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Correlation between physico-chemical parameters with soil of Citrus rhizosphere microbes

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Abstract
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, sulfur and micronutrients influences the microbial population as these nutrient elements are also needed for microbial growth and activity. 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.
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 know the correlation between physico-chemical parameters with soil rhizosphere microbes for which isolation of different microbes like bacteria, fungi was carried out.
The result showed that in healthy rhizosphere, moisture, Organic carbon, Copper and Phosphorous are positively correlated with bacterial and fungal population whereas Temperature, Manganese, EC, Iron, Zinc, pH, and Potassium were negatively correlated. In diseased rhizosphere of citrus, Temperature, Manganese, Phosphorous, Nitrogen, Iron and Moisture were positively correlated whereas P^H, Zn, Organic carbon and Potassium negatively correlated. The PCA analysis was carried out to see the influencing factor of Physio-chemical properties with microbial population at different locations. The result reported that the sampling sites Akola (AK) was influenced by Temperature, Moisture, Nitrogen, Copper and Zinc and Amravati (AM1) by Organic carbon, Moisture, Copper, Temperature, EC, Iron, Nitrogen, Potassium and Zinc. Whereas, Amravati (AM2) by Zinc, Nitrogen, Phosphorous, EC for microbial population in citrus rhizosphere ecosystem. In case of Wardha (WR) and Washim (WA), Temperature, Moisture, Nitrogen, Zinc, Iron, pH, are the most influencing factors whereas in Nagpur region NA1 and NA2, pH, Organic carbon, Moisture and Manganese are the factor which are responsible for the microbial population in the rhizosphere region of citrus. In Buldhana (BU) sampling sites, EC and Manganese whereas in Yavatmal (YA) sampling site pH, Iron and EC are the soil properties which influenced the microbial population in rhizosphere region of citrus.

Keywords: Rhizosphere, citrus, physico-chemical properties, correlation

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) [11] 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).

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

$$\% \text{ diseases incidence} = \frac{\sum \text{all reading}}{\text{No of observation} \times \text{maximum rating}} \times 100$$

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
Dextrose	20g
Agar	20g
Distilled water	1000 ml

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 litter. 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 1.04 kg/cm for 15 min. Glassware were sterilized in hot air oven at 180 ^{0}C for 2 hr.

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 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^{-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 pre poured 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

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

Physico-chemical properties of rhizosphere soil collected from healthy and diseased citrus plants at different sampling sites of Vidarbha region

The physico-chemical properties of healthy and diseased citrus rhizosphere soil for different sampling site of Vidarbha region were shown in Table 2 & 3. The physico-chemical properties of soil showed variations in different sampling sites. In case of healthy rhizosphere soil predicted in Table 2 the maximum temperature (30.60^0C) was recorded at Akola (AK) sampling site followed by Amravati (AM2) i.e. 28.42^0C. The moisture percentage of rhizosphere soil was in the range of 27.84 to 39.14%, however, maximum moisture percentage in AM1 (39.19%) sampling site. It was recorded that soil acidity of all sampling sites indicates the alkaline nature of soil (8.20 to 8.49). The electrical conductivity of healthy rhizosphere soil was in the range of 0.28 to 0.52 mg / dsm^{-1}. In case of macro and micro nutrient maximum nitrogen (239.14 kg/ha) was estimated from Wardha (WR) sampling site, however phosphorus was in the range of 27.58 to 31.77 kg/ha. In case of Potassium maximum Potassium i.e. 354.68 kg/ha was estimated from Akola (AK) sampling site. The organic carbon (0.88%), zinc (0.85ppm) was estimated maximum from Nagpur (NA2) while, Iron (10.83ppm) and Manganese (6.35ppm) was observed maximum in Buldhana (BU) sampling side. In diseases citrus rhizosphere soil, the data predicted in Table 3 resulted that the maximum temperature (28.95 ^{0}C) was recorded at Akola (AK) sampling site followed by Amravati (AM1) *i.e.* 27.65 ^{0}C. The moisture percentage of rhizosphere soil was in the range of 26.93 to 40.25%, however, maximum moisture percentage *i.e.* 40.25% was observed in AM1 sampling site. It was recorded that soil acidity of all sampling sites indicates the alkaline nature of soil (7.93 to 8.41). The electrical conductivity of healthy rhizosphere soil collected from different sampling sites was in the range of 0.16 to 0.5 mg / dsm^{-1}. In case of macro and micro nutrient, maximum nitrogen (190.72 kg/ha) and Copper (4.17 ppm) was estimated from Amravati (AM1) sampling site, however phosphorus was in the range of 27.51 to 31.12 kg/ha. In case of Potassium, maximum Potassium *i.e.* 344.78

