# First report of *Myrothecium inundatum* causing leaf spot of Dolichos bean (*Lablab purpureus* L.) in India

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# First report of *Myrothecium inundatum* causing leaf spot of Dolichos bean (*Lablab purpureus* L.) in India

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A hitherto unreported leaf spot disease of Dolichos bean (*Lablab purpureus* L.) caused by the pathogen *Myrothecium inundatum* was noticed in the vegetable field at College of Agriculture, Vellanikkara, Kerala, during 2021 October with a recorded disease severity of 46 %. The typical symptom appeared as brown circular spots with alternate reddish brown and light brown zonations, later forming pin cushion shaped sporodochia in concentric circles. The pathogen upon isolation on PDA, showed dull white mycelium and the pathogenicity was established within two days after inoculation. Microscopic examination revealed the mycelium as septate and branched with subhyaline, aseptate, one-celled conidia, cylindrical to ellipsoidal in shape with rounded ends measuring 4.76 - 7.11 µm length x 1.58 - 2.12 µm breadth. Molecular characterization by amplification of LSU specific sequences followed by in silico analysis revealed the pathogen to be *Myrothecium inundatum*. Preliminary studies on the efficacy of fungicides and biocontrol agents were attempted *in vitro* using poisoned food technique. The contact fungicides like Copper Hydroxide, Propineb, Mancozeb and Bordeaux mixture and the systemic fungicide Tebuconazole showed were found superior *in vitro* exhibiting hundred percentage inhibition at all the three tested concentrations. Among the combination fungicides evaluated, Tebuconazole (50 %) + Trifloxystrobin (25 %) at 0.04 % and 0.05 % and Carbendazim (12 %) + Mancozeb (63 %) at 0.25 % doses showed complete inhibition. The spore germination inhibition assay revealed the fungicides Chlorothalonil, Copper hydroxide, Propineb, Mancozeb, Tebuconazole, Carbendazim, Trifloxystrobin (25 %) +Tebuconazole (50 %), Azoxystrobin (18.2 %) + Difenoconazole (11.4 %) to be showing complete inhibition of *Myrothecium* spores at all three dosages. Among the biocontrol agents, *Trichoderma* showed 100 % inhibition of the pathogen followed by PGPR-II.

**Keywords:** *Lablab purpureus* , leaf spot disease, *Myrothecium inundatum*, symptomatology

## INTRODUCTION

Dolichos bean (*Lablab purpureus* L.) also known as hyacinth bean or Indian bean is an essential leguminous vegetable, whose cultivation is mainly limited to tropical and subtropical regions of India. It is generally known as “poor man’s meat” owing to its numerous nutritional benefits including vitamins, minerals, starch, dietary fibre, phytochemicals and is an ideal source of digestible vegetable protein (20 – 25 %). It is a multipurpose crop that can be utilized as a vegetable, pulse, or forage and is not only valued for its pods rich in proteins but also for the ability to fix nitrogen in the soil, contributing significantly to sustainable agriculture. However, like many other crops, Dolichos bean is susceptible to various fungal diseases including *Colletotrichum* stem and pod blight (Manjunath *et al*. 2013), web blight and collar rot caused by *Rhizoctonia solani* (Kader *et al.* 2022), Sclerotinia stem and pod rot of (Prova *et al.* 2018; Nahar *et al.* 2022) and stem rot by *Sclerotium rolfsii* (Vaniya*et al.*2022). Among the foliar diseases, Cercospora leaf spot (Dey *et al.* 2017; Kokare *et al.* 2020) is found to be major problem producing considerable yield losses, followed by *Alternaria* leaf blight and spot (Brajnath, 2006). A current discovery unveiling a new leaf spot caused by *Myrothecium inundatum* with disease severity of 46 % is an emerging threat to dolichos bean crop. The disease appeared as reddish-brown spots with concentric rings on the leaves of dolichos bean during October 2021 in the vegetable fields of College of Agriculture, Vellanikkara. The genus, *Myrothecium* was identified as a leaf spot and

