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Population dynamics of root inhibiting and invading micro-flora in citrus rhizosphere

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Abstract
Present investigation was carried out during 2014 - 2015 at Department of Plant Pathology, All India Co-ordinated Research Project on Fruits, Dr. P. D. K. V. Akola and Department of Soil Science and Agricultural Chemistry, Post Graduate Institute. Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (M.S.) by collecting soil samples from Akola (AK), Amravati (AM1 & AM2), Wardha(WR), Washim (WA),Yavatmal (YA), Nagpur (NA1&NA2) and Buldhana (BU) sampling site of Vidarbha region, to study the population dynamics of micro-flora in rhizosphere soil ecosystem of citrus. The maximum c.f.u. population of healthy rhizosphere was recorded in Wardha (WR) sampling sites *i.e.*99.22×10^5-cfu g^{-1} dry soil while in diseases, it was recorded in Amravati (AM2) *i.e.* 83.66 c.f.u. g^{-1} dry soil. The maximum fungal colonies i.e. 33.11×10^3 in healthy rhizosphere soil was recorded in Yavatmal (YA) sampling sites. The prospect of manipulating crop rhizosphere microbial populations by inoculation of beneficial organisms *i.e. Pseudomonas fluorescence* and *Trichoderma* spp. and managing soil physico-chemical properties of soil, increases plant growth and control of many soil borne diseases has shown considerable promise in laboratory and field.

Keywords: Citrus, rhizosphere, population dynamics, micro-flora

Introduction
Citrus is one of the most important tropical fruit crops of the world, considered native of Himalayan foothills of North-Eastern India, North Central China and its adjoining area. In India on commercial basis, it is grown in Assam, Maharashtra, Andhra Pradesh, Punjab, Kerala, Karnataka, Uttar Pradesh and Meghalaya, with an 1042.5 thousand ha area. In Maharashtra it is grown in Vidarbha, Marathwada and Western Maharashtra (Jagtap *et al.*, 2012) [13] occupying 27000 ha area under Nagpur mandarin in Vidarbha region of Maharashtra (National Horticulture Board, 2013). But now, the area under citrus cultivation is decreases due to citrus decline caused by *Phytopthora* spp. resulted in severe losses of citrus plants from nursery level to various stages of plant growth in the form of root rot, collar rot, crown rot, gummosis and brown rot in orchards, damping off and root rot in seed beds and nurseries appear to be the major cause of citrus decline. The survey of citrus nursery in central India revealed 24% mortality of nursery plants due to root rot and collar rot diseases in virgin areas (Naqvi, 1999; Gade and Armarkar, 2011).
The population dynamics of microorganisms in soil are extremely difficult to assess due to the complex nature of the soil environment. The diverse nutritional requirements of microorganisms in soil may not be easily estimated; hence, isolation of the soil microorganisms and studying them in the laboratory, as pure or mixed culture cannot be easily attained. The growth and colonization of soil microorganisms can be influenced by chemical, physical and biological properties of the soil. The availability of macro and micro nutrient element can limit microbial population growth in the particular soil ecosystem. Essential soil elements for plant growth such as nitrogen, phosphorous, sulphur and micronutrients influences the microbial population as these nutrient elements are also needed for microbial growth and activity. Rhizosphere is the site for harmful and beneficial organism where many key interactions from beneficial symbiotic relationships to detrimental pathogenic interaction takes place (Sylvia, 2005). Up to 15% of the root surface area is covered with rhizosphere specific microorganisms - providing many sites for biological interactions. Beneficial micro-organism *viz.*, *Trichoderma* spp., *Aspergillus* spp., *Bacillus* spp., *Pseudomonas* spp. *etc are*

