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Detection and survival of the *Alternaria* pathogen on *Bt* cotton

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ABSTRACT : In the present study, species of fungi *viz., Alternaria*, *Fusarium*, *Helminthosporium*, *Aspergillus*, *Penicillium*, *Rhizopus* and *Mucor* were detected in infected seeds. However, species of *Aspergillus* , *Penicillium* and *Rhizopus* were eliminated by surface sterilization. In the infected stalks kept under laboratory and refrigerator conditions, *Alternaria* spp survived upto 13 months and more than 14 months respectively. However in sterilized soil it survived upto 11 months, while in natural soil upto 9 months.

Key words : *Alternaria, Bt* cotton, infected seed, infected stalks, survival

Cotton is one of the most ancient and important commercial crops next only to food grains and is the principal raw material for a flourishing textile industry. The low productivity of cotton can be attributed to many factors, one of which is the losses due to diseases although insect pests continue to be a major production constraint. A large number of fungal, bacterial, viral and nematode diseases have been reported on cotton crop right from early stage to maturity (Anonymous, 2012). Among them, the economically most important ones are bacterial blight, Alternaria leaf spot, grey mildew, rust and vascular wilts which occur throughout the world. Even before the cultivation of *Bt* cotton, Alternaria leaf spot of cotton was one of the most important diseases noticed throughout the world. Yield losses upto 26 per cent due to Alternaria leaf spot have been reported (Chattannavar *et al.*, 2006). Keeping this in view, the present investigations were under taken to study the survival of this pathogen.

MATERIALS AND METHODS

Association of mycoflora with seeds of *Bt* cotton : The mycoflora associated with cotton seeds were isolated by following two methods *viz.,* Blotter paper method and Agar plate method.

Blotter paper method : Sterile petri plates were used in the study. Three blotter papers were moistened with sterile water and placed at the bottom of each petri plate. Twenty five seeds were placed at equidistance on blotter paper in each petri plate with and without surface sterilization by mercuric chloride (0.1%) separately. After incubation of the plates for 7 days fungal growth was observed under stereo binocular microscope and compound microscope and organisms associated were recorded (Anonymous, 1999).

Agar plate method : To detect the mycoflora of the seed, 100 seeds were placed @ 10 seeds / petriplate containing PDA. Similarly, 100 seeds were surface sterilized in 0.1 per cent mercuric chloride and placed on PDA for isolation of internal mycoflora associated with the seeds. The plates were incubated for 7 days. The mycoflora associated with *Bt* cotton (Bunny *Bt*) seeds were recorded after examining under stereo binocular microscope (Khare, 1996).

Identification of seed mycoflora : The mycoflora of the seed were identified by studying the morphological characters of the fungus and referring to the 'Illustrated Genera of Imperfect Fungi', 'Dematiaceous hypomycetes' and other incidental reference works.

Survival of *Alternaria* spp in crop debris : *Alternaria* spp infected stalks of *Bt* cotton (Bunny *Bt*) were collected from field and stored at a depth of 5 cm in soil under laboratory conditions for perpetuation study. The soil was sterilized and

filled in earthen pots and infected stalks were placed at 5 cm depth from the surface. The infected stalks were also placed in natural soil, laboratory and refrigerator conditions. Observations were recorded to know the longevity of the survival of *Alternaria* spp by regular tissue isolation at an interval of 30 days.

RESULTS AND DISCUSSION

Association of mycoflora with seeds : Totally seven fungi were recorded in both the methods of testing. Many fungi were eliminated with surface sterilization of seeds. *Alternaria* spp is found as major fungus associated with Bunny *Bt* cotton seeds (18.45 and 66.50 per cent in blotter and agar plate methods, respectively). Even after surface sterilization *Alternaria* spp was detected to the extent of 10.56 and 54.26 per cent, respectively. *Fusarium* spp was next important fungus identified on Bunny *Bt* cotton seeds and was recorded to an extent of 8.25 and 18.25 per cent in unsterilized while 5.26 and 11.45 per cent in sterilized seeds in blotter and agar plate, respectively. *Helminthosporium* spp was identified and observed to an extent of 2.20 and 2.65 per cent in unsterilized while 0.00 and 1.50 per cent in sterilized seeds in blotter and agar plate, respectively. Similarly, *Aspergillus* was observed to an extent of 3.65 and 16.50 per cent in unsterilized while 1.76 and 11.25 per cent in sterilized seeds in blotter and agar plate, respectively. *Penicillium* (2.47% and 4.00%) and *Rhizopus* (3.35% and 2.47%) and *Mucor* (5.64% and 3.75%), were other species detected. But these fungi were not detected when the seeds were surface sterilized, except *Mucor* (2.25%). The study indicated that, none of the seed samples tested were totally free from mycoflora. *A. macrospora, A. tenuis, Cladosporium* spp, *C. indicum, Curvularia lunata, Drechslera* spp, *Epicoccum purparescens, Fusarium* spp, *Nigrospora oryzae, Trichoderma viride, Trichothecium roseum, Verticillium* spp and species of *Aspergillus* and *Penicillium* were present in most of the seed samples (Table 1 and Plate 1).

