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Pathognes associated with Cotton Boll Rot and their Control with Fungicides

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Abstract : Among the various diseases infecting cotton crop, boll rots are of major significance as they directly affect the bolls and ultimately reduces the yield. Five distinct fungal colonies were isolated from rotted cotton bolls. Based on morphological characters, the fungi associated with boll rot disease of cotton were identified as *Fusarium* sp., *Colletotrichum* sp., *Diplodia* sp., *Aspergillus* sp. and *Rhizopus* sp. Under *in vitro* condition, carbendazim (50% WP @ 0.1%) showed maximum inhibition of *Fusarium* sp., *Colletotrichum* sp. and *Diplodia* sp. Propiconazole (25% EC @ 0.1%) and Pyraclostrobin (20% WG @ 0.1%) also gave promising control of myceial growth of all the five fugal pathogens.

Keywords: Boll rot, cotton, control, *in vitro*, pathogens

India is one of the major cotton growing countries contributing 37 per cent of world's cotton production. Among the different states of India, Maharashtra is the leading state in cotton cultivation with 42.23 lakh ha followed by Gujrat, Telangana, Karnataka and Rajasthan and (Anonymous, 2023). In Maharashtra, it is a main cash crop mostly grown GNES under rainfed condition which is characterized by unpredictable rainfall and it's uneven distribution. There are many reasons for low and stagnant production of cotton in our country. It includes heavy rain at the time of sowing, temperature fluctuation during flowering stage, weather adversaries, pest attack specially bollworm complex, various diseases and improper production technology. Among the many reasons of low productivity and production, diseases caused by pathogens contribute substantially to low yields of cotton. *Fusarium* wilt, *Verticillium* wilt, root rot, grey mildew, *Alternaria* leaf spot, *Cercospora* leaf spot, bacterial blight and boll rot are some of the major cotton diseases in India.

In cotton producing regions of the world, particularly in India, yield losses caused by boll rot disease has increased in recent few years. Boll rot is an extremely complex problem involving diversified disease symptoms and varying nature of damage. The disease reduces the quantity of produce as well as affects the quality. Yield reduction caused by boll rot estimated in different cotton growing areas throughout the world showed great economic losses. In USA, during 2010-2011 losses due to boll rot were estimated to the tune of 100,153 to 182,708 bales (Goldberg *et al.*, 2010). Cotton yield loss of 10-15 per cent was reported due to seed and boll rot in South Carolina (Kindall, 2013). In India also losses caused by boll rot of cotton are becoming serious threat to small and marginal farmers day by day. Nanda and Kulkarni (2020) reported 10.16 per cent disease index of boll rot disease at boll initiation stage in Uttar Kannada districts of Karnataka. Nearly five per cent of the cotton crop in Maharashtra was reported to be affected due to rotting cotton bolls. In Nanded and Ahmednagar districts of Maharashtra seven per cent incidence of boll rot was recorded (Anonymous, 2021). Number of pathogens, *viz.* *Alternaria* sp., *Botrydiplodia* sp., *Aspergillus* sp., *Colletotrichum* sp., *Fusarium* sp., *Myrothecium roridum*, *Pennicillium* sp. have been reported to be associated with the boll rot in cotton (Zancen *et al.*,

2011 and Perane *et al.,* 2015). The disease is however not been well understood and information on it's control is scanty. During present studies, pathogens associated with cotton boll rot and effectiveness of fungicides for their control under *in vitro* condition was investigated.

Isolation of pathogens causing boll rot

Infected cotton bolls showing the typical symptoms of rot disease were collected from cotton fields from various locations in Ahmednagar district (Maharashtra state). Isolation of fungi and bacteria associated with the rotted bolls was done on Potato Dextrose Agar (PDA) and Nutrient Agar medium, respectively by adopting standard laboratory procedure. The fungi isolated from rotted bolls were sub-cultured and pure culture of these fungi was obtained by hypal tip method. Pathogenicity of fungi was proved by inoculating the healthy cotton bolls under laboratory condition. For this, healthy cotton bolls were slightly injured with sterile needle and artificially inoculated with the spores of the respective fungus. Cotton bolls inoculated only with sterile water were maintained as control. These inoculated cotton bolls were placed in desiccator covered with moist cotton for 48 hours to maintain humidity to favour the disease development. Morphological characters of the pathogens were observed under microscope and based on these observations and description given by Dugan (2006), the isolated fungi were identified up to genera level.

