Occurrence of Some Bacteria from Rhizosphere of (Wheat) *Triticum aestivum* L. in South and West, Nganzun Townships, Mandalay Region

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Abstract

The present study was conducted on bacteria occurrence in rhizosphere of (wheat) *Triticum aestivum* L. in two different study sites. Bacteria were isolated and identified from rhizospheric soil of the studied plant species by biochemical test and morphological examination. Soil analysis was also done. A total of 10 species belonging to five genera and five families were recorded. Among them, nine species of bacteria in South and six species from rhizosphere of wheat plant in West Nganzun Township were isolated. Out of them five species occurred in both study sites. The four bacteria species isolated were namely *Agrobacterium ceramicola, Bacillus stearathermophiles, Flavobacterium ferrugineum, Pseudomonas cissicala* from rhizosphere of wheat in South and only one species *Pseudomonas radiciporda* was isolated in West Nganzun Township. Nitrogen fixation bacteria were recorded in two study sites. Total bacteria counts of rhizospheric bacteria of wheat plant species were made in different study sites. For (wheat) *Triticum aestivum* L*.,* the total viable counts were high in West, Nganzun Township.

Keyword: Bacteria, rhizosphere of wheat plant, Nganzun Township

Introduction

Several microorganisms are able to promote the plant growth directly and indirectly, playing an important role in soil processes that determine plant productivity (Trlak, 1993). Many are beneficial to plants, playing essential parts in the circulation of nutrients, such as plant growth promoting (PGP) bacteria directly (Jackson and Raw, 1966; Pinoton *et al.,* 2001).Wheat is one of the major crops cultivated all over the world. The different stages of the life cycle of a wheat consist of an elongation (30 days), a flowering stage (45 days),a fruiting stage (60 days) and ripened fruiting stage (75) days (Huddedar and Chopade, 2000; Huddedar *et al.,* 2002). It grows in a temperate climate and it is a staple food for 35% of world population.

PGPR activity has been reported for several genera such as *Pseudomonas, Acetobacter* and *Bacillus* (Kloepper, 1993).These microbes also play a significant role as plant growth promoting rhizobacteria (PGPR) in the biofertilization of crop (Afzal and Bano, 2008). So much bacteria used for crop inoculation hold great promise to reduce usage of chemical fertilizers in agriculture (Glick*,* 1995). Phosphobacterium, a phosphate solubilizing bacteria (PSB) which is

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able to convert the phosphate present in the soil from unavailable to an available-to-the plant form, has indirect but definite effect on the nodulation and yield of legume crops like a groundnut (Ghosh and Poi,1998).

In the present study, bacteria species from soil of wheat plant from two different study sites were studied with the following objectives:

- to enumerate and compare the total viable counts of bacteria isolated from rhizosphere of wheat plant in different areas
- to isolate and identify the bacteria from rhizospheric soil of wheat plant
- to analyze the physical and chemical properties of the rhizospheric soil of wheat plant

Materials and Methods

Study area

The study sites are located at the Southern and Northern parts of Nganzun Township, Mandalay Region. Site I (South Nganzun Township) is situated between latitude 21° 46′ 09″ N and longitude 95° 36′41″E. Site II (West Nganzun Township) is situated between latitude 21° 53′49″ N, and longitude 95° 40′17″E .

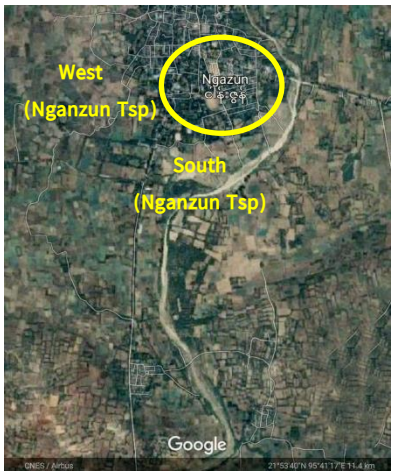


Fig.1 Location map of study areas (Source: Google Earth)

Study period

This study was carried out from July, 2018 to June, 2019.

