

Disease Incidence, Severity and Phenotypic Variation Among the Isolates of *Rhizoctonia Bataticola* Infected in Root Rot Disease of Mulberry in Different Mulberry Fields of Karnataka

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The present investigation was carried out in view of destructive nature of root rot disease of mulberry caused by *Rhizoctonia bataticola*. The pathogen is a soil inhabiting fungus which is causing serious threat to more than 500 plant species. This study will enable to have a clear view of the root rot pathogen, *R. bataticola* in respect to variability, distribution and economic impact on mulberry plant. Root rot disease of mulberry is a devastating disease among the mulberry diseases. The part of study was conducted in Ramanagara district of Karnataka which is the largest market for silk cocoons in Asia, followed by Kanakapura in Karnataka, India. A field survey was conducted on disease incidence in various mulberry cultivating fields in Kanakapura and Ramanagara areas of Karnataka. Disease incidence of 78% and 53.60% with an average leaf yield loss of 39.73% covering 80 mulberry gardens has been reported in Kanakapura and Ramanagara respectively. From the infected root samples the phytopathogen, *R. bataticola* was isolated and its phenotypic variation was studied by growing the fungal pathogen on different fugal isolation media such as potato dextrose agar, richard's agar and czapek dox agar under laboratory conditions. This study revealed the prevalence of the mulberry root rot disease in major mulberry growing locations in Karnataka. Incidence and severity of the mulberry root rot disease varied significantly among the locations under cropping seasons. This study was limited to southern Karnataka and did not cover other mulberry production locations in the different agro-ecological zones in Karnataka state. Therefore a study should be undertaken to evaluate the disease prevalence in other locations.

Keywords: Disease Incidence; Mulberry; Phenotypic Variation; Root Rot Disease; *Rhizoctonia Bataticola*.

The genus *Morus*, popularly known as mulberry, belongs to the family Moraceae. The plant is a fast growing, medium-size; deciduous woody tree species widely distributed in India, Japan, China, North Africa, and southern Europe¹.

². Mulberry is an important commercial crop grown under varied climatic conditions ranging from temperate to tropical conditions of the world. The plant is considered as an indispensable source for sericulture industry, as mulberry leaves particularly

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white mulberry is the sole food source of silkworm (*Bombyx mori*)³. It is well-known for its economic significance in the production of mori silk, which is used to make silk yarn. Mulberry leaves are also used as a source of food for livestock⁴. Mulberry has long been known as a plant used for silk production, contributing considerably to the livelihoods of many people across the world. India is the second largest producer of silk in the World followed by China. The sericulture industry is land based one concentrated in Karnataka, Andhra Pradesh and Tamil Nadu. The states such as Assam and West Bengal states are also practice sericulture to certain extent⁵.

Mulberry species has gained popularity as a versatile plant in recent years due to its medicinal properties⁶. Mulberry plants are attributed with many medicinal properties such as anti-inflammatory, antioxidant, antiviral, anti-hyperglycemic, and neuroprotective⁷. Phytochemicals contained in *Morus* spp., such as coumarins, flavonoids, and phenols, have been reported to help lower blood pressure and cholesterol levels in humans⁸. Mulberry leaves in its high-quality are valuable as herbal raw materials in the production of pharmaceutical and food products⁹.

Mulberry is attacked by various pathogens belonging to the group bacteria, fungi, viruses and nematodes that hinder mulberry cultivation in major mulberry growing areas. Damage to mulberry leaves caused by either microbial pathogens or plant protecting chemicals adversely affects on the quality of silk¹⁰. Root rot diseases continue to be a major global threat to agricultural crop productivity¹¹. They are sometimes referred to as a root rot complex since they are caused by multiple pathogens¹². The most common pathogens in the complex are fungi and oomycetes, however bacteria and viruses have also been linked to root rot diseases¹³. Among several diseases of mulberry, root rot disease caused by *Rhizoctonia bataticola* had become a serious threat in all mulberry growing areas. The disease was reported throughout the year in all soil types under different agro-climatic conditions and mortality occurs due to drying and death of plant¹⁴. The root rot disease of mulberry caused by *Rhizoctonia bataticola* in Karnataka, Andhra Pradesh and Tamil Nadu of South India has reported the highest leaf yield loss¹⁵.

