



Survey, Incidence and Pathogenicity of Root Rot Disease of Cotton in Different Districts of Haryana Incited by *Rhizoctonia* spp

Preeti Vashisht ^{a*} and N. K. Yadav ^{b++}

^a ICAR-Central Institute for Cotton Research, Regional Station, Sirsa, India.

^b Regional Research Station, Bawal (Rewari), India.

Authors' contributions

This work was carried out in collaboration between both authors. Author PV designed the study, performed the statistical analysis and wrote the protocol. Author NKY managed the analysis of the study and literature searches. Both the authors read and approved the final manuscript.

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ABSTRACT

Aim: Survey, incidence and pathogenicity of root rot disease of cotton in different districts of Haryana incited by *Rhizoctonia* spp.

Design: Survey.

Place and Duration of Study: Ten major cotton growing districts in Haryana during *Kharif* 2021 and *Kharif* 2022.

Methodology: A roving survey to record cotton root rot incidence was conducted in ten major cotton growing districts of Haryana during the month of June and July for consecutive two *Kharif* seasons 2021 and 2022. On an average 4 growing region of cotton in each district were visited and percent disease incidence was recorded.

Results: Among all the surveyed districts of Haryana the maximum mean disease incidence was

**Assistant Scientist;

*Corresponding author: E-mail: dhimanpreeti45@gmail.com;

recorded in Fatehabad (18.75%) followed by Hisar (15.25 %), Sirsa (15%), While the minimum disease incidence was recorded in Nuh and Charkhi dadri 9.25 per cent in both during *kharif* 2021. Among all the surveyed districts of Haryana the maximum mean disease incidence was recorded in Fatehabad (18.25%) followed by Sirsa (15.25%), Hisar (15.42%), While the minimum disease incidence was recorded Charkhi dadri (9.08%).

Conclusion: The results of the current study showed that important cotton-growing regions in Haryana have root rot disease incidence. It is clear from the overall study that root rot disease is common in significant mulberry-growing regions in Haryana. As a result, resistant cotton types are urgently needed to address the grower's issue, together with the best management techniques, such as effective biocontrol agents that can significantly reduce the illness. Consequently, research should be conducted to ascertain the incidence of the disease in different places.

Keywords: *Rhizoctonia*; disease incidence; cotton; root rot; survey.

1. INTRODUCTION

"The word 'cotton' refers to four species in the genus *Gossypium* (Family *Malvaceae*), namely *G. hirsutum* L., *G. barbadense* L., *G. arboreum* L. and *G. herbaceum* L. that are domesticated independently as sources of textile fibre. Globally, the *Gossypium* genus comprises about 50 species" [1]. "During the year 2022-23, Gujarat, Maharashtra and Telangana are the major cotton growing states covering around 68% (88.3 lakh hectare) in area under cotton cultivation and 63% (236 lakh bales) of cotton production in India" [2]. "India is the largest country in world in terms of area under cotton is 133.50 lakh hectare which is 41% of the world cotton area. In terms of production, the country sharing the leading position with China at 290 lakh bales of 480 lb (equivalent to 371 lakh bales of 170 kg). Cotton fibre is purest source of cellulose and the most significant natural fibre. The economic significance of cotton in the global market is evident by its majority share over (50%) among fibers for textile goods" [3]. "It is harvested as 'seed cotton' which is then 'ginned' to separate the seed and lint. The long 'lint' fibre are further processed by spinning to produce yarn that is knitted or woven into fabrics. The ginned seed is covered in short, fuzzy fibres, Known as linters. Cotton is currently the leading plant fibre crop worldwide and is grown commercially in the temperate and tropical regions of more than 50 countries" [4]. Countries like the United States, India, China, the Middle East, and Australia are among the regions where cotton is grown specifically because of their climates, which include hot, dry spells and appropriate rainfall that is frequently attained through irrigation.

The cotton crop is grown extensively with a limiting factor, that is infected by fungal diseases

like anthracnose (*Collectotrichum gossypii*), leaf blight (*Alternaria macrospora*), wilt (*Fusarium oxysporum* f.sp. *vasinfectum*), Ramularia leaf spot (*Ramularia areola*), root rot (*Rhizoctonia bataticola* and *Rhizoctonia solani*) etc. Out of all the diseases, root rot of cotton is the most devastating disease and now a days this disease has become a major limiting factor in cotton cultivation. The management of root rot of cotton is of major concern wherever, cotton is cultivated thus, keeping in view the importance of the crop and destructive nature of disease and extent of losses it is causing to cotton cultivation in the state, the present investigation studies were undertaken.

