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Pawan K Dhoke
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Manjusha Shiradkar
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Swapnil Bhosale
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Corresponding Author:
Pawan K Dhoke
Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra, India

Investigations on the efficacy of different bioagents and chemicals against grey mildew *in vivo*

Pawan K Dhoke, Manjusha Shiradkar and Swapnil Bhosale

Abstract
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. The objective of the study is to study the efficacy of different bioagents and chemicals against grey mildew *in vivo*.
A survey carried out during *kharif* 2018 revealed the incidence of the grey mildew in parts of Mahoor, Kinwat, Himayatnagar, Bhokar and Hadgaon talukas of Nanded district. The maximum per cent grey mildew disease incidence was recorded in Kinwat, Mahoor and Hadgaon talukas of Nanded district.
In a field evaluation of Difenconazole 25% EC (0.1%), Propiconazole 25% EC (0.1%), Propineb 70% WP (0.2%), Mancozeb 75%WP (0.2%), Carbendazim 12%
+ mancozeb 63% WP (0.2%), Captain 70% + Hexaconazole 5% WP (0.2%) and Sulphur 80% WP (0.2%), the treatment of Sulphur 80% WP and Captain 70% + Hexaconazole 5% WP gave better control and Difenconazole 25% EC and Carbendazim 12% + mancozeb 63% WP gave poor control against grey mildew disease.

Keywords: bioagents, chemicals grey mildew, biotechnological tools

Introduction
Cotton (*Gossypium* spp.) is the most important cash crop cultivated in over 60 countries in the World and also important renewable natural textile fibre and sixth largest source of vegetable oil in world. It is often called "White gold" and considered as "King of apparel fibre". Cotton belongs to the genus Gossypium from the family Malvaceae. Moreover, cotton plays vital role in India's economy, by providing livelihood for more than 60 million people through cotton production, processing, textiles and related activities. It contributes nearly 75 per cent of total raw material needs of textile industry in our country.
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).
Introduction of transgenic 'Bt cotton' has substantially brought down the cost of cultivation (Herz *et al*., 2000). Cotton is very specific to its climatic requirement and reacts unfavorably for any shift in dates of sowing from normal period. Delay in time of sowing resulted in reduced yield due to production of less number of buds and bolls but increased bollworm attack. The preliminary investigation on Bt cotton proved that Bt hybrids are early in maturity and resistant to bollworm, possibly for this reason, Bt cotton may perform better than others under delayed sowing condition.
Comparing various Bt-cotton hybrids with their non-Bt counter parts for their morpho physiological characters, Bt hybrids recorded significantly higher yield than the

corresponding non-Bt hybrids. The hybrids mature early and thus avoid unfavorable weather during later phases (Hofs *et. al*., 2006). The earliness index indicated that Bt had 10 percent higher index as compared to corresponding non-Bt cotton and Bt cotton had better early boll retention and shorter vegetative cycle than the conventional cultivars. Such studies help in identifying the limitations in Bt cotton plant type and finding out the solutions to overcome the same.

Cotton crop in India is known to suffer from number of fungal, bacterial and viral diseases *viz.*, Wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum*, *Verticillium* wilt caused by *Verticillium dahliae*, *Alternaria* blight c Anthracnose caused by *Colletotrichum gossypii*, Ascochyta blight caused by *Ascochyta gossypii*, Bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum*, Crown gall caused by *Agrobacterium tumefaciens* and Cotton leaf curl virus.

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. The objective of the study is to study the efficacy of different bioagents and chemicals against grey mildew *in vivo.*

Material and Methods

In vivo evaluation of fungicides

Details of experiment

Design: RBD Replication: Three Treatments: Eight Crop: Cotton

Variety: Jaadoo (KCH-14K59) BG II

Tr. No.	Treatments	Trade name	Concentration (%)
T1	Difencozole 25% EC	Score	0.1
T2	Propiconazole 25% EC	Tilt	0.1
T3	Propineb 70% WP	Antracol	0.2
T4	Mancozeb 75% WP	Indofil M-45	0.2
T5	Carnendazim 12%+Mancozeb 63%	Saaff 75 WP	0.2
T6	Captain 70% + Hexaconazole 5%	Taquat 75WP	0.2
T7	Sulphur	Sulfex	0.2
T8	Control(Untreated)	--	--

After first initiation of grey mildew disease three consecutive sprayings of above mentioned fungicides was undertaken with 15 days' interval in each spraying.

