Soil rhizosphere microbial properties of selected farmlands in Rumuokparali community

ABSTRACT

This study was conducted to determine the rhizosphere soil microorganisms associated with the cultivation of *Manihot esculentum*, *Comelina bengalensis*, *Talinum triangulare* and *Telfairia occidentalis*. The plants were obtained from newly cultivated, ready for harvest and fallowed farmlands. The rhizosphere microorganisms were enumerated and compared to the bulk soil microorganisms. The heterotrophic bacterial count for the newly cultivated farmland ranged from 2.9 $x10^7$-$6.3x10^8$$cfug^{-1}$ and total fungal ranged from 5.6 $x10^4$-7.0 $x10^6$ $sfug^{-1}$, while the bulk soil total heterotrophic bacterial count was 4.96 x 10^9 $cfug^{-1}$ and total fungal count was 5.87 x $10^6$$sfug^{-1}$. The heterotrophic bacterial count for the ready for harvest farmland ranged from 1.82 $x10^8$-1.80 $x10^9$ $cfug^{-1}$ and total fungal ranged from 2.3 $x10^4$-$3.57x10^6$ $sfug^{-1}$, while the bulk soil total heterotrophic bacterial count was $1.90x10^9$ $cfug^{-1}$ and total fungal count was $2.30x10^6$ $sfug^{-1}$. The heterotrophic bacterial count for the fallowed farmland ranged from 5.65 $x10^8$-$1.50x10^9$ $cfug^{-1}$ and total fungal ranged from 1.33 $x10^6$-$3.05x10^6$ $sfug^{-1}$, while the bulk soil total heterotrophic bacterial count was $1.74.0x10^9$$cfug^{-1}$ and total fungal count was $1.07x10^6$ $sfug^{-1}$. The rhizopsphere soil bacterial isolates belong to the genera isolated *Staphylococcus*, *Hafnia*, *Acinetobacter, Bacillus*, *Bacteroides, Klebsiella* , *Tatumella*, *Enterobacter*, *Corynebacterium* and *Pseudomonas* while the fungal isolates belong to the genera: *Aspergillus*, *Epicocum*, *Chrysosporium*, *Trichosporon*, *Cryptococcus*, *Fusarium*, *Penicillium* and *Chaetomium*. The bulk soil bacterial isolates belong to the genera: *Staphylococcus*, *Hafnia*, *Acinetobacter, Bacillus*, *Klebsiella*, *Tatumella*, *Corynebacterium* and *Pseudomonas* while the fungal isolates belong to the genera: *Aspergillus*, *Epicocum*, *Chrysosporium*, *Trichosporon*, *Cryptococcus* and *Chaetomium.* Microbial diversity of the rhizosphere soil was more compared to the bulk soil.

Keywords: Rhizosphere, bulk soil, microorganisms, farmlands

Introduction

The rhizosphere is the narrow region of soil that is in direct proximity to plants roots and is influenced by root secretions and actions of associated soil microorganisms [1]. The actual extent of the rhizosphere is dependent on the zone of influence of the plants roots and associated microorganisms. Soil which is not part of the rhizosphere is known as bulk soil. The rhizosphere is generally considered to be the most biodiverse and dynamic habitat on earth and also, a metabolically busier and more competitive environment than the surrounding bulk soil [2]. Unlike the soil associated with the rhizosphere, bulk soil is not penetrated by plant roots, and generally has lower microbial communities [3].

In the rhizosphere, symbolic relationships exist between plant roots and microorganisms. Different water soluble nutritive compounds containing sugars and amino acids are exuded

by plant roots which sustain and provide for microbial existence. Plants roots have strong impacts on the physical and chemical environment in the rhizosphere, such that a shift in this environment, which is influenced by the manipulations of plants roots, significantly impacts on the availability and forms of substances used for microbial metabolism, which in turn strongly affect the prevalence and diversity of different microbial species and functions, as well as their total biomass within the rhizosphere [1]. The microbial population in the rhizophere carries out process such as the transformation of carbon, nitrogen and other minerals, to the benefit of the plant [4]. In addition to this, soil bacterial species produce biofilms which act as aggregating mechanism for mineral particles and organic matter [5]. Also, mycorrhizal fungi have been shown to increase aggregation through the physical construction of fungal hyphae, as well through the secretion of their own exopolymer deposit.

