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Influence of Organic and Inorganic Inputs on Non-Rhizosphere Mycoflora Population and Species Diversity of Cotton Field

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Abstract: ***In present investigation a field experiment was conducted in a cotton field during 2010-2013, to study the effect of various organic inputs and inorganic inputs on Non-rhizosphere mycoflora population and species diversity in cotton field. The mycoflora population and diversity was studied by using serial dilution technique. Result observed that application of organic inputs like Farm yard manure, Beejamruth and Jeevamruth increases Non-rhizosphere mycoflora population and species diversity in organic field of cotton. The application of inorganic inputs lowers the Non-rhizosphere mycoflora population and species diversity compare to organic field of Cotton. The total 27 mycoflora species were isolated and identified from Non-rhizosphere of organic field and a total 23 mycoflora species from Non-rhizosphere of inorganic field. The isolated mycoflora species belongs to genera Aspergillus, Cephalosporium, Cladosporium, Curvularia, Penicillium, Trichoderma, Fusarium, Rhizopus, Cladosporium and Mucor in both organic and inorganic field. The species like Alternaria brassicae, Chaetomium globosm, Trichoderma Koningii, Drechslera bicolar, Drechslera tetramera and Helminthosporium spp. are found in Non-rhizosphere of organic field. Overall result shows organic inputs like Farm yard manure, Beejamruth and Jeevamruth increases mycoflora diversity which helpful for maintenance of soil fertility for sustainable development.***

Keywords: Cotton, Non-Rhizosphere, mycoflora, Diversity, Organic and Inorganic inputs.

1. Introduction

The changes in soil microbes depends on type of plant, age of plant, soil physico-chemical parameters, nature of root exudation, type of agricultural inputs mainly fertilizers and different environmental conditions. In modern agriculture there is indiscriminate use of inorganic fertilizer to increase the production. But various results revealed that over use of chemical fertilizers has been shown to have a direct effect on the composition of the soil microbial community (Katayama *et al*., 1998). It is Reported that application of Chemical fertilizer (Urea) @ 300 kg per ha to wheat field decreases the microbial population (Kumar *et al.*, 2010). The application of commonly used herbicides on non-target soil of maize field resulted in decreases in microbial counts (Ayansina and Oso, 2006). The lower fungal diversity may be due to there are many disturbances such as irrigation, fertilizer and agricultural practices (Yadav, 2014). It is found that 1% of the pesticides applied may contact the target organisms and remainder moves into the soil, thereby soil flora and fauna may be adversely affected (Misra and Mani, 1994). Inorganic treatment lowers the microbial population **(**Kapoor *et al.*, 2015).

To minimize the adverse impact of chemical fertilizer now a day's farmers using various organic inputs for sustainable and eco-friendly development. Various comparative research on soil microflora population under different organic and inorganic inputs applied field revealed that addition of organic manure as an organic fertilizer rich in bacterial diversity, fungal diversity and other number of microorganisms compared to inorganic field (Ishaq and Khan, 2011). It is recorded that highest fungal population in treatment of FYM 40.6X 10 4 g^{-1} compared to urea treatment 38.8X10 4 g $^{-1}$ (Raindra *et al.*, 2010). Organic fertilizers to soybean variety increases the microbial population compared to NPK and control (Das and Dkhar, 2010). A 10-26% increase in microbial biomass under organic management was reported (Fraser *et al.*, 1994).

During the last few years in organic farming farmers using liquid organic inputs like Panchagavya, Dasagavya, Beejamruth, Jeevamruth, Amritpani and Sanjivak as a bio-booster to hasten microbial population. These liquid boosters contain beneficial microorganism's mostly lactic acid bacteria, yeast, actinomycets, photosynthetic bacteria, nitrogen fixers, phosphorus solublisers and fungi (Sreenivasa *et al.*, 2010). Higher rhizosphere microbial population recorded by application Panchagavya and Beejamruth (Shubha, *et al*., 2014).

The present investigation was carried out to study the effect of organic inputs (FYM, Jeevamruth and Beejamruth) and inorganic inputs on soil Non-rhizosphere mycoflora population and species diversity in Cotton field.