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blight pathogen on many vegetables, pulses, ornamental plants and other crops of economic importance. Yamazaki *et al*. (2014) isolated *Myrothecium verrucaria* and recorded as a causal agent for rhizome spot in ginger from Japan. Withee *et al.* (2022) described *Paramyrothecium viginicola* from leaves of *Vigna mungo, V. unguiculata, Lablab purpureus, Coccinia grandis, Commelina benghalensis, Solanum virginianum; P. breviseta* from leaves and twigs of *Coffea arabica*; *P. eichhorniae* from *Eichhornia crassipes*; *P. foliicola* from *Tectona grandis* as leaf spot disease in Northern Thailand. From India, *Myrothecium verrucaria* is reported as pathogenic to the seeds of watermelon (Bharath *et al.* 2006) and *Myrothecium roridum* is reported to cause berry rot on *Coffea arabica* (Giri *et al.* 2019) and leaf spot on Bael (*Aegle marmelos* Correa.) (Kumar and Singh, 2021). In Kerala, as per the reports by Praveena and Naseema (2004) *Myrothecium advena* was observed to cause infection on the leaves of water hyacinth to an extent of 61.1 %. Praveen (2016) reported the pathogenic nature of *Myrothecium roridum* causing leaf spot on gerbera with a severity of 9.1 % from Thrissur district of Kerala. Perusal of literature shows that *Myrothecium* has not been reported to cause infection on Dolichos bean in India. Therefore, the study reveals the first documented evidence of *Myrothecium* leaf spot on Dolichos bean and provides an overview on its symptomatology, cultural, morphological and molecular characteristics of the pathogen and the *in vitro* efficacy of chemical fungicides and biocontrol agents against the pathogen.

## MATERIALS AND METHODS

The typical leaf spot symptoms were observed from dolichos fields of Vellanikkara and disease severity was assessed. Characteristic symptom development of the pathogen was studied and documented under both natural and artificial conditions. The fungus was isolated by standard tissue isolation technique on PDA medium and further purification done by single hyphal tip method.

The Mycelial Bit Inoculation method (Rocha *et al.* 1998) was used to establish the pathogenicity of the fungus. Dolichos seedlings grown on pots were artificially inoculated on to the leaves with mycelial bits of pathogen with a moist cotton swab placed over and were maintained in humid conditions at room temperature until symptom development along with control plants.

The pathogen was identified up to genus level based on cultural and morphological characteristics by comparing the description given in CMI Descriptions of Pathogenic Fungi and Bacteria. The molecular characterization was done by isolating the genomic DNA, followed by amplification of specific LSU regions using universal primers of LSU (LROR (5' ACCCGCTGAACTTAAGC 3') and LR7 (5' TACTACCACCAAGATCT 3')) by PCR and obtained sequence was analyzed in BLASTn program of NCBI.

Efficacy of five contact fungicides like Chlorothalonil (75 WP), Copper hydroxide (50 % WP), Propineb (70 % WP), Mancozeb (80 % WP), Bordeaux mixture (35 % WG), three systemic fungicides viz., Tebuconazole (250 EC), Difenoconazole (25 % EC), Carbendazim (50 % WP) and three combination fungicides like Tebuconazole (50 % WG) + Trifloxystrobin (25 % WG), Carbendazim (12 % WP) + Mancozeb (63 % WP), Azoxystrobin (18.2 % SC) + Difenoconazole (11.4 % SC) were evaluated against *Myrothecium inundatum* at three different dosages (lower, recommended, higher) by poisoned food technique and spore germination inhibition assay. The biocontrol agents like *Trichoderma asperellum, Pseudomonas fluorescens*, and microbial consortia viz., PGPR-II and PGPM were assessed for their antagonistic activity against the pathogen by dual culture assay and poisoned food technique. The per cent inhibition of pathogen over control was calculated by using the formula given by Vincent (1927) as follows:

Per cent inhibition = C-T/C*100, where C- growth of the pathogen in control (cm); T- growth of the pathogen in treatment (cm)

## RESULTS AND DISCUSSION

### *Symptomatology*

A leaf spot disease with 46 % severity was observed from the vegetable fields of Vellanikkara

during October in 2021. The symptoms initiated as small light brown spots with reddish brown margins, which later developed to form concentric circles of alternate reddish brown and light brown zones (Fig.1). In later stages, the spots coalesced and spread over the entire leaf lamina to form large blighted areas. Further, white mycelial knots were formed in concentric circles on older lesions, which later developed in to pin cushion shaped sporodochia bearing olive green to black spore masses on the top. Development of dark brown circular abscission zone was observed on the blighted portions which on further stages, got sloughed off leading to shot hole symptoms. The lesions further enlarged to form blights resulting in complete rotting or drying of the leaves depending on the weather conditions. The symptoms described were in line with *Myrothecium roridum* causing leaf spot on Beal (Kumar and Singh, 2021) and gerbera (Praveen, 2016).