The genus *Rhizoctonia*, which means “root killer,” is an anamorphic fungus that produces hyphae and sclerotia rather than spores (hyphal propagules - asexual stage of fungi)¹⁶. The species of *Rhizoctonia* are saprophytic, but some act as facultative plant pathogens infecting valuable crops^{17, 18}. Due to the existence of sclerotial phase in mulberry, *Rhizoctonia bataticola* is known as *Macrophomina phaseolina* (Tassi) Goid in its pycnidial condition^{19, 20}.

R. bataticola is a soil-borne phytopathogen with wider host range involving more than 500 plant species in seventy five families^{21, 22}. The ubiquitous, sclerotial fungus *R. bataticola* isolated from different host differs in their cultural characteristics. Despite its wide host range, the genus *Macrophomina* contains only one species which is *M. phaseolina*²³.

The field surveys conducted during 2010–2013 indicated widespread and increased incidence of root rot disease of mulberry in the southern states and central India²⁴. The disease was found in all soil types, cropping systems, and cultivars, with disease incidence ranging from 5% to 50% or more in severely contaminated soils. Because of the disease’s extensive geographic occurrence, it is likely to be a key disease in mulberry for in-depth studies intended at understanding fungal behaviour in the face of future climate change projections. Quality, quantity or productivity is affected due to infection caused by various biological agents. Hence plant disease diagnosis plays a significant role in the agriculture. Therefore the field survey plays an important role in diagnosis of any kind of plant diseases and to find out best integrated disease management practices. Considering all the facts and observation of root rot disease in mulberry cultivating gardens, a field survey was undertaken to know the disease incidence, severity and leaf yield loss in mulberry gardens of Kanakapura taluk and Ramanagara districts of Karnataka by adopting random sampling method.

METHODOLOGY

Survey on Incidence of Mulberry root rot disease

The incidence of root rot disease was recorded based on foliar symptoms of a disease, for the quantification of disease incidence severity

and estimated leaf yield loss, in each village (4-6) gardens were selected at random for the study.

A revoing survey was conducted to know the disease incidence, severity and leaf yield loss in major sericulture practicing areas of Karnataka viz., Ramanagara and Kanakapura during 2018 and 2019. At Kanakapura taluk ten villages such as Bapujinagar, Kaadujakkasandra, Kanchanahalli, Hosadoddi, Manchanadoddi, Sorekayidoddi, T.Bekuppe, Theranadoddi, Thigalarahosahalli, Venkataramanadoddi were surveyed. At Ramanagara district five villages viz., Acchalu, Devaradoddi, Kempgowdanadoddi, Kuruballidoddi, S.R.hills were surveyed. The infected root samples were collected randomly from mulberry fields in sterile polythene bags and brought to laboratory for further studies.

Disease index

Disease Incidence

The incidence of root rot disease in different mulberry gardens with V-1 variety was determined using Sharma and Gupta's formulae [26].

$$\text{Disease incidence (\%)} = [\text{No. of infected gardens} / \text{Total no. of gardens surveyed}] \times 100$$

Disease Severity

Disease severity was estimated in each mulberry garden by as follows, in each garden twenty five plants were selected at the rate five plants each at the four corners and five plants at the middle of the garden. Based on the foliar symptoms, disease severity was calculated based on the following formulae given by Sharma and Gupta²⁶.

$$\text{Foliar Infection (\%)} = [\text{No. of wilted leaves} / \text{Total number of leaves}] \times 100$$

Estimation of leaf yield loss

The quantitative leaf yield loss due to root rot disease in different mulberry gardens was estimated at field level in all surveyed villages as recommended by Evans²⁷, and Chowdary and Govindaiah¹⁵. Twenty-five infected plants were chosen at random from each garden at five different sites in the field. The diseased plants were classified as a, b, or c using the following scale based on the severity of the foliage damage.

Foliar infection	Disease severity	Group of the plant
0.1-25% wilting	Mild	a
25.1-50% wilting	Moderate	b
50.1-100% wilting	Severe	c

The following formula was used to compute the leaf yield loss in the infected garden²⁷.

$$\text{Leaf yield loss} = [(a + 2b + 4c) / 10]$$

Where a, b, and c represent the number of mild, moderate, and severe plants, respectively.

Isolation and Identification of fungi from infected root samples

The associated fungal pathogens were isolated from diseased root samples by 'root bit method'^{28, 29}. Infected root samples were cut into small pieces and surface sterilized with 0.1 percent mercuric chloride before being washed five times with sterile distilled water; the surface sterilized root bits were then blot dried using filter paper and kept on petridishes containing sterilized Potato Dextrose Agar (PDA), Czapek dox Agar (CDA) and Richard'sagar (RA) medium under aseptic condition. Inoculated plates were incubated for 5-7 days at 28±2°C³⁰.

