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New Report of Pythium Soft Root Rot in Mulberry and its Cultural and physiological Studies

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A B S T R A C T

Mulberry crop affected by many diseases and threatening the mulberry cultivation. Recently, some of the mulberry gardens were infected with soft root rot disease with symptoms like withering and drying of leaves, mucilaginous matrix on bark of the roots resulting roots become soft and ultimately the plant showed epinasty and wilting. The soft root rot disease was observed in Agara (43.76 %), Kebre (32.57%), Thattekere (18.79%) and Gerehalli (13.58%) villages of Ramanagara district and Maddur (7.89 %), Halaguru (16.78 %) and Malavalli (6.58 %) of Mandya district. The pathogen *Pythium* sp. was isolated from infected roots and identified as Pythium sp. based on morphological characters and proved pathogenicity. The maximum growth of the pathogen was found at 25 °C with pH 7. The maximum growth of the pathogen was found in PDA (90 mm) and V-8 agar (90 mm) media.

Keywords

Soft root rot, Pythium sp., Mulberry

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Introduction

Mulberry (*Morus alba*) is an important commercial crop grown under varied climatic conditions ranging from temperate to tropical region of the world. It is indispensable in the sericulture industry, as its leaves are the sole food of silkworm (*Bombyx mori* L.). Mulberry is a deciduous woody perennial plant and family of mulberry is Moraceae. Owning to all this significance mulberry plant can be designated as "Kalparuksha" also has gained its own importance among farming community by its utility. But mulberry cultivation has many hindrances in its cultivation, one such constraint is diseases. As mulberry plant is affected by diseases like root rot, leaf spot, powdery mildew, leaf rust, bacterial blight and and root knot. Among these diseases, root diseases with soil borne

pathogens are serious problems in mulberry cultivation because of its perennial nature. Dry root rot disease causes leaf yield loss about 14 per cent (Sharma *et al.,* 2003). Whereas, mortality of cuttings due to dry root rot reported up to 44.40 per cent (Gupta *et al.,* 1997).

Symptoms of the disease manifests in yellowing sudden withering and drying of leaves starting from the bottom branches and spreads upwards, followed by defoliation and finally resulting in death of the plants. The disease initiates in isolated patches later spreading throughout the mulberry field. Bark of root peels off easily and the plants die. The cortex of infected roots first turns brown and rot followed by darkening of xylem. Conspicuous mucilaginous matrix covers the mulberry root and hence the name soft root. The exact cause of soft root rot disease in Karnataka is identified as *Pythium* sp. As the soft root rot disease is spreads rapidly in mulberry gardens in Ramnagar and Mandya districts and damage the crop, so an attempt was conducted to know the occurrence and causal agent of soft root rot disease in Karnataka *viz*., Kolar, Mandya, Ramanagara and Chikkaballapur districts.

Materials and Methods

Survey for occurrence of mulberry soft root rot incidence in southern Karnataka

The roving survey was conducted during 2019-2020 to know the incidence of soft root rot disease of mulberry in Ramanagara, Mandya, Kolar and Chikkaballapur districts of Karnataka. The survey was conducted in places Agara, Kebre, Thattekere and Gerehalli (Ramanagara district); Maddur, Halaguru and Malavalli (Mandya district); Narasapura, Vakkaleri, Vemagal and Mandikal (Kolar district), Manchenhalli, Gudibande, Mandalahalli and Bagepalli (Chikkaballapur district).The number of wilted plants were counted and per cent disease incidence was calculated. The complete wilted plants were collected for isolation and other studies.

The per cent disease incidence was calculated using following formula.

$$\text{Per cent Disease Incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Collection and isolation of the pathogen

The standard tissue isolation technique was followed for isolation of soft root rot pathogen from infected roots collected from different locations. The diseased root portions along with some healthy parts were cut into small pieces or bits and were washed with tap water. These bits of roots were surface sterilized by using mercuric chloride (0.1 %). Later aseptically transferred to sterile Petri plates with potato dextrose agar (PDA). The plates were incubated at room temperature (27±1 ^{o}C) for about one week and observed for fungal growth and sporulation. The fungus Pythium identified based on morphological characters. The identified fungus was later transferred to sterile PDA slants and incubated at 27 ± 1 ^{o}C for further use.