2. Materials and Methods

2.1. Experimental Study site

Agricultural fields of Nanded district of Maharashtra were selected for the study of Non-rhizosphere mycoflora populations and species diversity under the influence of organic and inorganic inputs applied field of Cotton during the period 2010-2013.The selected experimental organic field was supplied with farm yard manure and organic liquid inputs like Jeevamruth and Beejamruth (Palekar, 2006).The Jeevamruth applied to field crop and Beejamruth applied to seed. The inorganic field supplied with regular chemical fertilizers.

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2.2. Collection of soil samples

Non-rhizosphere soil samples were collected from organic and inorganic crop fields of cotton crop by digging out soil around the zone (25 - 40 cm away from the plant). The five samples were collected from sampling site from each selected crop field and mixed together into a single. These soil samples were collected in sterile polythene bags and brought to the laboratory.

2.3. Enumeration of soil fungal population

The Non-rhizosphere fungi were enumerated by Serial dilution method (Waksman, 1992).The collected Non-rhizosphere soil samples from both the organic and inorganic inputs applied field were used for preparation of different serial Dilutions such as $10^{-2,}$ 10^{-3}, 10^{-4}, and 10^{-5}. Then transferred 1 ml aliquots from each dilution were used to isolate fungi on Martins Rose Bengal Agar Medium, potato dextrose agar and Czapek's Dox Agar containing One percent streptomycin and plates was incubated at 28 ºC for 4-7 days for fungi. After 6 days of incubation the different colonies were counted from different organic and inorganic soil plates.

2.4. Statistical analysis

The quantitative analysis of fungal population was studied at 10^{-3} dilution. The percentage contribution of each colony forming units (CFU) of different fungal isolate was calculated by using the formula.

Mean plate count X dilution factor

CFU/ g dry soil = dry weight of soil

Total no. of CFU of an individual species X 100

Percentage contribution = Total no. of CFU of all species

2.5. Observation and Identification

The fungal morphology were studied macroscopically by observing colony features (Colour and Texture) and microscopically by staining with lacto phenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores . The microphotograph was taken for isolated species by using Magnus camera. The fungi were identified with the help of literature identification of the species (Barnett, 1975; Gilman, 2001).

3. Results and Discussion

The results on Non-rhizosphere mycoflora population and species diversity in organic and inorganic field shows there is increase in Non-rhizosphere mycoflora population and species diversity in organic inputs applied field compared to inorganic inputs applied field.

The Non-rhizosphere mycoflora population was more in organic field of crop plants during the 2010-2013 (Table.1). In organic field Non-rhizosphere Population of fungi ranged from 23.2×10^{-3} to 42.6×10^{-3}CFU/g of soil during 2010-13. In inorganic field Non-rhizosphere Population of fungi ranged from 10.4×10^{-3} to 25.6×10^{-3}CFU/g of soil. (Table.1).

Application of FYM (5 t/ha) to soybean field had significantly increased the fungi 22.21 and 27.25 CFUx10^3/g (Meena and Ghasolia, 2013). Application of panchagavya and beejamruth treatment increases rhizosphere microbial population of maize (Shubha *et al.*, 2014). The soil bacterial, fungal, actinomycetes and N fixing bacteria were more in organic fields than inorganic field (Padmavathy and Poyyamoli, 2011). Inorganic fertilizer to crop field significantly lowers the rhizosphere microbial population and diversity (Nelson and Mele, 2006).

The results on soil Non-rhizosphere mycoflora species colonies, species number and percentage contribution in organic field of Cotton during 2010-13 are presented in table 2, 3 and 4.Results revealed that during 2010-11 the total 527 mycoflora colonies of different 18 species isolated from Non-rhizosphere. During 2011-12 the total 631 mycoflora colonies of different 21 species isolated from Non-rhizosphere. During 2012-13 the total 718 mycoflora colonies of different 23 species isolated from Non-rhizosphere of organic field.