present in rhizosphere, however soil invading harmful pathogen like *Phytopthora* spp., *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp., *Sclerotia* spp., etc are predominantly present in citrus rhizosphere. The fungi like *Pythium* and *Phytopthora* require continuous moisture condition of soil for their multiplication. Some of the species of *Phytopthora* requires high and low temperature of soil for their survival. Fungi are found in more acidic soils than alkaline and bacteria have a very broad pH spectrum where they can survive. The influencing effects of pH in the rhizosphere are critical in supporting a biologically diverse microbial community. Bacteria are colonizing new locations more readily in sandy soils than clayey soils. Sand has larger pores between each granule allowing microorganisms and exudates can travel. Therefore, the larger the granule size, the further the rhizosphere and microorganisms associated with it will extend into the surrounding soil (Sylvia, 2005). Keeping this in view, present investigation was undertaken to study the microbial population dynamic of citrus rhizosphere soil from different location of Vidarbha region.

Materials and Methods

Collection of soil sample

Soil sample were collected from different places of Vidarbha region of Maharashtra during September-December (2014) by using auger, up to 30 cm depth from healthy and diseases plants. Soil sample were stored in sterilized polythene bags, used for isolation of bacteria and fungi. The remaining soil sample air- dried and used for the determination of soil properties.

Table 1: Details of soil sample collected from citrus orchards in citrus growing areas.

Sr. No.	Districts	Location/Village	Abbreviations
1.	Akola	All India Co-ordinated Research Project on Fruits, Dr. P. D. K.V. Akola.)	AK
2.	Amravati	Regional Research Centre (RRC)Dr. P.D.K.V. Akola	AM 1
3.	Amravati	Nandgaon Khandeshwar (Farmer field)	AM 2
4.	Wardha	Karanja Ghadge (Farmer field)	WR
5.	Washim	Patur (Farmer field)	WA
6.	Yavatmal	Digras (Farmer field)	YA
7.	Nagpur	Katol (Farmer field)	NA 1
8.	Nagpur	Saoner (Farmer field)	NA 2
9.	Buldhana	Sonala (Farmer field)	BU

Estimation of feeder root rating (based on rating scale)

The Feeder Root Rating of diseases citrus plant was rated on a scale, from 1 (healthy) to 5(dead) as given below.

Feeder root rating (Grimm and Hutchinson, 1971)

1. No visible symptoms of feeder root rating
2. Few feeder roots showing rotting
3. Majority of feeder roots showing rotting and loss of few roots.
4. All feeder roots are infected and cortex sloughed from major roots.
5. All feeder roots are dead or missing Feeder root rating are calculate based on following formula

$$\% \text{ diseases incidence} = \frac{\sum \text{All reading}}{\text{No. of observation} \times \text{Maximum rating}} \times 100$$

Sterilization

Glassware likes petriplates; conical flasks, test tube etc. were sterilized in hot air oven at 180^0 C for 1 hr. Cultural media and distilled water were sterilized in autoclave at 1.04 kg/cm^2 for 15 min.

Preparation of Potato Dextrose Agar media (PDA)

The medium was prepared by using following ingredients.

Peeled Potato - 200g
Dextros - 20g
Agar - 20g
Distilled water - 1000ml

Peeled potato were sliced into pieces and boiled in 500 ml distilled water till properly cooked. The extract was strained through muslin cloth and measured. In the remaining water, after dissolving dextrose and agar, potato extract was added and the volume was made to one liter. The medium was distributed in flasks and tubes. The flasks and tubes were plugged with cotton and medium was sterilized in autoclave at 1.04 kg/cm^2 for 15 minutes.

Preparation of nutrient Agar media (NA)

The medium used for isolation of bacterial colonies was prepared by using following ingredients.

Composition

Peptone -5g.
Beef extract - 1 g.
Yeast extract - 2 g
Sodium chloride - 5 g.
Agar - 20 g
Distilled water - 1000 ml

Peptone, Beef extract, yeast Extract, sodium chloride and Agar were dissolved 1 ml distilled water boil with properly and then filtered through muslin cloth, distributed in flasks and tubes The flasks and tubes were plugged with cotton and medium was sterilized in autoclave at 1.04 kg/cm^2 for 15 minutes. These medium were prepared by following standard formula and autoclave at 1.04kg/cm for 15 min. Glassware were sterilized in hot air oven at 180^0C for 2hr.