1.1 The Pathogen

"*Rhizoctonia* is a widespread, destructive and versatile plant pathogen, distributed worldwide in both agriculture and forest soils and are known to cause root diseases of several crop plants. *Rhizoctonia bataticola* (Taub.) Butler as a plant pathogen was recognized by Halsted. Taubehaus gave the name of the genus as *Sclerotium* because of absence of spores and the species name as *bataticola* because it was pathogenic to *Ipomea batatas* (L.) Lam" [5]. According to the International Code of Botanical Nomenclature, "the binomial *Macrophomia phaseolina* was the valid name for the pycnidial stage of *R. bataticola*. Mycelia width varied from approximately 2-11µm and distance between two consecutive septa measured 46µm. However, the most important character regarding taxonomy and classification were the production size and composition of microsclerotia The fungus produces root like (rhizomorph) strands that grow through the soil until coming in contact with growing plant roots. Strands grow on roots toward the soil surface. Immediately below the soil surface in cotton, the fungus proliferates

around the hypocotyl, producing a cottony, mycelial growth". "The bark is destroyed by this mycelium and the fungus fills the vascular tissue of the plant. Sclerotia form in the strands following death of the plant. Sclerotia form from strands and the cells divide, grow and enlarge. These sclerotia are small (1/32 to 1/16 inch in diameter), densely compact masses of thick walled cells. Sclerotia enable the fungus to persist in fallow soil or soil planted to resistant crops for several years. Sclerotia have been found up to 12 feet deep in some soils. Sclerotia within plant parts were black, smooth, hard and varied in size from 100 µm-1mm while in culture, it varied from 50-300µm. These descriptions were given by Common wealth Mycological Institute (CMI), Kew, England. During the sclerotial formation, 50–200 individual hyphal cells aggregate to give multicellular bodies called microsclerotia. The microsclerotia were black and variable in size from 50–150 µm depending on the available nutrients of the substrate on which the propagules were produced" [6].

1.2 Symptomatology

"Symptoms are most likely to occur from June through September when soil temperatures reach 28°C (82°F). The first symptoms are slight yellowing or bronzing of the leaves. The upper most leaves wilt within 24 to 48 hours after bronzing, followed by wilting of the lower leaves within 72 hours. *Progressive wilting, premature dying, loss of vigour and reduced yield are characteristic features of M. phaseolina infection.* Permanent wilt occurs by the third day, followed by death. The leaves remain firmly attached to the plant. Affected plants die suddenly, often after excellent growth. Trees and shrubs may die more slowly. Roots are usually extensively invaded by the fungus by the time wilting occurs. Affected plants can be pulled from the soil with little effort. Root bark is decayed and brownish, bronze colored wooly strands of the fungus are frequently apparent on the root surface" [6]. "The fungus generally invades new areas by continual slow growth through the soil from plant to plant. It may also be moved about on roots of infected plants moved to new areas. The fungus can survive in the soil for many years and often is found as deep in the soil as roots penetrate. Affected areas often appear as circular patterns of dead plants. These areas gradually enlarge during the season or in subsequent years as the fungus grows through the soil from plant to plant. Infested areas in cotton may increase 5 to 30 feet per year in cotton. The pathogen *M.*

phaseolina generally affects the fibrovascular system of the roots and basal internodes, impends the transport of nutrients and water to the upper parts of the plant. The disease actually starts much early and its above ground manifestation in the form of wilting is a very late symptom. The affected plants can easily be pulled out of the ground. The bark of roots is broken into shreds and gives yellowish appearance as compared to healthy plants. Examination of affected parts reveals a dry rot, with many tiny black sclerotia distributed throughout the wood and softer tissues" [6].

