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Dactuliophora mysorensis sp. nov.: A New Species of Mycelia Sterilia Causing Zonate Leaf Spot on Cowpea in India

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Abstract

Cowpea is an important pulse crop extensively grown in arid and semi-arid tropics which is affected by a number of diseases. Fungi belonging to mycelia sterilia are known to cause many diseases on cereals and pulses. During the cowpea field survey in Mysore District of Karnataka (India), *Dactuliophora* sp. was identified as the major pathogen causing zonate leaf spot (ZLS) disease. The fungal pathogen was isolated from naturally infected cowpea leaves and identified as a member belongs to the genus *Dactuliophora*, which was previously described by CLA Leakey in the year 1964 on *Vigna unguiculata* from Africa. However, detailed morphological and cultural examinations of the pathogen revealed striking differences from that of *D. tarrii*. Based on differences in morphology with *D. tarrii*, a new species *Dactuliophora mysorensis* sp. nov. is described herein. The disease incidence as well as disease index was estimated for 3 years (2016–2018). The severity of the disease was high during August–November. High incidence and disease index of ZLS was recorded in Doddamaragowdanahally region. The pathogenicity tests demonstrated similar symptoms of ZLS. The ITS barcoding revealed that the pathogen is closely related to *Rhizoctonia bataticola* and *Macrophomina phaseolina*. Further, in vitro evaluation of fungicides was carried out by poisoned food technique. Among the five fungicides examined, only two systemic fungicides (Benomyl and Carbendazim) were effective against *D. mysorensis*. Thus, the present study recommends Benomyl and Carbendazim for management of ZLS disease caused by *D. mysorensis*.

Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. (Fabaceae) is an important staple food for millions of people in the arid and semi-arid tropics [3, 25]. It has been estimated that about 3.3 million metric tonnes of cowpea produced worldwide during 2000. The global production of cowpea is up to 5.59 million metric tonnes. The Western Africa contributes cowpea production up to 81% followed by Eastern Africa (8.68%) and Central Africa (4.37%) [1, 14, 33]. Cowpea is also a chief grain legume in the sub-tropics as food and forage in sub-Saharan Africa [9, 32]. The grains of cowpea besides providing proteins and carbohydrates, its tender leaves are also edible [23, 28, 33]. Cowpea serves as potential nutritious fodder for the livestock. It is a resourceful crop owing to restoration of nitrogen content in soils leading to enhancement of soil fertility to grow cereals as an alternate crop [5, 10, 21, 24].

In India, pulses have been regarded as source of protein and they play an important role in healthy diets, sustainable food production and in larger context the food security. India has produced 23.40 million tonnes of pulses during 2018–2019 crop year yet we import 26–28 million tonnes to meet the national requirements [2]. Karnataka State is one among the main producers of pulses. The area under cowpea production in Karnataka state is increasing, there are several constraints allied with cowpea production as a result we are experiencing a large quantity of production loss. A large number of plant diseases and pests are

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considered as major constraints to cowpea production and they affect the crop at various stages of growth resulting in significant yield loss [29, 31]. The important diseases threatening the cowpea production includes anthracnose, blights, charcoal rot, collar rot, leaf spots, rusts, powdery mildew, root rot, southern blight, and others.

Recently, various researchers have reported the incidence of root rot/dry root rot diseases in cowpea caused by *F. equiseti* [17], *F. oxysporum, F. proliferatum* [26, 27] in the United States; target leaf spot disease by *Corynespora cassiicola* in China [16], leaf spot diseases by *Pestalotiopsi*s sp., *Dactuliophora* sp., and collar rot by *Aplosporella hesperidica* [6, 18, 19] in Southern India. *Dactuliophora* sp. was considered causing minor leaf spot disease of cowpea in Tropical Africa [30]. However, the disease incidence and severity reported in this paper denotes that *Dactuliophora* sp. causes a major disease in Southern India. The field survey was carried out to determine the occurrence of the zonate leaf spot (ZLS) disease. Therefore, the present study aims at detailed study of its morphological and cultural features to establish and to document the pathogen identity, extent of distribution and severity of disease in southern districts of Karnataka, India.

Materials and Methods

Survey of Disease

During 2016–2018, the major cowpea producing regions of Mysore district of Karnataka State were visited and the occurrence of ZLS disease was observed in many fields. The disease incidence (DI) and per cent disease index (PDI) was estimated following the procedure proposed by Mahadevakumar et al. [20]. The survey was carried out during months of August–November in a farmers' field in main cowpea growing locations. Disease Incidence was assessed by expressing the number of ZLS diseased cowpea plants. To record the disease in an infected cowpea field, 5 m^2 area each at four corners and the centre (total, 5 plots) were selected. On each plot, a number of healthy plants and infected plants were recorded. Further, to measure the PDI, all the infected leaves were categorized into different grades of infection using the following grading scale: 0 = no infection; 1 = 1–5%; 2 = 6–25%; 3 = 26–50%; 4 = 51–75%; and 5 = 76–100% leaf lamina covered by infection [20]. The incidence and severity were the mean value of leaf spot infection of 3-year assessment (data expressed in percentage). The diseased leaf samples were collected for further investigation. The fungal pathogen was isolated from the infected leaves.