Isolation of bacteria by serial dilution method

1. Soil sample were collected from healthy and diseases rhizosphere region of citrus. One gram of soil sample was taken for enumeration of population of bacteria.
2. Water blanks were prepared by autoclaving 9ml of sterilized distilled water in autoclave at 15 lbs for 15 min.
3. One gram of soil sample was taken into first number water blank to make 1:10 dilution
4. To obtain the serial dilution of 10^{-1}, 1 gm of soil was mixed with 9ml of sterilized distilled water and the dilution was vigorously shaken mechanically. Subsequently the 1 ml from this test tube was added to next water blanks (9ml) to obtain the dilution of 10^{-2}, and likewise dilution of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were prepared.

5. The dilution of 10^{-5}, 10^{-6}, and 10^{-7} was used for the enumeration of population of bacteria by using Nutrient Agar (NA). The 0.1 ml of the aliquot from 10^{-7} was added to the prepoured nine petriplates containing 20ml of medium (NA) and equally spread and the petriplates were incubated at 30^0c for 24 hr for bacterial colony.

Identification of positive and negative bacteria was made by Gram staining

Procedure

- First a smear was prepared by bacterial cells by holding a clean slide by grapping at the edges.
- A loopful of bacterial suspension was transferred in the centre of slide, with the help of wire loop.
- The drop was smeared over slide and air-dried was fixed by passing the slide 3-4 times rapidly over the flame.
- The smear was flooded with crystal violet for 30 second, and rinsed with gentle flow of sterile distilled water.
- The smear was covered with immersed in potassium iodide/ Lugol's iodine solution for 30 seconds and rinsed with gentle flow of sterile distilled water.
- Then it was decolorized with 70% alcohol and rinsed gentle flow of sterile distilled water.
- Smear was counterstained with saffranin for 10 second, again washed gentle flow of sterile distilled water, observed under oil immersion objective.
- Cell which retain basic dye approved blue was Gram Positive and those taking counter strain approved Purple was Gram Negative.

Isolation of fungi

1. Soil sample were collected from rhizosphere of healthy and diseases citrus plant and dried in shade. One gram of soil sample was taken for enumeration of population of fungi.
2. Water blanks were prepared by autoclaving 9ml of sterilized distilled water in autoclave at 15 lbs for 15 min.
3. One gram of soil sample was taken into first number water blank to make 1:10 dilution.
4. To obtain the serial dilution of 10^{-1}, 1 gm of soil was mixed with 9 ml of sterilized distilled water and the dilution was vigorously shaken mechanically. Subsequently the 1 ml from this test tube was added to next water blanks (9ml) to obtain the dilution of 10^{-2}, and likewise dilution of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} were prepared.
5. The dilution of 10^{-3}, 10^{-4}, and 10^{-5} was used for the enumeration of population of fungi by using Potato Dextrose Agar (PDA). The 0.1 ml of the aliquot from 10^{-4} was added to the prepoured nine petriplates containing 20ml of medium (PDA) and equally spread and the petriplates incubated at 28^0c c for 24hr for fungus isolation.

Study of microbial population

Isolated bacterial and fungal colony was counted by most probable number method:

Most Probable Number Method is an important technique in estimating soil microbial population in soil, water and agricultural products (Halvorson and Ziegler, 1933).

MPN is a procedure to estimate the population density of viable microorganisms in a test sample. It is based upon the application of the theory of probability to the numbers of observed growth responses to a standard dilution series of sample inoculums placed into a set number of culture media tubes. Growth response after incubation indicated by the sample should be diluted in such a manner that higher dilutions of the sample will results in fewer positive culture tubes in the series serial dilution are used in either 3, 5 or 10 tube MPN series. When a higher number of tubes are inoculated in the series, the confidence limits of the MPN are narrowed. For particularly high microbial populations, the values obtained by MPN are generally not considered as precise as population numbers derived from direct plating methods; however, it should be emphasized that MPN values are only estimates while plate counts are direct counts of living organisms expressed in cfu/ml. MPN values are, however, particularly useful when low concentrations of organisms (<100/g) are encountered in such materials as milk, food, water and soil where particulate matter of the matrix may interfere with obtaining accurate colony counts.