Purification of fungal culture by hyphal tip method

Water agar plates were used for the hyphal tip isolation. The dilute suspension of mycelia was prepared in sterile distilled water. Such suspension of one ml was spread uniformly on water agar (2%) plates. Single mycelial bit was marked with ink on plate's glass surface. They were further kept for incubation at 27±1 ^{o}C. Later, bits of mycelium of fungus was placed on center of Petriplate containing potato dextrose medium and incubated for 10 days at 27 ± 1 ^{o}C. The culture was used for further studies. The pure culture of fungus was sub cultured on potato dextrose agar (PDA)

slants and allowed to grow at 27±1 ^{o}C for 7 days. Later, such slants were stored in a refrigerator at 4 ^{o}C and regularly subcultured once in a month to obtain the fungus in viable condition.

Cultural characters of *Pythium* sp.on different solid media

The cultural characters of *Pythium* sp. was studied on eleven different media viz., Potato dextrose agar, vegetable juice agar, potato Carrot agar, carrot agar, pectin yeast glucose agar, oat meal agar, corn meal agar, chloramphenicol rose bengal agar, malt extract agar, distil water agar and water agar. Twenty ml of each sterilized and cooled medium was poured aseptically into sterilized Petri plates. Five mm disc of the *Pythium* sp. was placed at the centre of Petri dish and then incubated at 27 ± 1 ^{o}C for 12 days. Each of this experiment was replicated thrice and observations regarding cultural characters such as the colour, diameter and pigmentation of colony were recorded.

Physiological studies

Effect of temperature on the growth of *Pythium* sp.

The fungal growth was tested at 15, 20, 25, 30 and 35 ^{o}C. For each treatment, four replications were maintained. 25ml PDB was added into each of 100ml conical flask and sterilized at 1.1 kgcm^{-2} pressure for 20minutes at 121 °C. The flasks were allowed to cool after sterilization. Later, the flasks were inoculated with 5mm disc of fungus which was collected from 12days old culture and incubated at respective temperatures. The mycelial mat was harvested by filtering through Whatman No.1 filter paper of 9cm diameter and dried. The dry mycelial weight was recorded and results were analysed statistically.

Effect of pH on the growth of *Pythium* sp.

The liquid medium used in this study was potato dextrose broth. Hydrogen ion (pH) concentration of the media was determined by using pH meter. Adjustment of pH was done using 0.1 N alkalis (Sodium hydroxide) or 0.1 N acids (Hydrochloric acid). Reaction of liquid media was adjusted to required pH *viz.*, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5.Twenty five ml of the medium was added to 100 ml conical flask and sterilized at 1.1 kgcm^{-2} pressure for 20 minutes at 121 °C. Each treatment was replicated thrice. Each flask, 5 mm fungal disc was inoculated aseptically and incubated at 27 ± 1 ^{o}C for 12 days. The ideal pH for growth of the fungus was determined by harvesting mycelial mat that was filtered through Whatman filter paper and dry mycelial weight (mg) was recorded.

Results and Discussion

Survey for occurrence of mulberry soft root rot incidence in southern Karnataka

During 2019-2020 roving survey was conducted to assess the soft root rot incidence of mulberry in major mulberry growing areas *viz.*, Kolar, Mandya, Ramanagara and Chikkaballapur districts of Karnataka. The soft root rot survey results are presented in Table 1. Soft root rot disease incidence in different districts ranged from 6.58 to 43.76 per cent. Among surveyed locations, highest incidence was recorded in Agara (43.76 %) of Ramanagara district and least incidence in Malavalli (6.58 %) of Mandya district.

Among the districts surveyed, highest soft root rot incidence was in Ramanagara district kanakapura taluk Agara (43.76 %) and least disease observed in Mandya district Malavalli 6.58 per cent and no disease found in kolar and Chikkaballapur

Symptomatology

Mulberry soft root rot symptoms are sudden withering and drying of leaves starting from the bottom of the branch and spreads upwards, followed by defoliation and finally resulting in death of plants. The disease initiates in isolated patches later spreading throughout the garden. The below ground symptoms are peeling and rotting of roots and plants die. The cortex of the infected roots first turns brown and rot followed by darkening of xylem. A conspicuous mucilaginous matrix covers the entire root system and hence the name soft root. The exact cause of soft root rot disease of mulberry was identified as *Pythium* sp.(Plate 1a and 1b)

Morphological characters and pathogenicity of mulberry soft root rot pathogen

Isolation of fungus was done from typical root rot infected mulberry plants by using standard tissue isolation technique. The pure culture of *Pythium* sp. was inoculated to the artificially wounded roots of healthy mulberry plant. Initially drying of leaves was noticed at 18 days after inoculation later withering and wilting after 21 to 25 days of plant was observed as above ground symptoms(Plate 3). Pathogen *Pythium* sp. identified on the bases of morphological characteristics on PDA medium. The colonies were white colour. It produced the aseptate mycelium and globose shape sporangium (Plate 2). Sporangium white to colourless. Zoospores releases from sporangium in the water suspension.