2. MATERIALS AND METHODS

A roving survey to record cotton root rot incidence was conducted in ten major cotton growing districts of Haryana during the month of June and July for consecutive two *Kharif* seasons 2021 and 2022. On an average 4 growing region of cotton in each district were visited and percent disease incidence was recorded by counting total cotton plant in 1x1m² area and total rootrot infected plants. Plants showing typical symptoms were also investigated for microscopic association of pathogen and final confirmation of pathogen by isolation, purification and characterization. Typical symptoms like straw coloured appearance of plants at pod formation, black rotted roots, shredding of bark and root broken easily with presence minute dark black sclerotial bodies on root surface were considered for identification of disease. The per cent disease incidence was calculated as per formula given below-

$$\% \text{ Disease Incidence} = \frac{\text{Number of Diseased plants}}{\text{Total number of plants}} \times 100$$

2.1 Survey for Incidence and Collection of Disease Samples

The diseased samples of cotton showing typical root rot symptoms were collected in *Kharif* 2021 and *Kharif* 2022 from farmer's fields of different cotton growing areas of Haryana viz., Hisar, Sirsa, Fatehabad, Bhiwani, Charki dadri, Mahendergarh, Rewari, Gurugram, Nuh, Palwal all from local land races. The main aim was to explore possibility of existence of different species and/or variables of root rot pathogen, incidence caused by them and the survey was conducted in four villages selected randomly from each district and four fields from each village. The infected plants were carefully

uprooted and placed in polythene bags, properly tagged and brought to the laboratory and subjected to microscopic examination and tissue isolation.

2.2 Isolation, Purification and Identification of Pathogen

The pathogens were isolated on potato dextrose agar (PDA) medium. Small pieces (1-2 mm) of diseased roots were cut, washed with sterilized water, surface sterilized with 0.1 percent sodium hypochlorite (NaOCl) solution for 1

minutes followed by three to four washings with sterilized distilled water and were transferred aseptically to 2 percent PDA (Potato Dextrose Agar) poured Petri plates. The plates were incubated in an incubator at $28 \pm 1^\circ\text{C}$ for 7 days. Hyphae coming out from the bits were sub-cultured on the fresh PDA in Petri dishes. From these bits mostly cultures of *Rhizoctonia* spp., *Sclerotium* spp. and *Fusarium oxysporum* were recovered. The culture of *Rhizoctonia* was purified by single hyphal tip method. A total of 21 isolates of *R. bataticola* and *R. solani* in which 16 were *R. bataticola* and 5 were *R. solani*.

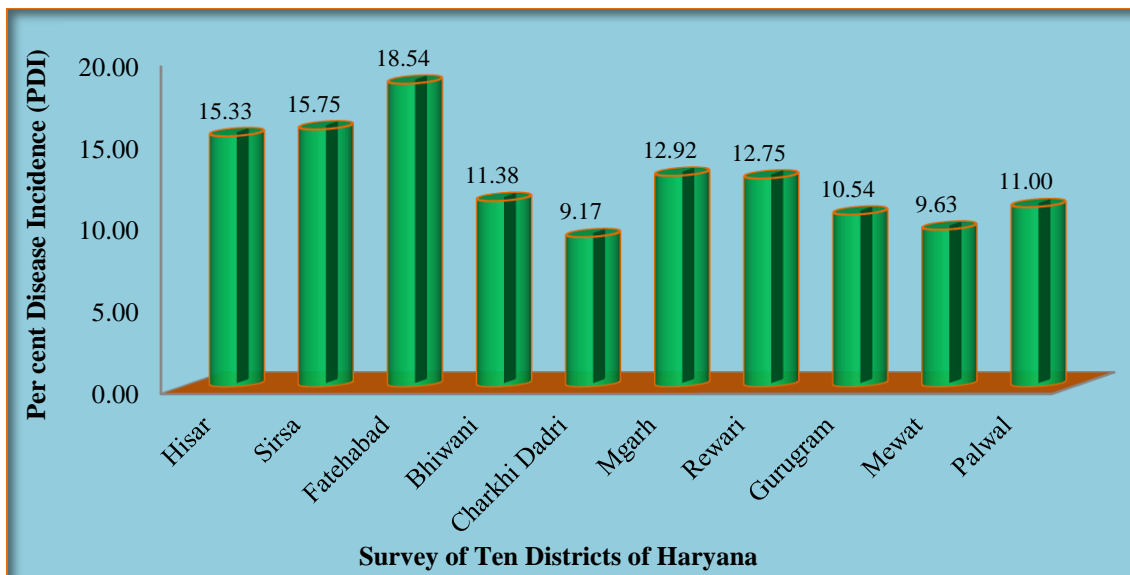


Fig. 1. District wise average disease incidence at different locations in Haryana