The results on soil Non-rhizosphere mycoflora species colonies, species number and percentage contribution in inorganic field of Cotton during 2010-13 are presented in table 5, 6 and 7. Results revealed that during 2010-11 the total 383 mycoflora colonies of different 17 species isolated from Non-rhizosphere. During 2011-12 the total 312 mycoflora colonies of different 16 species isolated from Non-rhizosphere. During 2012-13 the total 286 mycoflora colonies of different 14 species isolated from Non-rhizosphere of inorganic field of Cotton.

Overall diversity indicate that Non-rhizosphere of organic field shows more species diversity i.e. 27 mycoflora species in organic field and 23 species in inorganic field during 2010-2013.

The identified dominant Non-rhizosphere mycoflora species belongs to genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Rhizopus*, *Curvularia*, *Cladosporium* and *Mucor*. While genera like *Phoma*, *Verticillium* and *Cephalosporium are* least population diversity.

The species like *Alternaria brassicae*, *Chaetomium globosm*, *Trichoderma Koningii*, *Drechslera bicolar*, *Drechslera tetramera* and *Helminthosporium spp.* are found in Non-rhizosphere of organic field. The different fertilization changes in soil microfungal communities and fungal activities in agricultural soils (Rezacova *et al.*, 2007). It is observed that long-term effects of organic matter inputs on different cropping systems in a 10-year-old experiment enhances microbial activity (Chirinda *et al.*, 2008). It is observed that changes in frequency of mycoflora in agricultural fields are due to several factors like temperature, humidity, vegetation, organic and

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inorganic materials, soil type and texture (Gaddeyya *et al.*, 2012).

Overall results revealed that there is monthly and yearly variation in Non-rhizosphere total colonies and species diversity in organic and inorganic field of Cotton. The organic field shows more Non-rhizosphere population and species diversity compared to inorganic field.

4. Conclusion

The organic inputs like farm yard manure, Jeevamruth and Beejamruth increases the soil beneficial mycoflora population and species diversity compared to inorganic inputs applied field which adversely affect mycoflora diversity. It is due to organic inputs contains essential soil nutrient and microbial load which results increase in mycoflora population.

The Increase in soil mycoflora diversity enhances nutrient availability to crop ultimately increases growth and productivity of crop plants. From this result we can conclude that organic liquid manure can be used for increase in microbial population and species diversity for sustainable eco-friendly development.

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Table 1: Population of Non-Rhizosphere mycoflora (x 10^{-3} CFU/g soil) in organic and Inorganic field of cotton

Sr. No	*Months*	*Organic field*			*Inorganic field*		
		2010-2011	2011-2012	2012-2013	2010-2011	2011-2012	2012-2013
1	Jun	31.2	33.6	37.2	20.8	18.4	17.6
2	July	24.8	28.8	32.6	14.8	16.0	18.5
3	Aug	26.4	31.6	36.3	17.6	15.2	16.3
4	Sep	23.2	27.2	31.6	19.2	10.4	15.4
5	Oct	27.6	34.4	38.5	25.2	15.2	13.6
6	Nov	32.0	36.8	42.6	22.4	11.5	14.2
7	Dec	35.2	33.6	39.8	24.8	21.6	18.7
8	Jan	38.0	28.8	33.2	25.6	23.0	17.6
	Average	29.80	31.85	36.47	21.30	16.41	16.48
	S.D	±5.184	±3.325	±3.830	±3.923	±4.434	±1.934

Table 2: Monthly variation in soil Non-rhizosphere mycoflora and percentage contribution in organic field of cotton (2010-2011)