RESULTS AND DISCUSSION

Disease Incidence

The present study was conducted in different regions of Ramanagara district and Kanakapura taluk of Karnataka, India. The number of villages surveyed at Kanakapura was ten and five at Ramanagara. Total eighty mulberry gardens were surveyed, fifty at Kanakapura taluk and at Ramanagara district belonging to the different soils (red loamy and black cotton), farming systems in irrigated gardens with V-1 mulberry variety.

The root rot disease incidence observed in different villages at Kanakapura and Ramanagara as shown in table 1 and 2. In each village three to six mulberry gardens were selected for survey purpose. At Kanakapura taluk 100% of disease incidence was recorded in villages like Bapujinagar, Sorekayidoddi, T.Bekuppe, and Venkataramanadoddi followed by 83.3%

disease incidence in Manchanadoddi, 75% was recorded in kaadujakkasandra, Hosadoddi and Thigalarahosahalli. In Theranadoddi village 40% and least incidence of 20% recorded in Kanchanahalli village. At Ramanagara district

Kempegowdanadoddi and Kuruballidoddi showed 67% of disease incidence, followed by 50% in S.R. Hills and Acchalu village. Finally least percentage of 34% disease incidence was observed in Devara doddi village.

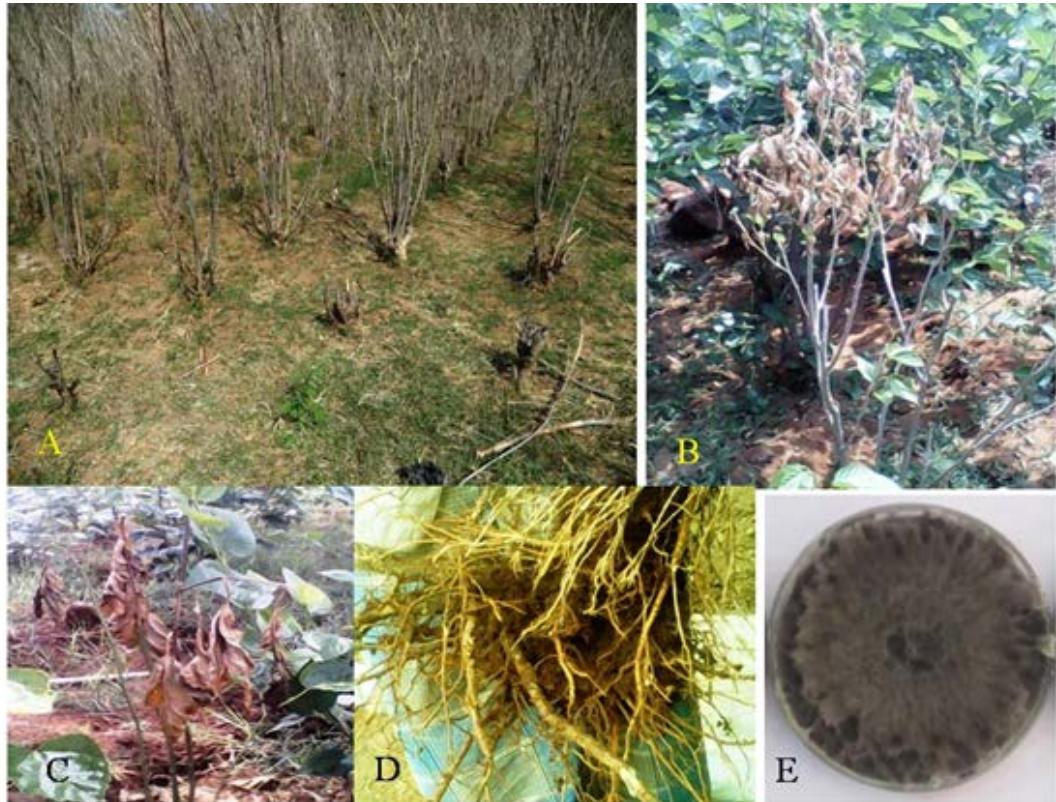


Fig. 1. Symptoms of mulberry root rot disease (A) field symptoms, (B) infected plant, (C) Foliar infection D) peeled off root (E) *Rhizoctonia bataticola* isolated from infected mulberry root

Table 1. Incidence of Root Rot Disease of mulberry in cultivating areas of Kanakapura area of Karnataka