Isolation and Identification

Infected cowpea leaves were cut into 1 cm^2 pieces, surface-sterilized (sodium hypochlorite, 2%) followed by rinsing thrice with sterile distilled water. The leaf pieces were blotter dried and inoculated on the surface of potato dextrose agar (PDA) medium. After incubation up to 5 days, fungal colonies developed from the infected leaf pieces were sub-cultured on fresh PDA medium to obtain pure cultures. The morphological features of the pathogen on the host plant and its cultural characteristics on the medium were considered for identification [4, 8]. A censorious examination of the symptoms and sclerotial characteristics by micrometry were considered for comparison with the *Dactuliophora tarrii* to characterize the new pathogen.

Pathogenicity Test

Pathogenicity tests were performed on healthy cowpea plants under green house conditions to ascertain the association of the isolated fungus causing ZLS disease. The experiments were conducted on 45 days-old healthy cowpea plants with 15 days-old suspension of *Dactuliophora* sp. [18]. The inoculated plants were kept under high humidity (80%) for 5 days and at the ambient conditions. Cowpea plants inoculated with distilled water served as control. The experiments were carried out in triplicates with two repetitions to confirm the pathogenicity and successful manifestation of symptoms on the host plant similar to naturally infected ones.

Molecular Identification

The genomic DNA was isolated using 10-day-old fungal cultures using the CTAB method [36]. The PCR was performed using Applied Biosystems Veriti Thermocycler (Foster City, CA, USA). The ITS-rDNA region was amplified employing ITS1 and ITS4 universal primers [35]. The PCR reaction was carried out in 25 µL reaction mixture containing 2 µL of DNA sample, 12.5 µL of ready-mix (Genei, Bangalore, India), 20 pmol of each forward and reverse primers (1.0 µL) (Sigma, Bangalore, India) and the final volume was made up to 25 µL with 8.5 µL of nuclease-free water. The PCR programme include initial denaturation (95 °C, 5 min) followed by 38 cycles of denaturation (94 °C, 1 min), primer annealing (55 °C, 30 s), extension (72 °C, 2 min) and the final extension (72 °C, 12 min). The amplified PCR products were sequenced using an ABI3730 x I DNA analyzer (Applied Biosystems, Foster City, CA, USA). The representative reference sequences were retrieved from the NCBI-GenBank Database. The DNA sequences were nBLAST searched against the NCBI nucleotide database for closely

matching relatives. A phylogenetic tree was constructed for further confirmation of the species in a combination of DNA sequences of our isolates and the reference sequence from the GenBank database. The reference sequences were retrieved from the GenBank database and phylogenetic tree was constructed using the Neighbour-Joining (NJ) method as implemented in MEGA 7.0 with Kimura-two-parameter model with 1000 bootstrap replications [13].

Sensitivity to Fungicides

In the present study, three systemic fungicides viz., Thiophanate-Methyl, Benomyl and Carbendazim; one contact—Mancozeb and one combi product—Mefenoxam, were used (Table 1). All the five fungicides were tested at three different concentrations (100, 150, 200 mg/L) using the poisoned food technique [22]. The requisite quantity of each fungicide was dissolved in PDA medium after autoclaving and well grown 7 day-old culture of *Dactuliophora* sp. (0.7 cm diam disc) was placed at the centre of PDA plates. The PDA medium with sterile distilled water served as control and plates were observed for the growth of fungi. On the day 5, the radial growth of the fungal colony was recorded and the per cent growth inhibition was calculated: $I = C - T/C \times 100$ (where, I, per cent inhibition; C, growth in control; T, growth in treatment) [34].

Statistical Analysis

The data on in vitro efficacy of fungicides were analysed statistically with One-Way ANOVA @ $P < 0.05$ level by using SPSS Inc. 16.0 and treatments means were separated by Tukey's Honestly Significant difference (HSD) tests.

Results

Diagnosis and Incidence of Disease

During the survey, the disease incidence (DI) of a new leaf spot disease was found with characteristic symptoms distinguishable from other diseases. The ZLS disease recorded on cowpea displayed characteristic rosette-like patches with whitish to tan spots on the upper leaf surface and dark-reddish brown bearing sclerotia attached through sclerotiophores on the lower surface. Leaf spots are zonate on upper as well as on the lower side of the leaf usually up to 2–3 cm in diameter, rosette-like with alternating whitish and tan bands on the upper leaf surface and light-brown on the lower leaf surface with sclerotiophores bearing dark-brown sclerotia. These sclerotia are the means of perennating structures which disseminates to considerable distance (Fig. 1).

The prevalence and severity of *Dactuliophora* species infected cowpea were evaluated in 17 farmers field in and around Mysore district. The ZLS was recorded only in four locations and the incidence ranged from 8 to 22% and the PDI ranged from 1.8 to 4.6, 1.1–6.8 and 0.8–6.3 during the years 2016–2018, respectively (Table 2). The prevalence of ZLS disease was severe in Doddamaragowdanahally village (Mysore, Karnataka State, India) up to 81.39%. The details of disease incidence and PDI of ZLS disease during the survey are presented in Table 2.