### *Isolation and Pathogenicity*

The samples when subjected to isolation on PDA medium, the fungus produced flattened creamy white coloured colony. In order to establish pathogenicity, mycelial bits taken from fifteen days old culture of the pathogen was inoculated on healthy leaves and was kept in humid conditions for disease development. The symptoms started to appear on the inoculated leaves after two days of inoculation (Fig.2). The symptom initiated as small brown spots, which further expanded to form light brown blight with alternate circles of light brown and reddish brown. Further, the size of the lesion increased and it attained the size of 3.4 $cm^2$ area by fourth day of inoculation. Initiation of formation of sporodochia was observed within three days after inoculation on both lower and upper leaf surfaces. Sporodochia was observed later from stem portions also. The pathogen was re-isolated from inoculated plants and the re-isolated fungal isolate was found to be morphologically similar to the initially isolated culture of *Myrothecium.*

### *Characterization and identification of the pathogen*

The pathogen cultured on PDA media, produced dense, circular, flattened, slightly raised, floccose creamy white coloured mycelium (Fig.3). The sporodochia were radially spread over the mycelia as irregular concentric dark black slimy drops, which gradually became dry and hardened when the culture was aged. The reverse side of the Petri plate was whitish with concentric circles. The culture attained full growth in the Petri plate (9 cm) within 14 days of incubation at room temperature and the growth rate was 0.64 cm per day.

The mycelium was branched, septate, and hyaline. Upon microscopic examination (Fig. 4), conidiophores were found hyaline and repeatedly branched, each branch bearing conidia terminally. Conidia were aseptate and subhyaline, one-celled, cylindrical to ellipsoidal with rounded ends. Dimensions of spores ranged from 4.76 - 7.11 µm length x 1.58 - 2.12 µm breadth. Based on cultural and morphological characters, the pathogen was identified at the genus level as *Myrothecium* sp.

The forward and reverse nucleotide sequence by LSU sequencing was compared with known sequences of nucleotides available in NCBI, and it revealed the sequence similarity of 99.5 % with 98 % query coverage and maximum score of 2348 with accession number AY489731.1 of *Myrothecium inundatum*.

### *In vitro evaluation of chemical fungicides and biocontrol agents against Myrothecium inundatum*

The *in vitro* analysis was conducted in Completely Randomized Design (CRD) with three replications each. Among the contact fungicides tested, Copper hydroxide (50 % WP), Propineb (70 % WP), Mancozeb (80 % WP) and Bordeaux mixture (35 % WG) were highly effective against *M. inundatum* exhibiting cent percentage inhibition at all three concentrations (Fig 5). The least effective among them was Chlorothalonil (75 % WP) recorded with 56.29 %, 60.74 % and 62.2 % inhibition at 0.1 %, 0.2 % and 0.3 % doses respectively. In case of systemic fungicides, the Tebuconazole (250 EC) showed higher efficacy (100 %) at all the three doses tested. The least effective fungicide was *Carbendazim* (50 % WP), which showed 45.5 %, 50.37 % and 55.92 %

