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Fungi associated with boll rot of cotton and their management

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ABSTRACT : Boll rot is an extremely complex problem involving diversified disease symptoms and varying nature of damage at any time from boll set to boll bursting. *Alternaria macrospora, Fusarium moniliforme, Colletotrichum gossypii, Aspergillus niger, Rhizopus stolonifer, Penicillium* spp, *Curvularia lunata*, *Dreschlera gossypii* and *Xanthomonas axonopodis* pv. *malvacearum* were found associated with rotted bolls of cotton, from which *A. macrospora, F. moniliforme, C. gossypii* and *X. axonopodis* pv. *malvacearum* were found pathogenic. Potato dextrose agar and Richard's media were found good for growth and sporulation of fungal pathogens, while *X. axonopodis* pv. *malvacearum* growth was excellent on nutrient agar medium. Potassium nitrate and sodium nitrate were good source of nitrogen for profuse growth and sporulation of *A. macrospora, F. moniliforme, C. gossypii,* while glucose and sucrose were good source of carbon for growth and sporulation of the pathogen. The fungicides propiconazole (0.1%), copper oxychloride (0.3%) and streptocycline (100 ppm) were effective against boll rot causing pathogens of cotton, whereas the bioagent *Trichoderma viride* (0.3%) was effective against *A. macrospora, F. moniliforme* and *T. harzianum* against *C. gossypii.*

Key words : Boll rot, biocontrol agent, chemicals, cotton, fungi

Cotton (*Gossypium* spp.); 'King of Fibre' is the most extensively cultivated money minting commercial crop which plays a key role in economic developmental affairs of the world. India is one of the major cotton growing countries in the world. Among several reasons of low productivity and production of cotton, diseases contribute substantially to low yields of cotton. Among other important diseases on *Bt* hybrids in Maharashtra, Alternaria blight (10.2-35.8%) and bacterial blight (2.2 to 22.2 %) was observed serious during 2010-2011 (Anonymous, 2010) and cultivation of high yielding American varieties and hybrids, as thick canopy provides excellent conditions for development of boll rots in cotton. Looking to the severity of the disease the study was undertaken to know pathogens associated with the boll rot of cotton.

MATERIALS AND METHOD

The affected rotten bolls were collected from the Research Trials of Cotton Improvement Project, MPKV, Rahuri to isolate the pathogens associated with boll rot. The common laboratory medium potato dextrose agar (PDA) was used for isolation of pathogens associated with boll rot and were identified on the basis of growth, colony characters and microscopic observations by comparing with available description from Mycological books. The fungal pathogens isolated from rotten bolls were grown on different media in order to study the growth characters and ability of pathogen to sporulate on different media. The carbon requirement of each pathogen was studied by replacing sucrose of Richard's medium with different carbon sources and the nitrogen requirement was studied by replacing potassium nitrate with different nitrogen sources.

The efficacy of different fungicides for controlling the pathogens responsible for boll rot was determined by poison food technique on potato dextrose agar and nutrient agar media. The per cent inhibition of mycelial growth of inoculated pathogens was worked out by using the formula;

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent inhibition on fungal growth

C = Growth in mm on the 7th day after inoculation in control

T = Growth in mm on the 7th day after

inoculation in treatment.

The antagonistic studies between the isolated pathogens and bioagents *Trichoderma viride*, *T. harzianum* and *T. koningii* were carried out in *in vitro* condition.

Isolation and pathogenecity : Isolations made from infected cotton bolls showing typical rotting symptoms, yielded 9 fungi and one bacterium. The pure cultures of these organisms were obtained and maintained for further studies and based on morphological characters the isolated organisms were identified as *A. macrospora, F. moniliformae, C. gossypii, Xam, A. niger, R. stolonifer, Penicillium* spp, *C. lunata* and *Dreschlera gossypii*. The result with regards to pathogenicity tests indicated that out of 10 microorganisms only 4 *viz.*, *A. macrospora, F. moniliforme, C. gossypii* and *X. axonopodis* pv. *malvacearum* were found pathogenic.

