

Exploration on Severity and Morphological Variations in Root Rot causing *Macrophomina phaseolina* (Tassi.) Goid and *Sclerotium rolfsii* (Sacc.) in Peanut (*Arachis hypogaea* L.)

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ABSTRACT: Peanut (*Arachis hypogaea* L.) is a major oilseed crop grown in many countries around the world as it provides good edible oil and protein. It is both leguminous and oilseed crop, but its production has been reduced over years due to the attack of pest and pathogen at field level. Among many pathogens, *Macrophomina phaseolina* (Tassi.) Goid (dry root rot) and *Sclerotium rolfsii* Sacc. (stem rot) are the destructive soil borne pathogens which cause severe infection and capable of producing resting structures, microsclerotium and sclerotium respectively. In this regard, survey has been carried out in various districts of Tamil Nadu which recorded an incidence ranging from 18.60 per cent to 64.00 per cent and 20.30 to 66.20 per cent respectively. In addition, different isolates of *M. phaseolina* and *S. rolfsii* were isolated from the diseased samples collected from different districts of Tamil Nadu. The isolates of the rot pathogens isolated were characterized both morphologically and molecularly. The size of the sclerotium produced by *M. phaseolina* and *S. rolfsii* were ranging from 29.416 to 84.896 µm and 480.95 to 778.42 µm. Molecular characterization of the pathogens resulted that the DNA of all the isolates of *M. phaseolina* amplified at an amplicon size of app. 550 - 560 bp and isolates of *S. rolfsii* were amplified at an amplicon size of app. 650 – 700 bp. Thus, in this study the incidence of the pathogens were recorded and different isolates of *M. phaseolina* and *S. rolfsii* were characterized.

Keywords: *Macrophomina phaseolina*, *Sclerotium rolfsii*, incidence, isolation and morpho – molecular characterization.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) is one of the most important oilseed and legume crop, which is highly used for edible purposes either as nuts or as oil extracted from nuts. It comes under the family Leguminaceae and originated from South Africa. Groundnut is of higher economic importance as it contributes to the Indian economy by exportation and exportation of groundnut is more than the domestic consumption. Although India is first in acreage, the yield/ ha is very low compared to china. The production of groundnut is declining over years and the main reason is considered as the yield loss caused by the economically important pathogens, *Macrophomina phaseolina* and *Sclerotium rolfsii* which infect the peanut crop and cause dry root rot and stem rot diseases respectively. These diseases are most destructive as it is soil borne and systemic, it infects the plants and cause complete destruction. Both the pathogens are soil borne and have a wide range of host infecting more than 500 crop species (Doley *et al.*, 2012). These pathogens are

considered to be detrimental due to the production of the fungal propagules, microsclerotium by *M. phaseolina* and sclerotium by *S. rolfsii* which survive in the soil for many years and it is difficult to destroy (Shifa *et al.*, 2015). Also, the somatic structures of the pathogens are distributed in the soil randomly, it is very challenging to control by any means (Pardeep *et al.*, 2015). The pathogens infect all the stages of peanut crop and their control has been difficult since. The incidence of dry root has been recorded as 33.33 per cent decayed seeds and post emergence mortality of 23.80 per cent (Moradiaand Khandar, 2011). And incidence of stem rot was recorded as 7.88 to 32.02% in various villages of Cuddalore district of Tamil Nadu (Sivakumar *et al.*, 2016). Hence, the present study was aimed on survey of incidence of dry root rot and stem rot of peanut, isolation and characterization of *M. phaseolina* and *S. rolfsii* based on morphological and molecular characterization.

MATERIALS AND METHODS

Survey on the occurrence of root rot. The incidence of the root rot diseases were surveyed by roving method of survey (Archana *et al.*, 2020) in major peanut growing districts of Tamil Nadu viz., Coimbatore, Dharmapuri, Krishnagiri, Salem and Cuddalore at different stages of the crop.