Sample collection

The rhizospheric soil of (wheat) *Triticum aestivum* L. were collected by circular random sampling method. The samples of soils were collected at the depth between 15.20 cm and 20.30cm from the rhizosphere of plant species at the two study sites.

Soil analysis

Physical and chemical analyses of soil samples were done at the Land Use Department; Ministry of Agriculture, Yangon Region.

Materials

The glassware were cleaned with 1% H_2SO_4 solution and rinsed with distilled water several times to remove all traces of acids. Used and contaminated glassware were autoclaved at 121^o C, at 15lb psi for 15 minutes. Subsequently they were dried in a hot air oven. Bacteriological culture media, ingredients and other chemicals used in this research were purchased from BDH Co.,Ltd.

Isolation of bacteria from soil

Ten gram each of soil sample was placed in the conical flask and mixed with 90ml of distilled water (DW) and shaken for 10 minutes in a shaker. Then, this solution was serially diluted into 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} levels and were separately transferred into three Petri dishes of nutrient agar under aseptic conditions using a pipette and incubated at 30ºC for three days. Conformation was carried out using various media.

Identification of bacteria

Biochemical tests for identification of isolated bacteria were made and bacteria colonies counts of isolates from the soil samples were done fallowed after Jensen, 1950, Alexander (1961), Collins (1964), Harrigan and McCane (1966), Cowan and steel, 1966, Cruickshank *et al.,* 1975, Cowan and steel (1981), Brad Shaw (1992) and Bisen and Verma (1997), Pettipher *et al.,* 1980**.**

Results

A total of ten isolate species, representing nine species in Site I and six species in Site II were recorded. The ten rhizospheric bacteria species were namely *Agrobacterium ceramicola, Azotobacter chroococcum, Bacillus stearathermophiles, Flavobacterium ferrugineum*, *Pseudomonas cattleyae, Pseudomonas cissicala, Pseudomonas effusa, Pseudomonas eridotryae, Pseudomonas radiciperda* and *Pseudomonas subcreta*. Out of them five species were isolated for both study sites. The species namely *Azotobacter chroococcum, Pseudomonas cattleyae, Pseudomonas effusa, Pseudomonas eridotryae* and *Pseudomonas subcreta* were recorded for two study sites, whereas four species namely *Agarbacterium ceramicola, Bacillus stearathermophiles, Flavobacterium ferrugineum* and *Pseudomonas cissicala* were recorded in Site I. Only one species of *Pseudomonas radiciperda* was not observed in Site I. The total viable bacteria counts of rhizosphere $3.54x10^5$, $4.43x10^5$, $5.26x10^5$ and $6.11x10^5$ CFU/ mL in Site I in contrast with Site II of rhizosphere $3.99x10^5$, $4.92x10^5$, $5.90x10^5$ and $6.76x10^5$ CFU/ mL in respectively were recorded. (Table.1,2,3 and 4, Fig.2, Plate 1)

The soil of both study sites had alkaline condition at pH (8.4) in Site I and pH (8.3) in SiteII. The texture was sandy loam with the relative contents of sand(61.65%),silt(24.80%),and clay (12.00%) in Site I, Nganzun Township, whereas sandy clay loam with the relative contents of sand(30.50%),silt(31.45%),and clay(36.00%) in Site II, Nganzun Township. The moisture values were 0.97% in Site I and 1.46% in Site II. The temperature was 30ºC for both study sites. The concentrations of exchangeable cations in the different soils were recorded as Ca^{++} (14.70 meq/100g), Mg^{++} (1.27 meq/100g), Na^{+} (12.51meq/100g) and K_2O (30.90 mg/100g) in Site I, whereas Ca^{++}(30.81 meq/100g), Mg^{++}(1.92 meq/100g), Na^{+}(13.04 meq/100g) and K_2O (46.06 mg/100g) in Site II. The concentrations of available nutrients were total N (0.194%), P(26.66

mg/L), K(0.65 meq/100g) in Site I and total N (0.301%), P(39.35 mg/L), K(0.98 meq/100g)in Site II, Naganzun Township. (Table. 5 and 6, Fig.3)