**Table1:** *In vitro* evaluation of fungicides against *Myrothecium inundatum*

| Fungicide | Concentration (%) | Inhibition (%) |
|---|---|---|
| Chlorothalonil | 0.1% | 56.29 (7.53$^{j}$) |
| | 0.2% | 60.74 (7.82$^{h}$) |
| | 0.3% | 62.2 (7.91$^{g}$) |
| Copper hydroxide | 0.1% | 100 (10.02$^{a}$) |
| | 0.2% | 100 (10.02$^{a}$) |
| | 0.3% | 100 (10.02$^{a}$) |
| Propineb | 0.1% | 100 (10.02$^{a}$) |
| | 0.2% | 100 (10.02$^{a}$) |
| | 0.3% | 100 (10.02$^{a}$) |
| Mancozeb | 0.1% | 100 (10.02$^{a}$) |
| | 0.2% | 100 (10.02$^{a}$) |
| | 0.3% | 100 (10.02$^{a}$) |
| Bordeaux mixture | 0.5% | 100 (10.02$^{a}$) |
| | 1 % | 100 (10.02$^{a}$) |
| | 1.5% | 100 (10.02$^{a}$) |
| Tebuconazole | 0.1% | 100 (10.02$^{a}$) |
| | 0.15% | 100 (10.02$^{a}$) |
| | 0.2% | 100 (10.02$^{a}$) |
| Difenoconazole | 0.05% | 81.85 (9.07$^{f}$) |
| | 0.1% | 82.96 (9.13$^{e}$) |
| | 0.15% | 84.81 (9.23$^{c}$) |
| Carbendazim | 0.05% | 45.55 (6.78$^{k}$) |
| | 0.1% | 50.37 (7.13$^{j}$) |
| | 0.15% | 55.92 (7.51$^{i}$) |
| Trifloxystrobin (25%) + Tebuconazole (50%) | 0.03% | 88.14 (9.4$^{b}$) |
| | 0.04% | 100 (10.02$^{a}$) |
| | 0.05% | 100 (10.02$^{a}$) |
| Carbendazim (12%) + Mancozeb (63%) | 0.15% | 85.92 (9.29$^{c}$) |
| | 0.2% | 88.88 (9.45$^{b}$) |
| | 0.25% | 100 (10.02$^{a}$) |
| Azoxystrobin (18.2%) + Difenoconazole (11.4%) | 0.05% | 84.81 (9.23$^{d}$) |
| | 0.1% | 85.55 (9.27$^{cd}$) |
| | 0.15% | 88.14 (9.41$^{b}$) |
| CD (0.05) | 0.046 | |
| CV | 0.3 | |

Data in parenthesis are square root transformed values

inhibition at 0.05 %, 0.1 %, 0.15 % doses. Among the combination fungicides evaluated, Tebuconazole (50 % WG) + *Trifloxystrobin* (25 % WG) at 0.04 % and 0.05 % and Carbendazim (12 % WP) + Mancozeb (63 % WP) at 0.25 % doses showed complete inhibition. Azoxystrobin (18.2 % SC) + Difenoconazole (11.4 % SC) was found to be comparatively less effective exhibiting inhibition at a range of 88 - 84 % (Table1). Vishwakarma *et al.* (2021) conducted studies on *in vitro* assessment of fungicides against *Myrothecium* sp. isolated from Bael, where cent

**Table 2**: *In vitro* evaluation of fungicides against *Myrothecium inundatum* by spore germination inhibition assay

| Fungicide | Concentration (%) | Inhibition (%) |
|---|---|---|
| Chlorothalonil | 0.1% | 100 (10.02 [a]) |
| | 0.2% | 100 (10.02 [a]) |
| | 0.3% | 100 (10.02 [a]) |
| Copper hydroxide | 0.1% | 100 (10.02 [a]) |
| | 0.2% | 100 (10.02 [a]) |
| | 0.3% | 100 (10.02 [a]) |
| Propineb | 0.1% | 100 (10.02 [a]) |
| | 0.2% | 100 (10.02 [a]) |
| | 0.3% | 100 (10.02 [a]) |
| Mancozeb | 0.1% | 100 (10.02 [a]) |
| | 0.2% | 100 (10.02 [a]) |
| | 0.3% | 100 (10.02 [a]) |
| Bordeaux mixture | 0.5% | 54.56 (7.4 [e]) |
| | 1 % | 78.5 (8.8 [b]) |
| | 1.5% | 100 (10.02 [a]) |
| Tebuconazole | 0.1% | 100 (10.02 [a]) |
| | 0.15% | 100 (10.02 [a]) |
| | 0.2% | 100 (10.02 [a]) |
| Difenoconazole | 0.05% | 100(10.02 [a]) |
| | 0.1% | 100 (10.02 [a]) |
| | 0.15% | 100 (10.02 [a]) |
| Carbendazim | 0.05% | 66.33 (8.17 [d]) |
| | 0.1% | 100 (10.02 [a]) |
| | 0.15% | 100 (10.02 [a]) |
| Trifloxystrobin (25%) + Tebuconazole (50%) | 0.03% | 100 (10.02 [a]) |
| | 0.04% | 100 (10.02 [a]) |
| | 0.05% | 100 (10.02 [a]) |
| Carbendazim (12%) + Mancozeb (63%) | 0.15% | 76.43 (8.7 [c]) |
| | 0.2% | 100 (10.02 [a]) |
| | 0.25% | 100 (10.02 [a]) |
| Azoxystrobin (18.2%) + Difenoconazole (11.4%) | 0.05% | 100 (10.02 [a]) |
| | 0.1% | 100 (10.02 [a]) |
| | 0.15% | 100 (10.02 [a]) |
| CD (0.05) | 0.037 | |
| CV | 0.23 | |

* Data in parenthesis are square root transformed values

percentage inhibition was recorded with Tebuconazole which is comparable to the present study.

