International Journal of Applied Research 2016; 2(8): 439-443

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(8): 439-443
www.allresearchjournal.com
Received: 05-06-2016
Accepted: 06-07-2016

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Qualitative and quantities analysis of rhizosphere mycoflora of conventional and organic farming

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Abstract
Soil microbial diversity and activity of microbes play an important role in plant health and soil quality. Functional microbiological analysis of the rhizosphere has given new insights into the role of microbial communities in plant nutrition and plant protection against diseases. The diversity of microbes associated with plant roots is enormous. This complex plant-associated microbial community is decisive for plant health. Recent advances in plant microbe interactions research revealed different cultural practices also influence on their rhizosphere microbiome, as evidenced by the fact that different microbial communities when grown on the same soil. For this study 3 nearby locations of conventionally and organically growing cotton fields selected. During this study major dominating and commonly occurring 22 pathogenic as well as non pathogenic like *Alternaria alternata, Alternaria macrospora, Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Cladosporium macrocarpum, Colletotrichum gossypii, Curvularia lunata, Fusarium moniliforme, Fusarium oxysporum, Penicillium chrysogenum, Penicillium notatum, Phoma exigua, Pyhium spp., Rhizoctonia solani, Rhizopus oryzae, Sclerotium rolfsii, Trichoderma asperellum, Trichoderma harzianum, Trichoderma viride* and *Verticillium dahlia* were occur qualitatively and quantitatively.

Keywords: Rhizosphere, mycoflora, cotton

1. Introduction
Rhizosphere is the region of soil which is the surrounds root of plant. Rhizospheric mycoflora is definitely distinct from the bulk soil mycoflora. Rhizosphere is the two distinct phenomenons. Rhizosphere mycoflora is defined as the fungi present in the surrounding soil of roots are termed as Rhizosphere whereas the fungi present in the surrounding soil of root is termed as the Rhizospheric mycoflora. The phenomenon of accumulation of microorganisms around the root zone was reported by a number of earlier workers (Agnihothrudu 1955; Starkey 1958; Rouatt 1957; Parkinson 1957) [31, 26]. Various compounds such as amino acids, vitamins, sugars, tannins etc. are exuded by the roots can contain up to 1011 microbial cells per gram root Egamberdieva, D. *et al*. (2008) [10] and more than 30,000 prokaryotic species Mendes R. *et al*. (2011) [18]. Green revolution was based on crops quick to respond to soil fertility. This influencing use of synthetic fertilizers and plant protection chemicals these synthetic chemicals plays pivotal role for increase the soil fertility and disease control but in other hand these are affecting the mycobiota of soil. Soil is a most valuable natural source providing the most diverse habitat to the microorganisms. Microbial populations in soil is of fundamental importance for ecosystem functioning in both conventional (managed agricultural) and organic (natural) soils (O'Donnell *et al*. 1994; Doran and Zeiss 2000) [20, 9] because they involve and plays pivotal role in all natural processes like nutrient cycling, decomposition of organic matter, toxic removal and soil structure formation (Van Elsas, 1997; Doran and Zeiss, 2000) [36, 9]. The community of soil flora and fauna is influenced directly or indirectly by management practices, e.g. cultivation and the use and application of organic and inorganic fertilisers (Bloem *et al*. 1994; Matson *et al*., 1997) [6, 17]. The presence of potentially toxic compounds, low availability of essential minerals and pathogens in the soil often restrict crop production Song, Y.Y. *et al*. (2010) [29], Shoresh, M. *et al*. (2010) [30].

Number of studies show that organic farming leads to higher soil quality and more biological activity than conventional farming (Droogers and Bouma, 1996; Mader *et al*. 2002; Girvan *et al.,* 2004) [8, 16, 12]. Microbial population size and community structure are sensitive to changes in chemical properties of the surrounding soil (Pansombat *et al.,* 1997; Tokuda and Hayatsu, 2002) [21, 34]. It was shown use of synthetics affect the microbiota Further, considerable evidence indicates that changes in the composition of a microbial community can be used to predict and dictate alteration in soil quality (Van Brugen and Semenov, 2000; Breure, 2005) [37, 4]. Comparison between conventional and organic farms had founded that organic methods improve the fertility and overall health of the soil (Mader *et al.* 2002; Fliessbach *et al.* 2007; Birkhofer *et al.* 2008) [16, 7, 5]. More investigations on the effect of fertilization showed that in farming systems with regular application of organic manure, higher concentrations of soil microbial biomass and diversity were observed in comparison to the systems where mineral fertilizers were used (Lundquist *et al.* 1999; Mader *et al.* 2002; Hartmann *et al.* 2006; Widmer *et al.* 2006) [15, 16, 14, 38]. Considering all these aspect present study was undertaken to focus a light on the diversity and abundance of fungal species to expose the distribution and diversity with special reference to fungi of rhizosphere of cotton fields.