Isolation of root rot pathogens. The symptomatic plants were collected for isolation from various districts of Tamil Nadu in which the incidence was surveyed. The symptoms were yellowing and wilting of plants, bark shredding in dry root rot infected plants and white mycelial propagules were grown on the infected parts in stem rot infected plants. Presence of sclerotia were observed on the infected parts of the plants and were visible to the naked eye.

The pathogens were isolated by tissue segment method (Rangaswami, 1972). The infected roots were cut into small pieces of 0.2 to 0.5 cm in size. The root bits were washed in distilled water twice to remove the soil and debris and these bits were surface sterilized using 1% sodium hypochlorite once and then washed with sterile distilled water twice. Excessive moisture present on the root bits were dried by placing them on sterile tissue paper. After drying, root bits were placed in fresh sterilized PDA (Potato Dextrose Agar) medium which was poured half plate for isolation. Also, brown to black, rounded sclerotium present on the roots that were apparent to the naked eye were collected and placed on fresh sterilized potato dextrose agar medium (Shim *et al.*, 1998).

Morphological characterization. The pure cultures of the rot pathogens were observed in the naked eye for their colony characters. The colour and nature of the colony growth (Fluffy/Sparse/Compact) were observed in naked eye.

The mycelial characters (hyaline/coloured, septate/coenocytic) and the sclerotial characters (size and shape of sclerotium) were observed under phase contrast microscope (LEICA DM30000, DST FIST lab, TNAU, Coimbatore) (Emayavarman *et al.*, 2019).

Molecular characterization. The DNA of the isolates of *M. phaseolina* and *S. rolfii* were extracted separately from 14 days old PD broth inoculated with pathogens. DNA extraction was done by CTAB method (Almeida *et al.*, 2003). The extracted DNA was amplified using the universal primers, ITS 1 and 4 with

a reaction mixture, 5 µl of Master mix, 2 µl of double sterile distilled water, 1 µl of forward primer, 1 µl of reverse primer. The reaction mixtures were prepared for all the isolates of *M. phaseolina* and *S. rolfii* and were subjected to PCR in Thermocycler (Eppendorf Master Cycler Nexus gradient). The conditions for PCR fixed was, initial denaturation at 95°C for 1 min, denaturation at 95°C for 1 min (35 cycles), annealing at 58°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 mins. After the cycles were completed, the PCR product was checked for amplification in agarose gel electrophoresis by loading them in 1.2 % agarose gel amended with 2 µl of Ethidium Bromide. Then the gel was visualized under UV transilluminator and images were documented in gel documentation unit (BIO RAD, Gel Doc™ EZ Imager, Bio-Rad Laboratories Inc.). To confirm the amplicon size at which the product were amplified, Molecular marker (DNA ladder, 100 bp) was used (Emayavarman *et al.*, 2019).

Statistical analysis. Design of experiment used was CRD and AGRES ANOVA package version 7.01 from Department of physical sciences, TNAU, India was used to analyse the statistical data (Senthilkumar *et al.*, 2009) and the percent values are arcsine transformed. ANOVA was used to analyse the data at significant level 0.05 and means were compared using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Survey on the incidence of root rot. The incidence of dry root rot was recorded from 18.60 per cent to 64.00 per cent and stem rot was recorded from 20.30 per cent to 66.20 per cent in various districts of Tamil Nadu. The highest incidence of dry root rot was recorded as 64 per cent in Vridhachalam, Cuddalore district and lowest incidence was 18.6 per cent in Pulikarai, Dharmapuri (Table 1). Moradia and Khandar (2011) surveyed the incidence of dry root rot in Saurashtra region of Gujarat and reported 33.33 per cent of seed decay and post emergence mortality of 23.80 per cent in peanut crop due to dry root rot. The dry root rot incidence in the major peanut growing district in Tamil Nadu, Cuddalore, the incidence was recorded ranging from 21.73 per cent to 31.68 per cent (Raja Mohan *et al.*, 2012).

Table 1: Survey on the incidence of dry root rot and stem rot of peanut in different districts of Tamil Nadu.