The spore germination inhibition assay against *M. inundatum* revealed that the fungicides like Chlorothalonil (75 % WP), Copper hydroxide (50 % WP), Propineb (70 % WP), Mancozeb (80 % WP), Tebuconazole (250 EC), Carbendazim (50 % WP), Tebuconazole (50 % WG) + Trifloxystrobin (25 % WG), Azoxystrobin (18.2 % SC) + Difenoconazole (11.4 % SC) showed complete inhibition to *Myrothecium* spores at all three dosages. Carbendazim and Carbendazim (12 % WP) + Mancozeb (63 % WP) showed slight germination of spores, but at higher concentrations complete inhibition of spores was noted. (Table 2).

The response of *Trichoderma* spp. against *M. inundatum* showed better control of pathogen by

**Table 3**: *In vitro* evaluation of biocontrol formulations against *Myrothecium inundatum*

| Biocontrol agent | Concentration (%) | Inhibition (%) |
|---|---|---|
| *Trichoderma asperellum* | 1 | 100.00 (10) [a] |
| | 2 | 100.00 (10) [a] |
| | 3 | 100.00 (10) [a] |
| *Pseudomonas fluorescens* | 1 | 32.2 (5.71) [g] |
| | 2 | 54.07(7.38) [f] |
| | 3 | 82.59 (9.1) [c] |
| PGPR - II | 1 | 70.740(8.4) [e] |
| | 2 | 87.03 (9.3) [b] |
| | 3 | 100.00 (10) [a] |
| PGPM | 1 | 80.74 (9.1) [d] |
| | 2 | 82.96 (9.1) [c] |
| | 3 | 87.40 (9.3) [b] |
| CD (0.05) | 0.086 | |
| CV | 0.569 | |

