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RESEARCH ARTICLE

MICROBIAL POPULATION CHANGES IN THE RHIZOSPHERE OF TOMATO SOLANUM LYCOPERSICUM VARIETIES DURING EARLY GROWTH IN GREENHOUSE

Chinakwe EC[1], Ibekwe VI[1], Nwogwugwu UN[1], Onyemekara NN[1], Ofoegbu J[2], Mike-Anosike E[1], Emeakaraoha M[1], Adeleye S[1], Chinakwe PO[3]

[1]Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria
[2]Department of Science Laboratory Technology, Federal University of Technology, Owerri, Imo State, Nigeria
[3]Department of Crop Science, Federal University of Technology, Owerri, Imo State, Nigeria
**Corresponding Author Email: eti_chukwumaeze@yahoo.com*

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ABSTRACT

The microbial population changes in the rhizosphere of two varieties of tomato: cherry and plum were studied. They were grown in a greenhouse for five weeks. Standard microbiological procedures were applied. Biochemical and cultural characteristics revealed the presence of *Bacillus, Enterococcus, Staphylococcus, Rhizobium* as bacterial species and *Penicillium, Mucor* and *Saccharomyces* as fungal species. Total Heterotrophic Bacterial Counts (THBC) ranged from 1.0 x 10^6 to 4.8 x 10^7 cfu/g; 7.0 x 10^7 to 4.5 x 10^9 cfu/g and 5.4 x 10^7 to 3.0 x 10^9 cfu/g for bare soil, rhizosphere soil of cherry tomato and rhizosphere soil of plum tomato respectively. Total Fungal Counts (TFC) were lower and ranged from 1.3 x 10^6 to 6.5 x 10^6 cfu/g, 1.2 x 10^6 to 8.7 x 10^6 cfu/g and 1.0 x 10^6 to 1.2 x 10^6 cfu/g for bare soil, rhizosphere soil of cherry tomato and rhizosphere soil of plum tomato respectively. The microbial succession pattern further revealed that *Bacillus* sp, *Enterococcus* sp, *Rhizobium* sp, *Mucor* sp and Saccharomyces sp were the predominant microorganisms present in bare soil and rhizosphere soils of cherry and plum tomatoes. The presence of plant growth promoting rhizobacteria e.g. *Bacillus* sp and *Rhizobium* sp, is of great advantage to the early growth of tomato plants as they play important roles in increasing soil fertility, plant growth , and suppression of phytopathogens for healthy plant development and sustainable agriculture.

KEYWORDS

Microbial succession, plant growth promoting rhizobacteria. rhizosphere, total fungal count, total heterotrophic bacterial count

1. INTRODUCTION

Soil is the uppermost layer of the earth's surface on which plants grow. It is made up of decomposed rock materials and minerals, organic matter, water, gases and living organisms that combine effectively to support life on earth [1]. Plant growth and productivity depends largely on the nature of the soil and microorganisms form a greater percentage of living organisms found in the soil, amongst other soil organisms such as worms and insects. Microbial communities play a pivotal role in the functioning of plants by influencing their physiology and development [2]. Soil microorganisms affect soil fertility positively and also make nutrients available to plants. They do this by several mechanisms of plant – microbe interactions helping in such processes as carbon sequestration and nutrient cycling [3]. Microorganisms in the soil also help in organic matter decomposition, soil degradation, and bioremediation of polluted soils [4,5].

The rhizosphere is that region of the soil, including plant roots and tissues colonized by microorganisms. Plant roots provide the soil microorganisms with exudates that serve as substrates (e.g. carbohydrate sources) and signaling molecules. These exudates initiate rhizospheric interactions (plant-microbe and microbe-microbe) and influence the soil microbial community. Rhizosphere interactions involving plant roots, soil, and microbes obviously change the physical and chemical properties of the soil and in turn, the entire microbial population of the rhizosphere environment [6-8]. The microbial community structure in the rhizosphere is usually different from that in bulk soil (i.e. non-rhizosphere soil) because of the nutrients available to the soil as a result of biological interactions between the roots and the microbial community of the soil [9].

Amongst rhizosphere microorganisms, bacteria have been found to strongly influence plant growth. This has been attributed to the predominance of amino acids and other growth factors required by bacteria that are readily available in the root exudates secreted by plants in the rhizosphere. During the early stage of plant growth, the roots grow into the soil and release organic materials in the rhizosphere leading to development of reasonable microbial population in the area and creates opportunity for plant-microbe interactions. Several factors affect the microbial flora of the rhizosphere which includes soil type and its moisture, soil pH, soil amendments, proximity of roots to the soil, the plant species, age of the plant, and root exudates [10].

This study was aimed at evaluating the microbial population changes occurring in the rhizosphere of two varieties of tomatoes: cherry and plum, during early growth of their seedlings.

2. MATERIALS AND METHODS

2.1 Study Area

The greenhouse of the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria (5.3905°N, 6.9907°E).

2.2 Collection of Samples

2.2.1 Soil Sample

Soil samples were randomly collected from uncultivated portion of the farmland using a spade at a depth of about 20cm and bulked. Five (5) kg each of the bulked soil were packed into separate 5- litre pots for use in planting of the tomato seeds.