Sl No.	Name of the Village (In Kanakpur)	No. of Gardens Surveyed	No. of Gardens infected	Disease incidence (%)
1.	Bapujinagar	6	6	100
2.	Kadujakkasandra	4	3	75
3.	Kanchanahalli	5	1	20
4.	Hosadoddi	5	4	75
5.	Manchanadoddi	6	5	83.33
6.	Sorekayidoddi	4	4	100
7.	T.Bekuppe	6	6	100
8.	Theranadoddi	5	2	40
9.	Thigalarahosahalli	4	3	75
10	Venkataramanadoddi	5	5	100
	Total	50	39	78.00

Table 2. Incidence of Root Rot Disease of Mulberry in Farmers Field at Ramanagara area of Karnataka

Sl. No.	Name of the Village (In Ramanagara)	No. of Gardens Surveyed	No. of Gardens infected	Disease incidence (%)
1.	Acchalu	6	3	50
2.	Devara doddi	6	2	34
3.	Kempegowdanadoddi	6	4	67
4.	Kuruballidoddi	6	4	67
5.	S.R.Hills	6	3	50
	Total	30	16	53.60

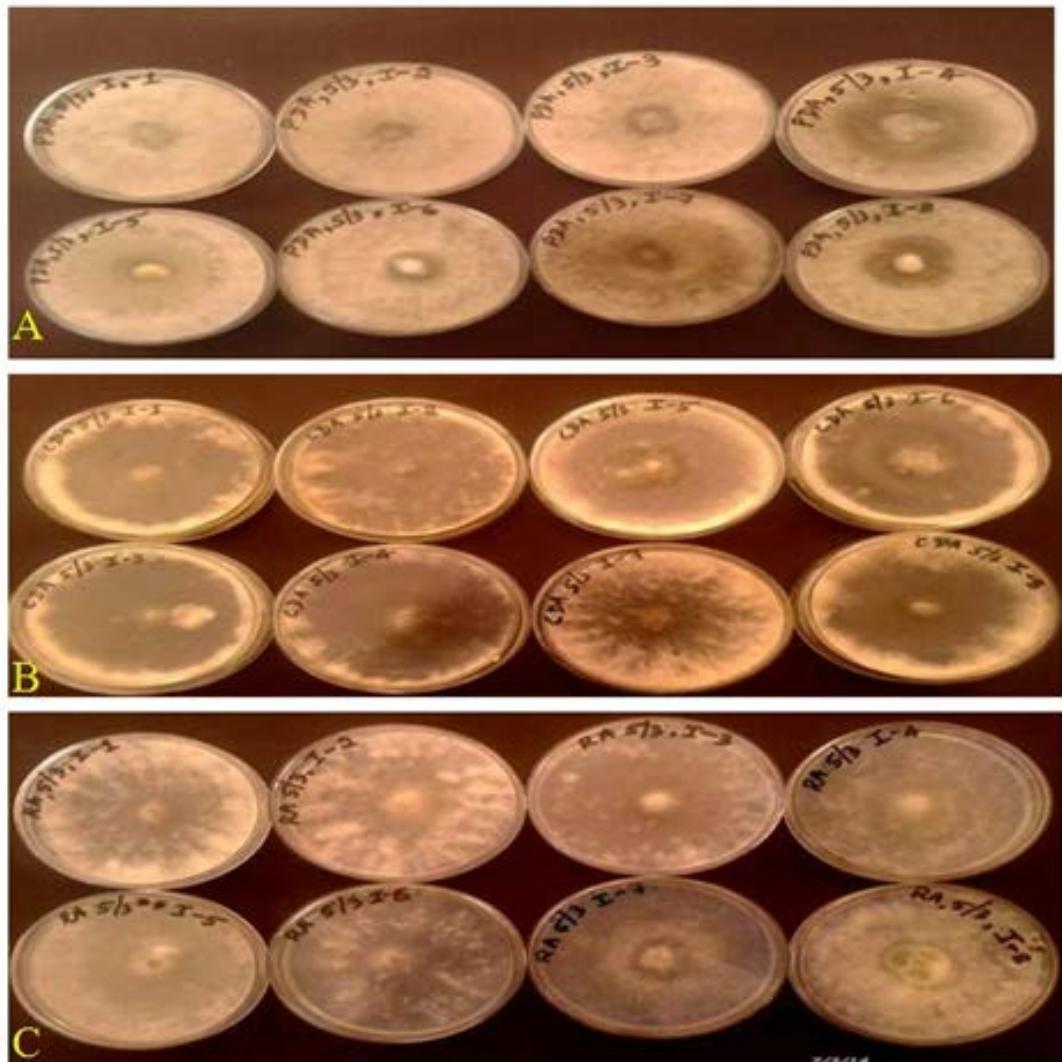


Fig. 2. Growth and phenotypic variation among the isolates of *Rhizoctonia bataticola* grown on A) Potato dextrose agar B) Czapek dox agar C) Richard's agar medium.