Table.1 Total viable bacteria counts (CFU/mL) recorded for wheat plant in different study sites

		Colony forming Unit (CFU/mL)	
No.	Dilution factors	Site I	Site II
1.	10^{-2}	3.54×10^5	3.99×10^5
2.	10^{-3}	4.43×10^5	4.92×10^5
3.	10^{-4}	5.26×10^5	5.90×10^5
4.	10^{-5}	6.11×10^5	6.76×10^5

Table 2. Bacteria isolated from rhizosphere of wheat plant in different study sites

Bacteria isolated	Site I	Site II
Agrobacterium ceromicola	+	-
Azotobacter chroococcum	+	+
Bacillus stearathermophiles	+	-
Flavobacterium ferrugineum	+	-
Pseudomonas cattleyae	+	+
P.cissicala	+	-
P.effusa	+	+
P.eridotryae	+	+
P.radiciperda	-	+
P.subcreta	+	+

+ = growth , - = no growth

Table.3 Colony morphology and cellular morphology of rhizospheric bacteria isolated from different study sites

Bacteria isolated	Colony morphology	Cellular morphology
Agrobacterium ceramicola	Circular, cream	Small rods, occurring singly or in pairs, star shaped clusters
Azotobacter chroococcum	Round, raised, gummy colonies white to creamy	Ovoid rods,0.5-2μm in diameter
Bacillus stearathermophiles	Round, raised colonies, white to creamy	Thin, rod-shaped, slightly bend, spore bearing 0.7 x 3.0 μm
Flavobacterium ferrugineum	Smooth entire yellow	Rod- shaped, 0.6-0.9 by 0.9-1 μm
Pseudomonas spp.	Luxuriant, glistening, moist, creamy, spreading growth, medium becomes greenish fluorescent	Round and rods shaped, slightly curved or straight, 0.4 by 1.7 μm

Table.4 Biochemical characteristics of isolated soil bacteria from different study sites

Soil bacteria isolated	Gram strain	Citrate	Motility	Indole	H_2S	MR	VP	Catalase	Urease	Gelatin	Fermentation of carbohydrates (TSI)		
											Glucose	Sucrose	Lactose
Agrobacterium ceromicola	-	-	-	-	-	-	-	+	+	-	+	+	+
Azotobacter chroococcum	-	-	+	-	-	-	-	+	-	+	+	-	-
Bacillus stearathermophiles	+	-	+	-	-	-	-	+	-	+	+	-	-
Flavobacterium ferrugineum	-	-	-	-	-	+	-	-	-	-	+	-	-
Pseudomonas cattleyae	-	+	+	-	-	-	+	+	-	+	+	+	+
P.cissicala	-	-	-	-	-	-	-	+	+	+	-	-	-
P.effusa	-	+	+	-	+	-	+	+	-	+	-	-	-
P.eridotryae	-	+	+	-	-	+	-	+	-	+	-	-	-
P.radiciperda	-	+	+	-	-	-	-	+	+	-	+	-	-
P.subcreta	-	+	+	-	-	+	-	-	-	+	+	+	+

+ = growth , - = no growth

Table.5 Physical properties of soil samples from different study sites

	Parameters	Study sites	
		Site I	Site II
	pH	8.4	8.3
	Temperature (ºC)	30	30
	Moisture (%)	0.97	1.46
Texture	Sand (%)	61.65	30.50
	Silt (%)	24.80	31.45
	Clay (%)	12.00	36.00
	Total (%)	98.45	79.95
	Others (%)	1.55	2.05
	Type	Sandy loam	Sand clay loam
	Colour	Dark brown	Brown

