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Studies on pathogenicity of grey mildew (*Ramularia areola* Atk.) in Bt cotton

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Abstract
In Maharashtra, grey mildew is becoming great threat to cotton growing areas in Marathwada, Vidarbha region and other cotton growing tract. It has been reported that the grey mildew disease reduces yield as much as 38 % from Akola (Shivankar and Anvikar, 1995). The occurrence of disease also been reported from Tamil Nadu, Andhra Pradesh and so many cotton growing states in India. Isolation of fungus from grey mildew infected leaves was made by tissue transfer method. Diseased leaves (grey mildew) of cotton collected from various fields during the survey were brought to the laboratory and washed thoroughly in running tap water. The maximum disease intensity was recorded in Gokunda village of Kinwat Taluka (14.50%) followed by Hiwar of Mahoor Taluka (13.70%).

The intensity of grey mildew of cotton in different places of Nanded district ranged from 7.30% to 14.50%. Pathogenicity of *Ramularia areola* Atk. was successfully proved on Bt cotton cv. Jadoo BG II under controlled conditions of screen house.

Keywords: pathogenicity, grey mildew, (Ramularia areola Atk.), Bt cottons

Introduction

Cotton is one of the most important fibre and cash crop of India and plays a dominant role in the industrial and agricultural economy of the country. It is cultivated on 332.58 lakh ha in world with the production of 1523 lakh bales and productivity of 779 kg/ha. In India, cotton is cultivated on area of 122.38 lakh ha with production of 361 lakh bales and productivity of 501 kg/ha. In Maharashtra, cotton is cultivated on area of 41.19 lakh ha with production of 81 lakh bales and productivity of 334 kg/ha. (Anonymous, 2019). The crop provides direct livelihood to 6 million farmers and about 50-60 million peoples are getting employment in cotton trade and its processing in the country.

In India, there are ten major cotton growing states which are divided into three zones, *viz*. north zone, central zone and south zone. North zone consists of Punjab, Haryana, and Rajasthan. Central zone includes Madhya Pradesh, Maharashtra and Gujarat. South zone comprises Andhra Pradesh, Telangana, Karnataka and Tamil Nadu. Besides these ten States, cotton cultivation has gained momentum in the Eastern State of Orissa. Cotton is also cultivated in small areas of non-traditional States such as Uttar Pradesh, West Bengal and Tripura.

Cotton (*Gossypium spp.*) is the most extensively cultivated commercial crop and is a major fiber crop of global importance. It is an important raw material of economy in term of both employment generation of foreign exchange and hence it is popularly known as "White gold or friendly fiber". Cotton is harvested as 'seed cotton' which is then 'ginned' to separate the seed and lint. The long 'lint' fibers are further processed by spinning to produce yarn which is knitted or woven into fabrics.

India is the only country in the world where all the four cultivated species of cotton, *viz*., *G. hirsutum*, *G. arboreum*, *G. herbaceum* and *G. barbadense*, are cultivated on commercial scale, besides their hybrid combinations. The diversity of cotton cultivars and cotton agro-climatic zones in India is considerably larger as compared to other major cotton growing countries in the world.

Advancement of biotechnological tools and genetic engineering paved the way for development of transgenic cotton (Bt cotton), which offers great promise in the control of

bollworms. The commercial cultivation of such transgenic cotton conferring pest resistance began by 2002 in India.

These genotypes are referred as transgenic Bt cotton genotypes. Numerous laboratory and field tests confirm that the most efficient and cheapest method of protecting cotton from pests is the use of transgenic cotton (Choudhary and Laroia, 2001). In Maharashtra, grey mildew is becoming great threat to cotton growing areas in Marathwada, Vidarbha region and other cotton growing tract. It has been reported that the grey mildew disease reduces yield as much as 38 % from Akola (Shivankar and Anvikar, 1995). The occurrence of disease also been reported from Tamil Nadu, Andhra Pradesh and so many cotton growing states in India. Objectives of the study are as follows,

1. To survey grey mildew of Bt cotton hybrids in Nanded district.
2. To prove pathogenicity of grey mildew in Bt cotton hybrid.