Observations were recorded on grey mildew intensity at first appearance of the disease symptoms. Subsequent two observations at one day before second and third spray treatments and last observation at 15 days after third spray treatment was recorded.

Five plants per treatment per replication was selected randomly, tagged and recorded foliage (bottom, middle, top) blight intensity. The grey mildew disease intensity was recorded by applying 0-9 disease rating scale (Mayee and Datar, 1986).

Disease rating scale (Mayee and Datar 1986)

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

Grey mildew disease intensity was recorded in all the treatments replicated by counting number of cotton plants exhibiting typical grey mildew symptoms and per cent disease intensity was calculated by applying following formula. (McKinney, 1923).

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

In vivo evaluation of bioagents. Details of experiment:

Design: RBD Replications: Three Treatments: Eight Crop: Cotton

Variety: Jaadoo (KCH-14K59) BG II

Tr. No.	Treatments	Concentration (%)
T1	*Trichoderma viride*	1
T2	*T. harzianum*	1
T3	*T. hamatum*	1
T4	*T. koningii*	1
T5	*Aspergillus niger*	1
T6	*Pseudomonas fluorescence*	1
T7	Biomix (Consortial product)	1
T8	Control	_

After first initiation of grey mildew disease three consecutive sprayings of above mentioned bioagents was undertaken with 15 days interval in each spraying.

Five plants per treatment per replication was selected randomly, tagged and recorded foliage (bottom, middle, top) blight intensity. The grey mildew disease intensity was recorded by applying 0-9 disease rating scale (Mayee and Datar, 1986).

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

Results and Discussion

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

Table 1: *In vivo* evaluation of bioagents against grey mildew (*R. areola* Atk.).

Tr. No	Treatments	Per cent Disease Intensity			
		Before first spraying	After first spraying	After second spraying	After third spraying
1	*Tricoderma viride*	14.00 (21.97)	12.00 (20.27)	10.50 (18.91)	10.00 (18.43)
2	*T. harzianum*	15.00 (22.79)	11.50 (19.82)	10.33 (18.75)	9.83 (18.28)
3	*T. hamatum*	14.50 (22.38)	12.33 (20.56)	11.33 (19.67)	10.33 (18.75)
4	*T. koningii*	14.25 (22.18)	12.83 (20.99)	11.83 (20.12)	10.83 (19.22)
5	*Aspergillus niger*	15.25 (22.99)	12.17 (20.41)	11.17 (19.52)	10.17 (18.59)
6	*Pseudomonas fluorescence*	14.50 (22.38)	11.83 (20.12)	10.83 (19.22)	10.50 (18.91)
7	Biomix (Consortial product)	14.00 (21.97)	13.00 (21.13)	12.00 (20.27)	11.00 (19.37)
8	Control	14.75 (22.59)	14.83 (22.65)	15.00 (22.79)	16.10 (23.66)
SE (m) ±		0.33	0.50	0.50	0.48
CD at 0.05%		NS	1.52	1.50	1.46

*Parentheses are arcsine transformed values.

