocated. BLAST search in GenBank revealed 99% homology of *Rhizoctonia solani* Lam isolate (MZ350064) with sequences of *R. solani* (accession number of JF 701750 and JF 701751) and *Thanatephorus* sp. LC494248 isolate showed 100% similarity. Phylogenetic analysis revealed that *R. bataticola* out grouped from *R. solani* on comparison with nucleotide sequences. Based on the symptoms, fungal morphology, pathogenicity test and ITS sequence comparison the pathogen was identified as *R. solani* as the isolate did not produce any sexual spores on the host.

R. solani is a common and important pathogen in the seedling disease complex of cotton. The symptom most often associated with *R. solani* was postemergence damping-off (Rothrock, 2001). The fungus, *R. solani* was found both externally and internally seed borne in cotton (Khatun *et al*., 2020; Siva Prasad *et al*., 2025). Earlier, Lakshmanan *et al*. (1988) evaluated different media for production of basidiospores from *Rhizoctonia solani* causing leaf spot and boll rot of cotton but they did not

get reproducible results and molecular confirmation was not conducted. Hence, this is the first report of *R. solani* causing leaf spot of cotton.

Conclusion

Identification of the pathogens causing leaf spots in cotton is very essential for developing management strategies at early stages. Since *R. solani* along with *Alternaria*, *Corynespora*, *Fusarium*, *Aspergillus* and *Phoma* were found internally seed borne (Siva Prasad *et al*., 2025) seed treatment with recommended fungicides and/or biocontrol agents (Prasad *et al*., 2025) to prevent seed borne pathogens and ensure vigourous plant stand is recommended to cotton farmers.

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Conflict of interests : Nil

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