Data and Methodology

A survey of farmer's cotton fields in the Nanded district was undertaken during *kharif*, 2018-19. The observations on grey mildew intensity was recorded. Disease samples collected from various locations during survey was brought to the laboratory and subjected for further studies. The survey was carried

out on the different places of Nanded district *viz.*, at Cotton Research Station, Nanded, Mahoor, Kinwat, Himayatnagar, Bhokar and Hadgaon.

In each field, plants were selected randomly and the intensity of disease was recorded. The per cent disease intensity was assessed by counting the numbers of plants showing grey mildew symptoms. The per cent disease intensity was calculated by using the formula given by (McKinney, 1923).

$$\% \text{ Disease Intensity (PDI)} = \frac{\text{Summation of numerical ratings}}{\text{No. of leaves / plants observed x Maximum rating}} \times 100$$

The severity of disease was estimated based on disease severity scale 0 to 9 (Mayee and Datar, 1986) [7].

Disease rating scale (Mayee and Datar 1986) [7]

Sr. No.	Leaf area infected (%)	Score
1.	Zero	0
2.	Less than 1%	1
3.	1 to 10%	3
4.	11 to 25%	5
5.	26 to 50%	7
6.	More than 50%	9

Isolation

Isolation of fungus from grey mildew infected leaves was made by tissue transfer method. Diseased leaves (grey mildew) of cotton collected from various fields during the survey were brought to the laboratory and washed thoroughly in running tap water. These diseased specimens (leaves) were blot dried and cut with sharp sterilized blade into small bits (5mm) keeping half healthy and half diseased portion intact. These pieces were surfaces sterilized with 0.1% aqueous solution of mercuric chloride ($HgCl_2$) for two minutes and then washed by giving three changes with sterile distilled water to remove traces of mercuric chloride and blot dried. The surface sterilized diseased pieces were inoculated on the solidified and cooled PDA (Potato Dextrose Agar) medium in petriplates under aseptic conditions of Laminar-air-flow cabinet, (Make: ACS, Bangalore). Inoculated plates were then incubated in BOD incubator (Make: MAC, Delhi) at 27 $\pm$ 2°C temperature. After two week of incubation, the well- developed mycelial growth free from any contaminant was obtained. Following single hyphal-tip technique, the fungus was transformed/ sub-cultured aseptically on the PDA slant in test tubes. Through frequent sub-culturing, the fungus was purified and pure culture was maintained on Agar slants in test tubes stored in refrigerator for further studies.

Result and Discussion

Present studies on the grey mildew (*Ramularia areola* Atk.) of cotton were undertaken during *kharif*, 2018-19 on the aspects *viz*., survey, isolation, identification, pathogenicity test, *in vivo* efficacy of different bio agents and chemicals and screen different cotton hybrids/varities against grey mildew disease. The results obtained on all these aspects are being interpreted and discussed under following sub-heads.

Survey

A roving survey was carried out during Sept-Oct, 2018 at different locations of Nanded district for recording the percent diseases intensity of grey mildew of cotton in field. Cotton fields were observed in each selected Talukas of Nanded district of Marathwada region.

Occurrence of grey mildew (*R. areola* Atk.) of cotton intensity in Nanded district.

Data pertaining to survey for the per cent disease intensity of grey mildew of cotton in different places of Nanded district are presented in (Table 1, Fig. 1 and PLATE- I).

During *kharif* 2018, survey of grey mildew disease intensity on cotton was undertaken in different places of Nanded district of Marathwada region. The maximum disease intensity was recorded in Gokunda village of Kinwat Taluka (14.50%) followed by Hiwar of Mahoor Taluka (13.70%).