kg/ha was estimated from Wardha (WR) sampling site. The organic carbon (0.66%), zinc (0.85 ppm) was estimated maximum from Nagpur (NA1) and Akola (AK) sampling sites, respectively while, Iron (9.61ppm) and Manganese (5.87 ppm) was observed maximum in Amravati (AM2) sampling side. The results regarding the range of physico-chemical properties are in accordance to the findings of Lu *et al*. (2012), Setiawati (2014) and Rao *et al*. (2014) [18].

Table 2: Physico-chemical properties of healthy rhizosphere soil at different sampling sites of Vidarbha region

Location	Temperature (^{O}C)	Moisture (%)	P^{H}	EC	N	P	K	O.C.	Fe	Mn	Zn	Cu
AK	30.60±2.63^{c}	35.43±2.56^{bc}	8.42± 0.22^{bc}	0.45±0.19^{cd}	185.8±44.61^{ab}	31.39±2.77^{a}	354.68±51.14^{c}	0.68±0.20^{ab}	10.18±2.48^{a}	5.27±1.45^{ab}	0.71±0.20^{ab}	4.03±1.18^{a}
AM1	26.97±2.77^{ab}	39.14±2.40^{c}	8.18±0.23^{a}	0.29±0.10^{ab}	185±28.75^{ab}	28.17±1.07^{a}	296.45±42.19^{ab}	0.75±0.14^{ab}	10.40±2.64^{a}	4.96±1.29^{ab}	0.68±0.12^{ab}	4.02±1.03^{a}
AM2	28.42±2.86^{bc}	34.18±4.46^{bc}	8.29±0.17^{abc}	0.41±0.05^{bcd}	160.23±29.39^{a}	29.08±3.06^{a}	330.57±38.91^{bc}	0.56±0.23^{a}	9.69±2.21^{a}	5.43±1.54^{ab}	0.71±0.17^{ab}	4.00±1.18^{a}
WR	25.23±3.54^{ab}	33.41±4.29^{b}	8.21±0.11^{ab}	0.35±0.08^{abc}	239.14±31.01^{c}	29.60±3.09^{a}	270.43±28.58^{a}	0.62±0.23^{a}	9.26±2.95^{a}	4.89±1.19^{ab}	0.66±0.16^{ab}	4.41±1.21^{a}
WA	26.13±1.38^{ab}	33.16±6.27^{b}	8.28±0.17^{abc}	0.25±0.10^{a}	216.53±0.67^{bc}	29.92±2.38^{a}	272.09±13.78^{a}	0.70±0.10^{ab}	9.48±2.77^{a}	4.53±0.09^{a}	0.85±0.01^{b}	4.38±1.33^{a}
YA	25.29±1.19^{ab}	32.28±2.63^{ab}	8.32±0.12^{abc}	0.25±0.07^{a}	182.9±28.43^{ab}	27.58±3.53^{a}	300.82±38.82^{abc}	0.58±0.22^{a}	9.45±2.89^{a}	4.06±1.39^{a}	0.67±0.18^{ab}	4.44±1.11^{a}
NA1	24.11±1.83^{a}	27.84±3.97^{a}	8.43±0.09^{bc}	0.52±0.10^{d}	173.82±29.38^{ab}	28.78±3.42^{a}	308.43±43.79^{abc}	0.65±0.14^{ab}	8.19±2.84^{a}	4±1.23^{ab}	0.69±0.12^{ab}	3.91±0.92^{a}
NA2	23.87±1.81^{a}	38.95±1.55c	8.49±0.14^{a}	0.28±0.10^{ab}	193.08±52.02^{ab}	31.77±2.86^{a}	293.49±45.90^{ab}	0.88±0.05^{b}	8.23±2.40^{a}	5.71±0.88^{ab}	0.79±0.12^{ab}	4.11±0.87^{a}
BU	24.72±1.40^{a}	31.28±2.60^{ab}	8.20±0.13^{ab}	0.44±0.09^{cd}	17.97±32.88^{ab}	31.20±3.33^{a}	314.24±42.16^{abc}	0.69±0.11^{ab}	10.83±3.09^{a}	6.35±0.09^{b}	0.59±0.10^{a}	3.94±0.79a
Total	26.15±3.01	33.96±4.87	8.31±0.18	0.36±0.14	190.05±39.12	29.72±3.11	304.58±45.45	0.68±0.18	9.52±2.71	5.11±1.26	0.70±0.15	4.14±1.05