RESULTS AND DISCUSSION

Cultural studies of fungi associated with boll rot : The culture media, evaluated for growth characters of *A. macrospora* exhibited varying degree of growth rates ranging from 6 to 72 mm in 7 days with maximum colony dia of 72 mm on potato dextrose agar (Fig. 1), abundant sporulation on potato dextrose agar and Richard's agar media and good sporulation on nutrient agar medium (Table 1). However, *F. moniliforme* showed significantly maximum colony dia of 88 and 78 mm, respectively on potato dextrose agar and Richard's agar media with abundant sporulation on both media. As regards to *C. gossypii*, abundant sporulation and maximum colony diameter of 82 mm was noticed on potato dextrose agar medium and no sporulation was observed on water agar medium. The excellent growth of *X. axonopodis* pv. *malvacearum* was observed on nutrient agar medium followed by potato dextrose agar medium (Table 2).

Utilization of carbon compounds by fungi associated with boll rot of cotton : The mean colony dia and sporulation of the pathogen on different carbon compounds revealed that the pathogen can grow on all carbon sources tested (Table 3). The maximum growth of *A. macrospore*; 83 mm was noticed on glucose followed by sucrose (71 mm) and good sporulation was observed on sucrose and moderate sporulation on dextrose, maltose, glucose and mannitol. The growth of *F. moniliforme* on various carbon sources revealed that the fungus could utilize all the carbon sources used in the experiment and excellent growth was observed on sucrose (82 mm), glucose (79 mm) and maltose (73 mm), whereas, abundant sporulation was observed on sucrose and good on dextrose, maltose and glucose. However, excellent growth of *C. gossypii* noticed on maltose (81 mm) while good on glucose (7 mm) and maltose (76 mm) and abundant sporulation on maltose.

Table 1. Cultural studies of fungi associated with boll rot of cotton on solid media

Cultural media	*A. macrospora*		*F. moniliforme*		*C. gossypii*	
	Mean colony diameter (mm)	Sporulation	Mean colony diameter (mm)	Sporulation	Mean colony diameter (mm)	Sporulation
T_1 Potato dextrose agar medium	72	++++	88	++++	82	++++
T_2 Oat meal agar medium	43	++	38	++	74	+++
T_3 Richard's agar medium	71	++++	78	++++	78	+++
T_4 Czapek's medium	30	++	70	+++	69	++
T_5 Water agar medium	6	-	7	+	12	-
T_6 Nutrient agar	20	+++	39	++	60	++
T_7 Lima bean agar medium	42	++	44	++	70	+++
S.E. ±	1.86		1.41		1.60	
C.D. (p=0.05)	5.64		4.28		4.85	

- = No sporulation, + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant

Table 2. Cultural characters of *X. axonopodis* pv. *malvacearum* on different agar media

Media	Cultural characters (7 DAI)
Potato dextrose agar	Good growth, filiform, slightly raised, glistening, pale yellow, secondary colonies began to develop along the margin
Nutrient agar	Excellent growth, filiform, slightly raised, glistening, dark yellow, secondary colonies began to develop along the margin

Utilization of nitrogenous compounds by fungi associated with boll rot : The growth of the fungi on various nitrogenous compounds revealed that the pathogens could grow well on various nitrogenous compounds (Table 3). The profuse growth of *A. macrospora* was observed on potassium nitrate (71 mm) and sodium nitrate (62 mm) and good growth on ammonium chloride (36 mm). However, *F. moniliforme* excellently grow on sodium nitrate (82 mm) and potassium nitrate (78 mm) and moderately on ammonium chloride (38 mm), ammonium sulphate (30 mm) and ammonium nitrate (28 mm), whereas abundant sporulation was observed on sodium nitrate and potassium nitrate and good sporulation on ammonium nitrate. The growth of *C. gossypii* on various nitrogenous compounds showed that good growth of the fungus noticed on potassium nitrate (78 mm) and sodium nitrate (65 mm) whereas moderate growth was observed on ammonium chloride, ammonium sulphate and ammonium nitrate. Moreover, abundant sporulation was noticed on potassium nitrate while good sporulation was observed on sodium nitrate.

***In vitro* evaluation of fungicides against fungi associated with boll rot of cotton** : The results of *in vitro* study regarding evaluation of fungicides against *A. macrospora* at recommended doses indicated that all fungicidal treatments showed significantly least colony dia as compared to control (Table 4). The 100 per cent growth inhibition of *A. macrospora* was observed by mancozeb followed by propiconazole (88.75 %), copper oxychloride (75 %) and propineb (67.50 %).