Effect of different solid media on growth of *Pythium* sp.

The growth of *Pythium* sp. was studied on eleven different synthetic solid media, results noted in Table 2 and Plate 4.

Pythium sp. growth varied among different media tested, it ranged from 11.10 mm to 90.00 mm.

The maximum radial growth of *Pythium* sp. was recorded on V-8 and PDA media (90.00 mm) which was on par with carrot agar (89.70mm) which was followed by potato carrot agar (87.00 mm), Pectin yeast glucose agar (86.80 mm), Corn meal agar (79.00 mm), Distil water agar (70.20 mm), water agar (59.50 mm), oat meal agar (45.00 mm), and Chloramphenicol Rose Bengal agar (13.80 mm), whereas least radial growth was observed on malt extract agar (11.10 mm),

The colour of mycelium varied from white to creamish white and type of growth was varied from flat irregular, fluffy raised to sparsely raised

Effect of temperature on *Pythium* sp. on solid and liquid media

The different temperature levels *viz.*, 15, 20, 25, 30 and 35 °C were studied for the growth of Pythium sp. The growth of *Pythium* sp. was gradually increased from 20 to 30 °C and later decreased at increasing temperature. The growth differences seen in all temperatures were statistically significant from each other.

The temperature of 25 °C and 30 °C was significantly superior to other temperature levels by recording the maximum radial growth (90.00 mm) followed by 35 °C (34 mm) and 20 °C(11 mm) and pathogen growth not seen at 15 °C.

The colour of mycelia was white at all temperature. In liquid media the experiment done by using potato dextrose broth at different temperature levels *viz.*, 15, 20, 25, 30 and 35 °C. In this maximum dry mycelial weight seen at 25 °C.

Table.1 Survey for the incidence of mulberry soft root rot during 2019-2020 in southern Karnataka

Sl. No.	District	Locations	Incidence of soft root rot (%)
1	Ramanagara	Agara	43.76
		Kebre	32.57
		Thattekere	18.79
		Gerehalli	13.58
2	Mandya	Maddur	07.89
		Halaguru	16.78
		Malavalli	06.58
3.	Kolar	Narasapura	0.00
		Vakkaleri	0.00
		Vemagal	0.00
		Mandikal	0.00
4	Chikkaballapur	Manchenhalli	0.00
		Gudibande	0.00
		Mandalahalli	0.00
		Bagepalli	0.00

Table.2 Effect of different solid media on the growth of *Pythium* sp.

Sl. No.	Different media	Radial growth (mm) *	Type of growth	Colour	Pigmentation
1	Potato dextrose agar	90.00	Fluffy and circular	White	White
2	V-8 juice agar	90.00	Suppressed and circular	White	White
3	Potato carrot agar	87.00	Fluffy, raised and circular	Creamy white	Creamy white
4	Carrot agar	89.70	Fluffy, flat and circular	White	Light brown to white
5	Pectin yeast glucose agar	86.80	Sparsely raised and circular	White	White
6	Oat meal agar	45.00	Raised and circular	Light white	White
7	Corn meal agar	79.00	Flat and irregular	White	Light white
8	Chloramphenicol rose bengal agar	13.80	Flat and irregular	White	White
9	Malt extract agar	11.10	Flat at center	White	Greyish white
10	Distil water agar	70.20	Sparsely raised and circular	Light white	White
11	Water agar	59.50	Flat and supressed	White	White
	SEm ±	**0.36**	-	-	-
	CD @ 1%	**1.03**	-	-	-

Table.3 Effect of temperature on growth of *Pythium* sp. in liquid and on solid media