Table.6 Chemical contents of soil samples from different study sites

	Contents	Study sites	
		Site I	Site II
Exchangeable cation (meq/100g)	Ca^{++}	14.70	30.81
	Mg^{++}	1.27	1.92
	Na^{++}	12.51	13.04
	K_2O (mg/100g)	30.90	46.06
Available nutrients	Total N (%)	0.194	0.301
	P (ppm)	26.66	39.35
	K (meq/100g)	0.65	0.98

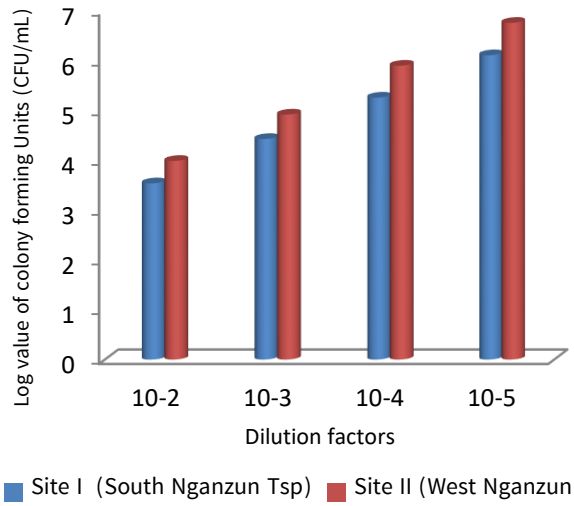


Fig.2 A Comparison between total bacteria counts (CFU/mL) of rhizospheric soil of bacteria of wheat plant from different study sites

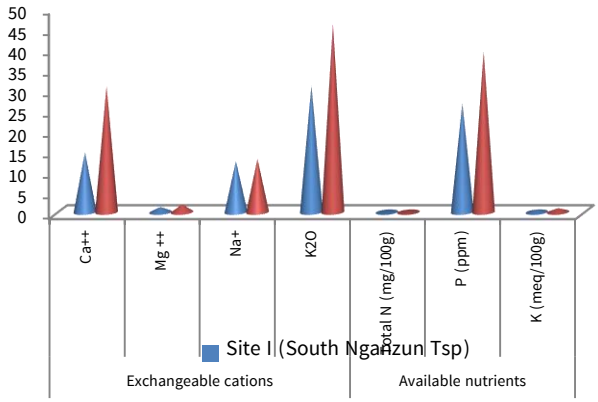
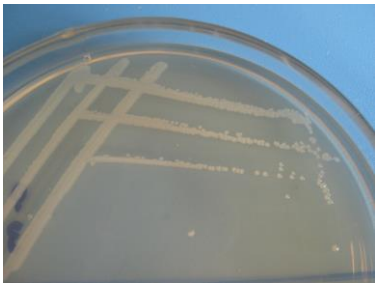
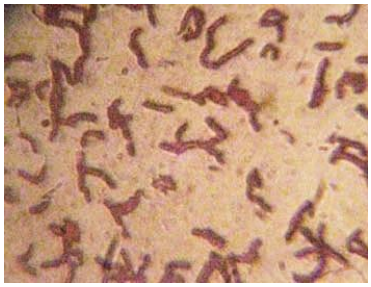


Fig.3 A Comparison between exchangeable cations and available nutrients of rhizospheric soil of wheat plant from different study sites

