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Rhizosphere bacillus diversity vis-à-vis fruit yield of citrus

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ABSTRACT

Role of microbes in soil fertility transformation is well established. But relationship of soil microbial diversity with crop yield is lesser studied, especially in perennial crop like citrus. Rhizosphere soil samples collected from the orchards of varying fruit yield levels, showed fungal count ranging from 2×10^3 to 7×10^{3}cfu/g in low yielding citrus rhizosphere soils, 6×10^3 to 13×10^3 cfu/g in optimum yielding citrus rhizosphere soils and10×10^3 to 28×10^3 cfu g^{-1} in high yielding citrus rhizosphere soils. While bacterial count ranged from 36x10^3 to 53x10^{3}cfu/g in low yielding,45x10^3 to 108x10^{3}cfu/g in optimum yielding and 86x10^{3}to132x10^{3}cfu/g in high yielding. These observations well supported by the population of different Bacillus species in high yielding trees (Bacillus subtilis 22.08cfu/g, Bacillus polymyxa 5.25 cfu/g, Bacillus licheniformis 6.38cfu/g,Bacillus mycoides 26.08 cfu/g,Bacillus pumilus 6.16cfu/g and Bacillus cereus 8.14cfu/g)being significantly higher over low yielding trees (Bacillus subtilis 11.75cfu/g, Bacillus polymyxa 7.25cfu/g, Bacillus licheniformis 10.66cfu/g, Bacillus mycoides 12.08 cfu/g, Bacillus pumilus 2.16cfu/g and Bacillus cereus 3.16cfu/g).These observations suggested strong role of rhizosphere microbial diversity in orchard performance.

Key words: Rhizosphere, bacilus diversity, fruit yield, citrus, central India

INTRODUCTION

Citrus (*Citrus spp.)* is one of the most important fruit crops grown in many tropical and subtropical countries. (Srivasatva *et al.* 2017, Srivastava and Malhotra, 2014).The rhizosphere of a plant is considèred as store house of all chemical transformations taking place as per plant metabolism (Srivastava, 2013a). Rhizosphere, the soil region nearest to plant root system, inhabits various microorganisms varying in their community structure and diversity, soil microbial biomass dominates fungal mycelium accumulates and retains mineral nutrients (Srivasatva and Ngullie, 2009; Ngullie*et al*2015). Rhizosphere properties indicate the causes against any possible depletion in either available supply of nutrients or loss of native soil microbial biomass as an indicator of rhizosphere health (Srivastava 2009). The rhizosphere microflora includes bacteria, fungi, nematodes, protozoa, algae and micro arthrops. Microbes residing in the rhizosphere also have key roles in ecosystems and influence a large number of important processes, including nutrient acquisition, nitrogen cycling, carbon cycling and soil formation (Srivastava, 2010a; 2010b). A number of species belonging to the genus *Bacillus* were found to be well adapted to the rhizoplane of established trees, including citrus (Srivasatva and Singh, 2009). *Bacillus* species are natural inhabitants of the phyllosphere and rhizosphere. Such microbial diversity are capable of producing enzymes, antibiotics, proteins, vitamins or secondary metabolites that exhibit the ability to promote growth or induce defense mechanisms in animals and plant. In this background efforts were made to study the bacillus diversity in relation varying levels of fruit yield.

MATERIALS AND METHODS

Four Nagpur mandarin (*Citrus reticulata* Blanco) orchards were selected at Hingna tahsil of Nagpur District representing typical sub-humid tropical climate of central India. Ten trees from each of these four orchards representing three categories of fruit yields were further elected. Trees with low yielding trees as< 500 fruits/tree (<30 kg/tree) optimum yielding trees indicated 500-800 fruits/tree (30-60kg/tree) and high yielding having more than 800 fruits/tree (>60 kg/tree). The rhizosphere soil samples from, within 0-15cm depth were collected from periphery of selected trees. Soil samples were brought to the laboratory for analysis. The data generated through analysis of 120 soil samples were partitioned into three categories for further results and analysis. Soil microbial count was determined by serial dilution and pour plate technique, following the procedure as outlined by

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Benson (2002). In this technique, one gram of soil sample was taken under aseptic condition in 10 ml of sterile test tube with 9 ml of distilled water the soil with distilled water was mixed thoroughly for uniform suspension. Then 1 ml suspension transferred from this tube to other with 9 ml distilled water to get the suspension of 10^{-2} dilution. It was further diluted to get desired levels of 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} dilutions, after dilution, 1ml of suspension was transferred in petridish with specific media (Potato dextrose agar: potato 200g,dextrose 20g,agar 20g and distilled water 1000 ml for fungi growth. Nutrient agar medium peptone 5g, beef extract 3g, agar 20g in 1000 ml distilled water for bacterial population) for specific growth of micro-organisms. The *Bacillus* diversity medium was used separated different species of Bacillus viz., *Bacillus subtilis, Bacillus cereus, Bacillus polymyxa, Bacillus licheniformis, Bacillus mycoides, Bacillus pumilus*.

