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Effect of Organic Bio-Booster and Inorganic Inputs on Rhizosphere Mycoflora Population and Species Diversity of Wheat

Shaikh N.F.[1], Gachande B.D.[2]

Botany Research Laboratory and Plant Disease Clinic, N.E.S. Science College, Nanded-431605 (MS), India

Abstract: ***In present investigation an experiment was conducted in a wheat field during 2010-2013, to study the effect of various liquid organic inputs and inorganic inputs on rhizosphere mycoflora population and species diversity. The soil rhizosphere mycoflora population and diversity was studied by using serial dilution technique. Result observed that application of organic liquid bio-booster like Farm yard manure, Beejamruth and Jeevamruth enhances rhizosphere mycoflora population and species diversity in organic field. The application of inorganic inputs lowers the rhizosphere mycoflora population and species diversity compare to organic field of wheat. The total 30 mycoflora species were isolated and identified from rhizosphere of organic field and a total 24 mycoflora species from rhizosphere of inorganic field. The isolated mycoflora species belongs to genera Aspergillus, Penicillium, Trichoderma, Fusarium, Rhizopus and Cladosporium in both organic and inorganic field. The Acremonium sp., Tichoderma pseudokonigii, Glomus sp., Cladosporium herbarum and Curvularia lunata are found in rhizosphere of organic field. Overall result shows organic bio-booster like Farm yard manure, Beejamruth and Jeevamruth increases mycoflora diversity which helpful for maintenance of soil fertility for sustainable development.***

Keywords: Wheat, Rhizosphere, mycoflora, Diversity, Organic and Inorganic inputs.

1. Introduction

Soil is an important panorama for wide range of microflora which includes fungi, bacteria and actinomycetes. Microflora increases nutrient content of soil via decomposition of crop residues, mineralization and immobilization of nutrients, biological nitrogen fixation and bioturbation. Rhizosphere is a zone of soil which directly influenced by root secretions and associated soil microorganisms. It is observed that rhizosphere having greater microbial activity. The changes in rhizosphere 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; Doran *et al.,* 1996). The lower fungal diversity may be due to there are many disturbances such as irrigation, fertilizer and agricultural practices (Yadav, 2014). 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). Inorganic treatment lowers the microbial population **(**Kapoor *et al.*, 2015). 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).

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). 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, Beejamrutha, Jeevamrutha, 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 (Swaminathan, 2007; Sreenivasa *et al.,* 2010)). Higher rhizosphere microbial population recorded by application Panchagavya and Beejamrutha (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 rhizosphere mycoflora population and species diversity in Wheat field.

2. Materials and Methods

2.1 Experimental Study Site

Agricultural fields of Nanded district of Maharashtra were selected for the study of rhizosphere mycoflora populations and species diversity under the influence of organic and inorganic inputs applied field of wheat during the period 2010-2013.The selected experimental organic field was supplied with farm yard manure and organic liquid booster 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.

2.2 Collection of Soil Samples

Rhizosphere soil samples were collected from organic and inorganic crop fields of Wheat crop by digging out soil around the rhizosphere area up to 20 cm from plant to a dimension of 15 cm height and 7 cm diameter. 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 rhizosphere fungi were enumerated by Serial dilution method (Waksman, 1992).The collected 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. One percent streptomycin solution was added to the medium before pouring into petriplates for preventing bacterial growth and plates were kept for incubation 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 individual colonies of fungi were selected based on morphology and purified by inoculation on PDA and Czapek's- Dox agar plates which were incubated for 7–14 days at 28ºC. Further slants were prepared and incubated at 28ºC for 7 to 10 days.
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; Nagamani *et al.,* 2006).