2.2.2 Tomato Seeds

Plum and cherry seed varieties were sourced from the Imo State Agricultural Development Programme (ADP) Office, Owerri, Imo State, Nigeria.

2.2.3 Seed Planting and Rhizospheric Soil Sample Collection

Nine pots were set up containing 5kg of the farmland soil, three each for the bare soil, plum tomato, and cherry tomato and placed inside a green house.

Five seeds, each of plum and cherry tomatoes, were planted in the pots labeled for plum and cherry tomato. Bare soil without these plants served as a control in this study.

The soils containing these seeds were watered daily to encourage germination. The seeds on germination were left to grow for five weeks and the microbial populations as well as the microbial succession patterns were determined.

The bare soil and rhizospheric soils from the roots of the tomato plants were collected using sterile container bottles and taken to the laboratory for analysis, weekly for five weeks. Bulk soil, defined a soil that does not adhere to plant roots, was obtained at least 20 cm from the plants. Bulk soils from five different spots were combined into one sample. Rhizosphere soil, defined as soil that adheres to the plant root after gentle shaking was obtained from five plants, using sterile brushes, and combined into one sample. Both rhizosphere and bulk soil samples were immediately transferred to the laboratory in a cool container (0–10°C) within 2 hours.

2.3 Determination of Microbial Population and Succession Patterns

One (1) gram each of dry samples of the bare soil and the rhizosphere soils from plum and cherry tomatoes were analyzed weekly after germination for five weeks to determine the microbial populations and microbial succession patterns in the samples.

Microbiological analysis was carried out using the dilution method and cultured on specific media [16]. Total heterotrophic count for bacteria was done on Nutrient Agar, Total Fungal Count on Potato Dextrose Agar and Total Rhizobium Count on Congo Red Yeast Extract Mannitol Agar (CREYEMA). Nutrient agar was incubated for 24 hours while Potato Dextrose Agar and Rhizobium Agar (CREYEMA) were incubated for 48-96 hours and at 28°C.

3.RESULTS

3.1 Microbiological Analysis

Microbiological analysis of bare soil and rhizospheric soils of plum and cherry tomatoes gave results as shown in Table 1. The bacteria isolated included *Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp and *Rhizobium* sp. Fungal species isolated from the soil samples included *Mucor*, *Penicillium* and *Saccharomyces*.

The Total Heterotrophic Bacterial Counts (THBC), Total Fungal Counts (TFC) and Total Rhizobium Counts (TRC) are shown in Tables 2, 3 and 4 respectively. There were fluctuations in these counts for the five- week period.

Table 1: Bacterial and Fungal Isolates from Bare Soil and Rhizospheric Soils of Plum and Cherry Tomatoes

Soil Type	Bacteria	Fungi
Bare Soil	*Bacillus* sp *Enterococcus* sp *Staphylococcus* sp *Rhizobium* sp	*Mucor* sp *Penicillium* sp *Saccharomyces* sp
Rhizosphere Soil from Cherry Tomato	*Bacillus* sp *Enterococcus* sp *Staphylococcus* sp *Rhizobium* sp	*Mucor* sp *Penicillium* sp *Saccharomyces* sp
Rhizosphere Soil from Plum Tomato	*Bacillus sp* *Enterococcus sp* *Staphylococcus sp* *Rhizobium sp*	*Mucor* sp *Penicillium* sp *Saccharomyces* sp

Table 2: Total Heterotrophic Bacterial Count (THBC) of Bare Soil and Rhizosphere Soils of Plum and Cherry Tomatoes during the Five- week Period of Growth.

Sample	Week 1 (CFU/g)	Week 2 (CFU/g)	Week 3 (CFU/g)	Week 4 (CFU/g)	Week 5 (CFU/g)
Bare Soil	9.8×10^7	1.0×10^6	1.2×10^7	3.2×10^7	4.8×10^7
Soil + Cherry Tomato	ND	7.0×10^7	1.1×10^8	3.8×10^8	4.5×10^9
Soil + Plum Tomato	ND	5.4×10^7	9.0×10^8	3.5×10^8	3.0×10^9

Key: CFU/g = Colony forming unit per gram of Soil; ND = Not Determined

Table 3: Total Fungal Count (TFC) of Bare Soil and Rhizosphere Soils during the Five -Week Period of Growth.

Sample	Week 1 (CFU/g)	Week 2 (CFU/g)	Week 3 (CFU/g)	Week 4 (CFU/g)	Week 5 (CFU/g)
Bare Soil	2.6×10^6	7.8×10^6	5.8×10^6	6.5×10^6	1.3×10^6
Soil + Cherry Tomato	ND	3.1×10^6	4.4×10^6	1.2×10^6	8.7×10^6
Soil + Plum Tomato	ND	6.5×10^6	7.6×10^6	1.0×10^6	1.2×10^7

Key: CFU/g = Colony forming unit per gram of Soil
ND = Not Determined

Table 4: Total Rhizobial Count of Bare Soil and Rhizosphere Soils during the Five- Week Period of Growth.