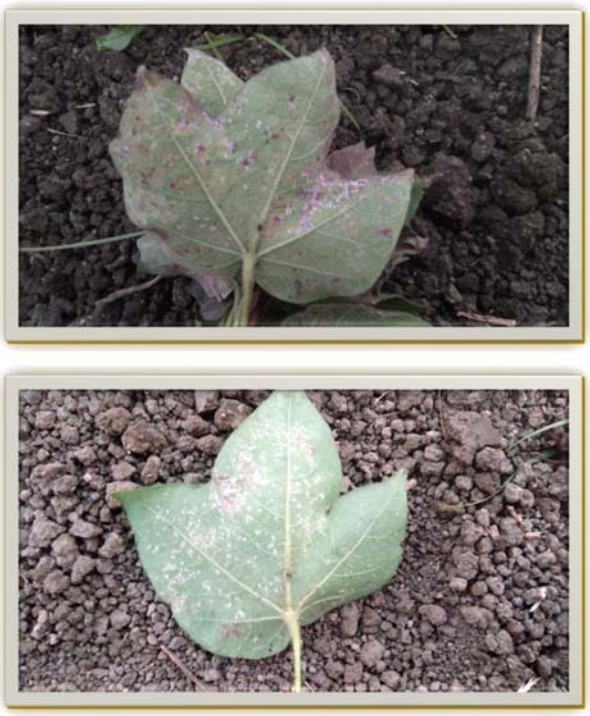
The maximum per cent disease intesity was recorded in Gokunda (14.50%) which was followed by Hiwar (13.70%), Godadi (13.50%), Kothari (12.95%), Kedarguda (12.65%), Dhanora (12.56%), Kasbag (12.50%), Shibdara (12.40%), Bhosa (12.30%), Divashi (12.02%), Met (11.33%), Palaj (11.25%), Umri (11.20%), Tamsa (11.00%), Dhanora (10.89%), Cotton Research Station, Nanded (9.65%), Andegao (9.44%), Padsa (9.40%), Ambadi (9.20%),

Table 1: Survey for the disease severirty and intensity of grey mildew of cotton caused by *R. areola* during *Kharif* 2018 in Nanded district.

Sr. NO	Talukas	Village/Places	Disease Severity Scale (Mayee and Datar 1986) [7]	Per cent Disease Intensity
1	Cotton Research Station, Nanded		**3**	**9.65**
2	Mahoor	Bhosa	**5**	12.30
		Kasbag	**5**	12.50
		Hiwar	**5**	13.70
		Padsa	**3**	9.40
		Met	**5**	11.33
Mean				**11.84**
3	Kinwat	Gokunda	**5**	14.50
		Ambadi	**3**	9.20
		Kothari	**5**	12.95
		Godadi	**5**	13.50
		Dhanora	**5**	12.56
Mean				**12.54**
4	Himayatnagar	Sarsam	**3**	7.56
		Borgadi	**3**	8.75
		Dhanora	**5**	10.89
		Andegao	**3**	9.44
		Shibdara	**5**	12.40
Mean				**9.80**
5	Bhokar	Divashi	**5**	12.02
		Kolagao	**3**	8.50
		Palaj	**5**	11.25
		Kini	**3**	8.36
		Kandali	**3**	7.30
Mean				**9.48**
6	Hadgaon	Kawatha	**3**	8.60
		Umri	**5**	11.20
		Banchincholi	**3**	9.12
		Tamsa	**5**	11.00
		Kedarguda	**5**	12.65
Mean				**10.51**

Leaf area infected (%) - Zero % - **0**, Less than 1% - **1**, 1 to 10% - **3**, 11 to 25% - **5**, 26 to 50% - **7**, More than 50% - **9**.

Plate I



Symptoms of grey mildew disease of cotton

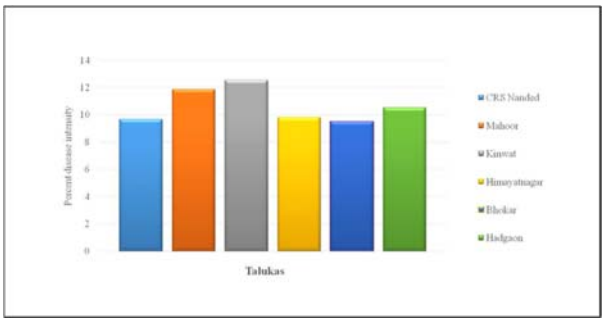


Fig 1: Survey for the per dent disease intensity of grey mildew of cotton caused by *R. areola* during *kharif* 2018 in Nanded district.