3. Results and Discussion

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

The rhizosphere mycoflora population was more in organic field of crop plants during the 2010-2013 (Table.1). In organic field rhizosphere Population of fungi ranged from 38.4×10^{-3} to 56.3×10^{-3}CFU/g of soil during 2010-13. (Fig.1).In inorganic field rhizosphere Population of fungi 20.5×10^{-3} to 36.8×10^{-3}CFU/g of soil. (Fig.2). Application of panchagavya and beejamrutha treatment increases rhizosphere microbial population of maize (Shubha *et al.,* 2014). 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). Inorganic fertilizer to crop field significantly lowers the rhizosphere microbial population and diversity (Nelson and Mele, 2006). The soil bacterial, fungal, actinomycetes and N fixing bacteria were more in organic fields than inorganic field (Padmavathy and Poyyamoli, 2011).

The results on soil rhizosphere mycoflora species colonies, species number and percentage contribution in organic field of Wheat during 2010-13 are presented in table 2, 3 and 4.Results revealed that during 2010-11 the total 534 mycoflora colonies of different 20 species isolated from rhizosphere. During 2011-12 the total 551 mycoflora colonies of different 22 species isolated from rhizosphere. During 2012-13 the total 514 mycoflora colonies of different 25 species isolated from rhizosphere of organic field.

The results on soil rhizosphere mycoflora species colonies, species number and percentage contribution in inorganic field of Wheat during 2010-13 are presented in table 5, 6 and 7. Results revealed that during 2010-11 the total 391 mycoflora colonies of different 19 species isolated from rhizosphere. During 2011-12 the total 373 mycoflora colonies of different 20 species isolated from rhizosphere. During 2012-13 the total 322 mycoflora colonies of different 17 species isolated from rhizosphere of inorganic field of wheat.

Overall diversity indicate that rhizosphere of organic field shows more species diversity i.e. 30 mycoflora species in organic field and 24 species in inorganic field during 2010-2013.

The identified dominant rhizosphere mycoflora species belongs to genera *Aspergillus, Penicillium, Trichoderma, Fusarium, Rhizopus* and *Cladosporium* in organic and inorganic field. While genera like *Botrytis, Nigrospora, Sclerotium* and *Humicola* are least population diversity.

The *Acremonium* sp., *Tichoderma pseudokonigii, Glomus* sp., *Cladosporium herbarum* and *Curvularia lunata* are found in rhizosphere of organic field. It is observed that changes in frequency of mycoflora in agricultural fields are due to several factors like temperature, humidity, vegetation, organic and inorganic materials, soil type and texture (Gaddeyya *et al.,* 2012). 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).

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

4. Conclusion

The organic liquid bio-booster contains microbial load and soil nutrient which results increase in microbial population. 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.

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

5. Acknowledgement

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

Sr. No	Months	Organic field			Inorganic field		
		2010-2011	2011-2012	2012-2013	2010-2011	2011-2012	2012-2013
1	Nov	39.2	42.4	48.6	32.8	29.6	24.5
2	Dec	45.6	49.2	54.3	36.0	32.8	33.0
3	Jan	48.8	47.2	56.3	31.2	36.8	29.6
4	Feb	38.4	39.2	44.9	26.0	24.8	20.5
5	Mar	43.2	42.4	47.8	30.4	25.2	22.3
	Average	43.04	44.08	50.38	31.28	29.84	25.98
	S.D	±4.35	±4.04	±4.75	±3.64	±5.10	±5.19

Table 2: Monthly variation of rhizosphere soil mycoflora colonies & percentage contribution in organic field of Wheat. (2010-2011)