Table 3. Estimated leaf yield loss in different villages of Ramanagara and Kanakapura area of Karnataka due to root rot disease in mulberry

Sl No.	Name of the Village	Estimated leaf yield loss (%)
1.	Acchalu	8.80
2.	Devara doddi	42.60
3.	Kempegowdanadoddi	29.80
4.	Kuruballidoddi	42.40
5.	S.R.Hills	41.20
6.	Bapujinagar	97.80
7.	Kadujakkasandra	55.40
8.	Kanchana halli	9.00
9.	Hosadoddi	18.80
10.	Manchanadoddi	67.20
11.	Sorekayidoddi	50.40
12.	T.Bekuppe	49.84
13.	Theranadoddi	16.69
14.	Thigalarahosahalli	10.20
15.	Venkataramanadoddi	27.30
Average		37.82

Disease Severity

The disease severity was recorded in different mulberry gardens based on the foliar infection. Disease severity at Acchalu and Devara doddi village was recorded as 50% and 34% respectively. However in Kempegowdanadoddi (67.0%), Kuruballidoddi (67.0%), Bapujinagar (57.20%), Kadujakkasandra (54.33%), Manchanadoddi (50.20%), S.R.Hills (50.0%), Sorekayidoddi (47.66%), Kanchanahalli (45.0%), Tehranadoddi (41.72%), T. Bekuppe (41.24%), Venkataramanadoddi (37.0%), Hosadoddi (35.0%) and Thigalarahosahalli (31.50%) were recorded.

Estimated Leaf Yield Loss

The maximum leaf yield loss of 97.50% was recorded at Bapujinagar followed by Manchanadoddi (68.0%), Kadujakkasandra (56.40%), Sorekayidoddi (51.20%), T.Bekuppe (48.89%), Devaradoddi (43.70%), Kuruballidoddi (43.20%), S.R.Hills (41.50%), Kempegowdanadoddi (30.30%), Venkataramanadoddi (28.80%), Hosadoddi (19.30%), Theranadoddi (17.78%), Thigalarahosahalli (11.70%), Kanchana halli (10.0%), Acchalu (9.70%). Among fifteen villages surveyed an average of 39.73% of leaf yield loss was recorded.

Our results were in accordance with the field survey conducted by Chowdary and

Govindaiah¹⁵ in Karnataka, Andhra Pradesh and Tamil Nadu, wherein the highest leaf yield loss in different mulberry varieties was reported as follows, V-1 variety (34.74%), MR-2 (32.90%), S-36 (32.06%), RFS-175 (31.75%), S-13 (29.0%), and K-2 are the most common varieties (28.54 percent). Further an average of 31.49% of leaf yield loss was observed due to *Rhizoctonia bataticola*.

Govindaiah and Sharma³¹ reported the leaf yield loss of 11.8% at field conditions due to root knot disease in mulberry. In a study conducted 22% of leaf yield loss was reported due to *Meloidogyne incognita* in mulberry³². The estimated leaf yield loss due to root rot disease in mulberry in this study was 37.82 percent. This indicates the damage caused by root rot disease and its impact on mulberry cultivation, which recommends the control measures to be adopted for further spread of disease at Kanakapura and Ramanagara on priority basis. The survey identified three hot spot villages such as Bapujinagar (97.50%), Manchanadoddi (68.0%) and Kadujakkasandra (56.20%) which needs immediate control measures to prevent the spreading of disease. The annual loss in leaf production (18,894 kg/ha), cocoon harvest (756 kg/ha of garden), and monetary (Rs. 90,720/ha) owing to root rot disease was observed in another study¹⁵. Figure 1 represents the symptoms of root rot disease of mulberry.

Isolation and phenotypic variation of *Rhizoctonia bataticola*

The associated fungi *Rhizoctonia bataticola* was isolated from mulberry roots collected from different fields showed phenotypic variation when grown on different fungal isolation media. Total eight isolates of *R. bataticola* were isolated from infected mulberry roots. The fungus is considered as a heterogeneous assemblage of filamentous taxa that do not produce asexual spores. The fungus belonging to this genus are generally soil borne, mostly associated with roots and usually pathogens, although there have been reports of a number of saprophytic and symbiotic taxa^{33, 34, 35}.