The rhizosphere is an environment that allows a diverse range of soil microorganisms to thrive. The aim of this study was to determine both quantitatively and qualitatively, the rhizosphere soil microorganisms associated with certain plants from different farmlands.

MATERIALS AND METHODS

Sample Collection

The rhizosphere soil samples were collected from farmlands at Rumuokparali community in Obio Akpor Local Government Area of Rivers State, Nigeria. The rhizosphere soils were obtained from the plant roots, ranging from 0-10 cm depth. The control for each of the farmland was obtained from the surface soil between 0-1cm thickness. Using the composite sampling technique, rhizosphere soil samples were aseptically obtained with a sterile spatula from *Manihot esculentum*, *Talinum triangulare, Telfairia occidentalis* and *Comelina bengalensis* from the newly cultivated and ready for harvest farmland. *Manihot esculentum, Talinum triangulare* and *Comelina bengalensis* rhizosphere soil samples were aseptically obtained from the fallowed farmland. The bulk soils from farmlands, which served as controls, were collected using the composite sampling technique, with the aid of a quadrate.

Microbiological Analysis

These dilutions were used to carry out the isolation and enumeration of the soil bacteria and fungi.

Enumeration of Total Heterotrophic Bacteria Count (THBC)

This test was done to screen for the total viable aerobic, mesophilic and heterotrophic bacteria present in each soil sample. The rhizosphere soil samples were serially diluted to 10^{-5} dilution using physiological saline. Aliquots 0.1ml from 10^{-3}, 10^{-4} and 10^{-5} dilutions were spread aseptically with a sterile glass rod on freshly prepared dry nutrient agar plates. The plates were incubated at 37ºC for 24 to 48 hours. After incubation, the TFC for each sample was recorded and expressed in colony forming unit per gramme ($cfug^{-1}$) after colony count.

Enumeration of Total Fungal Count (TFC)

This test was carried out to enumerate the total heterotrophic fungal species contained in the effluent samples. Aliquots of 0.1ml from 10^{-1}, 10^{-2} and 10^{-3} dilutions were spread aseptically with a sterile glass rod on freshly prepared dry potato dextrose agar plates and incubated at 28ºC for 3-5 days. After incubation, the TFC for each sample was recorded and expressed as spore forming unit per gram ($sfug^{-1}$) after colony count.

Purification and Characterization

After incubation, distinct colonies observed on both the nutrient agar plates and potato dextrose agar plates were isolated and purified by repeated sub-culturing. The bacterial isolates were characterized using their macroscopic, microscopic and biochemical attributes. The biochemical tests performed on the bacterial isolates were: catalase, oxidase, motility, hydrogen sulphide production (H_2S), indole, Voges-Proskauer (VP), Methyl red (MR), citrate, and sugar fermentation. The fungal isolates were characterized on the basis of their macroscopic and microscopic appearance with reference to standard manual.

Physicochemical Analysis

The physicochemical parameters monitored were temperature, pH, moisture content, total organic carbon and total nitrogen. The physicochemical parameters were assessed according to the methods as described by the American Public Health Association [6].

RESULTS

The results for the total heterotrophic bacterial count (THBC) and total fungal count (TFC) are shown in Table 1. The rhizophere THBC ranged from 2.9 $x10^{7}$-$1.80x10^{9}cfug^{-1}$ while the rhizophere TFC ranged from 2.3 $x10^{4}$-$7.0x10^{6}sfug^{-1}$. The bulk soil THBC ranged from 1.74-4.96.$0x10^{9}cfug^{-1}$, while the bulk soil TFC ranged from 1.07 $x10^{6}$-$5.87.0x10^{6}sfug^{-1}$.

Table 2 shows bacterial isolates from the rhizophere soil. The bacterial isolates belong to the genera: *Staphylococcus, Hafnia, Acinetobacter, Bacillus, Bacteroides, Klebsiella, Tatumella, Corynebacterium, Pseudomonas* and *Enterobacter*. The fungal isolates were characterized to belong to the genera: *Aspergillus, Epicocum, Chrysosporium, Trichosporon, Cryptococcus, Chaetomium, Microsporium, Penicillium* and *Fusarium* (Table 3).