S.No	Name of Mycoflora	Nov	Dec	Jan	Feb	Mar	Total colonies	%
1	*Acremonium* sp.	0	0	0	0	2	2	0.374
2	*Alternaria solani*	8	5	0	5	0	18	3.370
3	*Aspergillus flavus*	20	20	30	15	34	119	22.284
4	*Aspergillus fumigatus*	0	5	0	7	8	20	3.745
5	*Aspergillus nidulans*	6	5	6	8	0	25	4.681
6	*Aspergillus niger*	12	18	18	10	4	62	11.610
7	*Aspergillus oryzae*	6	7	8	0	0	21	3.932
8	*Aspergillus terrus*	4	4	4	5	8	25	4.681
9	*Botrytis ceneria*	3	3	0	4	0	10	1.872
10	*Cladosporium herbarum*	4	5	0	3	7	19	3.558
11	*Curvularia lunata*	4	2	0	0	0	6	1.123
12	*Fusarium oxysporum*	3	3	6	6	0	18	3.370
13	*Mucor* sp.	0	0	0	4	0	4	0.749
14	*Penicillium chrysogennum*	8	8	8	6	16	46	8.614
15	*Penicillium citrinum*	0	5	6	6	9	26	4.868
16	*Penicillium decumbens*	4	10	0	4	8	26	4.868
17	*Penicillium degitatum*	8	0	6	3	0	17	3.183
18	*Rhizopus stolonifer*	4	4	7	6	0	21	3.932
19	*Sclerotium* sp.	4	3	9	0	5	21	3.932
20	*Trichoderma harzianum*	0	7	10	4	7	28	5.243
	Total No. of colonies	**98**	**114**	**118**	**96**	**108**	**534**	**100**
	Total No. of species.	**15**	**17**	**12**	**16**	**11**		

Total colonies ($10^{-2,}$ 10^{-3} & 10^{-4}) dilution.

Table 3: Monthly variation of rhizosphere soil mycoflora colonies & percentage contribution in organic field of Wheat

(2011-2012)

S.No	Name of Mycoflora	Nov	Dec	Jan	Feb	Mar	Total colonies	%
1	*Alternaria solani*	5	8	0	4	0	17	3.085
2	*Aspergillus flavus*	22	23	29	14	22	110	19.963
3	*Aspergillus fumigatus*	0	6	0	6	6	18	3.266
4	*Aspergillus nidulans*	7	5	5	5	0	22	3.992
5	*Aspergillus niger*	10	18	12	8	10	58	10.526
6	*Aspergillus oryzae*	4	2	8	2	2	18	3.266
7	*Aspergillus tennues*	6	3	4	2	0	15	2.722
8	*Aspergillus terrus*	4	3	4	4	4	19	3.448
9	*Botrytis ceneria*	3	3	0	3	0	9	1.633
10	*Cladosporium herbarum*	3	4	0	4	9	20	3.629
11	*Curvularia lunata*	3	2	2	0	2	9	1.633
12	*Fusarium oxysporum*	3	5	6	5	3	22	3.992
13	*Mucor sp.*	0	0	0	3	0	3	0.544
14	*Mycelia sterilia 1*	0	0	2	0	1	3	0.544
15	*Mycelia sterilia 2*	2	0	0	0	0	2	0.362
16	*Nigrospora sphaerica*	0	6	4	5	0	15	2.722
17	*Penicillium chrysogennum*	6	4	6	4	11	31	5.626
18	*Penicillium citrinum*	0	5	4	3	6	18	3.266
19	*Penicillium decumbens*	4	6	0	3	5	18	3.266
20	*Penicillium degitatum*	3	4	5	7	0	19	3.448
21	*Penicillium oxalicum*	5	0	4	3	10	22	3.992
22	*Rhizopus stolonifer*	5	5	7	4	0	21	3.811
23	*Sclerotium* sp.	4	5	8	0	3	20	3.629
24	*Trichoderma harzianum*	7	6	8	9	12	42	7.622
	Total No. of colonies	**106**	**123**	**118**	**98**	**106**	**551**	**100**
	Total No. of species.	**19**	**20**	**17**	**20**	**15**		

Total colonies ($10^{-2,}$ 10^{-3} & 10^{-4}) dilution.