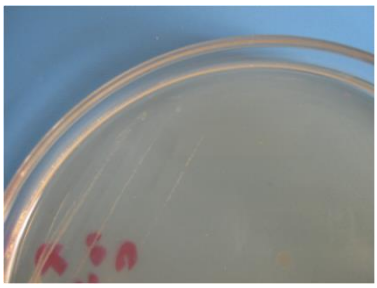


A. Agrobacterium ceramicola colonies on Nutrient Agar

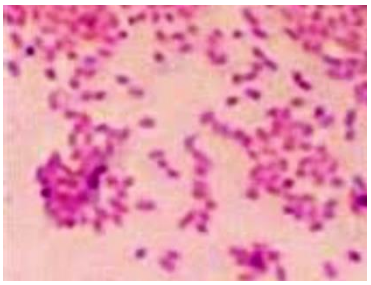


B. Cells of *Agrobacterium ceramicola* (1000x)

C. Biochemical test of *Agrobacterium ceramicola*

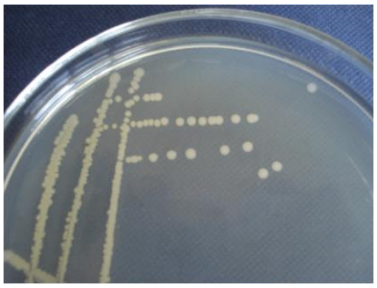


D.*Azotobacter chroococcum* colonies on AZA Agar

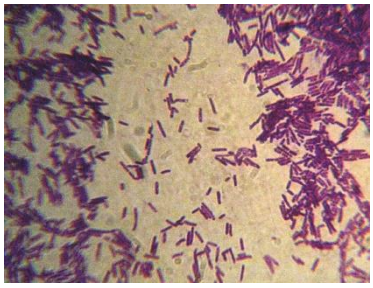


E. Cells of *Azotobacter chroococcum* (1000x)

F. Biochemical test of *Azotobacter chroococcum*

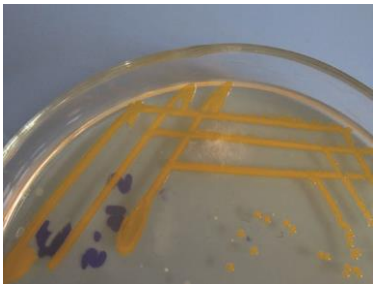


G.*Bacillus stearathermophiles* colonies on Nutrient Agar

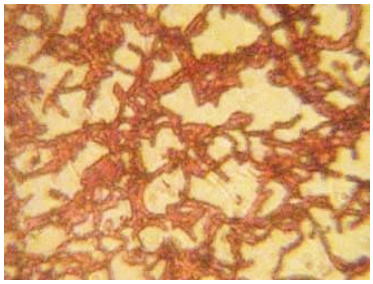


H. Cells of *Bacillus stearathermophiles* (1000x)

I. Biochemical test of *Bacillus stearathermophiles*

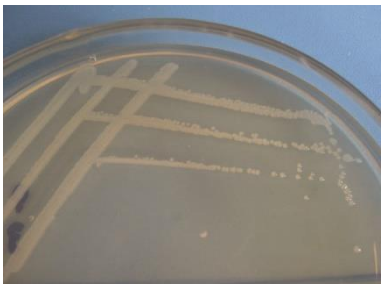


J. *Flavobacterium ferrugineum* colonies on Nutrient Agar

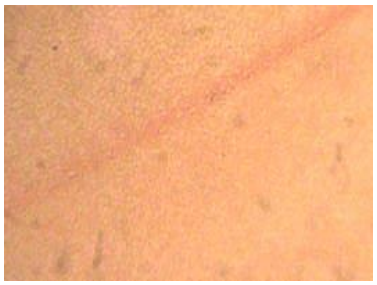


K. Cells of *Flavobacterium ferrugineum* (1000x)

L. Biochemical test of *Flavobacterium ferrugineum*

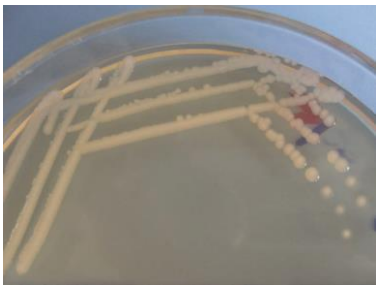


M. *Pseudomonas cattleyae* colonies on Nutrient Agar

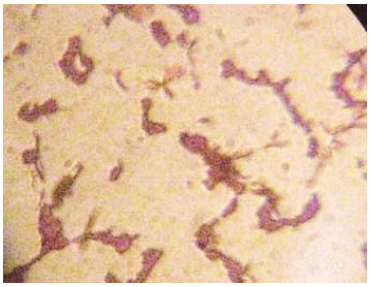


N. Cell of *Pseudomonas cattleyae* (1000x)

O. Biochemical test of *Pseudomonas cattleyae*

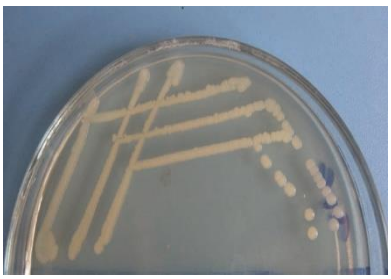