Values are mean of 9 replicates ± S.E.; EC: electrical conductivity (mg/ds.m^{-1}), N: Nitrogen (Kg/ha), P: Phosphorus (kg/ha),K:Potassium (kg/ha), O.C: Organic Carbon (%), Fe: Iron (ppm),Mn: Manganese (ppm),Zn: Zinc (ppm),Cu: Copper (ppm). 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)

Table 3: Physio-chemical properties of diseased rhizosphere soil at different sampling sites of Vidarbha region

	Temperature (^{O}C)	Moisture (%)	P^{H}	EC	N	P	K	O.C.	Fe	Mn	Zn	Cu
AK	28.95±0.86^{b}	34.47± 2.75^{bc}	8.30± 0.21^{b}	0.29± 0.07^{a}	155.17± 9.10^{ab}	30.69± 2.77^{a}	300.53±33.15^{c} de	0.62±0.19^{a}	9.47±2.68^{a}	4.86±1.27^{a}	0.85±0.03 b	3.85±1.00^{a}
AM1	27.65±1.89^{ab}	40.25±2.95^{e}	7.93±0.26^{a}	0.16±0.10^{a}	190.72±39.40 b	28.58±3.34^{a}	286.42±29.77 bcd	0.61±0.15^{a}	8.81±3.13^{a}	4.48±1.26^{a}	0.72±0.08 ab	4.18±1.12^{a}
AM2	26.84±2.95^{ab}	33.95±4.29^{b} c	8.29±0.18^{b}	0.32±0.08^{a}	173.96±49.77 ab	29.36±2.33^{a}	260.37±59.79^{a} bc	0.60±0.22^{a}	9.61±3.22^{a}	5.87±1.34^{a}	0.57±0.20 a	3.78±1.16^{a}
WR	27.56±1.90^{ab}	35.81±4.33^{c} d	8.41±0.19^{b}	0.28±0.08^{a}	184.72±34.90 b	27.80±3.20^{a}	243.88±27.52^{a} b	0.62±0.14^{a}	8.73±2.28^{a}	5.10±1.03^{a}	0.73±0.10 ab	3.94±1.24^{a}
WA	23.85±1.14^{a}	26.93±0.34^{a}	8.29±0.09^{b}	0.35±0.08^{a}	126.45±17.24 a	28.59±2.61^{a}	344.78±48.90^{e}	0.42±0.11^{a}	9.13±2.28^{a}	5.33±1.40^{a}	0.66±0.13 a	3.76±1.33^{a}
YA	27.33±3.06^{ab}	32.13±3.26^{b} c	8.23±0.24^{b}	0.16±0.12^{a}	190.23±38.15 b	27.51±4.60^{a}	224.05±228.68^{a}	0.54±0.29^{a}	8.89±2.48^{a}	4.09±1.30^{a}	0.65±0.15 a	3.97±0.93^{a}
NA1	26.14±3.73^{ab}	31.22±2.56^{b}	8.36±0.10^{b}	0.36±0.09^{a}	153.71±32.03 ab	29.37±2.65^{a}	326.49±36.74 de	0.66±0.24^{a}	8.29±2.81^{a}	4.98±1.53^{a}	0.60±0.14 a	4.03±1.53^{a}
NA2	28.52±3.68^{a}	38.53±1.78 de	8.19±0.21^{b}	0.35±0.08a	155.32±25.31 ab	30.06±2.30^{a}	256.79±23.27^{a} bc	0.65±0.16^{a}	8.06±2.19^{a}	4.82±1.34^{a}	0.67±0.13 a	3.94±0.61^{a}
BU	25.65±2.69^{ab}	33.66±3.34^{b} c	8.29±0.19^{b}	0.19±8.56^{a}	181.79±44.98 b	31.12±1.98^{a}	300.40±44.17^{c} de	0.61±0.08^{a}	6.28±1.63^{a}	5.22±0.05^{a}	0.65±0.03 a	3.54±0.05^{a}
Total	26.94±2.90	34.10±4.74	8.25±0.22	0.61±2.86	168.01±38.75	29.23±3.04	282.63±52.41	0.59±0.19	8.59±2.61	4.97±1.27	0.68±0.14	3.89±1.04