Table 4: Monthly variation of rhizosphere soil mycoflora colonies & percentage contribution in organic field of Wheat (2012-2013)

S.No	Name of Mycoflora	Nov	Dec	Jan	Feb	Mar	Total colonies	%
1	*Alternaria solani*	7	5	0	2	0	14	2.723
2	*Aspergillus repens*	3	2	2	1	0	8	1.556
3	*Aspergillus candidus*	0	5	3	0	3	11	2.140
4	*Aspergillus flavus*	12	23	20	14	26	95	18.482
5	*Aspergillus fumigatus*	2	5	0	6	4	17	3.307
6	*Aspergillus nidulans*	6	4	3	5	0	18	3.501
7	*Aspergillus niger*	15	14	8	9	7	53	10.311
8	*Aspergillus oryzae*	4	0	4	0	2	10	1.945
9	*Aspergillus terrus*	4	3	3	4	6	20	3.891
10	*Botrytis ceneria*	4	3	0	3	0	10	1.945
11	*Cladosporium herbarum*	0	5	0	3	10	18	3.501
12	*Curvularia lunata*	4	2	2	0	0	8	1.556
13	*Fusarium oxysporum*	3	5	3	1	0	12	2.334
14	*Glomus* sp.	2	4	5	0	4	15	2.918
15	*Humicola grisea*	2	0	0	0	0	2	0.389
16	*Mucor sp.*	0	0	0	3	0	3	0.583
17	*Mycelia sterilia 1*	2	0	0	0	1	3	0.583
18	*Mycelia sterilia 2*	0	0	2	0	0	2	0.389
19	*Nigrospora sphaerica*	0	6	3	4	0	13	2.529
20	*Penicillium chrysogennum*	8	7	5	6	9	35	6.809
21	*Penicillium citrinum*	0	6	4	3	5	18	3.501
22	*Penicillium decumbens*	5	10	0	2	3	20	3.891
23	*Penicillium oxalicum*	7	4	6	4	8	29	5.642
24	*Rhizopus stolonifer*	5	4	4	3	0	16	3.112
25	*Rizopus oryzae*	2	2	0	0	0	4	0.778
26	*Trichoderma harzianum*	8	9	6	7	5	35	6.809
27	*Trichoderma pseudokonigii*	6	8	5	3	3	25	4.863
	Total No. of colonies	**111**	**136**	**88**	**83**	**96**	**514**	**100**
	Total No. of species.	**21**	**22**	**18**	**19**	**15**		

Total colonies ($10^{-2,}$ 10^{-3} & 10^{-4}) dilution.

Table 5: Monthly variation of rhizosphere soil mycoflora colonies & percentage contribution in Inorganic field of Wheat (2010-2011)

S. No	Name of Mycoflora	Nov	Dec	Jan	Feb	Mar	Total colonies	%
1	*Alternaria alternata*	3	0	4	0	2	9	2.301
2	*Alternaria solani*	4	7	0	4	0	15	3.836
3	*Aspergillus flavus*	16	13	19	11	21	80	20.460
4	*Aspergillus fumigatus*	0	3	0	5	6	14	3.580
5	*Aspergillus nidulans*	8	3	4	3	0	18	4.603
6	*Aspergillus niger*	10	12	8	7	9	46	11.764
7	*Aspergillus oryzae*	5	7	4	0	5	21	5.370
8	*Aspergillus terrus*	0	4	3	3	5	15	3.836
9	*Biospora* sp.	4	0	4	0	4	12	3.069
10	*Botrytis ceneria*	3	2	0	2	0	7	1.790
11	*Fusarium oxysporum*	4	7	5	3	2	21	5.370
12	*Mucor* sp.	0	0	0	3	0	3	0.767
13	*Mycelia sterilia*	0	0	3	0	0	3	0.767
14	*Nigrospora sphaerica*	0	4	2	2	0	8	2.046
15	*Penicillium citrinum*	0	4	3	5	4	16	4.092
16	*Penicillium decumbens*	4	9	0	2	5	20	5.115
17	*Penicillium oxalicum*	3	0	6	4	6	19	4.859
18	*Penicillium rubrum*	5	3	0	5	0	13	3.324
19	*Rhizopus stolonifer*	5	3	7	3	3	21	5.370
20	*Trichoderma harzianum*	8	9	6	3	4	30	7.672
	Total No. of colonies	**82**	**90**	**78**	**65**	**76**	**391**	**100**
	Total No. of species.	**14**	**15**	**14**	**16**	**13**		