*Data in parenthesis are square root transformed values

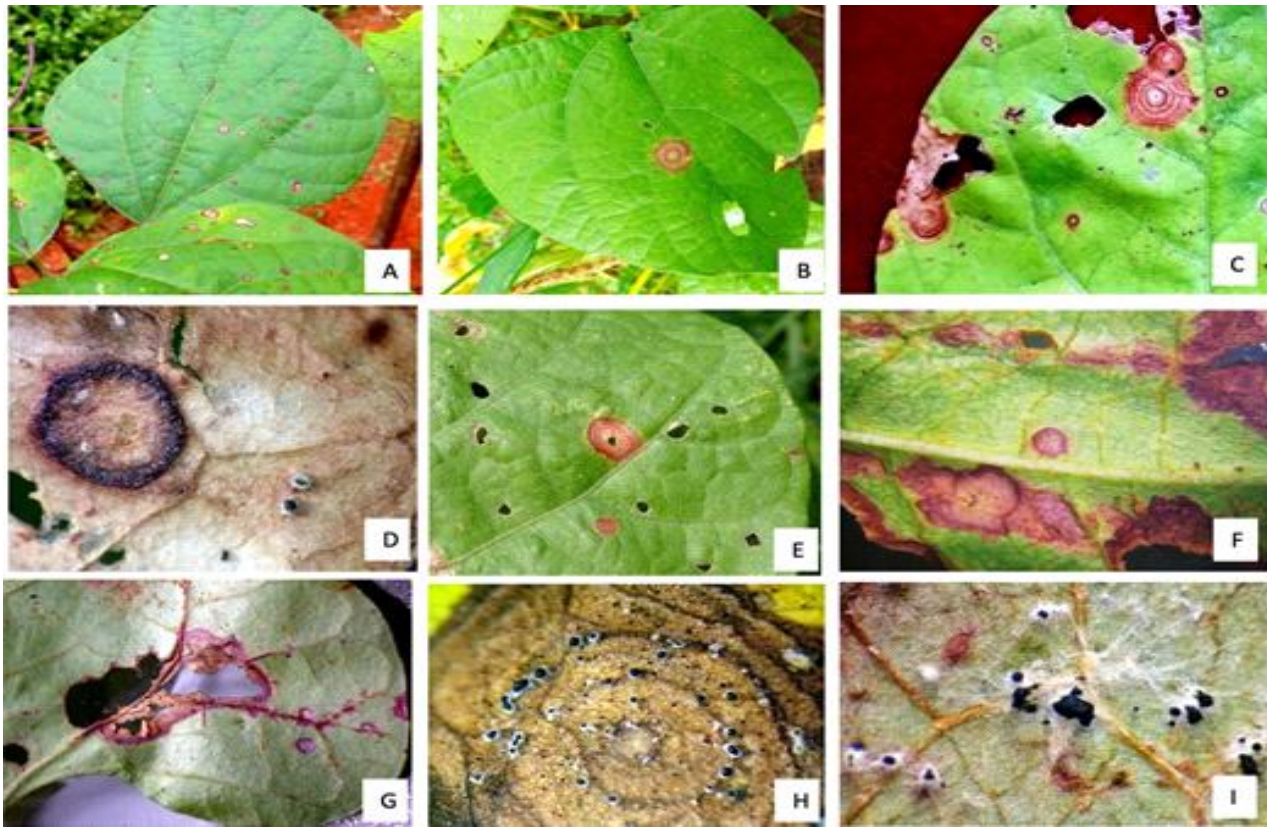


**Fig. 1:** Development of symptoms of *Myrothecium* leaf spot under field conditions. **A**.Initial light brown spots with reddish brown margins**B**.Development of concentric zones – target board symptom **C**. Coalescence of spots**D**.Development zone of abscission**E**.Formation of shot holes**F**.Development of leaf blight**G**.Enlarged shot holes**H**. Sporodochia formed in concentric circles **I**.Mature sporodochia