Isolation and Identification

The fungal pathogen associated with ZLS disease was isolated on PDA medium. Mycelia submerged, hyaline, diffused throughout the leaf tissue and accumulated in plectenchymic masses beneath the sclerotiophores. Sclerotiophores were erumpent and shaped a ring of dark coloured hyphae, which are consistent with a sub-epidermal plectenchymic mycelial mass. The sclerotia were scattered on abaxial surface of the infected leaves, solidly hypophyllous, circular to subspherical, measured 95–185 µm in diameter bearing 4–9 scattered setae. The sclerotial setae were cylindrical, often sinuate, measured 190 µm long, 3–4 µm width and marginally constricted distally to shape a blunt tip and setae with 0–3 septa (Figs. 1, 2). On PDA medium, the growth rate was 1.15–1.95 mm/day at 27 °C. Development of profuse sclerotia were seen, those were glomerate clusters scattered over the colonies. The aerial mycelia were tan and pale-brown underneath. Sclerotia developed on well differentiated sclerotiophores like those in live host plants. A

Table 1 Fungicides used for in vitro evaluation against *Dactuliophora mysorensis*

Fungicide	Active ingredient	Trade name	Manufacturer
S	Thiophanate-Methyl 70% W.P.	Roko®	Biostadt India Ltd.
SC	Mefenoxam (Metalaxyl-M)	Ridomil Gold®	Syngenta India Ltd.
S	Benomyl 50% W.P.	Benofit®	Universal crop protection Ltd.
C	Mancozeb 75% W.P.	Indofil M-45®	Indofil Chemical Company, Mumbai, India
S	Carbendazim (Benzimidazole)	Bavistin®	BASF Australia Ltd.

S systemic fungicide, *C* contact fungicide, *SC*, mixture of systemic and contact fungicides—combi product

Fig. 1 Zonate leaf spot (ZLS) disease of cowpea caused by *Dactuliophora mysorensis*: **a**, **b** typical ZLS symptoms observed on cowpea; **c**, **d** close view of ZLS spot—upper surface on cowpea; **e** lower surface of ZLS disease of cowpea showing sclerotia developed on the necrotic lesions; **f**, **g** stereo view of ZLS disease showing sclerotia developed along with sclerotiophores on the necrotic lesion (Scale = 5 mm); **h**, **i** pure cultures of *D. mysorensis* and microsclerotia on PDA medium

Table 2 Prevalence, disease incidence (DI) and severity of zonate leaf spot disease in different locations of Karnataka, India

Location	*N*/*N**	Prevalence (%)	Disease incidence (DI) (%)			Percent disease index (PDI)		
			2016	2017	2018	2016	2017	2018
DMG Hally	35/43	81.39	22.0	18.0	19.0	4.6	5.4	6.3
Kamarahally	03/18	16.66**	–	–	–	–	–	–
Yelwala	05/25	20.00**	–	–	–	–	–	–
Bannikuppe	00/21	00.00	–	–	–	–	–	–
Alanahally	00/32	00.00	–	–	–	–	–	–
Basavanahally	00/18	00.00	–	–	–	–	–	–
Rayanahally	03/25	12.00**	–	–	–	–	–	–
G. B. Sargur	20/48	41.66	18	16	15	3.4	3.8	7.2
Gollanabeedu	02/20	10.00**	–	–	–	–	–	–
Shindenahally	18/26	69.23	16	18	16	2.7	6.8	4.6
Jayapura	05/32	15.62**	–	–	–	–	–	–
Kyathanahally	00/28	00.00	–	–	–	–	–	–
Thumbasoge	00/18	00.00	–	–	–	–	–	–
Sagare	00/30	00.00	–	–	–	–	–	–
Singaramaranahally	30/38	78.94	12	8	9	1.8	1.1	0.8
Daaripura	02/40	05.00**	–	–	–	–	–	–
Kattemalalavadi	00/15	00.00	–	–	–	–	–	–

N* number of fields found infected, *N number of fields surveyed

***DI and PDI were not calculated due to small sample size

comparative account of *D. tarrii* and *D. mysorensis* is provided in Table 3.

These symptoms were persistent throughout the crop season and affected the overall yield of cowpea with sclerotia forming on the lower surface. On the lower leaf surface, sclerotiophores bearing sclerotia were produced from the immersed mycelium (Fig. 3). Sclerotia were generally globose to irregular in shape and dark to grey brown in colour measuring 139.5 × 89 µm. Upon germination, germ tubes were produced over the entire surface of the sclerotium measuring 69.9 × 6 µm.

The pathogenicity test revealed that, the fungus was pathogenic on healthy cowpea plants and produced a characteristic ZLS disease after 18 days of post-inoculation. The initial symptoms appeared after 15 days of post-inoculation and became prominent later with characteristic zonate spots on leaves.

Taxonomy

Dactuliophora mysorensis Deepika, Mahadevakumar, Amruthesh and Lakshmidevi

Mycobank # MB836147; Figs. 1 and 2

Holotype: UOM 2020-DM1, Herbarium, Department of Studies in Botany, University of Mysore, Karnataka, India.

Host: *Vigna unguiculata* (L.) Walp.

Distribution: The pathogen *Dactuliophora mysorensis* was recorded in different regions of the Mysore District. Geographical regions of Mysore District (Karnataka State, India) comprise dry deciduous regions with average rainfall in winter and the highest temperature recorded during summer (31 °C).

Etymology: The species named as *mysorensis* to assign and to commemorate the geographic location from where this species is collected and documented as new record from India on cowpea.

Type specimen: Holotype is available in the Herbarium, Department of Studies in Botany, University of Mysore, Mysore, Karnataka State, India.