Table 1. Isolates collected from different districts of Haryana

District	Village	Latitude	Longitude	Isolate
Hisar	Sadapur	29.1491	75.7216	<i>R. bataticola</i> , <i>R. solani</i>
	Balsamand	29.1564	75.7161	<i>R. bataticola</i>
	Muklan	29.0710	75.6624	<i>R. bataticola</i>
Sirsa	Nirwan	29.5571	75.0079	<i>R. bataticola</i> ,
	CICR	29.5365	75.0255	<i>R. bataticola</i> , <i>R. solani</i>
Fatehabad	Bodiakheda	29.6645	75.8396	<i>R. bataticola</i>
	Ramsara	29.5134	75.4531	<i>R. bataticola</i> , <i>R. solani</i>
	Dariyapur	29.5225	75.3558	<i>R. bataticola</i>
Bhiwani	Hassan	28.9244	76.5676	<i>R. bataticola</i>
	Jitakheri	29.0138	75.9971	<i>R. bataticola</i>
Charkhi Dadri	Ghasola	28.5530	76.2609	<i>R. bataticola</i>
Mahendargarh	Gudha	28.3208	76.2768	<i>R. bataticola</i> , <i>R. solani</i>
	Sigra	28.2850	76.1885	<i>R. bataticola</i>
Rewari	Bawal	28.0955	76.5927	<i>R. bataticola</i>
	Dahina	28.3059	76.4649	<i>R. bataticola</i> , <i>R. solani</i>
Gurugram	Pataudi	28.3255	76.7782	<i>R. bataticola</i>

Table 2. Cotton root rot average disease incidence at different locations of Haryana during Kharif 2021 and Kharif 2022

District	Village	Latitude	Longitude	Disease incidence (%)		Pooled mean
				(2021)	(2022)	
Hisar	Sadalpur	29.1491	75.7216	13.67	13.67	13.67
	Balsamand	29.1564	75.7161	16.67	16.00	16.33
	Muklan	29.0710	75.6624	15.33	16.33	15.83
	Saharwa	29.1518	75.7211	15.33	15.67	15.50
Mean				15.25	15.42	15.33
Sirsa	Panniwala Mota	29.6668	75.6269	15.00	15.67	15.67
	Odhan	29.533305	75.0166	15.00	17.00	16.00
	Nirwan	29.557179	75.0079	13.33	16.33	14.83
	Ludeshar	29.3672	75.0976	16.00	17.67	16.83
Mean				14.83	16.67	15.83
Fatehabad	Bodiakheda	29.6645	75.8396	19.67	18.33	19.00
	Ramsara	29.513446	75.4531	19.00	18.67	18.83
	Dariyapur	29.5225	75.3558	18.33	17.67	18.00
	Ahalisadar	29.5668	75.3339	18.33	18.33	18.33
Mean				18.83	18.25	18.54
Bhiwani	Hassan	28.9244364	76.5676	13.33	14.00	13.67
	Jitakheri	29.0138132	75.9971	9.33	9.33	9.33
	Bawanikhhera	28.84815	75.5464	10.00	11.67	10.83
	Sherpura	28.8241919	75.6368	10.67	12.67	11.67
Mean				10.83	11.92	11.38
Charki dadri	Pentawas Kalan	28.6382	76.2001	8.33	8.67	8.50
	Ghasola	28.5530	76.2609	8.67	9.33	9.00
	Modhi	28.592062	76.2652	8.67	8.00	8.33
	Chidiya	28.4614	76.2609	11.33	10.33	10.83
Mean				9.25	9.08	9.17
Mahendergarh	Badaph	28.268347	76.1509	14.33	15.33	14.83
	Gudha	28.320891	76.2768	12.33	13.67	13.00
	Sigra	28.28506	76.1885	14.00	12.67	13.33
	Ananas	28.262121	76.1556	10.67	10.33	10.50
Mean				12.83	13.00	12.92
Rewari	Budoli	28.24689	76.4476	12.33	10.33	11.33
	Siha	28.27206	76.4129	11.67	14.67	13.17
	Bawal	28.09558	76.5927	14.33	12.67	13.50
	Dahina	28.30597	76.4649	11.67	14.33	13.00
Mean				12.50	13.00	12.75
Gurugram	Saiyad Shahpur	28.2687	76.7703	9.67	9.00	9.33
	Manukalan	28.2667	76.7392	9.33	9.67	9.50
	Pataudi	28.3255	76.7782	11.33	12.00	11.67
	Jatauli	28.33055	76.717	12.33	11.00	11.67
Mean				10.67	10.42	10.54
Nuh	Salamba	28.1147	77.0464	7.67	9.67	8.67
	Gasheda	28.1362	77.0766	9.00	10.67	9.83
	Manki	28.1111	77.0674	8.67	9.67	9.17
	Chhapeda	28.1696	77.0651	11.67	10.00	10.83
Mean				9.25	10.00	9.63
Palwal	Mandori	28.1383	77.2046	11.33	12.67	12.00
	Rajolaka	28.1242	77.2678	10.00	10.33	10.33
	Karana	28.14873	77.3320	11.67	11.33	11.50
	Mahespur	28.1291	77.2434	10.33	10.33	10.33
Mean				10.83	11.17	11.00
Overall Average Of Haryana				12.51	12.89	12.70