***In vitro* evaluation of fungicides against the pathogens**

The efficacy of six fungicides was evaluated *in vitro* against each of the pathogen by poisoned food technique on PDA medium. The required quantity of fungicide was measured, mixed thoroughly with the medium then medium poured aseptically in Petri plates and allowed to solidify. The Petri plates poured only with PDA medium served as control. After solidification of the medium, fungal disc of 5 mm diameter was inoculated on to the centre of the each plate and allowed to incubate at 27±2°C. Observations were recorded on mycelial growth (colony diameter) of the test pathogens till it was fully grown in untreated control plate. The per cent inhibition of the growth of test pathogens was calculated by using the formula given.

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Mycelial growth in untreated control, T = Mycelial growth in treatment

Pathogens associated with cotton boll rot

Under field condition, boll rot symptoms observed were small light brown spots on cotton bolls at initial stage which later enlarged, became dark brown to black and coalesced together. Dark wet spots were also observed on some bolls which increased in size and covered entire boll. In severe cases the fungus growth was observed on petioles, stem and leaves. Heavily infected bolls remained unopened and premature fall of cotton bolls was also observed. Isolations made from the rotted bolls yielded five distinct fungi. The pathogenicity of these fungi was proved on healthy cotton bolls. Based on the cultural and morphological characters (Table 1) the isolated fungi were identified as *Fusarium* sp., *Colletotrichum* sp. *Diplodia* sp., *Aspergillus* sp. and *Rhizopus* sp.

The data on pathogens isolated from samples collected from various locations is presented in Table 2. Out of the 30 samples collected, *Fusarium* sp. was obtained from eleven samples, *Colletotrichum* sp. from nine samples, *Diplodia* sp. from eight samples, *Aspergillus* sp. from five samples and *Rhizopus* sp. from four samples while five samples did not yield any pathogen. Bacterium was not isolated from any of the sample. Several workers had reported a

Table 1. Characteristics of the pathogens isolated from rotted cotton bolls

Sr. No.	Cultural and Morphological characters	Identified as
1.	Mycelium hyaline, septate and profusely branched. Colony cottony white in colour and fluffy in nature. Microconidia oval and small in size, mostly 1-2 septate, hyaline, 8-10 µm. Macroconidia 1-2 septate, fusiform and slightly curved at the tip	*Fusarium* sp.
2.	Mycelium profuse, white, closely septate, irregularly branched and hyaline, which later turned slightly grey to light brown. Conidiophores arised in acervuli or from immersed hyphae, hyaline non-septate. Condia unicellular, straight or curved, and almost ablong, measuring 11-20 x 4-9 µm. Conidia formed on setae, typically tiny and oval.	*Colletotrichum* sp.
3.	Mycelium initially emerged as a white filamentous which turned greyish, finally into a black mass. Conidia initially appeard coloured and septate measuring 13.5 µm X 24 µm but later became hyaline and non-septate, marked with longitudinal striations, measuring 12.5 µm X 23 µm in diameter.	*Diplodia* sp.
4.	Colonies composed of compact white or yellow basal felt covered by a dense covering of dark-brown to black conidial heads Huge conidial heads (15-20 µm in diameter), globose, dark brown, radiate with age, and divide into multiple loose columns. Conidiophores with smooth walls, hyaline, or darkened. Conidial heads biseriate, with phialides on brown, frequently septate metulae. Conidia ranged in size from globose to subglobose (3.5-5.0 µm in diameter), dark brown to black in colour with rough walls.	*Aspergillus* sp.
5.	Mycelium growth initially radial with white filamentous colonies that turn dark greyish-brown, up to 10 mm high producing simple rhizoids. Sporangiophores brownish, up to 400 µm high and 10 µm wide and produced in groups of one to four, usually in pairs. Sporangia greyish-black, spherical, up to 100 µm in diameter.	Rhizopus sp.

number of pathogens associated with cotton boll rot. followed by *Aspergillus* and *Colletotrichum* sp. Belot and Zambiasi (2007) and Zancan *et al.,* (2011) isolated different fungi, *viz., Alternaria* sp., *Botrydiplodia* sp., *Colletotrichum* sp., *Fusarium* sp., *Myrothecium* sp., and *Penicillium* sp. associated with boll rotting in cotton. Perane *et al.,* (2015) identified the microorganisms associated with rotting as *Alternaria macrospora, Fusarium moniliforme, Colletotrichum gossypii, Aspergillus niger, Rhizopus stolonifer, Penicillium* spp*., Curvularia lunata, Dreschlera gossypii* and *Xanthomonas axonopodis* pv. *malvacearum.* In present studies five fungi, *viz. Fusarium* sp., *Colletotrichum* sp. *Diplodia* sp., *Aspergillus* sp. and *Rhizopus* sp. were found to be responsible for causing rotting of cotton bolls.