Sl. No.	Temperature	Potato dextrose broth	Potato dextrose agar		
	(^{0} C)	Dry mycelial weight (mg)*	Radial growth (mm)*	Mycelial colour	Type of growth
1	15	0.00	0.00	No growth	No growth
2	20	352.48	11	White	Flat
3	25	850.00	90	White	Fluffy, raised
4	30	734.70	90	White	Flat, raised
5	35	328.52	34	White	Raised
SEm ±		**0.065**	**0.258**		
CD @ 1%		**0.021**	**0.387**		

Table.4 Effect of hydrogen ion concentration (pH) on growth of *Pythium* sp. in potato dextrose broth medium

l. No.	pHlevel	Dry mycelial weight (mg)*
1	3.5	0.00
2	4.0	0.00
3	4.5	155.80
4	5.0	191.00
5	5.5	191.18
6	6.0	201.30
7	6.5	352.20
8	7.0	386.60
9	7.5	234.50
10	8.0	135.70
11	8.5	45.90
12	9.0	0.00
13	9.5	0.00
SEm ±		**0.067**
CD @ 1%		**0.187**

Plate.1a Above ground symptoms of mulberry soft root rot disease

Plate.1b Below ground symptoms of mulberry soft root rot disease

Plate.2 Microscopic observation of mycelia with sporangia development of *Pythium* sp.

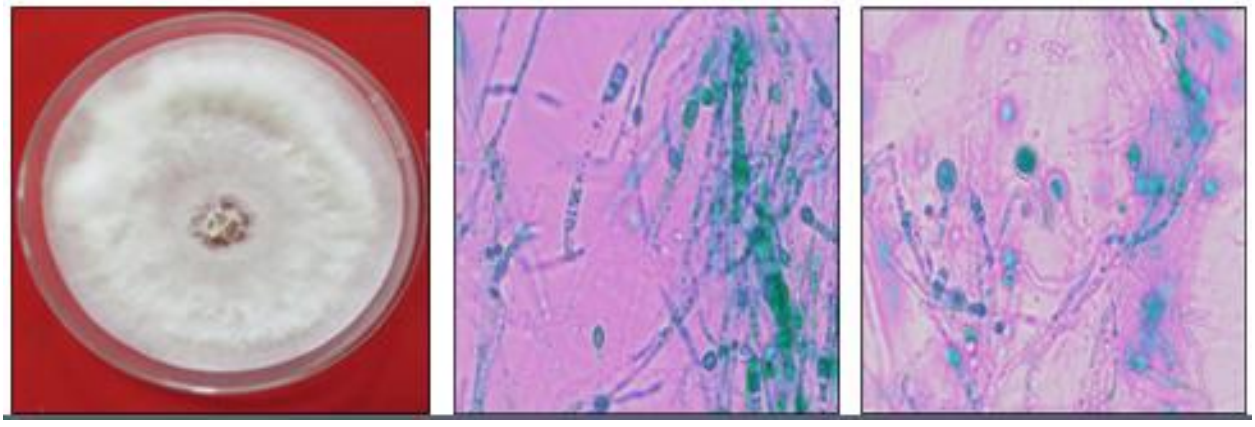
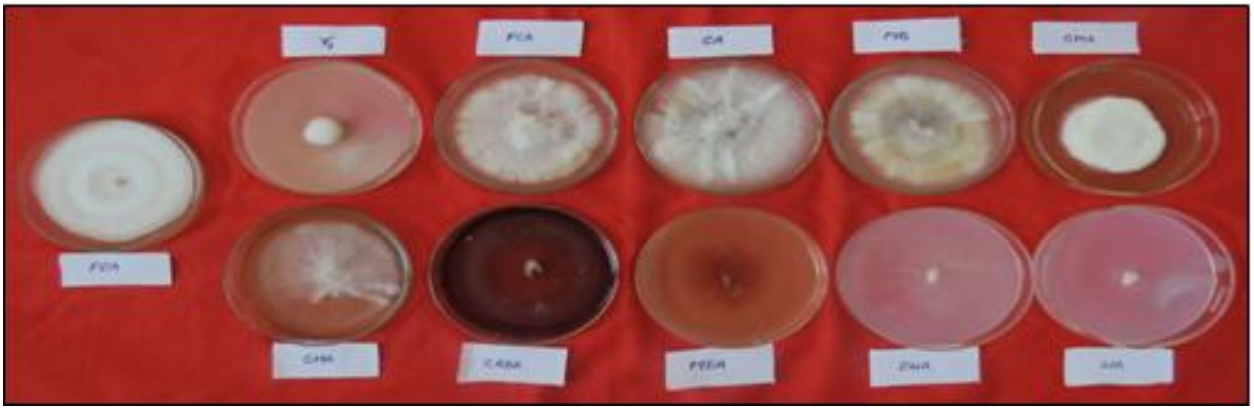