Under laboratory conditions, the fungus grows quickly on PDA, then CDA and RA, producing brown to grey mycelium that darkens with age. Young hyphae are slender, hyaline, aseptate, and dichotomously branching, producing classic black sclerotia later on. The sclerotia formed

are smooth, varying from spherical to irregular shapes. The fungus was identified on the basis of their morphological and cultural characteristics^{33,34}.

The fungus *R. bataticola* produced a abundant aerial mycelium, which was shown to be fully or partially repressed when it came into contact with the lids of culture plates. Similar kind of results was observed and has been reported^[36]. Several studies were also carried out by various researchers to determine the morphology, physiology, and pathogenicity of *R. bataticola* in various host plants, such as castor²¹, cowpea^{37,38}, sunflower^{39,40}, groundnut⁴¹, pearl millet, sesame and horsegram⁴², and bean⁴³.

The isolation of fungal pathogen confirmed the pathogen identity in all the infected root samples. The radial growth of eight isolates was tested on three different media viz., PDA, CDA and RA medium (figure 2). The results indicated that the fungus was fast growing and occupies entire petridish within three days on PDA medium. The observation on second and third day showed variation in growth, among the isolates, *R. bataticola* K1 and K2 has occupied 20.1 and 21.3 mm respectively, *R. bataticola* K4, K6 and K7 has occupied 17.0-17.5 mm whereas *R. bataticola* K5 and *R. bataticola* K8 has occupied 11.0-11.8 mm.

R. bataticola isolates showed different growth pattern on czapek dox agar, where the isolate K2 showed maximum growth of 38.5 mm followed by isolate K7 (18.3mm), K8 (17.2mm), K6 (14.3mm), K5 (11.8mm), K1 (11.3mm), K4 (8mm) and K3 (8mm) after 3 days of incubation period. The isolates reached 78 mm after 96 hours of incubation. It was found that isolates *R. bataticola* K1, K4, K6, K7 and K8 showed similar morphological growth pattern whereas Isolates *R. bataticola* K2, K3, and K5 showed light grey color mycelium with partial fluffy growth when compared to growth on PDA medium which was very less. The phenotypic variability of *R. bataticola* isolates from roots, leaf and pulses such as black gram, cowpea, green gram, redgram and soyabean were studied apart from mulberry^{21,44,45}.

The growth of all isolates on richard's agar medium was comparatively lesser than that of potato dextrose agar and czapek dox agar after 72 hours of incubation. The radial growth of *R. bataticola* was found to be as follows isolate

K1 (12.8 mm), K2 (6.5 mm), K5 (5.3mm), K3 (5.1 mm), K4 (4.8 mm), K6 (4.3 mm), K7 (8.3 mm) and K8 (7.8 mm). However the growth was increased after 48 and 72 hours which are similar to growth on PDA, and CDA. The isolates showed light-grey color mycelium with appressed growth. Similar result was reported from pigeon pea plant infected with *R. bataticola*⁴⁶. The results obtained in the present study were in confirmative with the investigation carried out by earlier researchers^{15, 44}. In their study they reported the isolation of *R. bataticola* from dry root rot diseased chick pea plant and variation in the growth of pathogen was studied on different solid media. The study highlights PDA medium was the best medium for growth of the pathogen followed by chickpea root extract agar medium and carrot root extract agar medium whereas the media such as richard's agar, oat meal agar, czapek dox agar, Asthana and Hawker's agar supported poor to very poor growth⁴⁷.

CONCLUSION

The present study concluded that incidence of root rot disease in mulberry was detected in prominent mulberry growing areas in Karnataka. The highest root rot disease was noticed in Kanakapura area whereas the lowest was recorded in Ramanagara district. A total of eight isolates of *Rhizoctonia bataticola* was isolated from the infected root sample. Phenotypic variability in *R. bataticola* isolates was detected, each and every isolate varied in its growth on different media. Three type of growth pattern such as fluffy, partially fluffy and appressed growth was observed. From the overall study it can be understood that there is a prevalence of root rot disease in major mulberry growing locations in Karnataka. Therefore, resistant mulberry varieties are need of the hour to address the grower's problem along with best management strategies including efficient biocontrol agent which can minimize the disease to a greater extent. This study was limited to southern Karnataka and did not cover other mulberry production locations in the different agro-ecological zones in Karnataka state. Therefore a study should be undertaken to determine the disease prevalence in other locations.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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