Molecular Detection

The genomic DNA isolated from the pure cultures and ITS-rDNA was amplified using ITS1-ITS4 primers. The sequence analysis by nBLAST search revealed that the ITS-rDNA sequences shared 100% sequence similarity with *Macrophomina phaseolina* (FJ415067.1, MK573366.1, GU046874.1) and 99.83% with *Rhizoctonia bataticola* (KX270356.1, KX270355.1, DQ339102.1, DQ222239.1). The ITS-rDNA sequences from the present study are deposited in the GenBank database with accession # KC568286.1 and KC568285.1. Further, phylogenetic analysis revealed that the sequences shared a common clade between *M. phaseolina* and *R. bataticola*

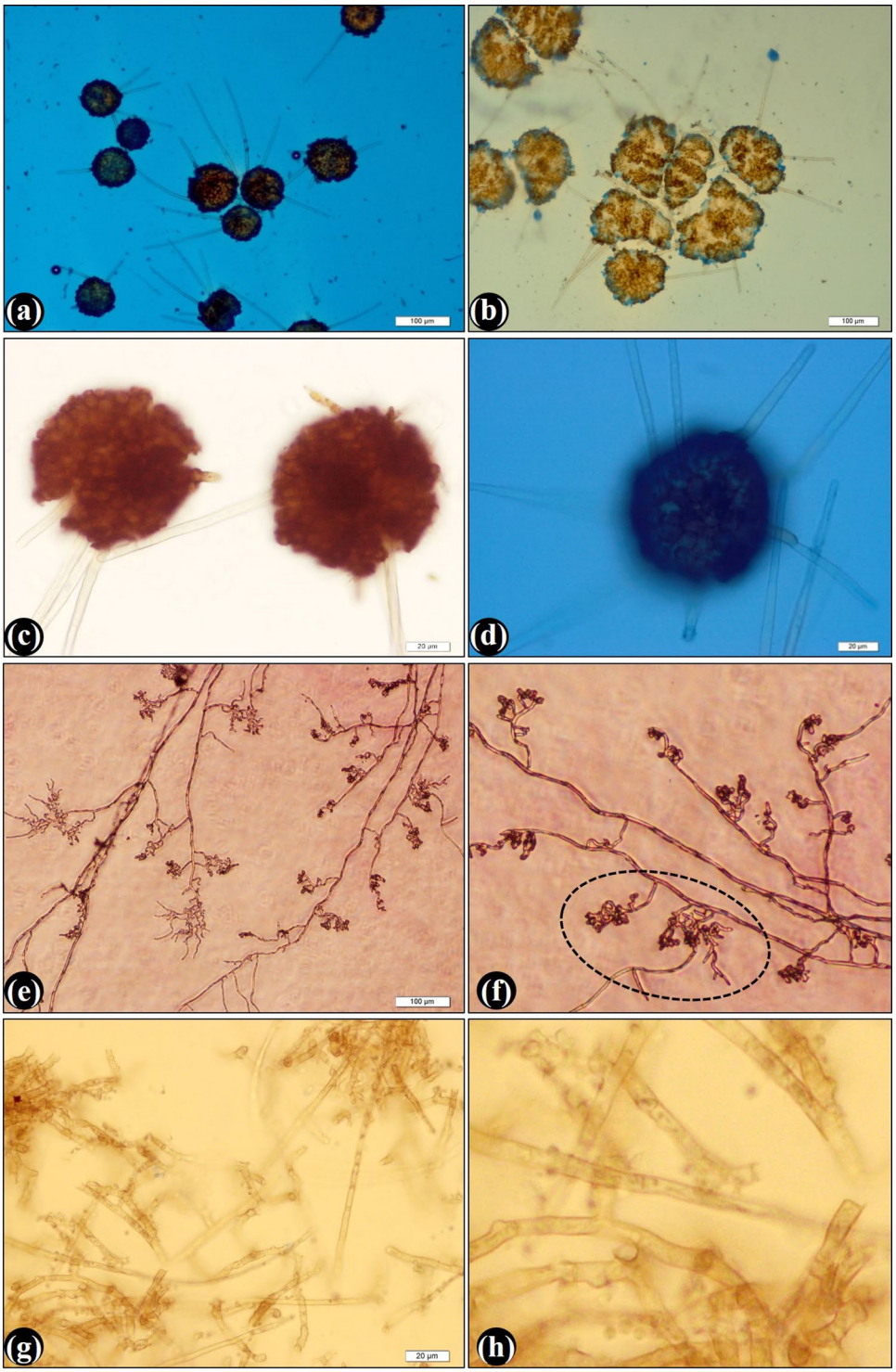

Fig. 2 Sclerotia and mycelium of *Dactuliophora mysorensis* under compound microscope: **a**–**d** sclerotia developed on host tissues observed under compound microscope showing the characteristic nature of long setae on the surface; **e**–**h** hyphae and mycelium of *D. mysorensis*

as depicted in phylogenetic tree (Fig. 3). There are other *Macrophomina* species placed on the same clade based on ITS-rDNA sequence, which indicates that the species though they share 100% sequence similarity, they distinctly differ in morphology and spore features.