2.3 Pathogenicity Test

The pathogenic ability of *Rhizoctonia* spp. was tested in screen house on cotton cultivars HD 432 and RCH 773. Culture of *Rhizoctonia* was raised in 250ml Erlenmeyer flask containing 50ml of PDB sterilized at 15 lbs per sq inch pressure for 20 minutes. The bits of 5mm size were cut with the help of sterilized cork borer from fresh pure culture plates (5 days old) and transferred into flasks with the help of sterilized needle under aseptic conditions. After 7 days of incubation in BOD incubator at $27\pm 1^{\circ}\text{C}$, mycelial mats were collected and dried between folds of blotting paper for further studies. Five gram fresh mycelial mat was homogenized in blender for 2 minutes at lowest speed in 1000 ml of sterilized water. The suspension was used to inoculate the pots containing 10 kg of sand: ground cotton seed mixture (9:1) which was sterilized by autoclaving at 15lbs/ inch pressure for one and half hours for two consecutive days. On the third day of inoculation fifteen seeds of cotton cultivars, HD 432 and RCH 773 were sown in each plot. A separate set of un-inoculated pots were kept as control. Pots were irrigated regularly to maintain moisture. After 45 to 60 days of sowing, the symptoms appeared and the infected plants exhibited elongated lesions at collar region which were later converted to dark brown to black and stem was completely girdled by the lesions. The affected plants wilted, dried up later and can be uprooted easily. Diseased plants were brought to laboratory and isolations were made on the PDA medium from diseased stem to confirm the identity of pathogen.

3. RESULTS AND DISCUSSION

The intensive survey was carried out during *kharif*, 2021 and 2022 in the cotton growing districts of Haryana viz., Hisar, Sirsa, Fatehabad, Bhiwani, Charkhi dadri, Mahendergarh, Rewari, Gurugram, Nuh, Palwal to record the incidence of root rot in different districts. Cotton root rot incidence during *Kharif*, 2021 ranged from 7.67 to 19.67. The data of Table 2 revealed that Bodiakheda village in Fatehabad district had maximum disease incidence (19.67%) followed by Ramsara (19.00%) and 18.33% Dariyapur and Ahalisadar, whereas, least disease incidence (7.67%) was recorded in village Salamba located in Nuh district. The results of the survey conducted in Hisar district showed that Balsamand had the highest disease incidence (16.67%) followed by Muklan (15.33%) and Saharwa (15.00%) and the minimum

disease incidence was recorded in Sadalpur village (14.00%). In Sirsa district, the maximum disease incidence was recorded in Ludeshar (16 %) followed by Panniwala Mota and Odhan (15.00%) while the minimum was recorded in Nirwan (13.33 %). In Bhiwani district, the maximum disease incidence was recorded in Hassan village (13.33%) followed by Sherpura (10.67%) and Bawanikhera (10.00%) while the minimum was recorded in Jitakheri (9.33%). In Charkhi Dadri district, the maximum disease incidence was recorded in Chidiya (11.33%) followed by Ghasola and Modhi (8.67%) while the minimum was recorded in Pentawas Kalan (8.33%). In Mahendergarh district, the maximum disease incidence was recorded in Badaph (14.33%) followed by Sигра (14%) and Gudha (12.33%) while the minimum was recorded in Ananas (10.67%) [7-9]. In Rewari district, the maximum disease incidence was recorded in Bawal (14.33%) followed by Budoli (12.33%) and least was recorded in both Dahina and Siha (11.67%) In Gurugram district, the maximum disease incidence was recorded in Jatauli (12.33%) followed by Pataudi (11.33%) and Saiyad Shahpur (9.67%) while the minimum was recorded in Manukalan (9.33%). In Nuh district, the maximum disease incidence was recorded in Chhapeda (11.67%) followed by Gasheda (9.00%) and Manki (8.67%) while the minimum was recorded in Salamba (7.67%). In Palwal district, the maximum disease incidence was recorded in Karana (11.67%) followed by Mandori (11.33%) and minimum was recorded in Mahespur (10.33%) and least was found in Rajolaka (10.00%). Among all the surveyed districts of Haryana the maximum mean disease incidence was recorded in Fatehabad (18.54%) followed by Sirsa (15.75%), Hisar (15.33%), Mahendergarh (12.92%), Rewari (12.75%), Bhiwani (11.38%), Palwal (11.00%), Gurugram (10.54%) and Nuh (9.63%) While the minimum disease incidence was recorded in Charkhi dadri 9.17 per cent during *kharif*, 2021 depicted in Table 1. Cotton root rot incidence during *Kharif* 2022 ranged from 8.00 to 18.67. Among the surveyed villages in different districts of Haryana, Ramsara village in Fatehabad district had maximum disease incidence as 18.67% followed by Bodiakheda and Ahalisadar both had 18.33% disease incidence in both villages and least was in Dariyapur (17.67%) whereas, over all least disease incidence was recorded in village Modhi (8.00%) located in Charkhi dadri district. The results of the survey conducted in the Hisar district showed that village Muklan had the highest disease incidence (16.33%) followed

