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Survey for incidence and severity of bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* in different districts of Marathwada region

G.P. Jagtap[a,*], A.M. Jangam[a], D. Utpal[a]
Department of Plant Pathology, College of Agriculture, M.K.V., Parbhani – 431 402 (MS)
[*]Corresponding author; Department of Plant Pathology, College of Agriculture, M.K.V., Parbhani – 431 402 (MS), India

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ABSTRACT

An extensive roving survey was conducted in different districts of Marathwada region of Maharashtra State to isolate the pathogen associated with the bacterial blight disease of cotton. In all 76 cotton fields were surveyed and average disease incidence (PI) to the tune of 51.12 per cent has been observed. Highest disease incidence noticed in Parbhani district (67%) followed by Hingoli (63%), Nanded (58%) and Latur (54%). The lowest disease incidence noticed in Jalna district (36%). Highest disease severity noticed in Parbhani district followed by Hingoli, Latur, Nanded and Aurangabad.

1. Introduction

Cotton (*Gossypium* SPP.) is important cash, fibre crop commonly grown in many parts of India. It is locally known as "White Gold". Cotton almost accounts 65 per cent of fibre production in India. Edible oil is extract from cotton seed and de-oiled cakes are used as a cattle feed, which is a good source of high quality protein for animals. India has the largest acreage 95.5 lakh/ha under cotton at global level and has the productivity of 591 kg lint/ha and ranks second in production 332 lakh bales after China during 2008-09 (Anonymous, 2009). In India, main cotton growing area is seen in the central zone of India comprising of Gujarat, Maharashtra and Madhya Pradesh. Cotton is grown in Maharashtra on an area of 31.24 lakh ha. While the production is near about 60 lakh bales, with an average productivity of 320 kg lint/ha during 2008-09 (Anonymous, 2009). But with the intensification of cotton production, however, there has been change in relative importance of different diseases affecting cotton. Bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* (E.F. Smith) is the major constraint and responsible for losses in seed cotton yields upto 30 per cent (Mishra *et al*., 2001). In India, disease observed in

Andhra Pradesh, Haryana, Madhya Pradesh, Maharashtra, Punjab, Rajasthan and Tamil Nadu (Verma, 1986; and Shrinivasan, 1994). Considering the importance of the bacterial blight in cotton cultivation, survey and surveillance have been advocated in this present study.

2. Materials and methods

Present investigations on bacterial blight of cotton caused by *X. axonopodis* pv. *malvacearum* were undertaken at Cotton Research Scheme Marathwada Agricultural University, Parbhani in the year 2008-09.

2.1. Survey and surveillance

A roving disease survey of the incidence and severity of bacterial blight disease of cotton during September to October 2008 was undertaken Latur, Aurangabad, Parbhani, Nanded, Hingoli Beed, Osmanabad and Jalna district of Marathwada region. Total seventy six cotton fields were inspected. Out of which 11, 10, 12, 10, 12, 10, 7 and 6 were from Latur, Aurangabad, Parbhani, Nanded, Hingoli Beed, Osmanabad and Jalna districts respectively.

In Latur district Latur and Chakur, in Aurangabad District Aurangabad and Paithan, in Parbhani district Parbhani, Jintur, Gangakhed and Pathri, in Nanded district Nanded and Biloli, in Hingoli district Hingoli and Basmat, in Beed district Beed, Kaij and Ambejogai, in Osmanabad district Osmanabad and Tuljapur and In Jalna district Jalna and Ambad tahsils were inspected for recording bacterial blight disease incidence and its severity. Incidence of bacterial blight disease was examined as severe, moderate, trace and free on the basis of percentage of severity of disease. Similarly, the per cent disease incidence of bacterial blight was calculated by using formula.

$$\text{Per cent disease incidence} = \frac{\text{Number of plant infected}}{\text{Total number of plant examined}} \times 100$$

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of all disease rating}}{\text{Number of ratings x Maximum disease grade}} \times 100$$

2.2. Isolation of pathogen

The diseased specimens of bacterial blight of cotton were collected from the experimental plots of Cotton Research Scheme, M.A.U., Parbhani. The specimens were cut into small pieces containing leaf areas adjoining the diseased spots and were surface disinfected in Petri plates containing 95 per cent alcohol for few seconds and immediately placed on blotting paper for the removal of excess alcohol. After this it was again disinfected in mercuric chloride ($HgCl_2$) solution for 20 to 30 seconds and subsequently washed in four changes of sterile water to remove the disinfectant. The disinfected pieces were transferred by means of sterile forceps to a drop of sterile water on a sterile glass slide and teased by means of sharp sterile razor blade. After about 10 minutes, a loopful of bacterial suspension was streaked by means of sterile inoculating needle on the nutrient agar in petriplates under aseptic condition. These plates were incubated at $27^{o}C$ in an inverted position. After 3 days yellow glistering colonies of organism appeared. Well isolated, distinct yellowish colonies of organism were picked up and transferred on Yeast Glucose Chalk Agar Medium (YGCA) slants for further use.