Sr. No.	District	Place	Crop Stage	Geographical position		Disease incidence (%)*	
				Latitude	Longitude	Dry root rot	Stem rot
1.	Coimbatore	Telugupalayam	Peg formation stage	11.02148°N	76.9281°E	58.00 ^b (49.6)	66.20 ^a (54.45)
2.	Dharmapuri	Pulikarai	Flowering stage	12.21317°N	78.12117°E	18.60 ^d (25.54)	35.80 ^c (36.74)
3.	Krishnagiri	Sokkadi	Vegetative stage	12.4824°N	78.1258°E	33.90 ^c (35.6)	45.00 ^b (42.12)
4.	Salem	Attur	Harvesting stage	11.5983°N	78.5974°E	33.33 ^c (35.25)	20.30 ^d (26.77)
5.	Cuddalore	Vridhachalam	Flowering stage	11.5159°N	79.3269°E	64.00 ^a (53.13)	35.60 ^c (36.62)

*Values are mean of four replications. Values in parentheses are arc sine transformed values. Means were compared by DMRT at p=0.05 and the values with same letter are not significantly different from each other.

Similarly, Telugupalayam, Coimbatore district, recorded highest stem rot incidence of 66.2 per cent and lowest incidence of 20.3 per cent was recorded in Attur village of Salem district. Archana *et al.* (2020) stated that the incidence of wet root rot in Coimbatore, Tamil Nadu was recorded as 86.6 per cent during roving survey. The yield loss by stem rot was normally upto 25 per cent but in conducive conditions the yield loss maybe upto 80-90 per cent (Deepthi & Reddy 2013). Sivakumar *et al.* (2016) recorded stem rot incidence from 7.88 to 32.02 per cent in various villages of Cuddalore district of Tamil Nadu. A roving survey was conducted in major peanut growing areas in *Kharif* 2014, in which the incidence of stem rot was recorded as 10.11 to 59.33 per cent (Haveri, 2017).

Isolation of rot pathogens. Dry root rot and stem rot infected specimens were collected and the pathogens were isolated separately under aseptic condition. The isolates were named as TNAU MP1, DPI MP2, KRI MP3, SLM MP4 and CUD MP5 for *M. phaseolina* isolates and TNAU SR1, DPI SR2, KRI SR3, SLM SR4 and CUD SR5 for *S. rolfii* isolates. The isolates of *M. phaseolina* and *S. rolfii* were periodically subcultured in fresh sterilized PDA plates for the maintenance of their axenic cultures. Similar isolation technique was used by Raja Mohan *et al.* (2012) for the isolation of *M. phaseolina*. The stem rot pathogen, *Sclerotium* was isolated by tissue segmentation method from the symptomatic stem parts by Archana *et al.* (2020).

Colony and Morphological characteristics of *M. phaseolina*. The colonies of isolates of *M. phaseolina*

were compact to fluffy in nature, the colour of the isolates varied from brown to black to olivaceous grey (Fig. 1). Similar observations made by Sarr *et al.* (2014) reported that the colony of *M. phaseolina* was fluffy, initially buff later turned to pale olivaceous grey and sclerotium were numerous produced. Dark brown to greyish coloured colonies were produced by *M. phaseolina* isolates on PDA medium (Pandey *et al.*, 2020).

The mycelium of all the isolates were observed under phase contrast microscope. The colour of the mycelium varies from light brown to dark brown and septations were present in all the isolates (Fig. 2). Emayavarman *et al.* (2019) reported the black coloured mycelium was produced by *M. phaseolina* and under microscope the mycelium was coloured and septate. The hyphae of *M. phaseolina* were found to be hyaline, with thin walls and the colour ranges from light brown to dark brown with more septa reported by Lakhra *et al.* (2018).

The microsclerotium were present in abundant numbers in culture plates of *M. phaseolina* and the colour of microsclerotium varies from dark brown to black and oval, spherical and irregular shapes of microsclerotium were also observed in different isolates (Fig. 3). Similarly, Marquez *et al.* (2021) described that themicrosclerotia were spherical, oval, or oblong masses of hardened fungal mycelium that are initially light brown in colour later turns to dark brown to black. Similarly, Sarr *et al.* (2014) reported that black coloured sclerotia were produced by *M. phaseolina*.