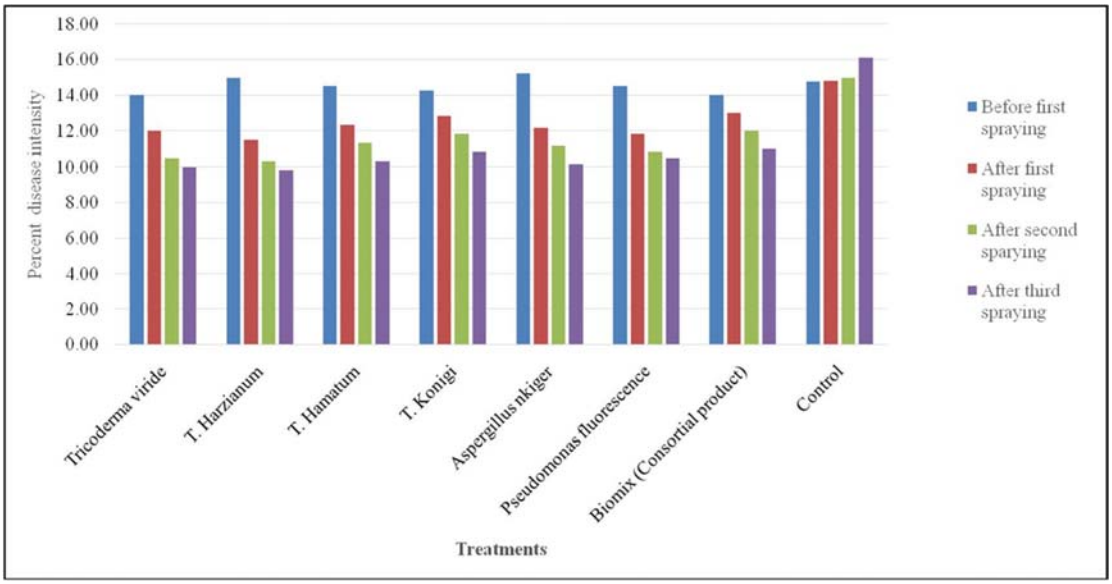

Fig 1: *In vivo* efficacy of bio-agents against grey mildew of cotton caused by *Ramularia areola* Atk.

After 8 days of first spraying minimum per cent disease intensity was found in case of *T. harzianum* (11.50) followed by *Pseudomonas fluorescence* (11.83) and *Tricoderma viride* (12.00) whereas moderate per cent disease intensity after first spraying was found in case of *Aspergillus niger* (12.17) followed by *T. hamatum* (12.33). And maximum per cent disease intensity after first spraying was found in case of *T. koningii* upto 12.83 followed by Biomix (13.00). The highest per cent disease intensity (14.83) was observed in untreated control plot after first spraying.

After 8 days of second spraying minimum per cent disease intensity was found in case of *T. harzianum* (10.33) followed by *Tricoderma viride* (10.50) and *Pseudomonas fluorescence* (10.83) whereas moderate per cent disease intensity after second spraying was found in case of *Aspergillus niger* (11.17) followed by *T. hamatum* (11.33). And maximum per cent disease intensity after second spraying was found in case of *T. koningii* upto 11.83 followed by Biomix (12.00). The highest per cent disease intensity (15.00) was observed in untreated control plot after second spraying.

After 8 days of third spraying minimum per cent disease intensity was found in case of *T. harzianum* (9.83) followed by *Tricoderma viride* (10.00) and *Aspergillus niger* (10.17) whereas moderate per cent disease intensity after third spraying was found in case of *T. hamatum* (10.33) followed by *Pseudomonas fluorescence* (10.50). And maximum per cent disease intensity after third spraying was found in case of *T. koningii* upto 10.83 followed by Biomix (11.00). The highest per cent disease intensity (16.10) was observed in untreated control plot after third spraying.

The use of bioagents has become important in controlling the grey mildew disease in the absence of suitable resistant cultivars. Bioagents were evaluated for their efficacy against the grey mildew disease in the field condition. The experiment conducted during *Kharif* 2018 revealed that *T. harzianum* (1%) and *Tricoderma viride* (1%) were effective in controlling the grey mildew disease significantly over control. Chattannavar *et al.*, (2000) [10] reported that the treatment of *Pseudomonas fluorescence* Migula gave 42.60 percent disease control (PDC) of grey mildew. Whereas, the treatment *P. fluorescence* (CHAO) gave 50.76 PDC of grey mildew.

Our results were collaborated with Chidambaram *et al.*, (2004) reported that the *T. viride* and *P. fluorescence* Pf1 consistently reduced the disease intensity of grey mildew from 24.47 to 83.2 per cent over the years as compared to the untreated check. However, Hosagoudar (2007) reported that the treatment of *Pseudomonas fluorescence* gave disease control of grey mildew.

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

The data regarding per cent disease intensity before spraying was found to be statistically non-significant. However, per cent disease intensity after 8 days of each spraying was found statistically significant. The treatment Sulfex 80% WP @ 0.2% found effective in reducing the disease intensity over control (Table-3, Fig. 3 and PLATE- VI).

Table 2: *In vivo* evaluation of fungicides against grey mildew (*R. areola* Atk.).