Sr. No	*Mycoflora*	*Months and No. of colonies*								*Total colonies*	*% Contri.*
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan		
1	*Alternaria solani*	5	7	6	0	0	4	3	0	25	4.74
2	*Aspergillus flavus*	8	9	10	11	15	13	14	16	96	18.21
3	*Aspergillus fumigates*	8	7	0	8	8	8	6	0	45	8.53
4	*Aspergillus nidulans*	7	6	7	5	6	7	7	7	52	9.86
5	*Aspergillus niger*	10	8	8	9	8	9	10	8	70	13.28
6	*Cephalosporium sp.*	0	0	1	0	0	0	2	0	3	0.56
7	*Clado. cladosporides*	3	0	0	0	0	3	4	0	10	1.89
8	*Cladosporium herbarum*	0	0	5	5	7	5	3	5	30	5.69
9	*Curvularia lunata*	0	0	0	0	5	5	4	0	14	2.65
10	*Drechslera bicolar*	0	3	0	0	0	3	3	2	11	2.08
11	*Fusarium oxysporum*	5	4	0	5	6	4	5	6	35	6.64
12	*Helminthosporium sp.*	4	0	3	0	4	5	3	5	24	4.55
13	*Mycelia sterilia (white)*	0	0	0	1	0	0	0	0	1	0.18
14	*Penicillium citrinum*	6	6	4	4	5	0	4	3	32	6.07
15	*Rhizoctonia solani*	0	4	0	0	0	4	3	4	15	2.84
16	*Rhizopus stolonifer*	4	5	0	0	0	6	4	4	23	4.36
17	*Trichoderma koningii*	0	0	4	0	5	4	4	0	17	3.22
18	*Trichoderma viride*	5	0	5	4	0	0	6	4	24	4.55
	Total No. of colonies	**65**	**59**	**53**	**52**	**69**	**80**	**85**	**64**	**527**	**100**
	Total No. of species.	**11**	**10**	**10**	**9**	**10**	**14**	**17**	**11**		

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Table 3: Monthly variation in soil Non-rhizosphere mycoflora and percentage contribution in organic field of cotton (2011-2012)

Sr. No	Mycoflora	Months and No. of colonies								Total colonies	% Contri.
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan		
1	*Alternaria solani*	8	7	9	0	0	7	6	0	37	5.86
2	*Aspergillus flavus*	12	14	12	13	13	12	14	18	108	17.11
3	*Aspergillus fumigates*	8	0	0	0	6	4	5	0	23	3.64
4	*Aspergillus nidulans*	7	7	6	5	5	7	4	6	47	7.44
5	*Aspergillus niger*	9	9	8	9	9	10	9	12	75	11.88
6	*Cephalosporium sp.*	0	0	3	0	0	0	1	3	7	1.10
7	*Chaetomium globosm*	0	0	0	4	6	4	0	2	16	2.53
8	*Clado. cladosporides*	5	0	0	5	6	5	3	3	27	4.27
9	*Curvularia lunata*	6	0	0	5	5	6	3	4	29	4.59
10	*Curvularia polyscens*	6	6	8	4	10	6	5	3	48	7.60
11	*Drechslera tetramera*	0	0	0	0	2	4	3	4	13	2.06
12	*Fusarium oxysporum*	0	5	0	3	7	4	4	5	28	4.43
13	*Fusarium roseum*	6	5	4	0	4	5	6	4	34	5.38
14	*Mucor sp.*	6	0	4	0	0	0	4	2	16	2.53
15	*Mycelia sterilia (white)*	0	0	0	0	0	0	0	1	1	0.15
16	*Penicillium citrinum*	8	6	7	6	6	5	0	5	43	6.81
17	*Phoma sp.*	0	0	3	0	2	0	3	0	8	1.26
18	*Rhizoctonia solani*	0	3	0	0	0	2	3	0	8	1.26
19	*Rhizopus stolonifer*	5	6	0	5	0	6	3	4	29	4.59
20	*Trichoderma viride*	4	0	5	5	0	5	6	0	25	3.96
21	*Verticillium sp.*	0	4	0	0	3	0	2	0	9	1.42
	Total No. of colonies	**90**	**72**	**69**	**64**	**84**	**92**	**84**	**76**	**631**	**100**
	Total No. of species.	**12**	**11**	**11**	**11**	**14**	**16**	**18**	**15**		

Table 4: Monthly variation in soil Non-rhizosphere mycoflora and percentage contribution in organic field of cotton (2012-2013)