Total colonies ($10^{-2,}$ 10^{-3} & 10^{-4}) dilution

Table 6: Monthly variation of rhizosphere soil mycoflora colonies & percentage contribution in Inorganic field of Wheat (2011-2012)

S.No	Name of Mycoflora	Nov	Dec	Jan	Feb	Mar	Total colonies	%
1	*Alternaria solani*	5	5	6	4	0	20	5.361
2	*Aspergillus candidus*	0	3	2	3	0	8	2.144
3	*Aspergillus flavus*	14	16	20	9	18	77	20.643
4	*Aspergillus fumigatus*	0	3	0	4	4	11	2.949
5	*Aspergillus nidulans*	4	4	4	5	0	17	4.557
6	*Aspergillus niger*	8	10	9	5	3	35	9.383
7	*Aspergillus oryzae*	2	0	5	0	0	7	1.876
8	*Aspergillus repens*	0	4	3	0	2	9	2.412
9	*Aspergillus terrus*	6	3	6	5	5	25	6.702
10	*Botrytis ceneria*	2	2	0	2	0	6	1.608
11	*Humicola grisea*	2	0	0	0	0	2	0.536
12	*Macrophomina phaeseolina*	0	2	3	3	3	11	2.949
13	*Mucor* sp.	0	0	3	2	0	5	1.340
14	*Mycelia sterilia*	2	0	0	0	1	3	0.804
15	*Penicillium chrysogennum*	5	8	5	4	9	31	8.310
16	*Penicillium citrinum*	0	4	9	4	6	23	6.166
17	*Penicillium decumbens*	3	0	0	2	4	9	2.412
18	*Penicillium rubrum*	5	3	0	6	0	14	3.753
19	*Rhizopus stolonifer*	5	4	6	4	2	21	5.630
20	*Sclerotium* sp.	3	5	6	0	3	17	4.557
21	*Trichoderma viride*	8	6	5	0	3	22	5.898
	Total No. of colonies	**74**	**82**	**92**	**62**	**63**	**373**	**100**
	Total No. of species.	**15**	**16**	**15**	**13**	**11**		

Total colonies ($10^{-2,}$ 10^{-3} & 10^{-4}) dilution.

Table 7: Monthly variation of rhizosphere soil mycoflora colonies & percentage contribution in Inorganic field of Wheat (2012-2013)

S.No	Name of Mycoflora	Nov	Dec	Jan	Feb	Mar	Total colonies	%
1	*Alternaria solani*	5	4	0	3	0	12	3.726
2	*Aspergillus candidus*	0	3	5	4	0	12	3.726
3	*Aspergillus flavus*	12	14	17	8	15	66	20.496
4	*Aspergillus fumigatus*	0	3	0	3	5	11	3.416
5	*Aspergillus nidulans*	6	3	6	4	0	19	5.900
6	*Aspergillus niger*	9	12	12	6	8	47	14.596
7	*Aspergillus oryzae*	4	6	5	0	0	15	4.658
8	*Botrytis ceneria*	3	3	0	2	0	8	2.484
9	*Macrophomina phaseolina*	2	2	0	2	1	7	2.173
10	*Mucor* sp.	0	0	0	3	0	3	0.931
11	*Mycelia sterilia*	0	0	0	0	2	2	0.621
12	*Penicillium chrysogennum*	5	7	6	3	7	28	8.695
13	*Penicillium citrinum*	0	4	4	4	6	18	5.590
14	*Penicillium decumbens*	3	7	0	2	3	15	4.658
15	*Penicillium rubrum*	4	3	8	4	4	23	7.142
16	*Rhizopus stolonifer*	5	2	7	3	2	19	5.900
17	*Sclerotium sp.*	3	5	4	0	3	15	4.658
18	*Trichoderma viride*	0	2	0	0	0	2	0.621
	Total No. of colonies	**61**	**80**	**74**	**51**	**56**	**322**	**100**
	Total No. of species.	**12**	**16**	**10**	**14**	**11**		

Total colonies ($10^{-2,}$ 10^{-3} & 10^{-4}) dilution.