Purification and maintenance of bacteria and fungi

Individual colonies of bacteria picked out and maintained by on NA medium. Culture were purified by following disc plate method by taking 5 mm disc of fungal culture, kept it in a medium containing PDA, and maintained at a room temperature by adopting sub sequent sub culturing at regular interval. Seven days old culture was used for further studies.

Results and Discussion

Isolation of bacteria and fungi by serial dilution method and estimation of population by C.F.U. count and Most Probable Number (MPN) method

The rhizosphere soil from healthy and diseased citrus plants at different sampling sites *viz.*, Akola (AK), Amravati (AM1 & AM2), Wardha (WR), Washim (WA), Yavatmal (YA), Nagpur (NA1 & NA2) and Buldhana (BU) were collected to isolate bacteria and fungi by serial dilution method, quantify the microbial population by C.F.U. at different dilutions and MPN method.

Estimation of soil bacterial population from citrus rhizosphere soil collected from healthy and diseased plants from different locations of Vidarbha region

The rhizosphere soil collected from healthy and diseased citrus plants from different sampling sites of Vidarbha region showed variation in bacterial population. The bacteria were found to be the most dominant species among all soil microbes. The c.f.u. at 10^5,10^6 and 10^7 of bacteria from rhizosphere of healthy and diseased citrus plants were presented in Table1 and fig 1 and 2, In healthy rhizosphere soil, the maximum c.f.u. population at 10^5,10^6 and 10^7 was recorded in Wardha (WR) sampling site *i.e.* 99.22 ± 6.50^{f}, 63.00 ± 2.74^{de} and 61.67 ± 8.23^{e} cfu g^{-1} dry soil, respectively, While in diseased rhizosphere soil maximum was recorded in Amravati (AM2) *i.e.* 83.66 ± 8.49^{f}, 65 ± 9.21^{e} and 51.33 ± 7.66^{d} cfu g^{-1} dry soil, respectively. The minimum c.f.u. population from healthy and diseased citrus plant was recorded in Washim (WA) *i.e.*17.56 ± 4.28^{a}, 17 ± 4.82^{a} and 9.33 ± 4.06^{a} cfu g^{-1} dry soil and 9.66 ± 4.21^{a}, 8.77 ± 4.27^{a} and8.56 ± 4.53^{a} at 10^5,10^6 and 10^7, respectively. Overall, the maximum bacterial population was recorded in healthy rhizosphere comprising the maximum colonies of *Bacillus spp.*, *Pseudomonas spp* and *Azotobacter spp.* The results are in conformity with Devi and Chhetry (2012), who reported that the total number of bacteria was high in rhizosphere of healthy plants as compared to diseases and non-rhizosphere soil. The maximum colonies of *Pseudomonas* spp. was recorded in healthy rhizosphere soil *i.e.* 20 x 10^6 $cfug^{-1}$. Ram *et al.* (2013) also recorded the maximum c.f.u. colonies of *Pseudomonas* 21.33x 10^3 $cfug^{-1}$ in normal soil as compared to sodic soil.