Sr. No	Mycoflora	Months and No. of colonies								Total colonies	% Contri.
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan		
1	*Alternaria brassicae*	0	0	0	0	3	0	4	4	11	1.53
2	*Alternaria solani*	7	14	8	6	4	7	5	0	51	7.10
3	*Aspergillus flavus*	10	14	15	11	16	14	16	18	114	15.87
4	*Aspergillus fumigates*	9	5	0	0	5	5	5	5	34	4.73
5	*Aspergillus nidulans*	6	6	8	8	3	5	7	4	47	6.54
6	*Aspergillus niger*	8	6	9	9	10	8	12	9	71	9.88
7	*Cephalosporium sp.*	0	0	4	3	0	4	2	3	16	2.22
8	*Chaetomium globosm*	0	5	0	0	0	6	5	6	22	3.06
9	*Clados. cladosporides*	5	0	0	6	3	2	6	5	27	3.76
10	*Curvularia lunata*	0	0	0	4	4	6	4	3	21	2.92
11	*Curvularia polyscens*	4	6	9	5	10	6	5	0	45	6.26
12	*Drechslera tetramera*	0	0	0	0	0	4	3	3	10	1.39
13	*Fusarium oxysporum*	6	6	0	5	6	4	4	5	36	5.01
14	*Fusarium roseum*	6	0	5	0	5	6	3	2	27	3.76
15	*Mucor sp.*	8	0	7	0	0	0	0	0	15	2.08
16	*Mycelia sterilia (black)*	0	0	1	0	0	0	1	0	2	0.27
17	*Mycelia sterilia (white)*	0	0	0	0	0	0	1	0	1	0.13
18	*Penicillium citrinum*	7	8	7	7	6	7	5	4	51	7.10
19	*Phoma sp.*	0	0	3	2	2	0	0	0	7	0.97
20	*Rhizoctonia solani*	2	2	0	0	5	5	0	2	16	2.22
21	*Rhizopus stolonifer*	5	6	0	3	6	4	5	5	34	4.73
22	*Trichoderma viride*	8	0	8	7	8	6	4	5	46	6.40
23	*Verticillium sp.*	0	4	3	0	0	3	4	0	14	1.94
	Total No. of colonies	**91**	**82**	**87**	**76**	**96**	**102**	**101**	**83**	**718**	**100**
	Total No. of species.	**15**	**12**	**13**	**13**	**16**	**18**	**20**	**16**		

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Table 5: Monthly variation in soil Non- rhizosphere mycoflora and percentage contribution in inorganic field of cotton (2010-2011)

Sr. No	Mycoflora	Months and No. of colonies								Total colonies	% Contri.
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan		
1	*Alternaria solani*	6	4	5	6	4	4	3	0	32	8.35
2	*Aspergillus flavus*	7	9	10	7	10	10	8	11	72	18.79
3	*Aspergillus fumigatus*	6	5	0	7	6	0	4	3	31	8.09
4	*Aspergillus niger*	8	4	7	6	7	8	7	5	52	13.57
5	*Cephalosporium sp.*	0	0	0	0	0	0	3	4	7	1.82
6	*Clados. cladosporides*	0	0	0	0	3	5	4	5	17	4.43
7	*Curvularia lunata*	0	0	0	0	4	3	2	3	12	3.13
8	*Fusarium oxysporum*	3	4	0	3	4	3	4	3	24	6.26
9	*Mucor sp.*	6	0	5	0	0	0	2	0	13	3.39
10	*Mycelia sterilia (brown)*	0	1	0	0	2	0	0	0	3	0.78
11	*Mycelia sterilia (white)*	0	0	2	1	1	0	0	0	4	1.04
12	*Penicillium citrinum*	4	4	4	0	5	0	3	4	24	6.26
13	*Phoma sp.*	0	0	3	3	0	0	0	0	6	1.56
14	*Rhizoctonia solani*	3	2	4	0	5	5	3	0	22	5.74
15	*Rhizopus stolonifer*	4	3	0	4	5	6	4	4	30	7.83
16	*Trichoderma viride*	3	0	3	4	5	0	4	0	19	4.96
17	*Verticillium sp.*	2	3	3	0	0	4	3	0	15	3.91
	Total No. of colonies	**52**	**39**	**46**	**41**	**61**	**48**	**54**	**42**	**383**	**100**
	Total No. of species.	**11**	**10**	**10**	**9**	**13**	**9**	**14**	**9**		