2. Materials and Methods

2.1 Collection of rhizosphere soil sample

Rhizosphere soil samples were collected from each selected Cotton plot of Conventional and Organic fields**,** the rhizosphere soils from the root zones of the plants were collected randomly and mixed together to obtain a homogenous soil sample. Composite soil samples from the plot of Conventional field and Organic cotton fields were further assessed for their fungal population. The soil samples were collected from the surface of underground roots and their surroundings. Surface sterilized scalpel was used to transfer rhizosphere soil to clean sterilized glass bottles and the bottles were sealed carefully. Same method was adapted to remaining cotton fields from each plot.

Organic Cotton fields were taken and survey was done to select the convenient farmers from Organic Grower Group (OGG), three locations (L1, L2 and L3) were selected from nearby to Aurangabad (Maharashtra) and from each location two organic Cotton fields were finalized. Also from nearby locations two conventional Cotton fields were selected. Soil samples were collected from these selected localities at different stages of Cotton crop.

2.2 Isolation of rhizosphere mycoflora by serial dilution agar plate method

Dilution plate method (Waksman, 1922) was used for isolation of rhizosphere mycoflora of cotton crop. For the isolation of fungi serial dilution factor 10^{-4} was used.

Soil samples were collected randomly from rhizosphere of cotton plants. Composite samples from each plot were used for fungal analysis during after each 15 days of growth stages. At the time of serial dilution labeled the dilution blank, as 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} marked with marker pencil. Soil sample suspension was added in three sterile poured petriplates. The inoculated petriplates were incubated in inverted position at room temperature 27 °C ± 2 for 2 -7 days.

2.3 Selection of media for isolation

During investigation usually Rose Bengal and Potato Dextrose Agar (PDA) medium were used for the isolation and maintenance of pure cultures.

2.4 Identification of fungal forms

Astana and Hawker's medium 'A' was used to maintain stock cultures. Isolated fungal forms were identified on the basis of available literature, including manuals and monographs as "A manual of the Aspergilli, by Thom and Raper (1945) [35]. "A manual of soil fungi" by Gilman, (1959) [11]. Illustrated genera of imperfect fungi" by H. L. Barnet and Bary B. Hunter (1965) [3]. "The Illustration of fungi" by Mukadam *et al*. (2006) [19]. The microphotographs and micro measurements were taken for every isolated fungi.

2.5 Counting of fungal colonies

Fungal colonies formed were calculated on per gram dry soil basis. Pure cultures of fungi were maintained in test tubes slants containing Czapex Dox agar medium (Raper and Thom 1949) and preserved in deep freezer at 20 ˚C. After 4 days of incubation numbers of distinct colonies were counted by using colony counter. The number of fungi per ml of original suspension was calculated. Distinguishing colonies were picked up and subculture on appropriate media. The subcultures were maintained for further observations on selected media.

3. Result and Discussion

During the *kharip* season study was carried out. In present study of fungal population as well as fungal occurrence 21 dominant and pathogenic as well as saprophytic fungal species *viz*. *Alternaria alternata, Alternaria macrospora, Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Cladosporium macrocarpum, Colletotrichum gossypii, Curvularia lunata, Fusarium moniliforme, Fusarium oxysporum, Penicillium chrysogenum, Penicillium notatum, Phoma exigua, Pyhium spp., Rhizoctonia solani, Rhizopus oryzae, Sclerotium rolfsii, Trichoderma asperellum, Trichoderma harzianum, Trichoderma viride and Verticillium dahlia* were observed in the rhizosphere of cotton.

Table 1: Percent frequency of fungal population ($X10^{-4}$) in rhizosphere of conventional and organic cotton fields

Fungi isolated	Conventional cotton field			Organic cotton field		
	L1	L2	L3	L1	L2	L3
Alternaria alternata	1.42	2.27	1.28	1.22	1.11	0.00
Alternaria macrospora	2.86	1.14	2.56	1.22	1.11	1.09
Aspergillus flavus	0.00	3.41	2.56	3.66	3.33	2.17
Aspergillus niger	1.42	2.27	3.85	2.44	1.11	3.26
Aspergillus terreus	0.00	1.14	1.28	1.22	2.22	1.09
Cladosporium macrocarpum	2.86	1.14	0.00	1.22	1.11	1.09
Colletotrichum gossypii	2.86	1.14	1.28	1.22	1.11	2.17

Curvularia lunata	0.00	2.27	1.28	0.00	0.00	1.09
Fusarium moniliforme	1.42	2.27	2.56	1.22	1.11	1.09
Fusarium oxysporum	2.86	1.14	1.28	1.22	0.00	0.00
Penicillium chrysogenum	1.42	0.00	0.00	1.22	1.11	2.14
Penicillium notatum	1.92	1.14	2.56	2.44	2.22	0.00
Phoma exigua	1.42	2.22	0.00	2.44	0.00	1.09
Pythium spp.	1.42	1.14	1.28	0.00	0.00	1.09
Rhizoctonia solani	1.42	2.27	1.28	1.22	2.22	1.14
Rhizopus oryzae	0.00	3.41	1.28	1.22	4.44	2.17
Sclerotium rolfsii	1.42	2.27	0.00	0.00	3.33	1.09
Trichoderma asperellum	0.00	0.00	1.28	2.44	1.11	1.09
Trichoderma harzianum	1.42	1.14	1.28	3.66	4.44	3.26
Trichoderma viride	1.42	0.00	0.00	1.22	3.33	1.09
Verticillium dahlia	0.00	1.14	1.28	1.22	0.00	0.00