Efficacy of fungicides against the pathogens under *in vitro* condition

The efficacy of six fungicides was evaluated under *in vitro* condition in order to identify the most efficient fungicide capable of inhibiting the growth of boll rotting. The results revealed that (Table 3) all six fungicides significantly inhibited mycelial growth over the untreated control. Among all the treatments, carbendazim (50% WP @ 0.1%) showed 100 per cent inhibitory effect for both *Fusarium* sp. and *Diplodia* sp. Similarly the least mycelial colony diameter (5.7 mm) of *Colletotrichum* sp. was recorded in the same treatment with 93.44 per cent inhibition over untreated control. Pyraclostrobin (20% WG @ 0.1%) showed complete inhibitory effect on *Aspergillus* sp. whereas both copper oxychloride (50% WP @ 0.25%) and streptocycline (0.05% + copper oxychloride 0.25%) showed complete inhibitory effect on *Rhizopus* sp.

Propiconazole (25% EC @0.1%) was the next effective fungicide and the least colony diameter of 9.5 mm, 9.0 mm and 7.0 mm for *Fusarium* sp., *Diplodia* sp. and *Aspergillus* sp., respectively with 89.9 per cent, 89.53 per cent and 91.95 per cent inhibition over control was recorded in this treatment. Copper oxychloride (50% WP @ 0.25%) and pyraclostrobin (20% WG @0.1%) also gave significant inhibition of

Table 2. Fungi isolated from rotted cotton bolls collected from various locations

Sample No.	Fungi obtained
1	*Fusarium* sp.,*Colletotrichum* sp.
2	*Colletotrichum* sp.
3	*Fusarium* sp., *Aspergillus* sp.
4	*Aspergillus* sp.
5	*Colletotrichum* sp. *Diplodia* sp.
6	*Fusarium* sp.,
7	*Fusarium* sp., *Aspergillus* sp.
8	-
9	*Diplodia* sp., *Colletotrichum* sp
10	*Colletotrichum* sp.
11	*Aspergillus* sp. *Fusarium* sp.
12	*Colletotrichum* sp.
13	-
14	*Diplodia* sp.
15	*Fusarium* sp. *Rhizopus* sp.
16	*Rhizopus* sp., *Diplodia* sp
17	*Colletotrichum* sp.
18	*Aspergillus* sp.
19	*Fusarium* sp., *Diplodia* sp.
20	*Diplodia* sp
21	-
22	*Fusarium* sp., *Rhizopus* sp.
23	-
24	*Fusarium* sp.
25	*Colletotrichum* sp.
26	*Diplodia* sp., *Fusarium* sp.
27	*Rhizopus* sp., *Diplodia* sp.
28	*Fusarium* sp.
29	*Colletotrichum* sp.
30	-

mycelial growth of *Colletotrichum* sp. and *Rhizopus* sp. with 87.35 per cent and 90.69 per cent inhibition over control, respectively.

Least inhibition of mycelial growth was observed with copper oxychloride (50% WP @ 0.25%) for *Diplodia* sp. and *Aspergillus* sp. Whereas, streptocycline (0.05% + copper oxychloride 0.25%), pyraclostrobin (20% WG @ 0.1%) and propineb (70% WP @ 0.25%) gave least inhibition of *Fusarium* sp. *Colletotrichum* sp. and *Rhizopus* sp. with 48.29, 29.88 and 47.67 per cent inhibition of mycelial growth, respectively.