Sensitivity to Fungicides

Efficacy of fungicides were tested in vitro for the fungal growth inhibition of the pathogen (Table 4). No growth was recorded in all three concentrations (100, 150 and 200 mg/L)

Table 3 Comparison of diagnostic features of *Dactuliophora tarrii* and *D. mysorensis* (CDA, Czapek Dox agar; potato carrot agar)

	Dactuliophora tarrii	*Dactuliophora mysorensis*
Diameter of leaf spot	Up to 1 cm	2.1 cm, sometimes it may reach to 2.5–3 cm in diam
	Rosette whitish and tan bands	Rosette whitish bands on adaxial surface
	Lower leaf surface covered with sclerotia and sclerotiophores	Brown rosette bands covered the entire spot by sclerotiophores with sclerotia
	Zonation less distinct in lower leaf surface	Zonation very clear and distinct on lower leaf surface
Nature of mycelia	Mycelia immersed, colourless, diffused throughout the tissues in the leaf spot and aggregated in plectenchymic masses beneath sclerotiophores	Mycelia immersed, pale-brown coloured, diffused throughout the leaf spot tissues in the leaf spot and aggregated in plectenchymic masses beneath sclerotiophores
Nature of sclerotia and sclerotiophores	Sclerotiophores consist of a fully erumpent ring of dematiaceous hyphae continuous with a sub-epidermal plectenchymic mycelial mass	Sclerotiophores consists of star-shaped dematiaceous hyphae on the lower leaf surface and continues with sub-epidermal plectenchymic mycelial mass
	Sclerotia scattered particularly on the paler parts of the leaf spot, generally hypophyllous but occasionally amphigenous, spherical to subspherical 35–135 µm diam, bearing 0–10 scattered setae, or sometimes glabrous, dematiaceous external cells, 8–12 µm diam, internal cells colourless, undifferentiated	Sclerotia scattered on the entire spot, strictly hypophyllous, spherical to subspherical, 95–189 µm in diam, bearing 2–5 scattered setae, external cells are dark-brown, internal cells are light-brown and undifferentiated
	Sclerotial setae when present more or less cylindrical but sometimes slightly sinuate up to 190 µm long, 3–4 µm wide, slightly attenuated distally to a blunt tip; dematiaceous or colourless, 0–13 septate	Sclerotial setae were present and are cylindrical and sometimes with sinuate tips up to 70 µm long and 2–4 µm wide with attenuated blunt tip. Dematiaceous and 1–3 septate
Cultural details	In culture on malt agar, PDA, PCA and CDA growth 0.5–1 mm/day at 27 °C	In culture on PDA growth of 1.95 mm/day at 27 °C
	On plates sclerotia develop abundantly in glomerate clumps scattered over the colonies	Sclerotia develop abundantly over the entire colony
	Aerial mycelia tan, reverse pale-brown. Sclerotia borne on well differentiated sclerotiophores similar to those on living hosts	Aerial mycelia pale-brown. Sclerotia borne but well differentiation of sclerotiophores were not clear on culture

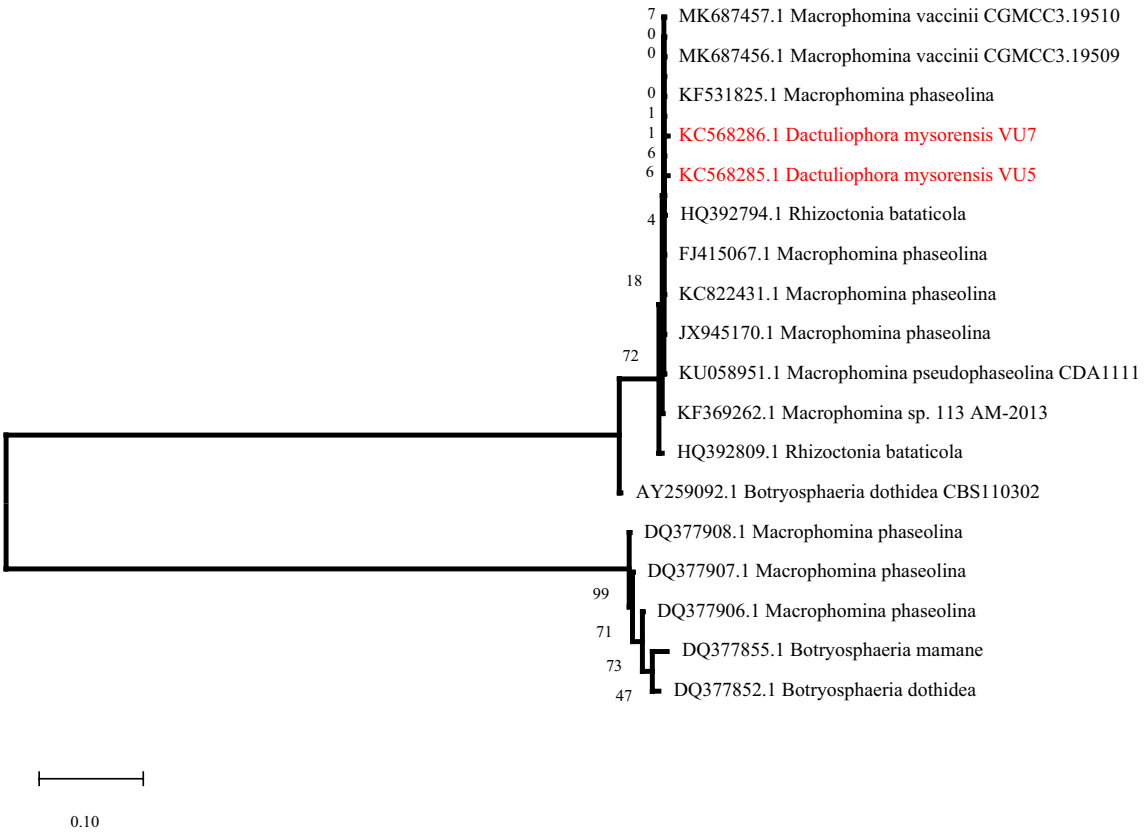

Fig. 3 Phylogenetic analysis by Neighbour-Joining method of *Dactuliophora mysorensis* based on ITS-rDNA with reference sequence from GenBank database. The tree is rooted to *Botryosphaeria dothidea* (Tamura-Nei Substitution model and nearest neighbour-interchange search options with 1000 bootstrap replicates were used)