The results of fungicidal treatments against *F. moniliforme* in *in vitro* revealed that all

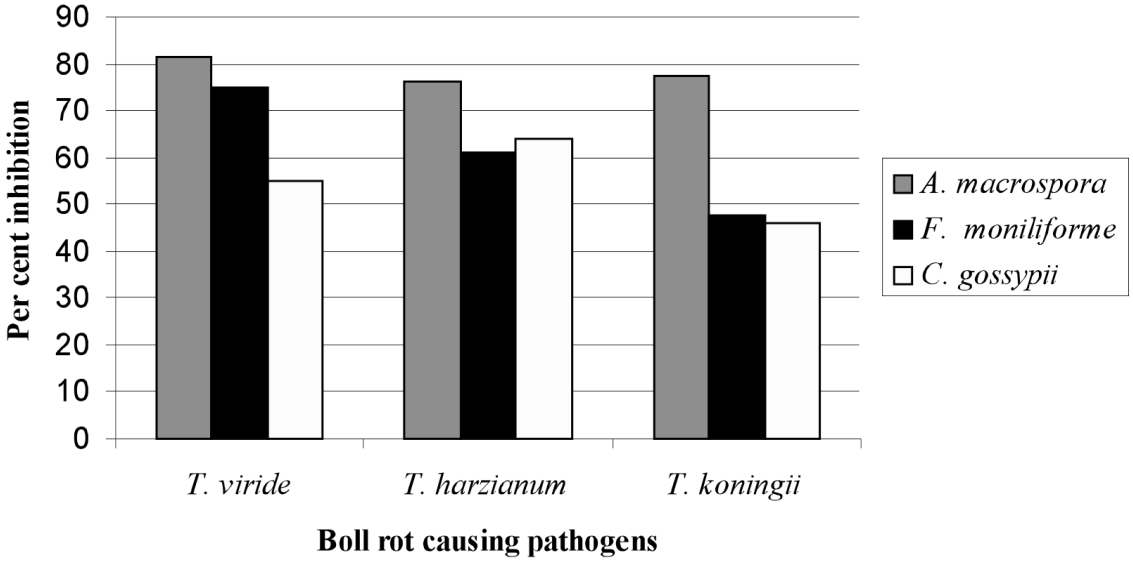

Fig. 1. Efficacy of Trichoderma spp against funing associated with boll rot of cotton

Table 3. Utilization of carbon sources and nitrogenous compounds of fungi associated with boll rot of cotton on solid media

Sources	*A. macrospora*		*F. moniliforme*		*C. gossypii*	
	Mean colony diameter (mm)	Sporulation	Mean colony diameter (mm)	Sporulation	Mean colony diameter (mm)	Sporulation
Carbon source						
Sucrose	71	+++	82	+++ +	72	+++
Dextrose	55	++	69	+++	68	+++
Maltose	50	++	73	+++	81	++++
Mannitol	40	++	65	++	76	++
Glucose	83	++	79	+++	78	+++
Control	12	-	10	-	8	-
Nitrogen source						
Potassium nitrate	71	+++	78	++++	78	++++
Sodium nitrate	62	++++	82	++++	65	+++
Ammonium nitrate	28	+++	28	+++	28	++
Ammonium sulphate	30	++	30	++	29	++
Ammonium cloride	36	++	38	++	35	++
Control	14	-	10	-	11	-

* = Average of three replications, - = No sporulation, + = Scanty, ++ = Moderate +++ = Good, ++++ = Abundant

fungicides tested were found significant over the control in inhibiting the colony growth of (Table 4). Among all the fungicides tested carbendazim (0.1%) was observed significantly superior, which recorded 100 per cent inhibition of the *F. moniliforme* followed by propiconazole, benomyl and copper oxychloride which recorded the pathogen inhibition by 87.50, 73.75 and 58.75 per cent, respectively. And as regards to *F. moniliforme* no sporulation was observed in case of carbendazim and poor in copper oxychloride, propineb, mancozeb, propiconazole and benomyl treatment. The fungicides studied *in vitro* against *C. gossypii* revealed that benomyl was found significantly superior showed complete inhibition of the pathogen, which was followed by carbendazim (92.30) and copper oxychloride (87.17) and were at par with each other in growth inhibition of *C. gossypii*. These results are in consonance with the work of Yadav and Kumar (2007) who reported that copper oxychloride was highly effective against *Colletotrichum* spp. Sensitivity of *X. axonopodis* pv. *malvacearum* studied against chemicals indicate that, there was no growth of *X. axonopodis* pv. *malvacearum* in copper oxychloride, propineb, mancozeb and

