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Impact of plant age on phyllospheric mycoflora of conventional and organic cotton fields

KEYWORDS: Cotton, Phyllosphere, organic, conventional, mycoflora.

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ABSTRACT *The phyllosphere is considered as suitable habitat for microorganisms, which is predominantly characterized by variable environmental conditions i.e. light, temperature, humidity and nutrient availability. The ability of microorganisms to immigrate and to colonize this environment mainly determines the composition of microbial phyllosphere communities. Immigration and colonization by the microorganisms in turn is linked to their ability to adapt to or to tolerate the adverse conditions in the phyllosphere. Total fungal population was higher in organic cotton fields with the age of cotton plant as compare to conventional cotton fields; While Pathogenic fungal population was higher in conventional cotton field as compare to organic one. Alternaria alternata, Alternaria macrospora, Aspergillus flavus, Aspergillus niger, Cercospora gossypina, Colletotrichum gossypii, Curvularia lunata, Drechslera tetramera, Helminthosporium gossypii, Penicillium notatum, Penicillium chrysogenum, Rhizoctonia solani, Stemphylium solani, Rhizopus oryzae, Trichoderma harzianum and Trichoderma viride were reported. It was reported that Alternaria, Penicillium and Aspergillus were the most common fungi observed in both the farming systems at crop maturity.*

Introduction

Cotton production in India reaching a record area of 12.3 million hectares in 2014/15, area in India is forecast down 5% to 11.6 million hectares, and production down 3% to 6.4 million tons in 2015/16, according to International Cotton Advisory Committee (ICAC, 2015). World area is projected to decrease 7% to 31.2 million hectares, and assuming a world average yield of 765 kg/ha, production is forecast down 9% at 23.9 million tons from 2014/15. The 2014-15 seasons began on October 1, 2014. The total cotton crop stood at 407.25 lakh bales (of 170 kg each) during 2013-14 Cotton Association of India (CAI data).

India makes dominant position in production and exports of cotton lint in the world. Cotton is major crop of Indian farming scenario it plays a vital role in farmers economy as well as country's economy. Therefore farmer's were relay on conventional farming system for more yielding for that using advance techniques of farming like heavy use of chemicals fertilizers, chemical pesticides and genetically modified seeds. It may influences on the soil fertility and also on the pests and microbes.

Organic farming systems have attracted increasing attention over the last one decade because they are perceived to offer some solutions to the problems currently besetting the agricultural sector. Organic farming has the potential to provide benefits in terms of environmental protection, conservation of non-renewable resources and improved food quality (Worthington, 2001; Haas et al., 2005).

At different stages of growth and ages the condition of plant and the leaf is different. This may be responsible for difference in surface conditions of the leaf. Hall and Jones (1961) showed that presence of wax the surface of growing leaf but it is being removed by natural brushing or weathering. In case of aging leaves, the age and growth stage may be responsible for difference in physiological aspects of the plant and the leaf. This may result in changes in the nature of leaf exudates and as a consequence of which the microflora of phyllosphere may change.

Ruinen (1961) found that, phyllosphere population became maximum at leaf maturity. He further observed that, as the leaf became older the microbial population also became progressively autonomous by mutual adjustment among themselves as the exudates decreased and ultimately leaf became simply a mechanical support. Plant species, age and health of the plant, season, climatic factors, location, agricultural operations like fertilizer applications, pest and disease control, all these factors cause a wide variation in quantitative and qualitative nature of phyllosphere microflora (Dickinson *et al.* 1976, Khara and Singh1, 1981, Kothandaraman, 1984).

Material and methods

Sample collection

Samples were collected from the different localities and different fields of cotton, from Marathwada region. Sample brought in the laboratory of plant pathology, Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Sample were randomly collected at different time interval from different fields and used for the isolation by different methods.

Phyllosphere mycoflora of cotton

Study of phyllosphere mycoflora by leaf impression method

This method was useful for superficial fungal spores. At the time of isolation fresh leaf were taken and pressed from its dorsal surface momentarily against the agar surface of petriplates at three places. Some leaf was placed from ventral surface against the agar surface in the same way like the first. Same procedure was repeated for other leaf samples. Inoculations of plates were carried out at 26 °C in an inverted position for 7 days.

Leaf washing method

Standard washing method for isolating microflora of leaf is used. The process involved the cutting of 3 mm diameter uniform discs at random from leaf surfaces samples and washing them in 2-3 changes of sterile water. Aliquots of the final washing were plated out with tap water agar and

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incubated to determine the efficacy of washing process. The results were taken after 3 days of incubation.