Values are mean of 9 replicates ± S.E.; EC: electric conductivity (ds.m^{-1}), N: Nitrogen (Kg/ha), P: Phosphorus (kg/ha), K: Potassium (kg/ha),O.C: Organic Carbon (%), Fe: Iron (ppm),Mn: Manganese (ppm),Zn: Zinc (ppm),Cu: Copper (ppm). 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)

In case of healthy rhizosphere soil, the maximum temperature (30.60^{0}C) was recorded at Akola (AK) sample. The moisture percentage of rhizosphere soil was in the range of 27.84 to 39.14%, which was maximum 39.14% in Amravati (AM1) sampling site. It was recorded that soil acidity of all sampling sites indicates the alkaline nature of soil (8.20 to 8.49). The electrical conductivity of healthy rhizosphere soil collected from different sampling sites was in the range of 0.28 to 0.52 mg /dsm^{-1}. In case of macro and micro nutrient maximum nitrogen (239.14 kg/ha) was estimated from Wardha (WR) sampling site, however phosphorus was in the range of 27.58 to 31.77 kg/ha. In case of Potassium maximum Potassium *i.e.*

354.68 kg/ha was estimated from Akola (AK) sampling site. The organic carbon (0.88%), zinc (0.85ppm) and Copper (4.44ppm) was estimated maximum from Nagpur (NA2), Washim (WA) and Yavatmal (YA) sampling sites while, Iron (10.83ppm) and Manganese (6.35ppm) was observed maximum in Buldhana (BU) sampling side.

The data revealed in diseased rhizosphere soil, that the maximum temperature (28.95^{0}C) was recorded at Akola (AK) sampling site followed by Amravati (AM1) *i.e.* 27.65^{0}C. The moisture percentage of rhizosphere soil was in the range of 26.93 to 40.25%, the soil acidity of all sampling sites indicates the alkaline nature of soil (7.93 to 8.41). The electrical conductivity of healthy rhizosphere soil collected from different sampling sites was in the range of 0.16 to 0.5 mg / dsm^{-1}. In case of macro and micro nutrient, maximum nitrogen (190.72 kg/ha) and Copper (4.17ppm) was estimated from Amravati (AM1) sampling site, however phosphorus was in the range of 27.51 to 31.12 kg/ha. In case of Potassium, maximum Potassium *i.e.* 344.78 kg/ha was estimated from Wardha (WR) sampling site. The organic carbon (0.66%), zinc (0.85ppm) was estimated maximum from Nagpur (NA1) and Akola (AK) sampling sites, respectively while, Iron (9.61ppm) and Manganese (5.87 ppm) was observed maximum in Amravati (AM2) sampling side.