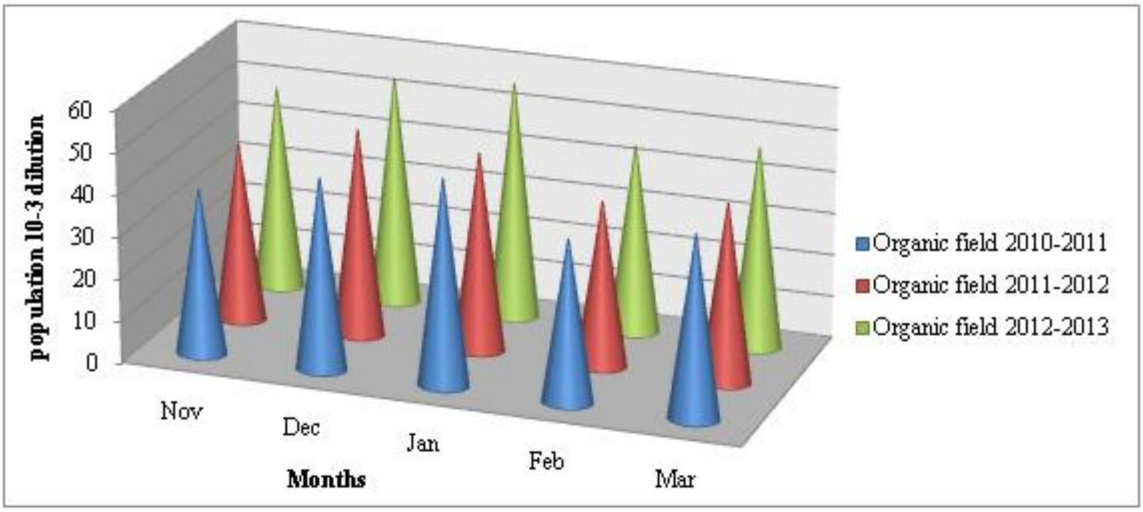


Figure 1: Rhizosphere mycoflora population in organic field of wheat

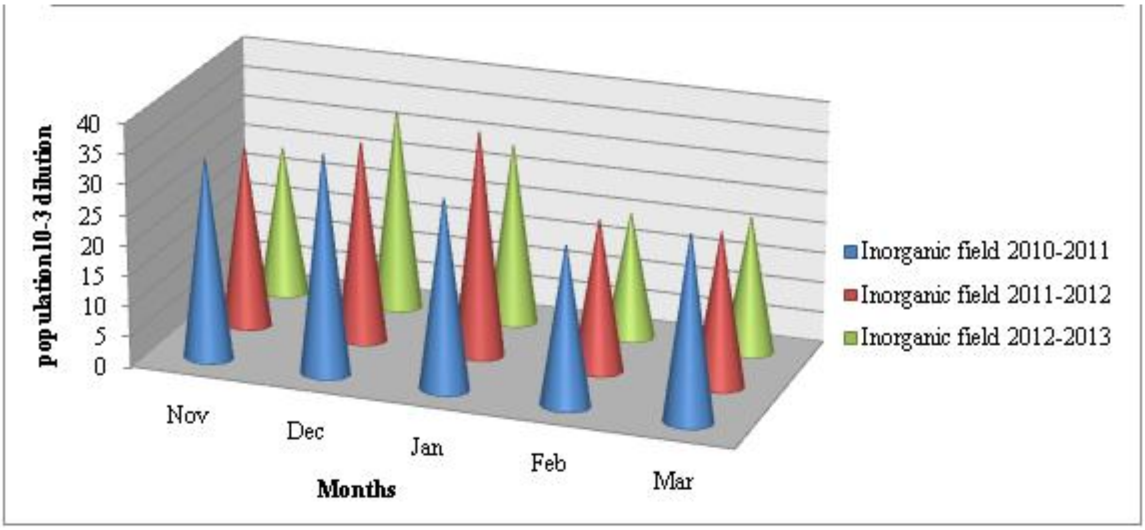
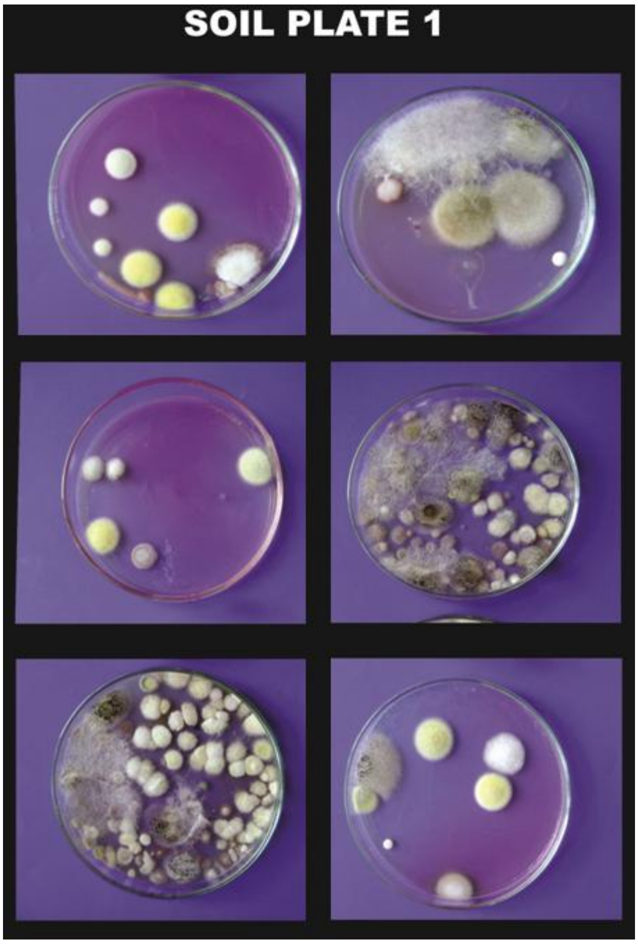