Results and Discussion

In the present investigation direct method includes isolation were done. In this study, 16 dominating as well as pathogenic fungal species were selected from intensive screening; i.e. *Alternaria alternata, Alternaria macrospora, Aspergillus flavus, Aspergillus niger, Cercospora gossypina, Colletotrichum gossypii, Curvularia lunata, Drechslera tetramera, Helminthosporium gossypii, Penicillium notatum, Penicillium chrysogenum, Rhizoctonia solani, Stemphylium solani, Rhizopus oryzae, Trichoderma harzianum and Trichoderma viride.* Shamsi et al (2014) reports the nine fungal species from phyllosphere of *Clitoria ternatea* i.e. *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium oxysporum, Colletotrichum gloeosporoides, Curvularia lunata, Fusarium oxysporum, Penicillium sp.* . Chikere and Azubuike, (2014) also shows Phyllospheric microbial composition of guava, hibiscus, mango and pumkin, they reported 13 fungal species belonging to five genera from this vegetables and fruits; i.e. *Aspergillus, Fusarium, Mucor, Penicillium* and *Rhizopus oryzae.* In the present study comparative analysis of Phyllospheric mycoflora of conventional and organic cotton fields at different crop age were shown. From the (table no. 1) fungal population is higher in organic cotton field than the conventional cotton fields at 30 day old plants; whereas the populations of pathogenic fungi were higher in the conventional cotton fields as compare to organic cotton fields. (Zhang et al, 2010) shows the comparative analysis of phyllosphere microbial community between various vegetable crop plants. They show the rate of microbial community in spinach and rape is higher than the celery, broccoli and cauliflower. Majorly saprophytic fungi were dominated in the organic cotton field as compare to pathogenic fungi. (Schmid *et al*, 2011) reported impact of organic farming the number of *in vitro* antagonists was enhanced due to an enrichment of *Aureobasidium pullulans* in grapevine. *Drechslera tetramera* and *Rhizoctonia solani* shows highest percentage of population 6.8% and 5.1% whereas *Rhizopus oryzae, Aspergillus flavus* and *Penicillium notatum shows* highest percentage of populations in organic fields 8.5, 6.8 and 6.8 % respectively in 30 days old cotton leaf sample.

Percent frequency of phyllosphere fungal population of conventional and organic cotton fields after 30 days

Name of fungi	Conventional cotton fields	Organic cotton fields
Alternaria alternata	1.7	1.7
Alternaria macrospora.	3.4	1.7
Aspergillus flavus	5.1	6.8
Aspergillus niger	1.7	0.0
Cercospora gossypina	1.7	1.7
Colletotrichum gossypii	5.1	0.0
Curvularia lunata	3.4	0.0
Drechslera tetramera	6.8	1.7
Helminthosporium gossypii	3.4	0.0
Penicillium notatum	0.0	6.8
Penicillium chrysogenum	1.7	1.7
Rhizoctonia solani	5.1	0.0
Stemphylium solani	3.4	0.0
Rhizopus oryzae	0.0	8.5
Trichoderma harzianum	0.0	5.1
Trichoderma viride	1.7	5.1

Percent frequency of phyllosphere fungal population of conventional and organic cotton fields after 60 days

Name of fungi	Conventional cotton field	Organic cotton field
Alternaria alternata	3.5	2.6
Alternaria macrospora.	5.2	1.3
Aspergillus flavus	1.7	2.6
Aspergillus niger	3.5	1.3
Cercospora gossypina	3.5	1.3
Colletotrichum gossypii	5.2	2,6
Curvularia lunata	1.7	1.3
Drechslera tetramera	0.0	6.7
Helminthosporium gossypii	8.7	0.0
Penicillium notatum	1.7	3.0
Penicillium chrysogenum	0.0	6.7
Rhizoctonia solani.	5.2	0.0
Stemphylium solani	3.5	1.3
Rhizopus oryzae	0.0	6.7
Trichoderma harzianum	0.0	5.3
Trichoderma viride	1.7	3.0

Helminthosporium gossypii show highest percent of population in conventional cotton field at 60 day old plants which is absent in organic cotton field at the age of 60 days of plant; whereas Drechslera tetramera were dominated at the age of 30 days (6.8%) in conventional cotton fields, which is completely existed in 60 as well as 90 day old plants (table 2 and table 3). Mishra and Srivastava (1971) studied the leaf surface fungi they found different dominant fungal species at seedling, preflowering and at preharvest stages in the phyllosphere. *It shows interesting results in organic cotton fields, it gradually increases with age of the crop (table 2). In conventional cotton fields Alternaria sps, Rhizoctonia solani and Stemphyllium solani gradually increases as the crop age, whereas in organic cotton field their population shows irrespectiveness.* Sharma and Sinha (1968), in a study of phyllosphere of sorghum, reported that the age of leaf affects the mycoflora population frequency and abundance. *Contrasting results were shown by Trichoderma sps in organic as well as conventional cotton fields. It shows consistent higher population in organic cotton fields at various ages of the plants whereas in conventional fields its shows negligible presence as compare to organic cotton fields (Table 1,2,3).*

Percent frequency of phyllosphere fungal population of conventional and organic cotton fields after 90 days

Name of fungi	Conventional cotton field	Organic cotton field
Alternaria alternata	3.3	0.9
Alternaria macrospora.	6.7	1.8
Aspergillus flavus	0.0	6.4
Aspergillus niger	1.7	5.5
Cercospora gossypina	5.5	0.0
Colletotrichum gossypii	4.4	1.8
Curvularia lunata	4.4	0.0
Drechslera tetramera	0.0	6.9
Helminthosporium gossypii	5.5	0.0
Penicillium notatum	1.1	8.3
Penicillium chrysogenum	2.2	5.6
Rhizoctonia solani.	6.6	1.8
Stemphylium solani	3.3	0.0
Rhizopus oryzae	1.1	7.4
Trichoderma harzianum	0.0	6.5
Trichoderma viride	1.1	5.5

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Conclusion

From the study and results it is concluded that the fungal population were higher in the organic cotton fields as compare to conventional fields. The frequency of fungi has been raises as increase in the plant age. Highest percentages of frequency were observed in 60 days old plant.

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