The distribution of soil microbial population was determined by different soil physico-chemical properties. Maximum significant correlation of microbial population with soil physico-chemical properties found in a Temperature, Moisture, pH, EC, Nitrogen, Phosphorous, Potassium, Organic carbon, Manganese and Zinc.

The result showed that in healthy rhizosphere, Moisture, Organic carbon, Copper and Phosphorous are positively correlated with bacterial and fungal population whereas Temperature, Manganese, EC, Iron, Zinc, pH, and Potassium are negatively correlated. In diseased rhizosphere of citrus, Temperature, Manganese, Phosphorous, Nitrogen, Iron and Moisture are positively correlated whereas, pH, Zn, Organic carbon and Potassium negatively correlated.

The PCA analysis was carried out to see the influencing factor of physio-chemical properties with microbial population at different locations. The result reported that the sampling sites Akola (AK) was influenced by Temperature, Moisture, Nitrogen, Copper and Zinc and Amravati (AM1) by Organic carbon, Moisture, Copper, Temperature, EC, Iron, Nitrogen, Potassium and Zinc. Whereas, Amravati (AM2) by Zinc, Nitrogen, Phosphorous, EC for microbial population in citrus rhizosphere ecosystem.

In case of Wardha (WR) and Washim (WA), Temperature, Moisture, Nitrogen, Zinc, Iron, Ph, are the most influencing factors whereas in Nagpur region NA1 and NA2, P^H, Organic carbon, Moisture and Manganese are the factor which are responsible for the microbial population in the rhizosphere region of citrus. In Buldhana (BU) sampling sites, EC and Manganese whereas in Yavatmal (YA) sampling site pH, Iron and EC are the soil properties which influenced the microbial population in rhizosphere region of citrus.

Conclusions

The sampling sites Akola (AK)was influenced by Temperature, Moisture, Nitrogen, Copper and Zinc and Amravati(AM1) by Organic carbon, Moisture, Copper, Temperature, EC, Iron, Nitrogen, Potassium and Zinc on soil microbial population. Whereas Amravati (AM2) by Zinc, Nitrogen, Phosphorous, EC for microbial population in citrus rhizosphere ecosystem. In case of Wardha (WR) and Washim (WA), Temperature, Moisture, Nitrogen, Zinc, Iron, pH are the most influencing properties whereas in Nagpur region NA1 and NA2, pH, Organic carbon, Moisture and Manganese are the factor which are responsible for the microbial population in the rhizosphere region of citrus. Buldhana (BU) sampling sites, physico-chemical properties like EC, and Manganese influenced the soil microbial population. 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 manegment for citrus diseases by enriching the soil Physico-chemical properties which are mainly essential for luxuriant growth of plant. However, the further work is necessary to enhance the disease control capability by managing the nutrient and ecological status of soil.