2.3. Pathogenicity test of *Xanthomonas* bacteria

Five seeds of the susceptible variety LRA-5166 were sown in each pot filled with soil and FYM in 2:1 proportion and immediately watered with sterilized distilled water. Two weeks after germination, only four seedlings were maintained in each pot. When the plants were 4-6 weeks old, a bacterial suspension (10^8 cfu/ml) was prepare as an inoculum for pathogenicity test. The underside of the leaf surface was sprayed with water and dusted with carborendum powder. Further, these leaves were smeared with bacterial suspension by means of sterile cotton swab. Simultaneously one of the plants was inoculated with sterile water as control.

3. Results and discussion

3.1. Survey and surveillance

An extensive disease survey of incidence and severity of bacterial blight disease of cotton during September - October, 2008-09 in Latur, Aurangabad, Parbhani, Nanded, Hingoli, Beed, Osmanabad and Jalna district of Marathwada region. Total seventy six cotton fields were observed, out of which 11, 10, 12, 10 12, 10, 7 and 6 were observed in Latur, Aurangabad, Parbhani, Nanded, Hingoli, Beed, Osmanabad and Jalna districts respectively.

3.2. Occurrence of bacterial blight of cotton severity in different districts of Marathwada region

Results (Table 1) showed that the severity of disease was maximum in Parbhani district followed by Hingoli, Latur, Nanded and Aurangabad. The disease severity observed minimum in Jalna district. The results clearly depicts that the variety Kanak BT is highly susceptible to disease in all districts followed by NHH 44 and Mallika BG II.

Table 1
Survey of bacterial blight severity of cotton in different districts of Marathwada region.

Sr. No.	District	No. of field surveyed	Variety	Disease severity				
				Severe	Moderate	Trace	Free	Total
1	Latur	11	NHH-44	2	1	1	-	4
			Mallika Bg-II	1	1	-	1	2
			Kanak Bt	2	1	1	-	5
			Total	5	3	2	1	11
2	Aurangabad	8	NHH-44	1	1	1	-	3
			Mallika Bg-II	1	-	-	1	2
			Kanak Bt	1	1	1	-	3
			Total	3	2	2	1	8
3	Parbhani	12	NHH-44	2	1	1	-	4
			Mallika Bg-II	2	1	-	-	3
			Kanak Bt	3	2	-	-	5
			Total	7	4	1	-	12
4	Nanded	10	NHH-44	1	1	1	-	3
			Mallika Bg-II	1	1	-	1	3
			Kanak Bt	2	1	1	-	4
			Total	4	3	2	1	10
5	Hingoli	12	NHH-44	2	1	1	1	5
			Mallika Bg-II	2	1	-	-	3
			Kanak Bt	2	1	1	-	4
			Total	6	3	2	1	12
6	Beed	10	NHH-44	1	1	1	-	3
			Mallika Bg-II	1	1	-	1	3
			Kanak Bt	1	2	1	-	4
			Total	3	4	2	1	10
7	Osmanabad	7	NHH-44	1	1	-	1	3
			Mallika Bg-II	-	1	-	-	1
			Kanak Bt	1	1	1	-	3
			Total	2	3	1	1	7
8	Jalna	6	NHH-44	-	-	1	-	1
			Mallika Bg-II	-	1	-	1	2
			Kanak Bt	1	1	1	-	3
			Total	1	2	2	1	6

Severe > 50 %
Moderate > 10 % to < 50 %
Trace < 10 %

3.3. Occurrence of bacterial blight of cotton incidence in different districts of Marathwada region

Results (Table 2) clearly revealed that the maximum per cent disease incidence in Parbhani district (67 per cent) followed by Hingoli (63 per cent), Nanded (58 per cent), Latur (54 per cent), Beed (48 per cent), Aurangabad (44 per cent) and Osmanabad (39 per cent). The minimum per cent disease incidence of bacterial blight of cotton was found in Jalna district (36 per cent). Due to this disease the cotton yield and quality of fibre was reduced substantially. The average per cent disease incidence in Marathwada region was about 51.12 per cent. The grower followed regular spray schedule of Bordeaux mixture, copper oxychloride, carbendazim, streptocycline and clean cultivation, proper sanitation had no or low disease incidence in fields. Bacterial blight disease incidence on cotton fields was previously reported by Govindappa *et al*. (2008) from Karnataka region.