The soil fungal community in species composition of the in particular variants of fertilization appeared to be more diversified. The quantitatively more fungal count was observed in organic cotton field. In soils subjected to organic fertilization, Shannon *et al*. (2002) [28] reported that long term positive influence of organic farming on soil quality and microbial activity in comparison with conventional farming. Ritz *et al*. (1997) [25] reported bacteria, actinomycetes and fungi were increase in the count due to enhanced biological activity in soil with an prominent soluble C in soil, lesser amount with that of N. particularly favourable for changes affecting an increase in the population of beneficial microflora as specific, biological protection of plants against pathogens Widner *et al*. (1998) [40], Szczech (1999) [33]. In table no. 1 *Curvularia lunata* shows the dominance in conventional field as compare to organic field. A reduction was observed in the count of pathogens in the soil upon fertilization with organic as compared to the conventional cultural practice with synthetic fertilization Gorodecki and Hadar (1990) [13] confirmed the inhibiting effect of fertilization with farm manure on the growth of *S. sclerotiorum*, and that of *R. solani*. Admir Araujo *et al*. (2010) [1], confirmed that organic input increases the soil microbial biomass. It was clear from the table quantitatively and qualitatively fungal population as well as fungal occurrence of saprophytic fungi like *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Trichoderma asperellum, Trichoderma harzianum, Trichoderma viride, Penicillium chrysogenum, Penicillium notatum, Phoma exigua* and *Rhizopus oryzae,* some of them are showing antagonistic action against pathogens shown more in organic field. Similar results in phyllosphere Mane and Chavan (2015) [27]. *Trichoderma* genus was quantitatively and qualitatively dominated fungal population as well as fungal occurrence in organic field.

Table 2: Percent frequency of fungal occurrence in rhizosphere of conventional and organic cotton fields

Fungi isolated	Con			Frequency of occurrence	Org			Frequency of occurrence
	L1	L2	L3		L1	L2	L3	
Alternaria alternata	+	+	+	99.99	+	+	-	66.66
Alternaria macrospora	+	+	+	99.99	+	+	+	99.99
Aspergillus flavus	-	+	+	66.66	+	+	+	99.99
Aspergillus niger	+	+	-	66.66	+	+	+	99.99
Aspergillus terreus	-	+	+	66.66	+	+	+	99.99
Cladosporium macrocarpum	+	+	-	66.66	+	+	+	99.99
Colletotrichum gossypii	+	+	+	66.66	+	+	+	99.99
Curvularia lunata	-	+	+	66.66	-	-	+	33.33
Fusarium moniliforme	+	+	+	99.99	+	+	+	99.99
Fusarium oxysporum	+	+	+	99.99	+	-	-	33.33
Penicillium chrysogenum	+	-	-	33.33	+	+	+	99.99
Penicillium notatum	+	+	+	99.99	+	+	-	66.66
Phoma exigua	+	+	-	66.66	+	-	+	66.66
Pythium spp.	+	+	+	99.99	-	-	+	33.33
Rhizoctonia solani	+	+	+	99.99	+	+	+	99.99
Rhizopus oryzae	-	+	+	66.66	+	+	+	99.99
Sclerotium rolfsii	+	+	-	66.66	-	+	+	66.66
Trichoderma asperellum	-	-	+	33.33	+	+	+	99.99
Trichoderma harzianum	+	+	+	99.99	+	+	+	99.99
Trichoderma viride	+	-	-	33.33	+	+	+	99.99
Verticillium dahlia	-	+	+	66.66	+	-	-	33.33

4. Conclusion

During the present investigation pathogenic fungi like *Alternaria alternata, Alternaria macrospora, Cercospora gossypina, Colletotrichum gossypii, Curvularia lunata* and *Rhizoctonia solani* shows highest percentage of occurrence in conventional cotton field. Where saprophytic fungal genera like *Trichoderma, Penicillium, Rhizopus, Aspergillus* dominated. From the study and results it is concluded that the fungal population qualitative composition of the soil fungi were a greater extent than its quantitative structure. were superior in the organic cotton fields as compare to conventional fields. The dominancy of saprophytic fungi in organic cotton field was observed. Significant positive correlations between fungal populations were observed in all

the organic fields. The organic matter level in the organically managed soil systems can play a pivotal role in fungal growth, sporulation and diversity.

5. Acknowledgment
Authors are graceful to Dr. Ashok M. Chavan for providing valuable guidance and required information to this work and also thankful to Head Department of Botany Dr Babasaheb Ambedkar Marathwada University, Aurangabad for giving facilities and support this research work.

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