References

1. Abd El-Motty EZ, Selim M, Abou YR, Farahat SA. Studies on growth, nutritional and biological status of citrus seedling infested with root rot diseases. Nat. and Science 2010;8(4):112-121.
2. Ahmad K, Sajam K, Hashim H, Rosli Z, Abdu A. Field assessment of calcium, copper and zinc ions on plant recovery and disease severity following infection of Huanglongbing (HLB) disease. African Journal of Microbiology Research 2011;5(28):4967-4979.
3. Bhattacharyya PN, Jha DK. Seasonal and depth-wise variation in microfungal population number in Nameri forest soil, Assam, Northeast India. Mycosphere 2011;2(4):297-305.
4. Black CA. American Soc. of Agro. Madison, USA 341-344.
5. Carney KM, Matton PA. Plants communities, soil microorganism, and soil carbon cycling: does altering the world below ground matter to ecosystem functioning. Ecosystems 2005;8:928-940.
6. Devi TR, Chhetry GKN. Rhizosphere and non rhizosphere microbial population dynamics and their effect on wilt causing pathogen of Pigeonpea. Inter. J of Scintific and Res. Publication 2012;2(5):26-29.
7. Etebu E, Osborn AM. Areview of indicators of healthy agricultural soils with Pea Footrot Diseases suppression potentials. Sustain. Agri Res 2012;1(2):235-250.
8. Gade RM. Biological and chemical manegment of root rot/ coller rot in citrus nursery. The Bioscan 2012;7(4):631-635.
9. Gomez UE, Bayon JR, Castro D, Coupe SJ. Effeciency of MPN method to indicate hydrocarbon biodegradation process within Permeable pavements.11th International Conference on Urban Drainage 2008, 1-10.
10. Healther MD, Alexandra GS, Richard PD. Compost and manure mediated impact on soil born pathogens and soil quality. Soil Science Society of American, J 2006;70:347-358.
11. Jagtap GP, Dhavale MC, Dey U. Evaluation of natural plant extracts, antagonistists and fungicides in controlling root rot, fruit (brown) rot and fungicides of citrus caused by *Phytopthora* spp. *in vitro*. Scientific Journal of Microbiology 2012;1(2):27-47.
12. Karam DS, Arifin A, Radiziah O, Shamshuddin J, Ahmad H. Impact of enrichment planting activity or soil physico-chemical properties of degraded forest land. International Journal of Environment Science 2013;5(2):407-422.

13. Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil sci. soc. Am. J 1978;42:421-428.
14. Marschner H. Mineral nutrition of higher plants. 2nd Edition. Academic Press, London 1995.
15. Mary CL, Sujatha R, Chozhaa AJ, Navas P. Influence of organic manures (Biofertilizers)on soil microbial population in the rhizosphere of mulberry (*Morusindica* L.). Int. J S Appl. Sci. Biotechnol 2015;3(1):61-66.
16. Naher UA, Othaman R, Panhwar QA. Culturable total and beneficial microbial occurrences in long-term nutrient deficit wetland rice soil. Australian J Crop Sci 2013;7(12):1848-1853.
17. Ramamoorthy SM, Kumar DR. Impact of Conventional, sustainable and organic farming system on soil microbial population and soil biochemical properties, Puducherry, India. Inter J Environ. Sci 2013;4(1):0976-4402.
18. Rao Y, Nigam N, Varma R, Venkatesh KR, Kumar A. Microbial and nutrient study in alkaline soil used for cultivation of different verities of Mulberry plants. Inter. J Agri. Sci. and Res 2014;4(6):81-94.
19. Sing HV. Soil Carbon Sequestration and Rhizospheric Microbial Population in Apricot Orchards Following Plastic Film Mulching Under Cold Arid Region. Inter. J Horti 2013;3(8):35-41.
20. Singh RP, Duveiller E, Nicol JM. The challenges of maintaining wheat and productivity pest, diseases and potential epidemics. Euphytica 1999;157:417-430.
21. Spann TM, Schumann AW. The role of plant nutrients in disease development with emphasis on citrus and Huanglongbing. Proc. Fla. State Hort. Soc 2009;122:169-171.
22. Srivastava AK. Nutrient manegment in Nagpur mandarin frontier development. Sci. J agri 2013;2(1):1-14.
23. Sudhakaran MR, Kumar DR. Impact of Conventional, sustainable and organic farming system on soil microbial population and soil biochemical properties, Puducherry, India. Inter J Environ. Sci 2013;4(1):0976-4402.
24. Yadav A, Yadav K. Seasonal population dynamics of rhizosphere and non rhizosphere soil microorganism of Chir pine seedlings (*Pinus roxburghii* Sarg.). British Microbiol. Res J 2013;3(4):664-677.