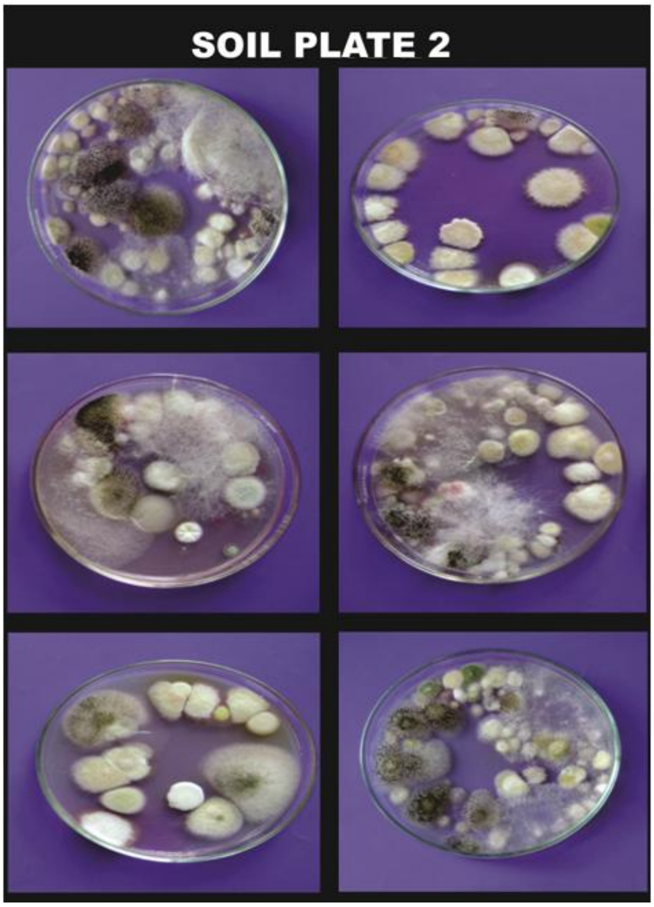


Figure 2: Rhizosphere mycoflora population in inorganic field of Wheat


SOIL PLATE 1


SOIL PLATE 2

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