of Benomyl and Carbendazim (100% of inhibition of mycelial growth), followed by Thiophanate-methyl at 200 mg/L with inhibition up to 70.93%. Similarly, 52.99% and 58.97% growth inhibition offered at 100 mg/L and 150 mg/L of Thiophanate-methyl, respectively. Metalaxyl-M showed 31.33%, 33.9% and 39.6% in 100, 150 and 200 mg/L concentration, respectively. Compared to other fungicides, the Mancozeb showed the least mycelial growth inhibition: 15.88%, 22.26% and 26.84% at three increasing concentrations (100, 150 and 200 mg/L) tested, respectively (Fig. 4).

Discussion

Emerging diseases caused by the fungi and fungi-like organisms being increasingly reported in many crops [11]. The emerging and new fungal diseases on cowpea caused by fungal pathogens are becoming major constraints in production of cowpea. A survey conducted in the Mysore District of Karnataka (India) revealed that cowpea was infected with leaf spot disease. The infection becomes severe after rain, leading to substantial death of plants. The disease was first found during September 2010 and initially, spots were appeared as large lesions with concentric rings with whitish on the upper leaf surface and pinkish-grey on lower leaf surfaces. These symptoms were persistent throughout the season with production of sclerotia on the lower surface. Sclerotia were generally globose to irregular, dark to grey brown. Sclerotiophores develop from the mycelia immersed in the leaf tissue and produce sclerotia. Sclerotia germinate by the germ tubes over the entire surface [18]. Leakey [15] performed a comparative study to distinguish the isolated species *Dactuliophora tarrii* from the Tropical Africa. Although the occurrence of *Dactuliophora* sp. associated with ZLS disease of cowpea from Mysore region was reported earlier, there was no clarity on its species identity. The pathogen was isolated from the Mysore District in India are distinct from that of *D. tarrii* recorded by CLA Leaky during 1964. Based on differences in morphology with *D. tarrii*, the fungus isolated in the present study was recorded as new species and named as *Dactuliophora mysorensis*.

The ITS-rDNA sequence analysis revealed that the fungus shared 100% similarity with *R. bataticola* and *M. phaseolina* as per phylogenetic placement as well as nBLAST analysis. It is very clear that the fungus may be genetically related to *R. bataticola* and *M. phaseolina*. The taxonomic placement

Table 4 Effect of different fungicides on the mycelial growth of *Dactuliophora mysorensis*

Treatments	Concentration (mg/L)	*Dactuliophora* sp.	
		Growth (cm) ± SE*	Growth inhibition* (%) ± SE
Benofit	100	0.00 ± 0.00^{k}	100.00 ± 0.00^{a}
	150	0.00 ± 0.00^{k}	100.00 ± 0.00^{a}
	200	0.00 ± 0.00^{k}	100.00 ± 0.00^{a}
Roko	100	3.93 ± 0.06^{h}	52.95 ± 0.79^{d}
	150	3.43 ± 0.03^{i}	58.94 ± 0.40^{c}
	200	2.43 ± 0.06^{j}	70.79 ± 0.79^{b}
Bavistin	100	0.00 ± 0.00^{k}	100.00 ± 0.00^{a}
	150	0.00 ± 0.00^{k}	100.00 ± 0.00^{a}
	200	0.00 ± 0.00^{k}	100.00 ± 0.00^{a}
Indofil M-45	100	7.03 ± 0.03^{b}	15.87 ± 0.39^{j}
	150	6.50 ± 0.02^{c}	22.25 ± 0.34^{i}
	200	6.11 ± 0.01^{d}	26.84 ± 0.20^{h}
Ridomil Gold	100	5.75 ± 0.07^{e}	31.02 ± 0.91^{g}
	150	5.53 ± 0.03^{f}	33.82 ± 0.40^{f}
	200	5.05 ± 0.02^{g}	39.60 ± 0.34^{e}
Control		8.36 ± 0.06^{a}	00.00 ± 0.00^{k}

*Values are the means of four independent replicates

Means followed by the same letter(s) with in the column are not significantly different according to Tukey's HSD ($P < 0.05$)

of mycelia sterilia is solely based on morphological features and may or may not be substantiated by sequence analysis. However, as previously predicted the relationship between *R. bataticola* and *M. phaseolina* as they are similar genetically and differ morphologically (former represent the sclerotial form and the latter representing the pycnidial form). The *R. bataticola* and *M. phaseolina* are recognized as two asexual sub-phases of which, the former has been known to form microsclerotia and the latter, forms pycnidia on host tissues and microsclerotia on culture [7, 12]. This scenario illustrates that the genetic relatedness between *M. phaseolina* and *R. bataticola* (although both are same, where *M. phaseolina* is pycnidial form and *R. bataticola* is sclerotial form) exhibits similar relationship, where *Dactuliophora* may represent sclerotial form of *M. phaseolina* or related species.