Table 2

Percent disease incidence of bacterial blight of cotton in different districts of Marathwada region.

Sr. No.	District	Total number of field surveyed	Total number of plant examined	Infected plants with bacterial blight	Per cent disease incidence (%)
1	Latur	11	250	135	54
2	Aurangabad	8	250	110	44
3	Parbhani	12	300	201	67
4	Nanded	10	250	145	58
5	Hingoli	12	300	189	63
6	Beed	10	250	120	48
7	Osmanabad	7	200	78	39
8	Jalna	6	200	72	36
				Total	51.12

3.4. Isolation of *X. axonopodis* pv. *malvacearum* bacteria from diseased leaf

The pathogen was isolated from the diseased leaves by streaking method on the nutrient agar medium. Yellow glistening colonies with smooth, profuse and mucoid growth began to appear after 48 to 96 hrs of incubation at $27^{o}C \pm 2^{o}C$. The purified *Xanthomonas* bacteria were maintained at $4^{o}C$ on YGCA slants for further investigations.

3.5. Pathogenicity

Pathogenicity was be tested by using cotton seedlings of variety LRA 5166 in pots at ten to twelve leaf stage plants, after predisposition to humid condition for 24 hours were selected for leaf inoculation. The leaves were inoculated with the homogenized culture of *X. axonopodis* pv. *malvacearum*. Prior to automization of the culture on leaf, the leaves were slightly injured with carborendum powder. Seedlings were then retained under humid condition for 24 hours by covering the pots with plastic bag. Pots were adequately watered. Also uninoculated plants were maintained as control.

The symptoms initiated in the form of lesions, distinctly visible and appeared within 3-4 days after inoculation. The spots initially appeared to be water soaked lesions which later turned into brown to black spots. These infected leaves were further used for reisolation and isolated pathogen was found identical as used for inoculation. The identification was further confirmed with the description of *Xanthomonas* given by Akhtar and Khan (1988) and Supriadi *et al*. (1996).

3.6. Cultural studies

Results (Table 3, 4 and 5) revealed that the moderate growth was obtained on Yeast extract mannitol agar broth and Glucose Chalk Agar Broth of susceptible variety LRA 5166. All the three media proved to be significantly superior in favoring the growth of *X. axonopodis* pv. *malvacearum* than the rest of the media tested. Observed elongated, greyish to sooty black lesions on petioles and stems. Small, round, water soaked raised spots observed on the bolls. Abundant growth, filiform nature, glistening, butyrous, pale yellow colonies observed on nutrient agar medium *in vitro*. Fairly good growth, filiform, slightly raised, glistening, whitish yellow, unbanate, secondary colonies observed on PDA medium. Also filiform, glistening, yellowish, fairly good growth observed on chalk agar medium *in vitro* study.

Table 3

Growth and cultural characters of *X. axonopodis* pv. *malvacearum* on different agar media.

Sr. No.	Medium	Growth characters after 48 hrs of incubation
1.	Nutrient agar	Growth abundant, filiform, slightly raised, glistening, butyrous, pale yellow, secondary colonies begin to develop along the margin
2.	Potato dextrose agar	Growth fairly good, filiform, slightly raised, glistening, whitish yellow, unbonate, secondary colonies begin to develop along the margin
3.	Yeast extract mannitol agar	Growth abundant, colonies were filiform, citron yellow, raised circular entire margin, secondary colonies begin to develop along the margin
4.	Glucose Chalk agar medium	Growth fairly good, filiform slightly raised with entire margin, glistening, yellowish, medium slightly turn brown

Table 4

Growth and cultural characters of *X. axonopodis* pv. *malvacearum* on different agar slants.

Sr. No.	Medium	Growth characters after 48 hrs of incubation
1.	Nutrient agar	Colonies of first were filiform and light yellow then becoming waxy yellow, growth was abundant
2.	Potato dextrose agar	Colonies at first were light yellow, filiform, unbanate good growth
3.	Yeast extract mannitol agar	Colonies were filiform, citron yellow, growth was abundant
4.	Glucose Chalk Agar Medium	Colonies were filiform, slight brown colour, growth was abundant

Table 5

Growth and cultural characters of the *X. axonopodis* pv. *malvacearum* in different broth.

Sr. No.	Name of broth	Surface growth	Turbidity	Amount of growth
1.	Nutrient broth	Slightly growth, slightly fluorescent	Light cloudy	Poor
2.	Potato dextrose broth	Membranous	Light cloudy	Scanty
3.	Yeast extract mannitol agar broth	Pellicle	Light cloudy	Moderate
4.	Glucose Chalk Agar Broth	Pellicle	Light cloudy	Moderate

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