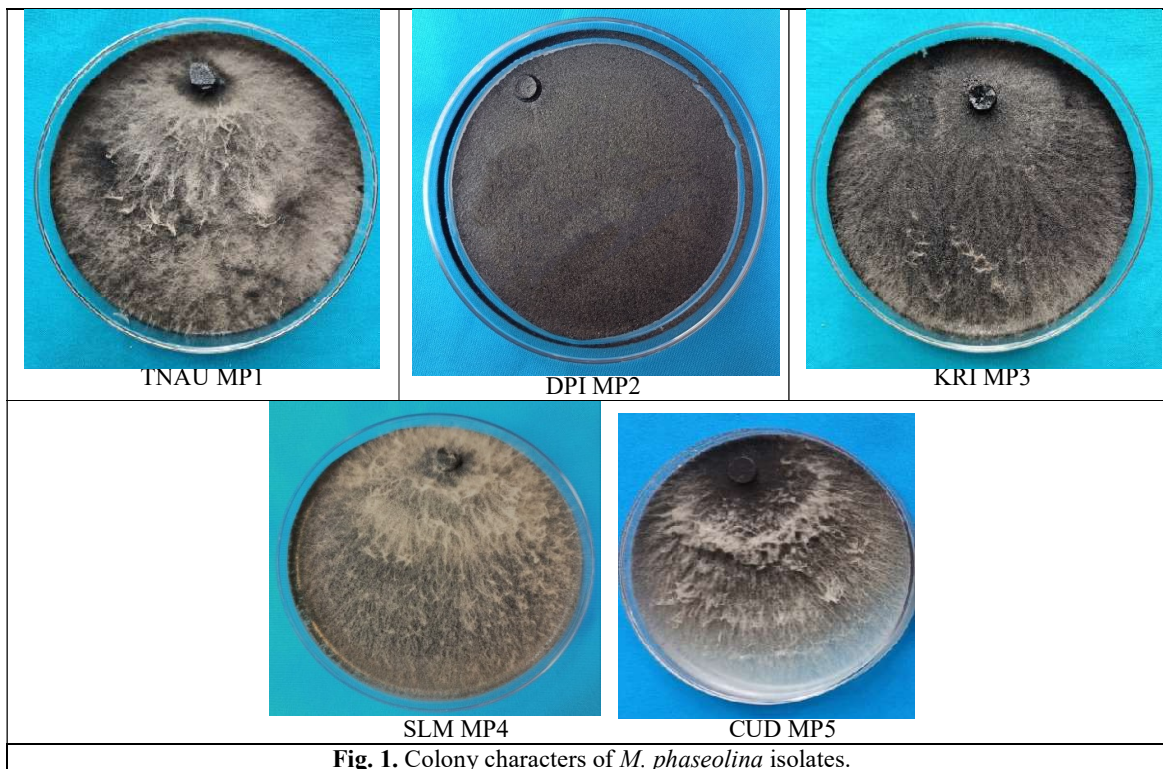
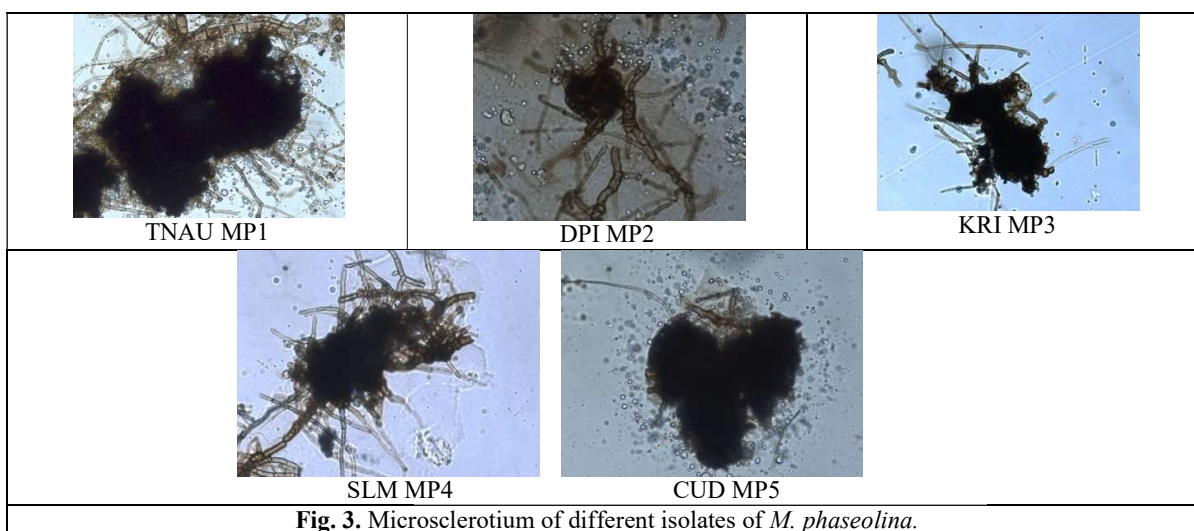