The fungicides used in the present study are generally advised to manage various fungal diseases like anthracnose, powdery mildews, leaf spot and fruit rots of vegetables and pulses (https://www.agritech.tnau.ac.in). The Benomyl, Mancozeb and Carbendazim are usually applied to treat various fungal diseases associated with vegetables, flower crops against sheath blight, loose smut, leaf spots and powdery mildew of pulses, oil seeds and vegetables (https://agritech.tnau.ac.in/crop_protection/pdf/6_Major_use_fungicides.pdf; [6]). Among the five combinations of fungicides evaluated, Benomyl and Carbendazim performed well by 100% growth inhibition followed by Thiophanate-methyl (71% inhibition). Further work needs to be carried out to evaluate the efficacy of systemic fungicides in field trials to confirm the strategies of disease management.

The new ZLS disease caused by *D. mysorensis* described from Southern India is distinct from the previously described *D. tarrii* from Africa. In order to have a better comprehension of the host range for *D. tarrii*, the host details available from IMI herbarium were retrieved and compiled in Table 5. The *D. tarrii* has been reported on various pulse crops from Africa region including Sudan, Uganda, Tanzania, Nigeria, Zambia, Zimbabwe and Malawi. Important pulses recorded to be associated with *D. tarrii* are *Vigna unguiculata*, *V. coerulea*, *V. mungo*, *V. sesquipedalis*, *V. vexillata*, *Phaseolus aureus*, *P. lunatus* and *Crotalaria juncea*. The *D. mysorensis* identified and isolated from the naturally infected leaf samples of cowpea was pathogenic and successful manifestation of symptoms were established in the plants inoculated with pure cultures in green house conditions. Since the *D. mysorensis* showed distinct morphology and cultural features, the erection of new species is justified. Management of the newly emerged disease is necessary to reduce the loss of production of protein-rich pulse. In the present study, the performance of systemic fungicides was better compared to contact fungicides against *Dactuliophora* sp. The Benomyl and Carbendazim were proved to be the most effective fungicides for complete inhibition of *Dactuliophora* sp. All the three concentrations of Mancozeb were less effective, while the performance of Metalaxyl-M was poor. The Benomyl and Carbendazim were effective against *Dactuliophora* sp. than the Mancozeb, Thiophanate-methyl and Metalaxyl-M. The Benomyl and Carbendazim may be recommended in field trials for the management of ZLS disease of cowpea caused by *Dactuliophora* species.