Table 6: Monthly variation in soil Non- rhizosphere mycoflora and percentage contribution in inorganic field of cotton (2011-2012)

Sr. No	Mycoflora	Months and No. of colonies								Total colonies	% Contri.
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan		
1	*Alternaria solani*	4	5	3	0	3	3	2	0	20	6.41
2	*Aspergillus flavus*	7	8	8	4	5	7	7	5	51	16.34
3	*Aspergillus fumigatus*	4	2	0	0	2	0	4	4	16	5.12
4	*Aspergillus nidulans*	5	6	7	5	4	6	5	3	41	13.14
5	*Aspergillus niger*	6	5	5	6	3	5	5	4	39	12.50
6	*Cephalosporium sp.*	0	2	4	0	2	0	0	2	10	3.20
7	*Curvularia lunata*	0	0	4	3	4	4	3	3	21	6.73
8	*Fusarium oxysporum*	0	4	0	3	3	3	4	4	21	6.73
9	*Mucor sp.*	3	0	3	0	0	0	3	0	9	2.88
10	*Mycelia sterilia (brown)*	2	0	0	0	0	0	0	0	2	0.64
11	*Mycelia sterilia (white)*	0	0	0	0	0	1	0	0	1	0.32
12	*Penicillium citrinum*	0	4	5	4	2	0	3	3	21	6.73
13	*Phoma sp.*	0	0	2	2	3	0	0	0	7	2.24
14	*Rhizoctonia solani*	5	3	0	0	3	3	3	0	17	5.44
15	*Rhizopus stolonifer*	3	6	0	4	0	4	2	3	22	7.05
16	*Trichoderma viride*	5	0	0	4	2	0	3	0	14	4.48
	Total No. of colonies	**44**	**45**	**41**	**35**	**36**	**36**	**44**	**31**	**312**	**100**
	Total No. of species.	**10**	**10**	**9**	**9**	**12**	**9**	**12**	**9**		

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Table 7: Monthly variation in soil Non- rhizosphere mycoflora and percentage contribution in inorganic field of cotton (2012-2013)

Sr. No	Mycoflora	Months and No. of colonies								Total colonies	% Contri.
		Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan		
1	*Alternaria solani*	3	5	3	0	0	2	4	4	21	7.34
2	*Aspergillus flavus*	9	6	6	5	6	3	6	6	47	16.43
3	*Aspergillus fumigatus*	6	4	0	0	5	0	3	0	18	6.29
4	*Aspergillus nidulans*	0	6	5	4	3	3	5	2	28	9.79
5	*Aspergillus niger*	6	5	6	4	5	2	4	3	35	12.23
6	*Cladosporium herbarum*	0	0	0	0	3	3	3	4	13	4.54
7	*Curvularia polyscens*	3	0	4	3	3	2	4	3	22	7.69
8	*Fusarium oxysporum*	0	4	0	2	3	3	4	3	19	6.64
9	*Mycelia sterilia (white)*	0	0	0	0	0	1	0	0	1	0.34
10	*Penicillium citrinum*	0	3	2	0	4	0	2	2	13	4.54
11	*Pythium middletonii*	4	3	2	2	2	3	3	0	19	6.64
12	*Rhizopus nigricans*	3	0	3	3	0	2	2	0	13	4.54
13	*Rhizopus stolonifer*	4	3	4	0	4	2	2	3	22	7.69
14	*Trichoderma viride*	5	0	3	3	0	0	4	0	15	5.24
	Total No. of colonies	**43**	**39**	**38**	**26**	**38**	**26**	**46**	**30**	**286**	**100**
	Total No. of species.	**9**	**9**	**10**	**8**	**10**	**11**	**13**	**8**		

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