Table 2: Soil bacterial population (cfug^{-1}) of citrus rhizosphere soil collected from healthy and diseased plants at different locations of Vidarbha region

Location	Healthy plant			Diseased plant		
	CFU(10^5)	CFU (10^6)	CFU (10^7)	CFU (10^5)	CFU (10^6)	CFU (10^7)
AK	50.89±2.76^{c}	34.00±2.74b^{c}	19.00±2.74^{b}	34.00±2.73^{b}	31.00±2.74^{c}	14.00±2.74^{a}
AM1	62.22±7.19^{d}	39.00±2.74^{c}	22.00±2.74^{b}	31.00±2.47^{b}	30.33±4.15^{c}	29.56±4.42^{c}
AM2	93.00±6.36^{f}	89.11±5.18^{f}	62.56±8.05^{e}	83.66±8.49^{f}	65.00±9.21^{e}	51.33±7.66^{d}
WR	99.22±6.50^{f}	63.00±2.74^{de}	61.67±8.23^{e}	53.66±4.72^{d}	31.22±6.38^{c}	23.11±2.62^{bc}
WA	17.56±4.28^{a}	17.00±4.82^{a}	9.33±4.06^{a}	9.66±4.21^{a}	8.77±4.27^{a}	8.56±4.53^{a}
YA	33.88±3.72^{b}	32.00±4.87^{b}	30.22±5.65^{c}	29.00±8.62^{b}	20.00±4.03^{b}	9.78±5.52^{a}
NA1	70.56±4.77^{e}	67.11±4.41^{e}	54±4.58^{d}	66.22±7.77^{e}	62.66±7.09d	60.00±8.92^{e}
NA2	74.11±6.19^{e}	59.78±4.89^{d}	30.56±5.13^{c}	44.77±10.93^{c}	56.22±8.54^{d}	44.56±8.02^{d}
BU	57.89±8.57^{cd}	61.67±4.03^{de}	18.89±3.06^{b}	57.33±3.97^{d}	22.00±5.70^{b}	21.44±6.27^{b}
Total	62.15±25.43	51.40±21.56	34.24±19.66	45.48±22.11	36.35±19.94	29.15±18.66

All values are in cfug^{-1}

Mean ± SD; for each column different lower case letter significant differences among different sampling sites $p \leq 0.05$ level, as analysis by two sided Tukey's HSD between different sampling sites. AK: Akola (AICRP on Fruits), AM1: Amravati (Regional Research Station), AM2: Amravati (Nandgaon Khandeshwar), WR: Wardha (Karanja Ghadge), WA: Washim (Patur), YA: Yavatmal (Digras), NA1: Nagpur (Katol), NA2: Nagpur (Saoner), BU: Buldhana (Sonala)

Estimation of soil fungal population from citrus rhizosphere soil collected from healthy and diseased plants at different locations of Vidarbha region

The c.f.u. Population of rhizosphere soil showed that the colonies of *Aspergillus spp.*, *Penicillium spp.* and *Trichoderma spp*. were dominant in the rhizosphere of healthy citrus plant. Whereas, in rhizosphere soil collected from diseased plants comprise *Fusarium spp*, *Rhizoctonia Spp*, *Pythium spp* and *Phytophthora spp*. fungal population in healthy and diseased rhizosphere soil was ranged between 5.00-33.11 cfug-1 dry soil (Table 3 and Fig. 3 and 4). The maximum fungal colonies at 10^3,10^4 and 10^5(33.11±4.94^{c}, 23.89±3.92^{c}, 17±4.66^{b} and 28.11±9.02^{b}, 18.33±6.08^{b}, 8.11±4.17^{ab} respectively was recorded in healthy and diseased rhizosphere soil at different dilutions in Yavatmal (YA) sampling site. Whereas, the minimum fungal population in healthy rhizosphere soil was recorded in Wardha (WR) *i.e.* 10.22±4.02^{a} x 10^3, Amravati (AM2) 7.89±4.37^{a} x 10^4 and 7.77±4.29^{a} x 10^5 Buldhana (BU), while in diseased rhizosphere minimum c.f.u. population was recorded in Amravati (AM1) and Akola (AK) *i.e.* 7.22±3.83^{a}x 10^3 and 5.22±2.44^{a}x10^4 and 5.00±2.74^{a}x 10^5, respectively. The results are in agreement with the findings of Devi and Chhetry (2012) who recorded the maximum fungal population comprising *G.virens*, *T.viride*, *A.niger* and *Penicillium citrinum* from healthy rhizosphere soil than diseased and non-rhizosphere soil, this may be due to the availability of nutrients released by the root exudates around the vicinity of root zone of citrus. The same finding was also recorded by Gesheva (2001), that the population dynamics of fungi *Penicillium*, *Aspergillus*, *Trichoderma*, *Fusarium*, *Mucor*, *Actinomycets* were more numerous (0.63 to 11.01x 10^5c.f.u/ g of soil) in the orange rhizosphere.