P. *Pseudomonas cissicala* colonies on Nutrient Agar

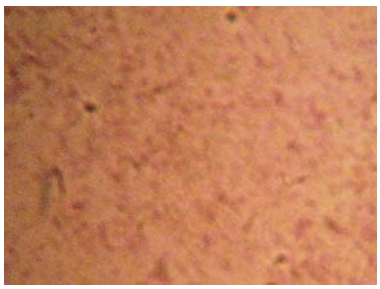


Q. Cell of *Pseudomonas cissicala* (1000x)

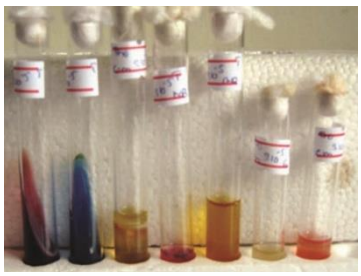
R. Biochemical test of *Pseudomonas cissicala*



S. *Pseudomonas effusa* colonies on Nutrient Agar

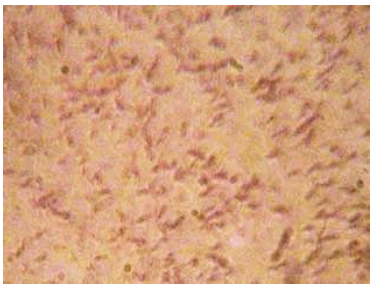


T. Cell of *Pseudomonas effusa* (1000x)



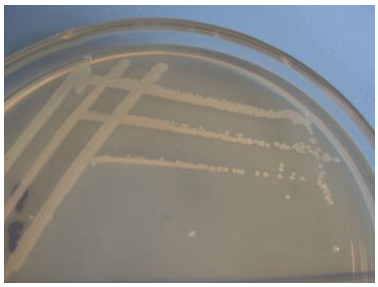
U. Biochemical test of *Pseudomonas effusa*

V.*Pseudomonas eridotryae* colonies on Nutrient Agar

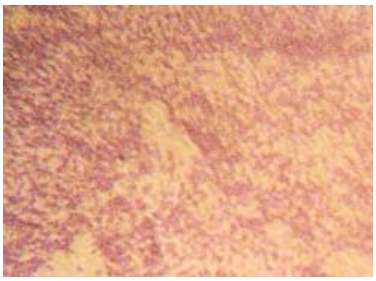


W. Cells of *Pseudomonas eridotryae* (1000x)

X. Biochemical test of *Pseudomonas eridotryae*



AB. *Pseudomonas subcreta* colonies on Nutrient Agar



AC. Cell of *Pseudomonas subcreta* (1000x)

AD. Biochemical test of *Pseudomonas subcreta*

Plate 1. Continued

Discussion

In the present study, a total of 10 species belonging five genera and five families were recorded. Among these, nine species of bacteria in Site I and six species in Site II were isolated from samples of rhizoshperic soil of *Triticum aestivum* L. wheat plant growing of the two studied

sites. Out of them five species occurred in both study sites. Four diverse bacteria species were observed of wheat plants in Site I which had higher species than that of Site II.