Table 1: Bacterial and fungal counts obtained from the rhizosphere soil samples

S/N	Soil sample	Bacterial count ($cfug^{-1}$)	Fungal count ($sfug^{-1}$)
1.	NME	3.1×10^8	1.91×10^6
2.	NCB	1.55×10^8	7.0×10^6
3.	NTT	6.3×10^8	5.6×10^4
4.	NTO	2.9×10^7	1.6×10^6
5.	RME	18.2×10^8	3.57×10^6
6.	RCB	180×10^9	2.0×10^6
7.	RTT	9.0×10^8	2.3×10^4
8.	RTO	6.2×10^8	3.32×10^6
9.	FME	1.5×10^9	1.83×10^6
10.	FCB	6.55×10^8	3.05×10^6
11.	FTT	5.65×10^8	1.33×10^6
12.	NC	4.96×10^9	5.87×10^6
13.	RC	1.9×10^9	2.30×10^6
14.	FC	1.74×10^9	1.07×10^6

Keys: NME - Newly Cultivated *Manihot esculentum*

NCB - Newly Cultivated *Comelina bengalensis*

NTT - Newly Cultivated *Talinum triangulare*

NTO - Newly Cultivated *Telfairia occidentalis*

RME - Ready for Harvest *Manihot esculentum*

RCB - Ready for Harvest *Comelina bengalensis*

RTT - Ready for Harvest *Talinum triangulare*

RTO - Ready for Harvest *Telfairia occidentalis*

FME - Fallowed *Manihot esculentum*

FCB - Fallowed *Comelina bengalensis*

FTT - Fallowed *Talinum triangulare*

NC - Nearly Cultivated Control

RC - Ready for Harvest Control

FC - Fallowed Control

Table 2: Bacterial isolates obtained from the different soil samples

S/N	Soil sample	Bacterial Isolates
1.	NME	*Staphylococcus* sp, *Hafnia* sp, *Acinetobacter* sp, Bacillus sp, *Bacteroides* sp
2.	NCB	*Staphylococcus* sp, *Hafnia* sp, *Corynebacterium* sp, *Klebsiella* sp, *Bacillus* sp
3.	NTT	*Klebsiella* sp, *Bacillus* sp, *Corynebacterium* sp
4.	NTO	Bacillus sp, *Enterobacter sp*, *Tatumella* sp
5.	RME	*Klebsiella* sp, *Hafnia* sp, *Acinetobacter* sp, *Tatumella* Sp, *Bacteroides* sp, Bacillus sp
6.	RCB	*Klebsiella* sp, *Tatumella* Sp, *Acinetobacter* sp, *Bacillus* sp
7.	RTT	*Bacillus* sp, *Hafnia* sp
8.	RTO	*Bacillus* sp, *Enterobacter sp*, *Tatumella* sp, *Klebsiella* sp, *Hafnia* sp, *Acinetobacter* sp
9.	FME	*Staphylococcus* sp *Bacillus* sp, *Corynebacterium* sp, *Hafnia* sp, *Tatumella* sp, *Klebsiella* sp
10.	FCB	*Pseudomonas sp*, *Klebsiella* sp, *Hafnia* sp, *Acinetobacter* sp, *Tatumella* Sp, *Bacillus* sp
11.	FTT	*Bacillus* sp, *Hafnia* sp, *Tatumella* sp, *Klebsiella* sp
12.	NC	*Bacillus* sp, *Acinetobacter* sp, *Staphylococcus* sp *Tatumella* Sp
13.	RC	*Bacillus* sp, *Acinetobacter* sp, *Staphylococcus* sp *Tatumella* Sp, *Klebsiella* sp
14.	FC	*Bacillus* sp, *Acinetobacter* sp, *Staphylococcus* sp *Klebsiella* sp, *Pseudomonas* sp, *Corynebacterium* sp, *Tatumella* sp