**Fig.2 (A-D):** Development of symptoms on artificially inoculated plants

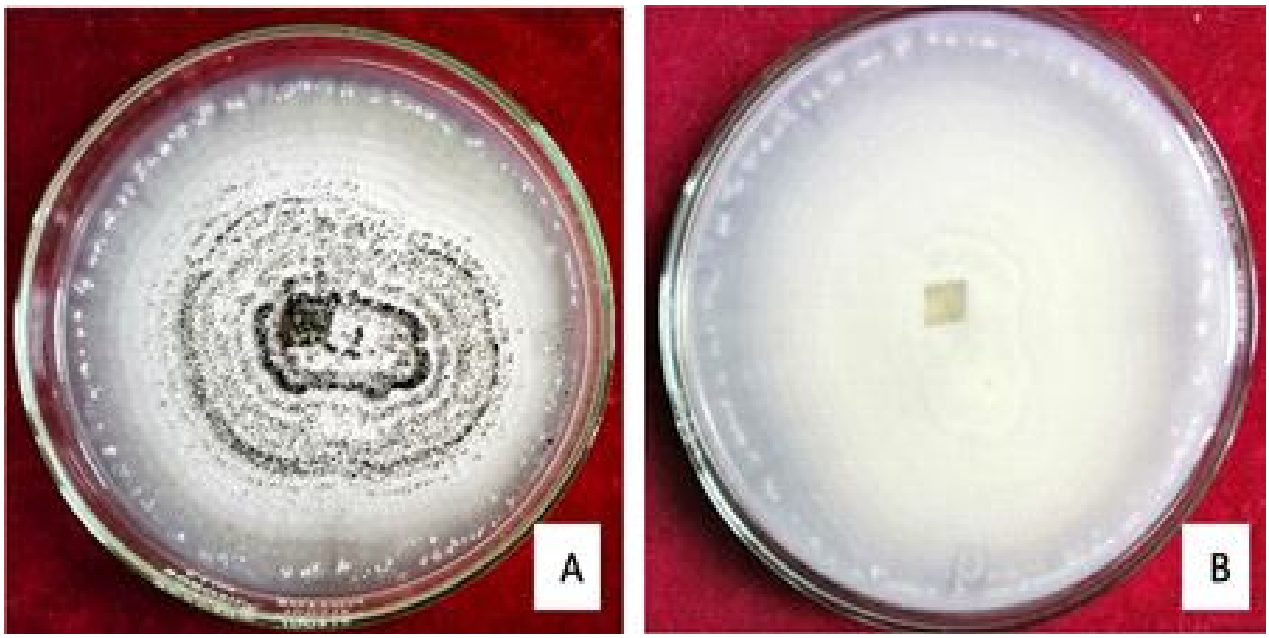


**Fig.3:** Growth of *Myrothecium roridum* on PDA

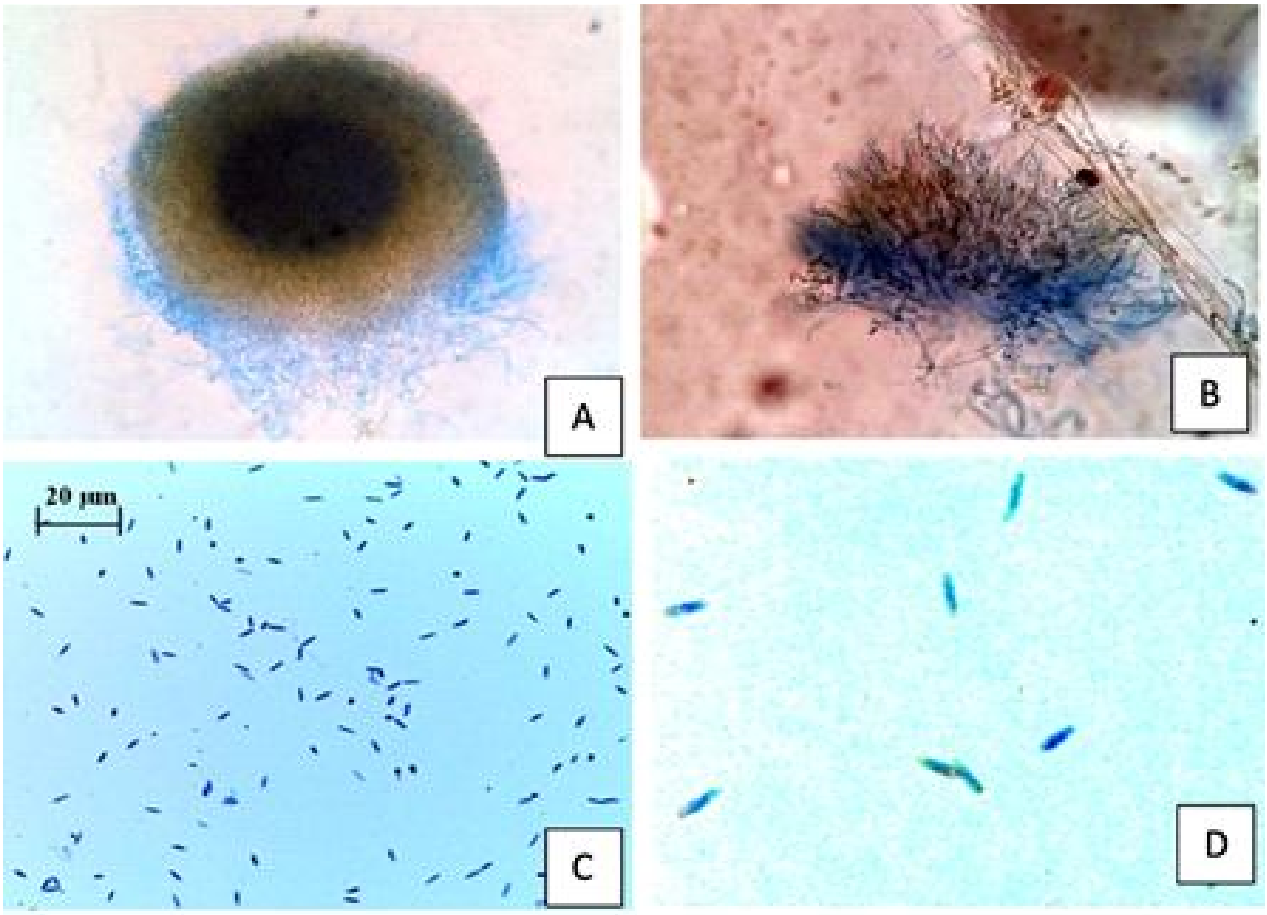


**Fig.4:** Sporodochia, conidiophores and conidia of *M. inundatum* **A**. Sporodochia of *M. inundatum***B**.Conidiophores of *M. inundatum* **C**.Conidia of *Myrothecium* (400 X) **D**. Conidia of *Myrothecium* (1000 X)