Table 3: Soil fungal population (cfug-1) of citrus rhizosphere soil collected from healthy and diseased plants in different locations of Vidarbha region

Location	Healthy plant			Diseases plant		
	CFU (10^3)	CFU (10^4)	CFU (10^5)	CFU (10^3)	CFU (10^4)	CFU (10^5)
AK	10.55±3.97^{a}	9.44±4.39^{ab}	8.00±3.81^{a}	10.22±4.29^{ab}	5.22±2.44^{a}	5.00±2.74^{a}
AM1	18.55±6.91^{ab}	10.11±5.01^{ab}	8.78±3.70^{a}	7.22±3.83^{a}	6.88±4.14^{a}	6.78±4.12^{ab}
AM2	23.33±8.09^{b}	7.89±4.37^{a}	8.88±4.59^{a}	17.00±4.42b^{c}	5.00±2.74^{a}	8.55±5.50^{ab}
WR	10.22±4.02^{a}	11.00±5.22^{ab}	8.67±4.09^{a}	11.67±5^{ab}	7.77±4.06^{a}	7.00±4.24^{ab}
WA	10.88±4.96^{a}	8.22±3.27^{a}	8.44±3.68^{a}	9.22±4.02^{a}	8.77±4.27^{a}	8.77±4.26^{ab}
YA	33.11±4.94^{c}	23.89±3.92^{c}	17±4.66^{b}	28.11±9.02^{b}	18.33±6.08^{b}	8.11±4.17^{ab}
NA1	10.66±5.79^{a}	9.22±4.60a^{b}	9.11±3.92^{a}	11.33±4.66^{ab}	8.88±5.09^{a}	9.11±4.20^{ab}
NA2	16.00±6.25^{ab}	16.00±6.08^{b}	12.44±4.67^{ab}	19.44±6.62^{c}	8.66±5.55^{a}	9.00±4.58^{ab}
BU	14.66±6.63^{a}	9.44±5.73^{ab}	7.77±4.29^{a}	14.66±3.61^{abc}	9.33±4.72^{a}	12.44±4.67^{b}
Total	16.44±9.17	11.69±6.69	9.90±4.87	14.32±7.93	8.76±5.65	8.31±4.54

All values are in cfug^{-1}

Mean ± SD; for each column different lower case letter significant differences among different sampling sites $p \leq 0.05$ level, as analysis by two sided Tukey's HSD between different sampling sites. AK: Akola (AICRP on Fruits), AM1: Amravati (Regional Research Station), AM2: Amravati (Nandgaon Khandeshwar), WR: Wardha (Karanja Ghadge), WA: Washim (Patur), YA: Yavatmal (Digras), NA1: Nagpur (Katol), NA2: Nagpur (Saoner), BU: Buldhana (Sonala)

Estimation of bacterial population by Most Probable Number Method

MPN method developed for the quantitative estimation of bacteria (log of cells g^{-1} soil). Estimation can be made by using 10^6 dilutions- nine numbers of tubes per dilution were used for adjusting accuracy. The upper and lower 95% confidence limit was calculated according to Alexander (1982).

The results obtained using MNP method, revealed that in citrus soil the number of bacteria were significantly higher in Yavatmal healthy (3.42) sample and least in Akola (0.53) diseases citrus plant at a 95% confidence level (Table 4). Papen and Berg (1998) also isolated different heterotrophic nitrifying bacterial strains and recorded MPN of surface soil are higher *i.e.*10 log of cell/ gm of dry weight soil at 95% confidence level.