The present results showed that the nitrogen fixing bacteria of *Azotobacter chroococcum* were recorded in two study sites. *Azotobacter* sp. is heterotrophic, aerobic bacteria and their main property is the ability to fix nitrogen non-symbiotically, and distributed in soils, water and sediments. Azotobacter synthesizes and secretes considerable amount of biologically active substances like vitamin, nicotinic acid, biotin, hetero auxins, gibrellins etc, which enhance root growth of plants (Becking, 2006). Volker and Birnstiel (1989) showed that microorganisms isolated from the soil and the rhizosphere of wheat plants produced growth promoting substances gibrellins.

The Gram strain reaction of the bacteria species showed that only one species of gram positive *Bacillus stearathermophiles* were occurred in Site I. Ubiquitous in nature, Bacillus includes both free living (non-parasitic) and parasitic species, phosphate solubilizing bacteria and also made the denitrification. It promotes plant growth and suppression of pathogen (Madigan *et al.,* 1997). Bacteria of diverse genera were identified as PGPR of which *Bacillus* and *Pseudomonas* are predominant. PGPR exert a direct effect on plant growth (Podile and Kishore,2007).

In the present study, the largest number of species occurred under genus *Pseudomonas* from wheat plant for both study sites. Sivasakthi *et al.,* (2014) reported that *Pseudomonas* is also ubiquitous in soil and rhizosphere and one of the dominant PGPR genus with diverse traits. The plant growth promoting bacteria (PGPR) include both free living and symbiotic bacteria, typically found in the soil which facilitates the growth and development of plant. The total bacteria counts were slightly higher in Site II than Site I, Nganzun Township.

In the present study, the soil condition for wheat plantations had alkaline soil condition for both study sites. The values of exchangeable cations of Ca^{2+}, Mg^{2+} ,Na^{+} and K_2O was high in Site II than of Site I and available nutrients of total N, P and K were higher in Site II than that of Site I.

The soil pH is important in determining the availability of soil nutrients. Different plants have differing optimum soil pH requirements. Soil pH strongly affects the microbial activities, as at below pH 5.0 bacterial as well as fungal activities are reduced. Highly acidic and highly alkaline soils often remain injurious for plant growth, microorganisms etc. Neutral (or) slightly acid soil remains best for the growth of majority of plants (Verma and Agarwal, 1999). The concentration of nutrients (P,K, Mg, Ca and Na) in the seed were also significant affected by the application of PSB and P fertilizer (Flahaut *et al.*, 1997). Therefore, wheat plantation, at Site II, Nganzun Township could also be affected by adequate available nutrients and exchangeable ions in the soil.

According the present work, in wheat plant fields, the texture of soil was sandy loam (Dark brown) and sandy clay loam with brown colour in all study sites. Clay content of Site II was higher than that of Site I. The clay is the main source of nutrition in the soil. Clay controls the most important properties of soils, including plasticity and exchangeable ions between soil particles and soil solution (Verma and Agarwal, 1999). Therefore, the soil texture conditions in Site II were well suited for wheat plants growth than that of Site I.

In the present study, the soil temperature of two study sites was 30°C. These findings were similar to that of Atlas and Batrha (1993) who reported that most soil microbes grow best at temperature between 15°C-30°C and the growth rates increase with increasing temperature up to a certain point. Grayston (1988) reported that effect on the plant growth of microbial populations and their actions on soils depend on the interaction between plant species and soil. Therefore, the soil conditions of different agricultural lands of South and West, Nganzun Township were analysed. It assumed that rhizosphere provided more favourable conditions when bacteria inhabited in it.

Conclusion

The values of available nutrients and exchangeable ions of phosphorus, calcium, and potassium oxide were high in West, Nganzun Township compared to the South, in Nganzun Township. It is the reason that the largest diversity of microbes occurred in the rhizosphere of West Nganzun Township. In West, Nganzun Township, the agricultural land typically provides the soil which facilitates the growth and development of wheat plants when it is compared to the South Nganzun Township.

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