Thus the result indicated that among the all fungicides tested *in vitro* against five boll rotting fungi, carbendazim (50% WP @ 0.1%) showed maximum efficacy and least mean colony diameter (8.54 mm) of all fungal pathogens was recorded in this treatment. It was followed by propiconazole (25% EC @ 0.1%) showing 18.00 mm mean colony diameter for five pathogens whereas least efficacy was exhibited by streptocycline (0.05% + copper oxychloride 0.25%) recording maximum *i.e.* 45 mm mean colony diameter of five fungal pathogens.

Fungicides are being widely used worldwide for control of fungal pathogens infecting the various crops. Gaikwad (2010) reported that among the various fungicides tested, carbendazim was the most efficient in inhibiting the growth of *Fusarium* sp, followed by propiconazole and copper oxychloride. Kumar *et al.,* (2021) and Yerukala *et al.,* (2021) also reported that carbendazim showed 100 per cent inhibition of mycelial growth of *Fusarium oxysporum*. Fungicide benomyl totally suppressed the growth of *C. gossypii* demonstrating its highest effectiveness against the pathogen under *in vitro* condition. It was followed by carbendazim, copper oxychloride, mancozeb and propineb with streptocycline performing the least well. Mateo *et al.,* (2011) screened three fungicides, *viz.* mancozeb, copper oxychloride and sulfur against cotton boll rot caused by *Aspergillus* sp. It was reported that mancozeb was the best to hinder *Aspergillus* spp. growth followed by copper oxychloride and sulphur compound. Jiahuai Hu (2018) reported that effective chemical for control of boll rot complex includes triazole fungicides (tebuconazole, tetraconazole, prothioconazole, and flutriafol), thiophanate methyl, succinate dehydrogenase inhibitor (boscalid), and strobilurin fungicide (picoxystrobin).

The present studies thus revealed that five fungi, *viz. Fusarium* sp., *Colletotrichum* sp., *Diplodia* sp., *Aspergillus* sp. and *Rhizopus* sp. were responsible to cause boll rot in cotton crop. Under *in vitro* condition, carbendazim (50% WP @ 0.1%) showed maximum efficacy against most of the boll rotting pathogens, followed by propiconazole (25% EC @ 0.1%).

Table 3. *In vitro* evaluation of fungicides against boll rotting fungi

Tr No.	Treatments	*Fusarium* sp.		*Colletotrichum* sp.		*Diplodia* sp.		*Aspergillus* sp.		*Rhizopus* sp.		Mean colony diameter of five pathogens (mm)
		Mean colony diameter (mm)	Per cent inhibition	Mean colony diameter (mm)	Per cent inhibition	Mean colony diameter (mm)	Per cent inhibition	Mean colony diameter (mm)	Per cent inhibition	Mean colony diameter (mm)	Per cent inhibition	
T1	Copper oxychloride (50%) WP @ (0.25%)	35.0	59.92 (50.72)*	11.0	87.35 (69.17)*	84.0	2.32 (8.76)*	83.0	4.59 (12.37)*	0.00	100 (90)*	42.0
T2	Carbendazim (50%) WP @ (0.1%)	0.00	100 (90)	5.50	93.44 (75.11)	0.00	100 (90)	12.0	86.2 (68.19)	25.0	70.93 (57.37)	8.54
T3	Propiconazole (25%) EC @ (0.1%)	9.50	89.19 (70.80)	41.0	52.87 (46.65)	9.00	89.53 (71.12)	7.00	91.95 (73.51)	24.0	72.09 (58.11)	18.1
T4	Streptocycline (0.05%) + copper (0.25%) oxychloride	45.5	48.19 (43.96)	21.0	75.86 (60.57)	78.0	9.3 (17.80)	79.0	9.19 (17.65)	0.00	100 (90)	45.0
T5	Propineb (70%) WP @ (0.25%)	40.5	53.77 (47.12)	51.0	41.37 (40.03)	15.0	82.55 (65.31)	65.0	25.28 (30.18)	45.00	47.67 (43.66)	43.0
T6	Pyraclostrobin (0.1%) (20%) WG @	15.5	82.35 (65.16)	61.0	29.88 (33.14)	10.0	88.37 (70.06)	0.00	100 (90)	8.00	90.69 (72.23)	19.0
T7	Control	88.0	-	87.0	-	86.0	-	87.0	-	86.0	-	86.8
	S. Em. ±	0.42		0.55		0.56		0.76		0.70		
	CD (p-0.05)	1.79		2.37		2.35		3.18		2.95		

* : Figures in parentheses are arc sine transformed values

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