RESULTS AND DISCUSSION

Rhizosphere microbial load

Soil microbial is considered an important index of soil fertility (Srivasatva *et al.,* 2012; Keditsu and Srivastava, 2014). The fungal count ranged between 2-7×10^3 cfu/g in low yielding citrus rhizosphere soils with an average value of 4.33 ×10^3cfu/g of soil. While in optimum yielding citrus rhizosphere soils, the fungal count ranged as 6-13x 103 cfu/g soils with an average value of 9.81x10^3cfu/g soil. These values were significantly higher in high yielding citrus rhizosphere soils as 10-28x10^3 cfu /soil with an average value of 16.58x10^3cfu/g soil. These were the reasons, why healthy rhizosphere soil supported higher fruit yield (Malhotra and Srivasatva, 2015; Srivasatva and Singh, 2015).The bacterial count, most of the times, is registered invariably higher in population compared to fungal count (Joseph *et al.,* 2015). The bacterial count ranged between from 36-53×10^3cfu/gin low yielding citrus rhizosphere soils and 86-132x10^3cfu/g in high yielding citrus rhizosphere soils, with corresponding average values of 43.08x10^3cfu/g soil, 72.75 cfu/g soil and 109.08x10^3cfu/g soil.

Bacillus diversity of rhizosphere

Bacillus diversity analysis of differentially yielding citrus trees expressed their reorientation in a specific manner (Srivasatva*et al.,* 2015). The *Bacillus subtilis*count ranged from 9×10^3 to 20×10^3 cfu g^{-1} in low yielding citrus rhizosphere soils (Mean 11.75 cfu / gsoil) in optimum yielding citrus trees, the *Bacillus subtilis* count was observed to vary from 8x10^3 to 23x10^3cfu/g withan average value of 15.37x 10^3 cfu/g soil. While in high yielding citrus trees, the *Bacillus subtilis* count ranged between 14-33x10^3cfu/g. An important phosphate solubilising *Bacillus species*, *Bacillus polymyxa* count ranged from 4-12×10^3 cfu/g, 3-12x10^3cfu/g,and 2-8x10^3cfu/gin low yielding, optimum yielding and high yielding rhizosphere soil, respectively with corresponding average of 7.3x10^3 cfu/g,7.6x10^3cfu/g and 5.3x10^3 cfu/g. The *Bacillus licheniformis* count ranged from 7- 23×10^3cfu/gin low yielding citrus rhizosphere soils. The mean as 10.7×10^3cfu/g soil.4-20×10^3 cfu / gin optimum yielding citrus rhizosphere soils. (Mean as 10.3×10^3cfu/g soil and4-12×10^3 cfu/gin high yielding citrus rhizosphere soils (Mean as 6.6x10^3cfu/g soil). On the other hand, *Bacillus mycoides* count ranged as 8-28×10^3 cfu/gin low yielding citrus rhizosphere soils, with an average value of 12.1×10^3cfu/g soil. The population was much higher in optimum yielding rhizosphere ranging as 11-25x10^3cfu/g within an average of 17.4x103cfu/g soil.

Table1: Rhizosphere soil microbial load Bacillus diversity vis-à-vis yield of Nagpur mandarin in summarized form representing average of 40 rhizosphere soil samples under each of the three categories of orchard yield

	Orchards yield		
	Low (<30kg/kg)	Optimum (30-60kg/kg)	High (>60kg/ha)
Bacterial count (10^3xcfu/g)	43.08 (36-53)	72.75 (45-108)	109.0 (86-132)
Fungal count (10^3xcfu/g)	4.33(2-7)	9.81(6-13)	16.58(10-28)
Bacillus subtilis(10^3xcfu/g)	11.75(9-20)	15.37(8-23)	22.08(14-33)
Bacillus polymyxa(10^3xcfu/g)	7.25(4-12)	7.56 (3-12)	5.25 (2-8)
Bacillus licheniformis(10^3xcfu/g)	10.66 (7-23)	10.25(4-20)	6.58 (4-12)
Bacillus mycoides(10^3xcfu/g)	12.08 (8-28)	17.43 (11-25)	26.08 (16-39)
Bacillus pumilus(10^3xcfu/g)	2.16 (1-6)	4.68 (1-11)	6.16 (2-8)
Bacillusecreus(10^3xcfu/g)	3.16 (1-12)	6.06(1-14)	8.41(6-14)

** Figures in paraenthes is indicate the range*

The *Bacillus mycoides* count ranged from 16- 39×10^3 cfu/gin high yielding citrus rhizosphere soils indicating their strong contribution toward better rhizosphere health vis-à-vis fruit yield (Srivastava *et al.,* 2007; Srivastava and Malhotra, 2014) The average value of Bacillus mycides count was 26.1x10^3cfu/g soil in those high yielding rhizosphere soils. The *Bacillus pumilus* count ranged from1.6x10^3 cfu/gin low yielding citrus rhizosphere soils. While it count ranged from 1×10^3 to 11×10^3 cfu/gin optimum yielding citrus rhizosphere soils.The *Bacillus pumilus* count ranged from 2×10^3 to 8×10^3 cfu/ g in high yielding citrus rhizosphere soils. 2.3 x 10^3 cfu/g, 4.6 x 10^3 cfu/g and 6.2 ×10^3cfu /g in low, optimum and high yielding rhizosphere soils, respectively. The *Bacillus cereus* count ranged from 1-12×10^3 cfu/gin optimum yielding citrus rhizosphere soils with an 6.1x10^3cfu/g soil. While these counts of were much higher 1-14 x10^3cfu/g in high yielding citrus rhizosphere soils. *Bacillus cereus* count ranged from 1×10^3 to14×10^3cfu/gin high yielding citrus rhizosphere soils withan average of 8.4x10^3cfu/g soil. These observations warrant greater in depth studies to pinpoint effective rhizosphere microbes contributing towards orchard performance.

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