Table 1. Association of different mycoflora with *Bt* cotton seeds

Fungus isolated	Per cent mycoflora detected			
	Blotter paper method		Agar plate method	
	Un-sterilized	Steri-lized	Un-sterilized	Steri-lized
Alternaria spp	18.45	10.56	66.50	54.26
Fusarium spp	8.25	5.26	18.25	11.45
Helminthosporium spp	2.20	0.00	2.65	1.50
Aspergillus spp	3.65	1.76	16.50	11.25
Pencillium spp	2.47	0.00	4.00	0.00
Rhizopus spp	3.35	0.00	2.47	0.00
Mucor spp	5.64	0.00	3.75	2.25

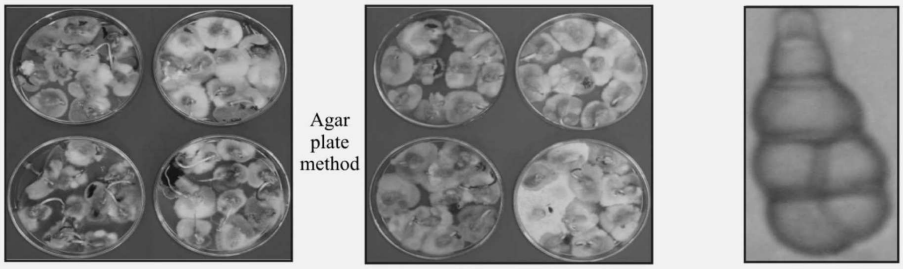
Survival of *Alternaria* spp in crop debris : The infected stalks were tested for the survivability of *Alternaria* spp in sterilized soil, natural soil, laboratory and refrigerator. The Table 2 and Plate 2 revealed that the infected stalks kept in the laboratory were viable for 12 months. On the 13th month the *Alternaria* spp could not be isolated from infected stalks kept in laboratory. However, stalks kept in the natural soil lost their viability after 9 months. When infected stalks were kept in sterilized soil, *Alternaria* spp was viable upto 11 months and when kept in refrigerator it remained viable even after more than 14 months (Amaresh, 2000). The present studies indicated that *Alternaria* spp can survive in cotton stalks which

Table 2. Survival of *Alternaria* spp in plant debris

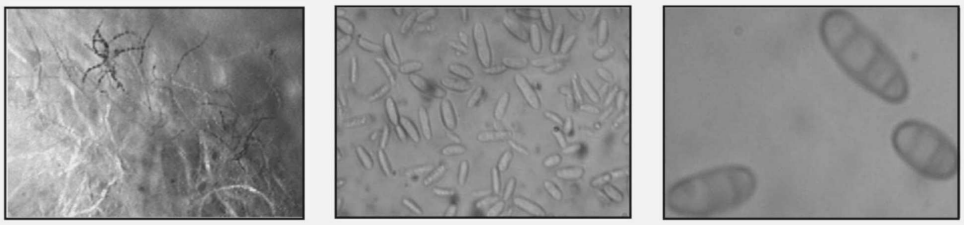
Months	Sterilized soil	Natural soil	Laboratory	Refrigerator
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	-	+	+
11	+	-	+	+
12	-	-	+	+
13	-	-	-	+
14	-	-	-	+