Banchincholi (9.12%), Borgadi (8.75%), Kawatha (8.60%), Kolagao (8.50), Kini (8.36%), Sarsam (7.56%), Kandali (7.30%).

The maximum mean per cent disease intensity was recorded at Kinwat (12.54%) which was followed Mahoor (11.84%), Hadgaon (10.51%), Himayatnagar (9.80%), Cotton Research Station, Nanded (9.65%) and Bhokar (9.48%).

Per cent disease intensity of grey mildew (*R. areola* Atk.) of cotton.

The data presented in table-2 revealed that the intensity of grey mildew of cotton in different places of Nanded district ranged from 7.30% to
14.50%.

Our results were collaborated with Holey and Somani (1980) when he reported that the occurrence of grey mildew and severe outbreak of grey mildew disease in variety SRT-1 and Hybrid 4 at Narkhe, Sawargaon, Mawad, and Katol tahsil of Nagpur district with an intensity up to 20 to 40 per cent on SRT-1 and 40 to 60 per cent on Hybrid 4.

Our results were collaborated with Ponnanna (1998) when he reported that the cotton crop suffers from many fungal diseases. Among these, grey mildew caused by *R. areola* Atk. was of great concern. The survey work revealed that maximum per cent of intensity of disease was noticed at ARS, Dharwad Farm during *kharif* 1996.

A roving survey conducted by Harlapur *et al.*, (2004) on the fields during 2002-03 for cotton disease problems in Ghataprabha Left Bank Canal Command Area Region of Karnataka, revealed the prevalence of disease, namely grey mildew, during different crop growth periods. The severe intensity of grey mildew was noticed during October to November months.

Similar results were also found when Hosagoudar (2007) [1] conducted a survey during *kharif* 2006 which revealed that the intensity of Grey mildew (5- 50%) in parts of Dharwad, Haveri, Belgaum, Bagalkot, Gadag, Bellary, Raichur and Gulbarga districts of North Karnataka. He observed that the maximum intensity of Grey mildew disease was recorded in Dharwad, Haveri, Belgaum and Gadag districts.

Our results were collaborated with Chattannavar *et al.*, (2009) revealed that the grey mildew (caused by *Ramularia areola*) was present in all cotton cultivated areas and he also noticed the disease intensity ranged from 5 to 30%. It was more pronounced in Dharwad, Haveri and Gadag districts.

Symptomatology

The symptoms of grey mildew disease (PLATE- I) started at 90 days after sowing on older leaves of the plants. On older leaves, small light green to yellowish, angular, pale translucent spots that are limited by the veins appeared on the upper surface and whitish grey powdery growth just beneath the spots on lower surface of leaves were observed. The severely affected leaves become necrotic, dry, showed red brown colour and drop prematurely. The infected boll loose strength and open prematurely (PLATE- I).

Similar results were given by Hosagoudar (2007) [1] that the grey mildew disease generally appears on older leaves as the plants reach maturity, in the form of irregularly angular, pale translucent spots, 1 to 10 mm (usually 3-4 mm) in diameter and with a definite and irregular margin formed by the veins of the leaf. The lesions are light to yellowish green on the upper surface. As the spots grow older, the leaf tissues turn yellowish brown while a whitish frosty growth appears chiefly on the under surface but occasionally also on the upper surface. This is the conidial stage of the causal fungus. The affected leaves are finally dropped. Lesions occur on the bracts subtending the bolls.

Isolation of the pathogen *R. areola.*

The pathogen was isolated from naturally infected cotton plants showing typical symptoms of grey mildew by tissue transfer method on Potato dextrose agar (PLATE- II).

Microscopic observations

The fungus formed hemispherical, slow growing frosty white

Plate II

Pure culture of Ramularia areola Atk. on PDA

colonies. The aerial mycelium is slightly fluffy consisting of thin hyaline hypha. Conidiophores were arranged in short hyaline clusters and formed a sporulating layer at the surface of the colony. Conidia often longer in size than those obtained from host. The conidia were observed to become deformed and rod shape as they become old, forming rounded uneven cells in old culture, thick walled, special, brown to dark brown cells were observed (PLATE-III).