Table 3: Fungal isolates obtained from the different soil samples

S/N	Soil Sample	Fungal Isolates
1.	NME	*Aspergillus* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp
2.	NCB	*Trichosporon* sp, *Cryptococcus* sp, *Aspergillus* sp
3.	NTT	*Aspergillus* sp, *Epicocum* sp, *Trichosporon* sp, *Chaetomium* sp
4.	NTO	*Aspergillus* sp, *Chrysosporium* sp *Chaetomium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Epicocum* sp
5.	RME	*Aspergillus* sp, *Trichosporon* sp, *Epicocum* sp, *Chrysosporium* sp, *Cryptococcus* sp, *Chaetomium* sp
6.	RCB	*Aspergillus* sp, *Microsporium* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Chaetomium* sp
7.	RTT	*Aspergillus* sp, *Epicocum* sp, *Trichosporon* sp, *Chaetomium* sp
8.	RTO	*Aspergillus* sp, *Microsporium* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Chaetomium* sp
9.	FME	*Aspergillus* sp, *Microsporium* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Chaetomium* sp
10.	FCB	*Aspergillus* sp, *Microsporium* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Chaetomium* sp *Penicillium* sp
11.	FTT	*Aspergillus* sp, *Epicocum* sp, *Chaetomium* sp, *Trichosporon* sp, *Cryptococcus* sp

12.	NC	*Aspergillus* sp, *Microsporium* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Chaetomium* sp
13.	RC	*Aspergillus* sp, *Microsporium* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus sp, Chaetomium sp*
14.	FC	*Aspergillus* sp, *Microsporium sp, Epicocum sp, Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Chaetomium* sp

Table 4 shows the physicochemical parameters of the farmlands. The rhizosphere soils from the *Manihot esculentum* for the three farmlands had their temperature as 27°C, while pH ranged from 5.265-6.190; moisture content, 10.8-12.9%; total carbon content, 0.927-1.614 mgL^{-1} and total nitrogen content, 1.40-1.68 mgL^{-1}. The rhizosphere soils from the *Comelina bengalensis* for the three farmlands had their temperature values as 27°C, while their pH values ranged from 5.901-6.682; moisture content, 41.0-43.6%, total carbon content, 12.232-17.001 mgL^{-1}; total nitrogen content, 4.321-4.968 mgL^{-1}. The rhizosphere soils from the *Talinum triangulare* for the three farmlands had their temperature values as 27°C, while their pH values ranged from 4.987-6.230; moisture content, 16.29-33.20%, total carbon content, 8.002-12.280 mgL^{-1}; total nitrogen content, 3.200-5.212 mgL^{-1}. The rhizosphere soils from the *Telfairia occidentalis* for the three farmlands had their temperature values as 27°C, while their pH values ranged from 4.890-6.009; moisture content, 28.2-32.0%, total carbon content, 3.210-7.980 mgL^{-1}; total nitrogen content, 5.101-5.210 mgL^{-1}. The bulk soil samples from the three farmlands had their temperature values as 27°C, while their pH values ranged from 6.628-6.983; moisture content, 6.69-7.21%, total carbon content, 10.01-11.29 mgL^{-1}; total nitrogen content, 11.68-12.90 mgL^{-1}.

Table 4: Physicochemical properties of farmland soil samples

S/N	Soil **Sample**	**Temperature (^{0}C)**	**pH**	**Moisture content (%)**	**Total carbon content ($mgkg^{-1}$)**	**Total nitrogen content (%)**
1.	NME	27	5.265	12.9	0.927	1.40
2.	RME	27	6.190	12.6	1.080	1.68
3.	FME	27	5.695	10.8	1.614	1.555
4.	NCB	27	6.682	43.6	12.232	4.968
5.	RCB	27	6.002	43.2	14.621	4.712
6.	FCB	27	5.908	41.0	17.001	4.321
7.	NTT	27	4.987	33.20	12.280	5.212
8.	RTT	27	6.230	16.29	8.002	3.200
9.	FTT	27	5.760	21.30	10.621	5.111
10.	NTO	27	4.890	32.0	3.210	5.210
11.	RTO	27	6.009	28.2	7.980	5.101
12.	NC	27	6.983	7.21	10.82	12.92
13.	RC	27	6.801	7.20	10.01	11.68
14.	FC	27	6.628	6.69	11.29	11.98