+ = Viable - = Non viable

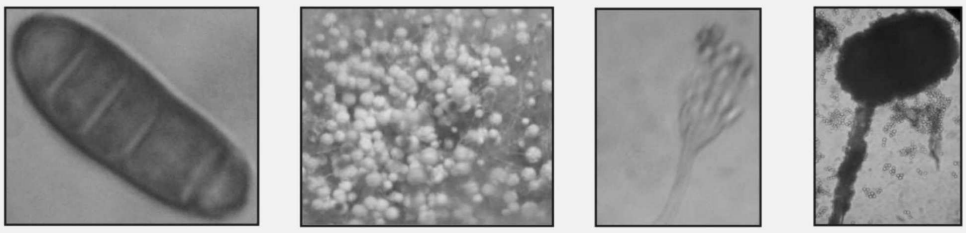
Surface unsterilized **Surface sterilized** **Conidia of *Alternaria* spp.**



Surface unsterilized **Surface sterilized** **Close up view of *Alternaria* spp.**

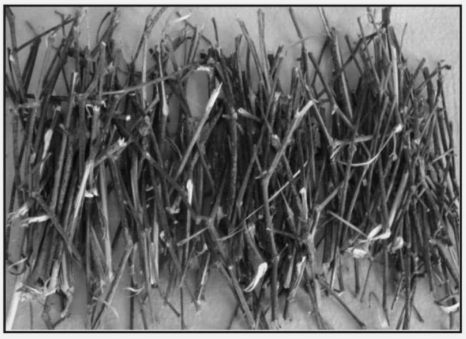


Chains of *Alternaria* conidia ***Fusarium* spp.** ***Helminthosporium* spp.**

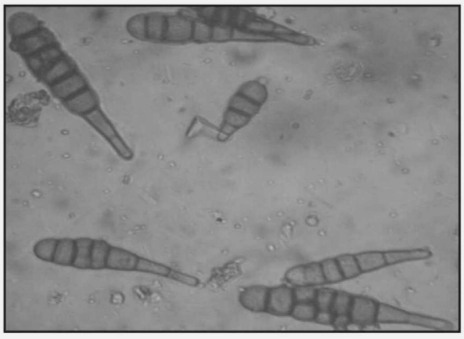


Close up view of *Helminthosporium* spp. **On seed *Aspergillus flavus*** ***Pencillium* spp.** ***Mucor* spp.**

Plate 1 : Association of different mycoflora with *Bt* cotton seeds

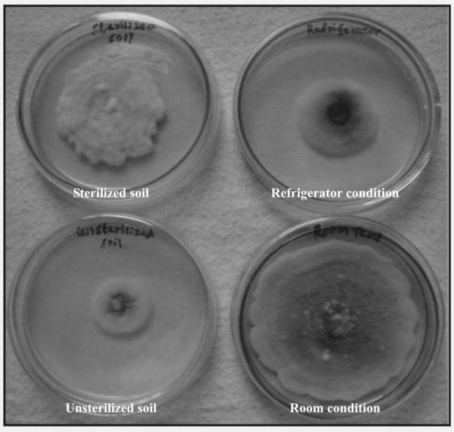


Alternaria infected stalks collected from field

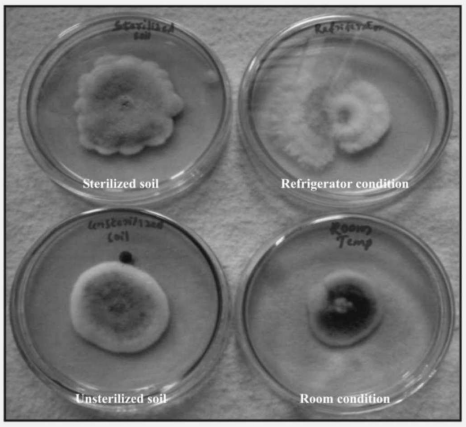


Conidia observed from the stalks

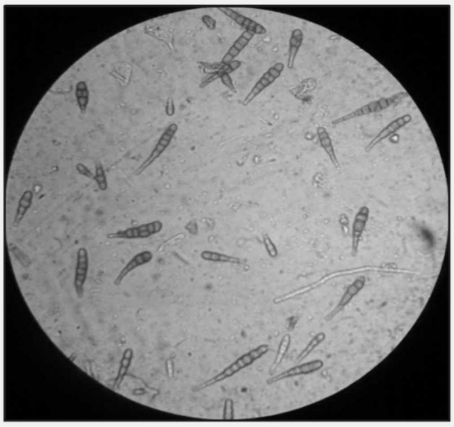
Alternaria infected stalks kept in pots



After 1 month of survival study



After 9 month of survival study



Conidia observed from the colony growth

Plate 2 : Survival of *Alternaria* spp. in plant debris

can serve as inoculum source during the next season.

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