Pathogenicity test

Pathogenicity test was attempted by spray inoculating the spore suspension on healthy growing, 90 days old plants of cotton cultivar revealed *R. areola* as pathogenic to cotton, causing grey mildew. Seedlings incubated in the screen house, where relative humidity above (66%) and optimum temperature (20-
28^0C) were maintained for the further development of symptoms. After fourteen days of incubation, typical symptoms grey mildew on foliage of artificially inoculated cotton plant were observed. However, the control plant sprayed with only sterilized distilled water remained healthy and did not produce any kind of symptoms throughout the period of observation (PLATE-IV and V).

Reisolation

The fungus was reisolated from the inoculated leaves and was compared with original culture of the test pathogen. The same was found identical to that of original culture, thereby confirming the test of pathogenicity.

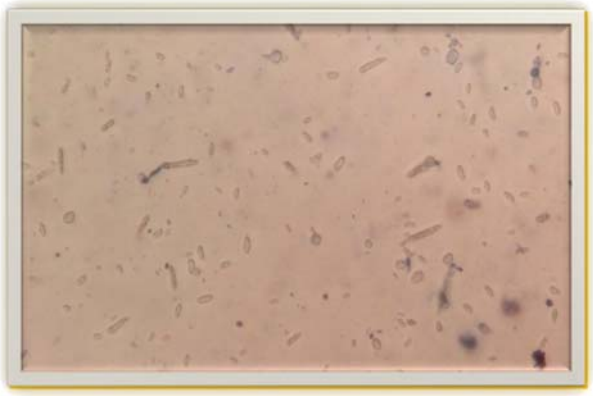
Disease management strategies

In vivo* efficacy of bioagents against grey mildew *(R. areola Atk.).

A total of seven bioagents, those found effective against *R. areola* in present *in vivo* studies were evaluated for the management of grey mildew of cotton under field conditions, during *Kharif*, 2018-19. The results obtained on grey mildew intensity was being narrated and discussed herein under following subheads.

The data regarding per cent disease intensity before spraying was found to be statistically non-significant. However, per cent disease intensity after 8

Plate III



Microphotograph showing conidia of *R. areola*

Plate IV

Mass multiplication of *Ramularia areola* in potato dextrose broth for pathogenicity test

Plate V

Pathogenicity test of *Ramularia areola* on Bt cotton

days of each spraying was found statistically significant. The treatment *T. harzianum* @ 1 % found effective in reducing the disease intensity over control.

Summary and Conclusions

Cotton is one of the most important fibre and cash crop of India and plays a dominant role in the industrial and agricultural economy of the country. It is cultivated on 332.58 lakh ha in world with the production of 1523 lakh bales and productivity of

779 kg/ha. In India, cotton is cultivated on large area of 122.38 lakh ha with production of 361 lakh bales and productivity of 501 kg/ha. In Maharashtra, cotton is cultivated on area of 41.19 lakh ha with production of 81 lakh bales and productivity of 334 kg/ha. (Anonymous, 2019). The crop provides direct livelihood to 6 million farmers and about 50-60 million peoples are getting employment in cotton trade and its processing in the country.

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Cotton crop in India is known to suffer from number of fungal, bacterial and viral diseases. Amongst the several factors responsible for reduction in yield and

quality deterioration of cotton in India, diseases occupy a vital place. Among the various diseases occurring on cotton, the grey mildew disease commonly known as Dahiya disease of cotton is caused by *R. areola* Atk. gaining more importance in recent years because of its increasing incidence. It has been known to occur more in all type of cottons and more probably in desi cotton, since many years, in an epiphytotic form leading to severe defoliation and substantial yield loss. Survey conducted during the *kharif* 2018-19 revealed that the maximum per cent disease intensity of grey mildew of cotton was recorded in Kinwat and Mahoor talukas of Nanded district.

The pathogen *Ramularia areola* Atk. was successfully isolated on PDA from naturally diseased foliage of Bt cotton collected during the survey from farmers field and then it was purified and maintained for further studies.

Pathogenicity of *Ramularia areola* Atk. was successfully proved on Bt cotton cv. Jadoo BG II under controlled conditions of screen house.

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