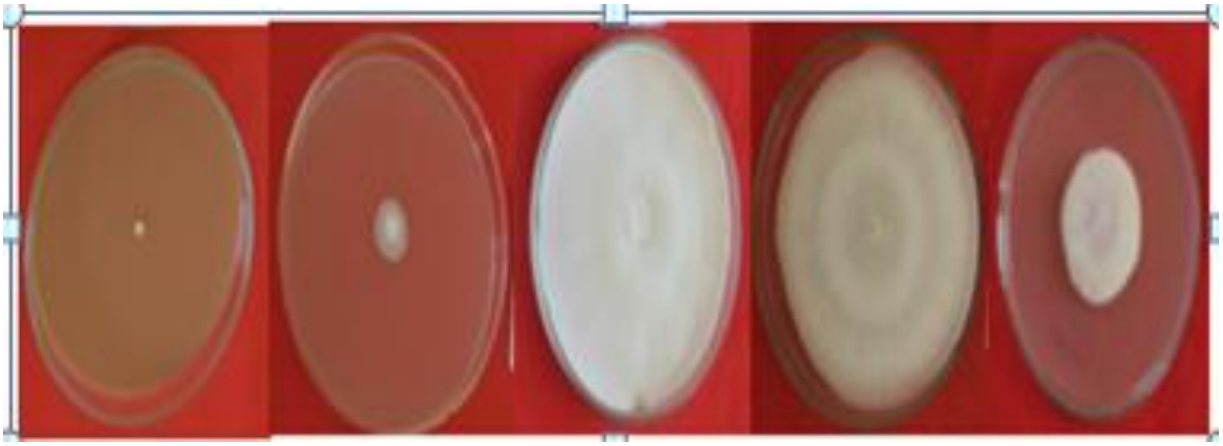
Plate.3 Pathogenicity of mulberry soft root rot pathogen *Pythium* sp.

Plate.4 Growth of pathogen *Pythium* sp. on different nutrients media



PDA: Potato dextrose agar, V-8: V-8 juice agar, PCA: Potato carrot agar, CA: Carrot agar PYG: Pectin yeast glucose agar, OMA: Oat meal agar, CMA: Corn meal agar, CRBA: Chloramphenicol rose bengal agar, MEA: Malt extract agar, DWA: Distilled water agar WA: Water agar

Plate.5a Effect of temperature on the growth of *Pythium* sp. on potato dextrose agar



15 °C **20 °C** **25 °C** **30 °C** **35 °C**

Plate.5b Effect of temperature on the growth of *Pythium* sp. on potato dextrose broth

Plate.6 Effect of hydrogen ion concentration (pH) on growth of *Pythium* sp. in potato dextrose broth

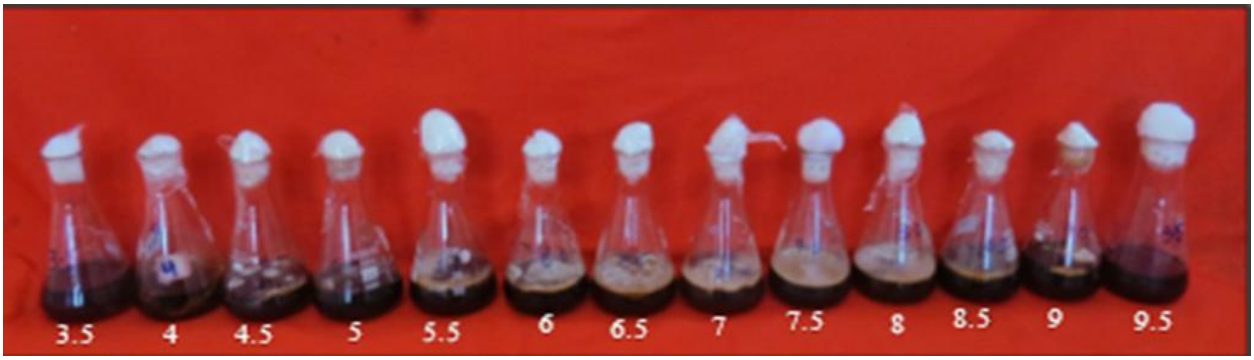
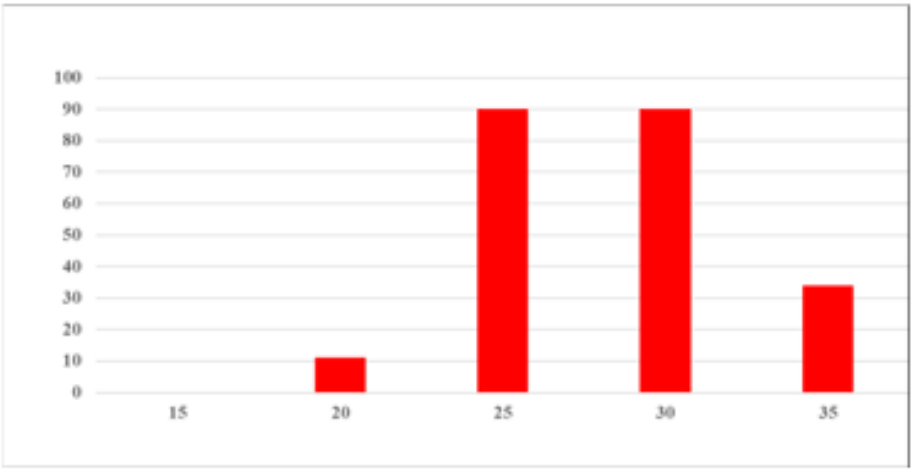