Discussion

This study was conducted to determine, both quantitatively and qualitatively, the diversity of the rhizosphere soil microorganisms associated with *Manihot esculentum*, *Comelina bengalensis*, *Talinum triangulare* and *Telfairia occidentalis* obtained from newly cultivated, ready for harvest and fallowed farmlands. The heterotrophic bacterial count obtained from the different rhizosphere soils for the different plant species in the ready for harvest farmland ranged from 1.82 $x10^8$-1.80 $x10^9$ $cfug^{-1}$ and the total fungal count ranged from 2.3 $x10^4$-3.57$x10^6$ $sfug^{-1}$, while the bulk soil had heterotrophic bacterial count was 1.9 $x10^9$ and fungal count was 2.3$x10^6$$sfug^{-1}$. The ready to harvest soil had more bacteria load than the bulk soil which had more fungal load. For the fallowed farmland, the heterotrophic bacteria count ranged from 5.65$x10^8$-1.50$x10^9$$cfug^{-1}$ and the total fungi ranged from 1.33-3.05$x10^6$ sfug-1, while the heterotrophic bacteria count for the bulk soil sample was 1.74$x10^9$$cfug^{-1}$ and the total fungi count was 1.07$x10^6$ $sfug^{-1}$. Just like in the ready to harvest soil, the fallowed farmland had more bacteria load than the bulk soil which had more fungal load.

The microbial count obtained from the different rhizosphere soils of the different plant species from the newly cultivated farmland ranged from 2.9$x10^7$-6.3$x10^8$$cfug^{-1}$ for heterotrophic bacteria and 5.6 $x10^4$-7.0 $x10^6$ $sfug^{-1}$ for the total fungi, while the bulk soil had heterotrophic bacterial count of 4.96 x $10^9$$cfug^{-}$1 and the total fungal count 5.87 x $10^6$$sfug^{-1}$. Soil root system affect soil microbial population [1].The heterotrophic bacterial count range was lower compare to the other farmlands. The lower heterotrophic bacterial content of the rhizosphere soil could be as a result of the regulatory mechanisms of the root system of crops in order to maximize environmental properties to enhance their well being.

The newly cultivated bulk soil had highest bacteria load compared to the rhizosphere soils. This might be due to the organic load present in the soil before cultivation, rather than to microorganisms-plant roots interaction. Plant derived compounds provide nutrient to a large variety of soil microorganisms [1]. The soil around *Manihot esculentum* in the ready to harvest farmland had the highest bacterial load (1.8$x10^9$ $cfug^{-1}$) among the rhizophere soils. *Manihot esculentum* is a root crop with several branches, having a long duration for maturity before harvest. It follows that the root exudates would have provided material for the proliferation of the associated bacteria. The soil around *Comelina bengalensis* in the newly cultivated farmland had the highest fungal load (7.0x1010^6 $sfug^{-1}$). The high fungal load from *Comelina bengalensis* a flowering plant and a common farmland weed in the area of study may be due to the nature of the plant material under decomposition within the soil vicinity.

Bacteria were the most dominant in the soil given their population and diversity. The rhizosphere bacteria differ with the plant species. The bacteria isolated from the different rhizosphere soils belong to ten genera namely: *Staphylococcus, Hafnia, Acinetobacter, Bacillus, Bacteroides, Klebsiella, Tatumella, Corynebacterium, Pseudomonas* and *Enterobacter*. Seven bacterial isolates were obtained from the bulk soil which were *Staphylococcus* sp, *Acinetobacter* sp, *Bacillus* sp, *Klebsiella* sp, *Tatumella* sp, *Corynebacterium* sp and *Pseudomonas* sp. The most common rhizosphere bacteria genera are *Pseudomonas, Bacillus, Arthrobacter, Rhizobia, Agrobacterium, Alcaligenes, Azotobacter,*

Mycobacterium, Flavobacter, Cellulomonas and *Micrococcus* [7]. Panaiyadiyan and Chellaia [8] reported *Acinetobacter* sp, *Klebsiella* sp, *Staphylococcus* sp, *Bacillus* sp and *Pseudomonas* sp among the 18 species of bacteria isolated from rhizosphere soil. *Bacillus* sp and *Pseudomonas* sp are generally found in most rhizosphere soil, with their composition dependent on the plant species [9]. In this study, *Bacillus* sp was dominant and present in both rhizosphere and bulk soil. *Bacillus* sp had been reported as the most numerous rhizobacteria associated with rice and wheat [10].