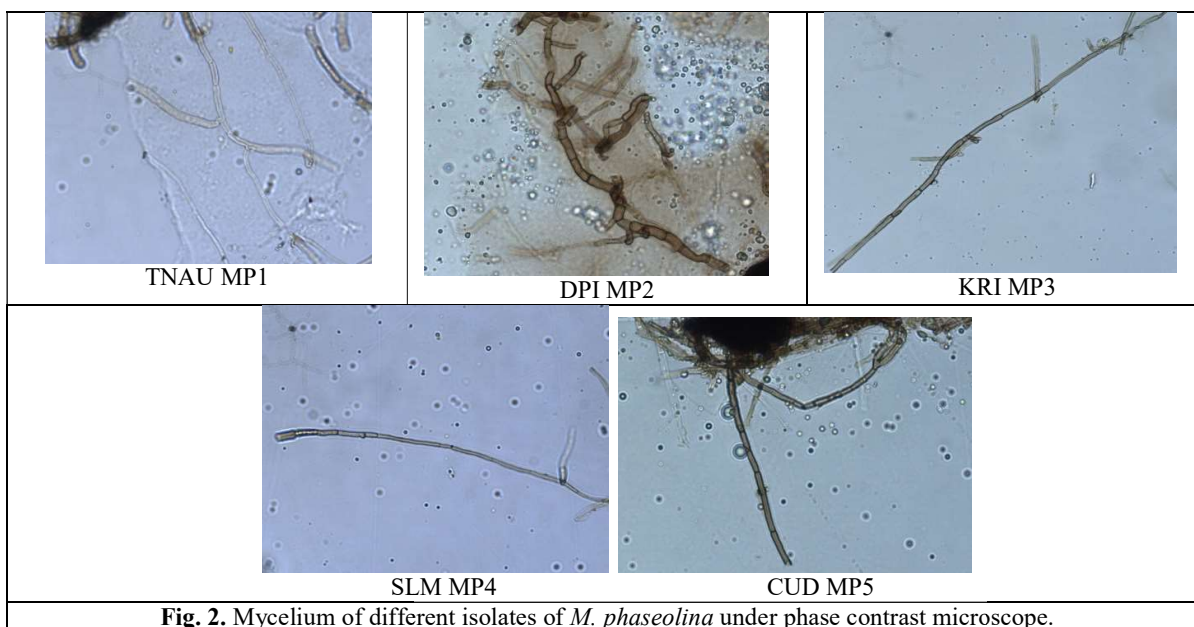