Fig.1 Effect of temperature on the growth of *Pythium* sp. on potato dextrose agar



Higher dry mycelial weight of the fungus (850.00 mg) was observed at temperature 25 °C which was followed by temperature level of 30 °C(734.70 mg), 20 °C(352.48 mg) and 35 °C(328.52 mg). There was no growth of the fungus was observed at 15 °C, results were presented in Table 3 and Plate 5b (Fig. 1).

Effect of pH on the growth of *Pythium* sp. in liquid media

The study was carried out to know the optimum pH required for the growth of *Pythium* sp.dry mycelial noted at different pH levels *viz.,* 3.5, 4, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9 and 9.5, results shown in Table 4 and Plate 6.

The highest *Pythium* sp. dry mycelial weight was recorded at the pH of 7.0 (386.60 mg) which is on par with the pH 6.5 (352.20 mg) and these are followed by pH 7.5 (234.50 mg), pH 6.00 (201.30 mg), pH 5.5 (191.18 mg), pH 5.0 (191.00 mg), 4.5 (155.80 mg), 8.0 (135.70 mg), 8.5 (45.00 mg) and no growth was seen at 3.5 pH, 4.0 pH and 9.5 pH

Mulberry is an important commercial crop of India, affected by many diseases caused by various pathogens, which affect the yield loss of leaves. The research done with reference to survey of soft root rot disease of mulberry in southern Karnataka to effectively manage of disease. During 2019-2020 survey done in sothern Karnataka for occurrence of mulberry soft root rot, revealed that Ramanagara district (27.17 %) had highest mean disease incidence.

Soft root rot pathogen was isolated from infected mulberry root and identified as *Pythium* sp. based on morphological and pathogenicity test. It produced aseptate mycelium. Colonies of *Pythium* sp. were initially creamy white and gradually turned white colour. It produced globose shaped sporangia reported by Dow and Lumsden (1975) and which were white to colourless reported by Alhussaen (2019). Pathogenicity was proved by inoculating mycelial plugs of fungus *Pythium* sp.on wounded root of healthy mulberry plant. The soft root rot, root peeling and wilting symptoms were observed 45 days after inoculation reported by Linde *et al.*, (1994)

At 25 ^{o}C maximum radial growth (90 mm) and dry mycelial weight (850 mg) was recoded by Bolton (1980) and *Pythium* sp. grew at different pH levels (4.5 to 8.5 pH). However, the highest dry mycelial weight (386.60 mg) was observed at pH7.0 reported by Alhussaen *et al.*,(2011). Among eleven cultural media tested for the growth of *Pythium* sp. The fungus grew well on all cultural media. But, the highest radial growth (90 mm) was observed on Potato dextrose agar reported by Conway (1985) and V-8 juice agar media.

Mulberry Soft root rot pathogen was confirmed as *Pythium* sp. based on morphological characteristics and pathogenicity and is a new report. Optimum temperature and pH for the growth of pathogen were 25^{o}C and 7 respectively.

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