Table 4: Estimation of viable bacterial count by Most Probable Number Method (MPN) at 95% confidence limit for various combination of positive result of healthy and diseases sampling sites

Location	MPN	CF Lower	CF Upper	LH Lower	LH Upper	Bias	Conf. Level
AK(H)	1.87	1.03	3	0.98	2.98	1.79	95%
AK(D)	0.53	0.3	0.94	0.21	0.86	0.48	95%
AM1(H)	2.04	1.14	3.29	0.93	3.65	1.95	95%
AM1(D)	1.41	0.73	2.29	0.65	2.6	1.36	95%
WR(H)	3.58	2.08	5.98	1.96	4.91	3.34	95%
WR(D)	1.92	1.06	3.09	0.98	3.14	0.184	95%
WA(H)	1.83	1	2.94	0.82	3.32	1.75	95%
WA(D)	1.39	0.72	2.26	1.34	1.41	1.34	95%
YA(H)	3.42	1.99	5.69	1.47	5.89	3.2	95%
YA(D)	3.3	1.92	5.47	1.82	4.51	3.09	95%
NA1(H)	2.75	1.58	4.47	1.31	4.41	2.6	95%
NA1(D)	2.3	1.31	3.71	1.24	3.56	2.19	95%
AM2(H)	2.57	1.47	4.16	1.5	3.67	2.44	95%
AM2(D)	1.86	1.02	2.98	1.22	2.46	1.78	95%
NA2(H)	3.42	1.99	5.69	1.47	5.89	3.2	95%
NA2(D)	2.51	1.43	4.06	1.77	4.24	2.38	95%
BU(H)	2.29	1.3	3.7	1.06	3.84	2.18	95%
BU(D)	1.28	0.65	2.09	0.57	2.44	1.23	95%

H-Healthy rhizosphere soil, D- Diseased rhizosphere soil, AK: Akola (AICRP on Fruits), AM1: Amravati (Regional Research Station), AM2: Amravati (Nandgaon Khandeshwar), WR: Wardha (Karanja Ghadge), WA: Washim (Patur), YA: Yavatmal (Digras), NA1: Nagpur (Katol), NA2: Nagpur (Saoner), BU: Buldhana (Sonala)

Conclusions

The bacteria were found to be the most dominant species among all soil microbes. The maximum c.f.u. population at 10^5 was recorded in Wardha (WR) sampling site *i.e.* 99.22 whereas in diseased rhizosphere soil, maximum population of c.f.u. was recorded in Amravati (AM2) *i.e.* 83.66. Healthy rhizosphere comprising the maximum colonies of *Bacillus spp.*, *Pseudomonas spp* and *Azotobacter spp.*as compared with diseases.

The fungal population of rhizosphere soil showed that the colonies of *Aspergillus spp.*, *Penicillium spp.* and *Trichoderma spp*. were dominant in the rhizosphere of healthy citrus plant. Whereas, in rhizosphere soil collected from diseased plants comprise *Fusarium spp*, *Rhizoctonia Spp*, *Pythium spp* and *Phytophthora spp.*

Fungal population in healthy and diseased rhizosphere soil was ranged between 5.00-33.11 $cfug^{-1}$ dry soils. The maximum fungal colonies (33.11×10^3c.f.u./g of soil) was recorded in healthy citrus plant of Yavatmal (YA) whereas the minimum fungal population in healthy rhizosphere soil was recorded in Wardha (WR) *i.e.* $10.22 \pm 4.02^a \times 10^3$ c.f.u./g of soil. Overall the potentiality of the antagonistic fungus like *Trichoderma spp.*, *Aspergillus spp* and bacterial such as Bacillus, Pseudomonas will be incorporate in the integrated diseases management for citrus diseases by in enriching the soil Physico-chemical properties which are mainly essential for luxuriant growth of plant. However further work is necessary to enhance the disease control capability by managing the nutrient and ecological status of soil.

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