Fig. 1. Colony characters of *M. phaseolina* isolates.



TNAU MP1 isolate produced larger sized microsclerotium of an average size of 84.896 μm and the growth of TNAU MP1 was also rapid at a growth rate of 36.68 mm/day compared to other isolates (Table 2). Raja Mohan *et al.*, (2012) recorded that size of microsclerotium produced by *M. phaseolina* MP₅ was

85.70 μm . The size of the microsclerotium for 64 isolates of *M. phaseolina* was recorded as 72.8 to 127.8 μm by Manici *et al.* (1992). Lakhari *et al.* (2018) have reported that the size of microsclerotium varied from 82.5 to 105 μm .

Table 2: Cultural and morphological characters of different isolates of *M. phaseolina*

Sr. No.	Isolate	Place of collection	Colony type	Mycelium	Microsclerotium	Size of microsclerotium (μm)*	Growth (mm/day)*
1.	TNAU MP1	Coimbatore	Fluffy, grey to black colour	Hyaline to grey colour, septate	Black, irregular	84.896 ^a	36.68 ^a
2.	DPI MP2	Dharmapuri	Compact, jet black colour	Hyaline to brown colour, septate	Dark brown, oval to irregular	29.416 ^e	23.75 ^d
3.	KRI MP3	Krishnagiri	Compact, black colour	Hyaline to brown, septate	Dark brown, irregular	62.657 ^b	15.22 ^e
4.	SLM MP4	Salem	Fluffy, brown colour	Hyaline to grey colour, septate	Black, irregular	56.046 ^c	27.34 ^c
5.	CUD MP5	Cuddalore	Fluffy, grey to black colour	Hyaline to brown colour, septate	Brown to black, irregular	37.362 ^d	29.78 ^b

*Values are mean of four replications. Means were compared by DMRT at $p=0.05$ and the values with same letter are not significantly different from each other.

Colony and Morphological characteristics of *S. rolfsii*. The colonies of *S. rolfsii* were sparse to fluffy in nature and spreading radially in vein like manner. The mycelium were initially resembled silk white threads later turned to cotton white colour with sparse to fluffy growth (Fig. 4). Mahadevakumar *et al.* (2018) reported that the colonies of *S. rolfsii* were white in colour with dense, fluffy growth. Sivakumar *et al.* (2016) investigated the colony characters of *S. rolfsii* and reported that the colonies were initially silk white which later changed to dull white colour and profuse mycelium which grow radially to give fan like appearance.

The mycelium of different isolates were hyaline and highly septate when observed under phase contrast microscope (Fig. 5). The sclerotium of all the isolates were observed in naked eye and were dark brown, reddish brown, golden brown and black colours (Fig. 6). The size of the sclerotium for different isolates was varied from 480.95 μm to 778.42 μm . The isolate TNAU SR1 was found to produce sclerotium on 5 days

after inoculation (DAI). The isolates DPI SR2, SLM SR4 and CUD SR5 produced sclerotium on 7 DAI and KRI SR3 isolate produced sclerotium on 8 DAI. The number of sclerotium produced by different isolates also varies from 77.68 to 434.22 on 9 DAI in which TNAU SR1 isolate produced the higher number of sclerotium and the mycelial growth is also rapid at a growth rate of 33.70 mm/day (Table 3). Pandi *et al.* (2017) reported that light brown, dark brown and reddish brown coloured sclerotia were produced by 8 isolates of *S. rolfsii* and the size of the sclerotium varied from 1002 to 1224 μm . The number of sclerotium produced by eight isolates ranged from 274 to 360/plate and the isolates grown with a growth rate of 21.62 to 31.45 mm/day. Sarma *et al.* (2002), reported that the colonies produced by 18 isolates of *S. rolfsii* were fluffy, 8 isolates were compact with the growth rate of 23-31 mm/day and the no. of sclerotium produced were 80 to 500 sclerotia/plate. The average diameter of the sclerotium was recorded as 1 – 1.2 mm and were dark to reddish brown in colour.



Fig. 4. Colony characters of *S. rolfsii* isolates.