by Balsamanad (16.00%), Saharwa (15.67%) and the minimum disease incidence was recorded in Sadalpur village (13.67%). In Sirsa district, the maximum disease incidence was recorded in Ludeshar (17.67%) followed by Odhan (17.00%) and Nirwan (16.33%) while the minimum was recorded in Panniwala mota (15.67%). In Bhiwani district, the maximum disease incidence was recorded in Hassan (14.00%) followed by Sherpura (12.67%) and Bawanikhera (11.67%) while the minimum was recorded in Jitakheri (9.33%). In Charkhi dadri district, the maximum disease incidence was recorded in Chidiya (10.33%) followed by Ghasola (9.33%) and Pentawas Kalan (8.67%) while the minimum was recorded in Modhi (8.00%). In Mahendargarh district, the maximum disease incidence was recorded in Badaph (15.33%) followed by Gudha (13.67%) and Sigr (12.67%) while the minimum was recorded in Ananas (10.33%). In Rewari district, the maximum disease incidence was recorded in Siha (14.67%) followed by Dahina (14.33%) and Bawal (12.67%) while the minimum was recorded in Budoli (10.33%). In Gurugram district, the maximum disease incidence was recorded in Pataudi (12%) followed by Jatauli (11%) and Manukalan (9.67%) while the minimum was recorded in Saiyad Shahpur (9%). In Nuh district, the maximum disease incidence was recorded in Ghaseda (10.67%) followed by Chhapeda (10.00%) and least was recorded both in Salamba and Manki (9.67%). In Palwal district, the maximum disease incidence was recorded in Mandori (12.67%) followed by Karana (11.33%) and Rajokalan (10.33%) while the minimum was recorded in Mahespur (10.33%). Among all the surveyed districts of Haryana the maximum mean disease incidence was recorded in Fatehabad (18.25%) followed by Sirsa (16.67%), Hisar (15.42%), Mahendargarh (13.00%), Rewari (13.00%), Bhiwani (11.92%), Palwal (11.17%) and Gurugram (10.42%), Nuh (10.00%). While the minimum disease incidence was recorded Charkhi dadri (9.08%) as depicted in table 2. The Table 2 also revealed that maximum average mean disease incidence during both *Kharif*, 2021 and *Kharif*, 2022 was recorded in Fatehabad district (18.54% and 18.25%) followed by Sirsa (15.75% and 16.67%) and least was recorded in Charkhi dadri (9.17%) [10-12].

4. CONCLUSION

The present study concluded that incidence of root rot disease in cotton was detected in prominent cotton growing areas in Haryana. The

highest average disease incidence was noticed in Fatehabad district during both *Kharif* 2021 and *Kharif* 2022 followed by Hisar district and least average mean disease incidence was recorded in Chrkhi Dadri and Nuh during *Kharif* 2021 and Charkhi dadri during *Kharif* 2022. A total of Twenty one isolates of *Rhizoctonia bataticola* and *Rhizoctonia solani* was isolated from the infected root sample. From the overall study it can be understood that there is a prevalence of root rot disease in major mulberry growing locations in Haryana. Therefore, resistant cotton 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. Therefore a study should be undertaken to determine the disease prevalence in other locations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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