Tr. No	Treatments	Per cent Disease Intensity			
		Before first spraying	After first spraying	After second spraying	After third spraying
1	Difenconazole 25% EC	14.00 (21.97)	13.67 (21.70)	12.83 (20.99)	10.83 (19.22)
2	Propiconazole 25% EC	13.67 (21.70)	13.17 (21.28)	11.67 (19.97)	09.50 (17.95)
3	Propineb 70%WP	14.67 (22.52)	12.50 (20.70)	10.50 (18.91)	08.50 (16.95)
4	Mancozeb 75%WP	14.67 (22.52)	12.67 (20.85)	11.17 (19.52)	09.17 (17.62)
5	Carbendazim 12% + Mancozeb 63% WP	13.83 (21.83)	13.72 (21.74)	12.00 (20.27)	10.33 (18.75)
6	Captain 70% + Hexaconazole 5% WP	14.17 (22.11)	11.33 (19.67)	10.00 (18.43)	08.33 (16.78)
7	Sulphur 80% WP	14.67 (22.52)	10.67 (19.06)	09.50 (17.95)	06.83 (15.15)
8	Control	14.17 (22.11)	14.92 (22.72)	15.50 (23.18)	16.17 (23.71)
SE (m) ±		**0.32**	**0.37**	**0.40**	**0.34**
CD at 0.05%		**NS**	**1.13**	**1.21**	**1.03**

*Parentheses are arcsine transformed values.

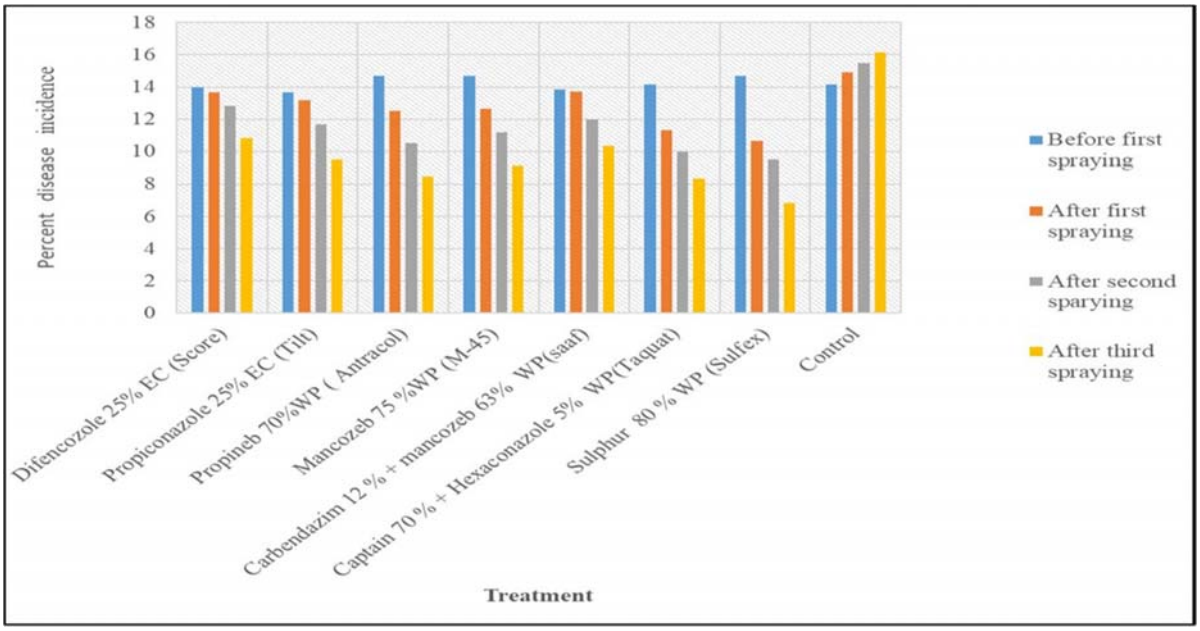

Fig 2: *In vivo* efficacy of fungicides against grey mildew of cotton caused by *Ramularia areola* Atk.

After 8 days of first spraying minimum per cent disease intensity was found in case of Sulphur 80% WP (10.67%) followed by Captain 70% + Hexaconazole 5% WP (11.33%) and Mancozeb 75% WP (12.67%) whereas moderate per cent disease intensity after first spraying was found in case of Propineb 70% WP (12.50%) followed by Propiconazole 25% EC (13.17%). Whereas Difenconazole 25% EC and Carbendazim 12%+ mancozeb 63% WP recorded comparatively maximum per cent disease intensity of 13.67% and 13.72%, respectively. The maximum per cent disease intensity (14.92%) was observed in untreated control plot after first spraying.

After 8 days of second spraying minimum per cent disease intensity was found in case of Sulphur (9.50%) followed by Captain 70% + Hexaconazole 5% WP (10.00%) and Propineb 70% WP (10.50%) whereas moderate per cent disease intensity after second spraying was found in case of Mancozeb 75% WP (11.17%) followed by Propiconazole 25% EC (11.67%). and maximum per cent disease intensity after second spraying was found in case of Carbendazim 12% + mancozeb 63% WP upto (12.00%) followed by Difenconazole 25% EC (12.83%). The highest per cent disease intensity (15.50) was observed in untreated control plot after second spraying.