Table 4. *In vitro* evaluation of fungicides against fungi associated with boll rot of cotton

Name of chemical	*A. macrospora*			*F. moniliforme*			*C. gossypii*		
	Mean colony diameter (mm)	Inhibition (%)	Sporulation	Mean colony diameter (mm)	Inhibition (%)	Sporulation	Mean colony diameter (mm)	Inhibition (%)	Sporulation
T_1 Copper oxychloride (0.3%)	20	75.00	-	33	58.75	+	10	87.17	-
T_2 Propineb (0.2%)	26	67.50	+	42	47.50	+	49	37.17	++
T_3 Mancozeb (0.3%)	0	100.00	-	39	51.25	+	40	48.71	++
T_4 Carbendazim (0.1%)	42	47.50	-	0	100.00	-	6	92.30	-
T_5 Propiconazole (0.1%)	0	88.75	-	10	87.50	+	45	42.31	++
T_6 Benomyl (0.1%)	48	40.00	+	21	73.75	+	0	100.00	-
T_7 Streptocycline (100ppm)	72	10.00	+++	72	10.00	+++	74	5.13	+++
T_8 Control	80	-	++++	80	-	++++	78	-	++++
S.E.±	1.57			1.74			1.82		
C.D. (p=0.05)	4.76			5.28			5.52		

* = Average of three replications, - = No sporulation, + = Scanty, ++ = Moderate, +++ = Good, ++++ = Abundant

Table 5. Efficacy of *Trichoderma* spp against fungi associated with boll rot of cotton

Bioagent	*A. macrospora*		*F. moniliforme*		*C. gossypii*	
	Mycelial growth (mm)*	Inhi-bition (%)	Mycelial growth (mm)*	Inhi-bition (%)	Mycelial growth (mm)*	Inhi-bition (%)
Trichoderma viride	15	81.25	20	75.00	35	55.13
Trichoderma harzianum	19	76.25	31	61.25	28	64.10
Trichoderma koningii	18	77.50	42	47.50	42	46.15
Control	80	-	80	-	78	-
SE ±	1.02		1.54		1.15	
C.D. (p0.05)	3.15		4.75		3.56	

*Average of five replications

streptocycline chemicals. The similar results of streptocycline against *X. axonopodis* pv. *malvacearum* was also reported by Mali *et al.,* (2007).

Efficacy of biocontrol agents against fungi associated with boll rot : The results of the inhibitory effects of an antagonistic bioagent (*Trichoderma* spp) against *A. macrospora* revealed that, the treatment differences in mycelium growth of pathogen and bioagents were statistically significant (Table 5). The bioagents in order of superiority in arresting growth of *A. macrospora* were *T. viride* and *T. koningii* by 81.25 and 77.50 pre cent inhibitions, respectively (Fig. 1). *Trichoderma* spp. tested against *F. moniliforme* revealed that, all were found significantly superior over the control in inhibiting the colony growth of *F. moniliforme* in *in vitro*. Amongst which *T. viride* was found most effective which recorded significantly lowest colony diameter (20 mm) i.e 75.00 per cent growth inhibition of pathogen. The bioagents *T. harzianum* and *T. koninigii* were also found effective in arresting the growth of *F. moniliforme* by 61.25 and 47.50 per cent, respectively (Fig. 1). The results in respect of biological control of *F. moniliforme* were also reported by Sangle and Bambawale (2004). Moreover, the studies on the inhibitory effect of bioagents against *C. gossypii* indicated that, *T. harzianum* was found significantly by arresting the colony growth of 64.10 per cent followed by *T. viride* (55.13 %) and *T. koningii* (46.15 %).

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