The fungal rhizophere isolates and the bulk soil isolate belong to the genera *Aspergillus, Epicocum, Chrysosporium, Trichosporon, Cryptococcus, Chaetomium, Microsporium, Penicillium* and *Fusarium*. There were nine fungal isolates in total. Fungi are more tolerable to acidic soil. The rhizosphere soil had more fungal diversity, with all the fungal genera represented. The bulk soil had *Microsporium* sp, *Aspergillus* sp, *Epicocum* sp, *Chrysosporium* sp, *Trichosporon* sp, *Cryptococcus* sp, *Chaetomium* sp, with *Penicillium* and *Fusarium* not detected. *Aspergillus* sp was the dominant fungal isolate. Panaiyadiyan and Chellaia [8] similarly isolated *Aspergillus* sp from rhizosphere soil, which they described as one of the most important soil fungi because of their benefits to plants.

The results of physicochemical analyses of the samples showed variations. The pH values were generally mildly acidic. Soil pH affects soil chemistry and extends to virtually all characteristic of soil that are affected by multiple interactions within the rhizophere. Respiration by the roots macro and microorganisms, releases carbon dioxide which generate bicarbonate/carbonic acid which impact on the soil acidity. Increased microbial activity also increases microbial transformations of chemical species to nitrate acid and sulphuric acid production [11]. The soil contributed to the soil microbial population and diversity. According to Kent and Triplett [5], soil pH affects the availability of nutrients and influence the abundance and diversity of associated microorganism. The soil temperature (27^{o}C) supported the mesophilic microorganism present in the soil. The soil moisture content which ranged from 16.29-33.20% was adequate for both bacteria and fungi to thrive.

Conclusion

It has been established that rhizosphere soil microbes play various roles in the development of plants, and these roles confer additional benefits to the plants. Several bacteria and fungi species are closely associated with plant roots, which together with the soil environment influence microbial population and diversity.

REFERENCES

1. Bakker PA, Berendsen RL, Doornbos RF, Wintermans PC, Pieterse CM. “The rhizosphere revisited: Root microbiomics”. *Frontiers in plant science*. 2013; 4:165. Doi:10.3389/fpls.2013.00165.

2. Cartmill AD, Valdez-Aguilar LA, Bryan DL, Alarcón A. Arbuscular mycorrhizal fungi enhance tolerance of vinca to high alkalinity in irrigation water. *Scientia Horticulturae*. 2008; 115 (3): 275-284.

3. Bonkowski M, Villenave C, Griffith B. Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots.*Plant and soil*. 2009;321:20.

4. Philippot L, Raaijmakers JM, Lemaceau P, van der Putten WH. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology*. 2013; 11(11): 789-99.

5. Kent AD, Triplett EW. Microbial communities and their interactions in soil and rhizosphere ecosystems. *Ann. Rev. Microbiol*. 2002; 56: 211-236.

6. American Public Health Association (APHA): Standard methods for the examination of water and waste water. 16th edition, American Public health association. Washington, D.C.; 1985.

7. Prashar P, Kapoor N, Sachdeva S. Rhizosphere: its structure, bacterial diversity and significance. *Rev Environ Sci Biotechnol*. 2014; 13: 63-77. DOI 10.1007/s11157-013-9317-z.

8. Panaiyadiyan P, Chellaia SR.. Biodiversity of microorganisms isolated from rhizosphere soils of Pachamalai Hills, Tamilnadu, India. *Research Journal of Forestry*. 2011; 5(1): 27-35.

9. Kundu BS, Nehra K, Yadav R, Tomar M. Biodiversity of phosphate solubilizing bacteria in rhizosphere oo chickpea, muatard and wheat grown in regions of Haryana. *Ind . J. Microbiol*. 2009; 49: 120-127.

10. Joshi P, Bhatt AB. Diversity and function of plant growth promoting rhizobacteria associated with wheat rhizosphere in north Himalayan region. *Int J Environ Sci*. 2010; 1:1135–1144.

11. Scow, K. Soil Microbiology class notes. Winter 2015, University of California, Davis.