After 8 days of third spraying minimum per cent disease intensity was found in case of Sulphur (6.83%) followed by Captain 70% + Hexaconazole 5% WP (8.33%) and Propineb 70% WP (8.50%) whereas moderate per cent disease intensity after third spraying was found in case of Mancozeb 75% WP (9.17%) followed by Propiconazole 25% EC (9.50%). And maximum per cent disease intensity after third spraying was found in of Carbendazim 12% + mancozeb 63% WP upto (10.33%) followed by Difenconazole 25% EC (10.83%). The highest per cent disease intensity (16.17%) was observed in untreated control plot after third spraying.

The use of fungicides has become inevitable in controlling the grey mildew disease in the absence of suitable resistant cultivars. Fungicides were evaluated for their efficacy against the grey mildew disease in the field condition. The experiment conducted during *kharif* 2018 revealed that Sulphur (0.2%) and Captain 70% + Hexaconazole 5% WP (0.2%) were effective in controlling the grey mildew disease significantly over control. Chattannavar *et al.,* (2000) [10] tested seven fungicides namely propiconazole (0.1%), octave (0.05%), carbendazim (0.1%), mancozeb (0.2%), copper oxychloride (0.2%), tridemorph (0.1%) and wettable sulphur (0.3%) for the control of R. areola. And reported that carbendazim showed maximum disease control followed by tridemorph. However, Chattannavar *et al.*, (2006) tested seven fungicides namely tridemorph, copperoxy-chloride, mancozeb, propineb, ziram, wettable sulphur and carbendazim for the control of *R. areola.* And carbendazim showed maximum disease control followed by ziram.

Our results were collaborated with Randive (2004) reported that Sulfex 80% WP (0.25%) significantly increased the seed cotton yield. Hosagoudar (2007) concluded that, propineb @ 0.2% gave better control of the grey mildew disease with maximum yield of 1752.99 kg/ha.

Similar results were reported by Shastry and Tomar (2008) that propiconazole treatment (14.39%) was most effective and significantly superior over all treatments in reducing the per cent disease intensity of grey mildew.

Khodke and Raut (2009) reported that propiconazole (0.05%) stood first and recorded maximum reduction of the disease (49.50%). Tridemorph (0.07%) recorded 19.92 per cent disease intensity, while it was equally effective with other test chemicals except mancozeb (0.25%) and neem seed extract (5%). Chattannavar *et al.,* (2013) reported that Taqat 500g/ha, Taqat 750g/ha and propiconazole @ 0.1 per cent were effective in controlling the foliar diseases.

Summary and Conclusions

A survey carried out during *kharif* 2018 revealed the incidence of the grey mildew in parts of Mahoor, Kinwat, Himayatnagar, Bhokar and Hadgaon talukas of Nanded district. The maximum per cent grey mildew disease incidence was recorded in Kinwat, Mahoor and Hadgaon talukas of Nanded district.

In a field evaluation of Difenconazole 25% EC (0.1%), Propiconazole 25% EC (0.1%), Propineb 70% WP (0.2%), Mancozeb 75%WP (0.2%), Carbendazim12%
+ mancozeb 63% WP (0.2%), Captain 70% + Hexaconazole 5% WP (0.2%) and Sulphur 80% WP (0.2%), the treatment of Sulphur 80% WP and Captain 70% + Hexaconazole 5% WP gave better control and Difenconazole 25% EC and Carbendazim12% + mancozeb 63% WP gave poor control against grey mildew disease.

In a field evaluation of *Tricoderma viride* (1%), *T. harzianum* (1%), *T. hamatum* (1%), *T. koningii* (1%), *Aspergillus niger* (1%), *Pseudomonas fluorescence* (1%) *and* Biomix (1%), the treatment of *T. harzianum* and *Tricoderma viride* gave better control and Biomix and *T. koningii* gave poor control against grey mildew disease.

Pathogenicity of *Ramularia areola* Atk. was successfully proved on Bt cotton cv. Jadoo BG II under controlled conditions of screen house. Among the seven bioagents treated against *Ramularia areola*, *T. harzianum* and *Tricoderma viride* was found most effective. Among the seven fungicides treated against *Ramularia areola*, Sulphur and Captain 70% + Hexaconazole 5% WP was found most effective.

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