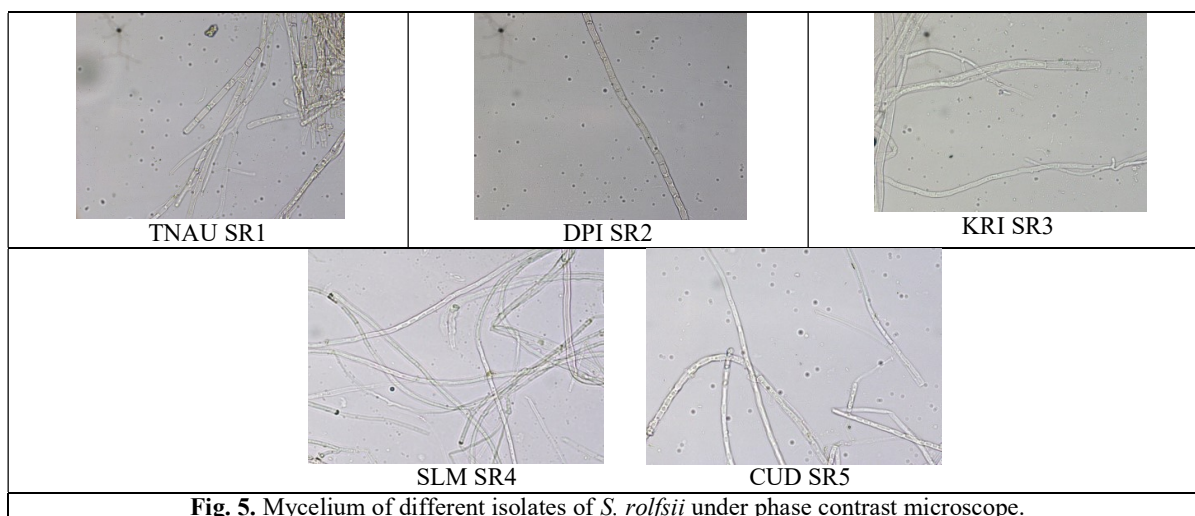


Fig. 5. Mycelium of different isolates of *S. rolfsii* under phase contrast microscope.