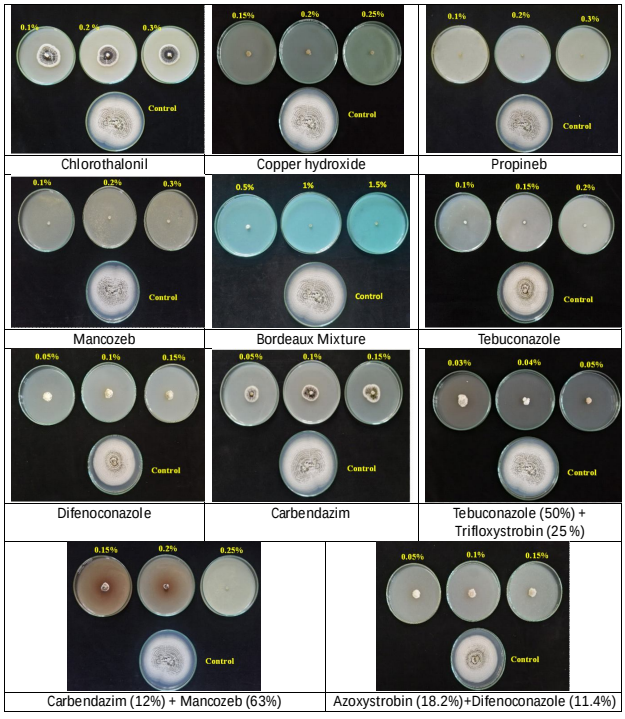


**Fig.5:***In vitro* efficacy of fungicides against *Myrothecium inundatam*

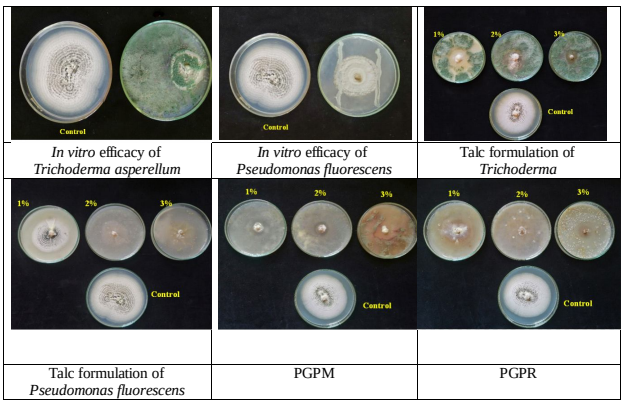


**Fig.6:** *In vitro* efficacy of bioagents and their formulations against *M. iunundatum*

overgrowth mechanism and 66.29 % inhibition was found against *P. fluorescens* antagonist in dual culture assay (Fig.6). When tested with *Trichoderma* talc formulation (Table3), the antagonist completely covered the pathogen by six-to-eight days recording cent per cent inhibition. However, it showed 82.59 %, 54.07 % and 32.2 % inhibition at 3 %, 2 % and 1 %

concentrations of Pseudomonas fluorescens talc formulations. Studies conducted by Ranjini and Naika (2019) revealed that the *T. viride* recorded an inhibition of 85.9 % and *P. fluorescens* with 50.6 % in dual culture assay of Myrothecium roridum. Dighule*et al.* (2011) noted an inhibition percentage of 75 % for *T. viride* against *Myrothecium roridum* from cotton. The PGPR-II microbial consortia showed cent per cent inhibition against pathogen at 3 % and 87.03 % and 70.74 % inhibition at 2 % and 3 % concentrations. Talc based PGPM formulation was observed to show inhibition of 87.4 %, 82.96 %, and 80.74 % at 3 %, 2 and 1 % concentrations. An overview of literature reveals that Myrothecium has not so far been reported from Dolichos bean in India. Hence this appears to be the first report of incidence of Myrothecium leaf spot on Dolichos bean from India. The nucleotide sequence of the fungus was deposited in gene bank of NCBI with accession number QQ345810.

## CONCLUSION

The paper reveals the incidence and symptomatology of leaf spot associated with dolichos bean from Kerala. The pathogen associated was identified based on cultural and morphological characters and the identity was confirmed as *Myrothecium inundatum* via molecular characterization. From the study, it was concluded that contact fungicides like Copper hydroxide (50 % WP), Propineb (70 % WP), Mancozeb (80 % WP), systemic chemical like Tebuconazole (250 EC) and the combination fungicide Tebuconazole (50 % WG) + Trifloxystrobin (25 % WG) were effective *in vitro* against *M. inundatum*. Among the biocontrol agents, *Trichoderma* was found to be highly effective against Myrothecium.

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