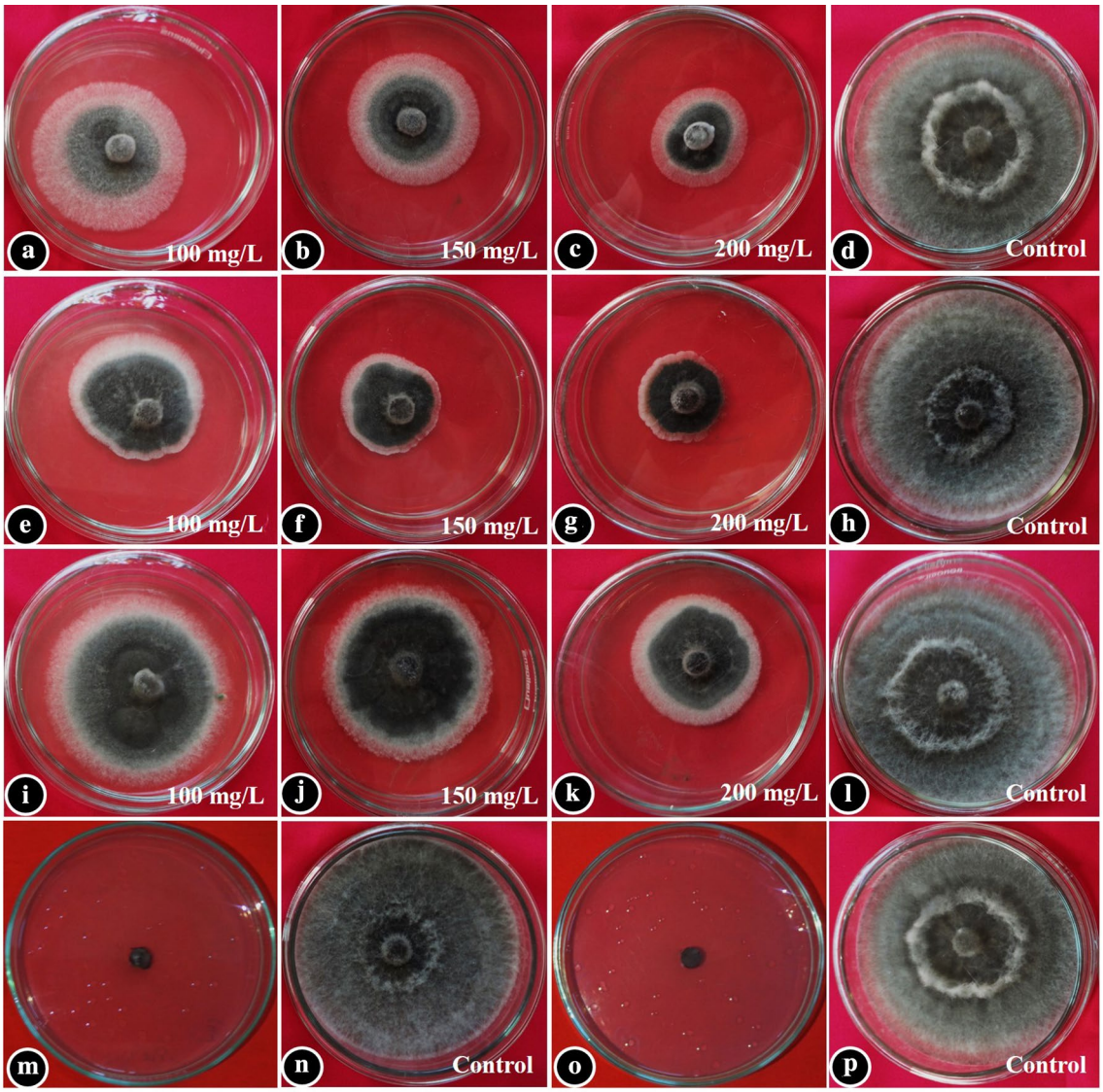

Fig. 4 In vitro efficacy of fungicides evaluated against *Dactuliophora mysorensis* at 100, 150 and 200 mg/L concentration: **a**–**d** Thiophanate-methyl (Roko); **e**–**h** Metalaxyl-M (Ridomil Gold); **i**–**l** Mancozeb (Indofil M-45) showed varied degree of inhibition. **m**, **n** Benomyl (Benofit), and **o**, **p** Carbendazim (Bavistin) showing 100% growth inhibition at lowest concentration tested (100 mg/L)

Table 5 World collection of *Dactuliophora* and their locality with voucher details

IMI number	*Dactuliophora* sp.	Host	Locality	Collector
IMI114373	*Dactuliophora tarrii*	*Vigna coerulea*	Uganda	C. Leakey 1964-10-08
IMI134066a	*Dactuliophora tarrii*	*Vigna sinensis*	Tanzania	Buckland, B.A. 1968-06-12
IMI134068	*Dactuliophora tarrii*	*Phaseolus mungo*	Tanzania	Buckland, B.A. 1968
IMI139065	*Dactuliophora tarrii*	*Vigna sinensis* var. *kwarva*	Nigeria	M. Dransfield
IMI147325	*Dactuliophora tarrii*	*Vigna unguiculata*	Tanzania	R.V. Billington 1970-03-03
IMI148181	*Dactuliophora tarrii*	*Phaseolus aureus*	Tanzania	1970-04-09
IMI153805	*Dactuliophora tarrii*	*Vigna coerulea*	Uganda	Leakey, C.L.A. 1964-08-22
IMI153806	*Dactuliophora tarrii*	*Vigna unguiculata*	Uganda	Leakey, C.L.A. 1964-07
IMI162950	*Dactuliophora tarrii*	*Vigna sinensis*	Zambia	E.A. Riley
IMI198855	*Dactuliophora tarrii*	*Vigna unguiculata*	Togo	Steiner, K.G. 1975-11-13
IMI281101	*Dactuliophora tarrii*	*Vigna unguiculata*	Mozambique	Plumb-Dhindsa, P.1981-12-10
IMI296501	*Dactuliophora tarrii*	*Vigna unguiculata*	Zambia	1985-03
IMI36319	*Dactuliophora tarrii*	*Vigna unguiculata*	Sudan	Tarr, S.A.J. 1949-09
IMI39957	*Dactuliophora tarrii*	*Vigna mungo*	Sudan	Tarr, S.A.J
IMI39958	*Dactuliophora tarrii*	*Crotalaria juncea*	Sudan	Tarr, S.A.J
IMI39959	*Dactuliophora tarrii*	*Phaseolus aureus*	Sudan	Tarr, S.A.J
IMI39967	*Dactuliophora tarrii*	*Phaseolus lunatus*	Sudan	Tarr, S.A.J
IMI39968	*Dactuliophora tarrii*	*Vigna mungo* var. max	Sudan	Tarr, S.A.J
IMI39971	*Dactuliophora tarrii*	*Vigna unguiculata*	Sudan	Tarr, S.A.J
IMI39973	*Dactuliophora tarrii*	*Vigna vexillata*	Sudan	Tarr, S.A.J
IMI39975	*Dactuliophora tarrii*	Leguminosae	Sudan	Tarr, S.A.J
IMI40390	*Dactuliophora tarrii*	*Vigna unguiculata*	Malawi	Wiehe, P.O
IMI63251	*Dactuliophora tarrii*	*Vigna unguiculata*	Zimbabwe	Whiteside, J.O
IMI74074	*Dactuliophora tarrii*	*Vigna sinensis*	Zambia	Angus, A
IMI75778	*Dactuliophora tarrii*	*Vigna mungo*	Nigeria	Harris, E. 1958-10-11
IMI75958	*Dactuliophora tarrii*	*Vigna sinensis*	Nigeria	Barley, A.G. 1958-11-04
IMI90059	*Dactuliophora tarrii*	*Vigna sesquipedalis*	Zambia	Angus, A. 1961-05-26
IMI98272	*Dactuliophora tarrii*	*Vigna unguiculata*	Uganda	Leakey, C.L.A
UOM2020DM1	*Dactuliophora mysorensis*	*Vigna unguiculata*	Mysore	Mahadevakumar, 2018

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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