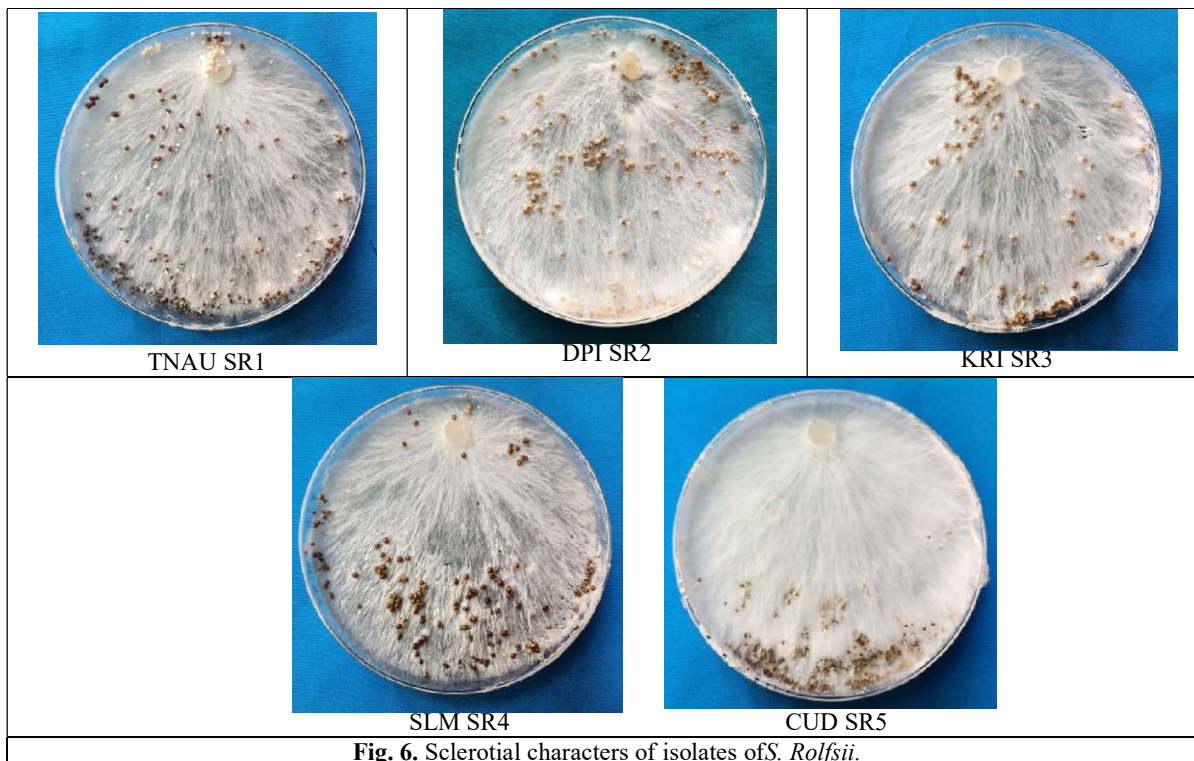


Fig. 6. Sclerotial characters of isolates of *S. rolfsii*.

Table 3: Cultural and morphological characters of different isolates of *S. rolfsii*.

Sr. No.	Isolate	Place of collection	Colony type	Mycelium	Growth rate (mm/day)	Sclerotium colour	No. of sclerotium/plate*			Sclerotium diameter (µm)*
							5 DAI	7 DAI	9 DAI	
1.	TNAU SR1	Coimbatore	Sparse	Hyaline, septate	33.70 ^a	Dark brown to black	89.25 ^a	226.34 ^a	434.22 ^a	768.95 ^a
2.	DPI SR2	Dharmapuri	Fluffy	Hyaline, septate	27.45 ^b	Light to Reddish brown	0.00 ^b	28.50 ^d	110.25 ^d	565.44 ^c
3.	KRI SR3	Krishnagiri	Fluffy	Hyaline, septate	24.50 ^c	Golden brown	0.00 ^b	0.00 ^e	77.68 ^e	778.42 ^a
4.	SLM SR4	Salem	Sparse	Hyaline, septate	18.57 ^e	Dark brown to black	0.00 ^b	52.75 ^c	149.50 ^c	631.50 ^b
5.	CUD SR5	Cuddalore	Fluffy	Hyaline, septate	23.60 ^d	Golden brown	0.00 ^b	76.00 ^b	185.00 ^b	480.95 ^d

*Values are mean of four replications. Means were compared by DMRT at $p=0.05$ and the values with same letter are not significantly different from each other.

Molecular characterization. The DNA of all the isolates of *M. phaseolina* and *S. rolfsii* were extracted separately and they were subjected to PCR and agarose gel electrophoresis was also performed for each isolate to find the amplicon size and were visualized under UV illuminator. All the *M. phaseolina* isolates were amplified and the amplicon were visible at app. 550 – 560 bp and for all the *S. rolfsii* isolates the amplicon were visible at app. 650 – 700 bp. The positive control with fungal DNA shown amplicon and in the negative control with bacterial DNA amplicon was not obtained. This confirms that the pathogens were fungi. The DNA of four isolates of *M. phaseolina* were extracted and 560 bp amplicon size were obtained in agarose gel electrophoresis in ITS 1&4 primers (Emayavarman *et al.*, 2019). Similarly, Chakraborty *et al.* (2011) also obtained an amplicon size of app. 550 bp for the *M. phaseolina* isolates in ITS 1&4 primers. In case of *S. rolfsii*, Jebaraj *et al.* (2017) observed the amplicon size of 650 – 700 bp in ITS 1&4 universal primers. Similar

results were also reported by Durgaprasad *et al.* (2008). Yu *et al.* (2019) extracted DNA of *S. rolfsii* on PCR and agarose gel electrophoresis reported that it was amplified at an amplicon size of 683bp in ITS 1&4 universal primers.

CONCLUSION

Hence, these findings revealed the diversity of colony and morphological traits among the different isolates of *M. phaseolina* and *S. rolfsii* collected from different districts of Tamil Nadu.

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Conflict of Interest. None.

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