Sample	Week 1 (CFU/g)	Week 2 (CFU/g)	Week 3 (CFU/g)	Week 4 (CFU/g)	Week 5 (CFU/g)
Bare Soil	1.2×10^6	1.8×10^7	3.6×10^6	2.0×10^6	9.8×10^5
Soil + Cherry Tomato	ND	2.6×10^7	6.9×10^7	4.1×10^7	7.0×10^7
Soil + Plum Tomato	ND	1.7×10^7	3.7×10^7	9.7×10^7	9.3×10^7

Key: CFU/g = Colony forming unit per gram of Soil ND = Not Determined

3.2 Microbial Succession Pattern of the Isolates during Early Growth of Plum and Cherry Tomatoes

The microbial succession patterns for bacteria and fungi isolated from the soil samples are shown in Tables 5 and 6 respectively. *Bacillus* sp and *Saccharomyces* sp were present in all the soil samples all through the study period. *Rhizobium* sp was also present in the rhizosphere soils of cherry and plum tomatoes all through the study period. There were fluctuations in the occurrence of the other bacteria (*Enterococcus* sp and *Staphylococcus* sp) and fungi (*Penicillium* sp and *Mucor* sp) from weeks 3 – 5 isolated during the study period.

Table 5: Succession Pattern of Bacteria and Rhizobium during the Five Weeks Period of Growth

Samples	*Bacillus* sp	*Enterococcus* sp	*Staphylococcus* sp	*Rhizobium* sp
Week 1				
Bare Soil	+	+	+	+
Week 2				
Bare Soil	+	+	+	-
Soil + Cherry Tomato	+	+	+	+
Soil + Plum Tomato	+	+	-	+
Week 3				
Bare Soil	+	+	+	-
Soil + Cherry tomato	+	-	-	+
Soil + Plum Tomato	+	-	-	+
Week 4				
Bare Soil	+	-	+	-
Soil + Cherry tomato	+	-	+	+
Soil + Plum Tomato	+	-	-	+
Week 5				
Bare Soil	+	+	-	+
Soil + Cherry tomato	+	-	-	+
Soil + Plum Tomato	+	+	-	+

Key: - = Absent
+ = Present

Table 6: Succession Pattern of Fungi during the Five-Week Period of Growth

Samples	*Mucor* sp	*Saccharomyces* sp	*Penicillium* sp
Week 1			
Bare Soil	+	+	-
Week 2			
Bare Soil	+	+	+
Soil + Cherry Tomato	+	+	-
Soil + Plum Tomato	+	+	-
Week 3			
Bare Soil	-	+	+
Soil + Cherry Tomato	-	+	-
Soil + Plum Tomato	+	+	-
Week 4			
Bare Soil	-	+	-
Soil + Cherry Tomato	-	+	-
Soil + Plum Tomato	-	+	-
Week 5			
Bare Soil	-	+	-
Soil + Cherry Tomato	-	+	+
Soil + Plum Tomato	+	+	+

Key: - = Absent
+ = Present

4. DISCUSSION

Microbial diversity in soil is considered important for maintaining the sustainability of agriculture production systems. The quantity and type of microorganisms are determining factors of the productivity of any kind of soil [11]**.** The isolation of *Bacillus* sp, *Enterococcus* sp, *Staphylococcus* sp, *Rhizobium* sp, and *Penicillium* sp were in line with reports from studies by a scholar [12]. They isolated *Bacillus* sp, *Rhizobium* sp, *Enterococcus* sp, and *Streptomyces* as part of the microbial community present in a study to determine the endophytic community of roots of *Phaseoulus vulgaris*. A scholar also reported the presence of Bacillus sp, *Fusarium*, and *Penicillium* sp in rhizosphere of bean plant [13]. *Penicillium* and *Mucor*, which are also filamentous fungi were isolated from bare soil and rhizosphere soils of cherry and plum tomatoes. The presence of these filamentous fungi may be useful in absorption of nutrients and water thereby helping in early growth of the tomato plants [14].

Enterobacter and *Bacillus* species had been identified as plant growth promoting rhizobacteria (PGPR). The isolation of these organisms in the rhizosphere of growing tomato is also very significant as they help improve plant growth and health. These bacteria are however provided with amino acids and growth factors by the root exudates of the plants.

Saccharomyces sp isolated could be as a result of soluble sugars in the plant root exudates which act as chemo attractants for the microorganisms.

The succession pattern of the population of microorganisms in the bare soil and rhizosphere soils of plum and cherry tomatoes (Tables 5 and 6) showed that *Bacillus* sp and *Rhizobium* sp were more abundant during the five-week period of study. There were fluctuations in the occurrences of *Enterococcus* sp and *Staphylococcus* sp in the bare soil as well as rhizosphere soils studied. *Bacillus* sp was present in the soil samples studied; and this could be as a result of their ability to resist biotic and abiotic stresses (as in the case of being present in the bare soil and through), or availability of nutrients (from interactions with plant root exudates, which contain compounds such as malic acid, that recruit such bacteria as *Bacillus* sp; as well as sugars and amino acids which the bacteria require for growth). *Rhizobium* sp are nitrogen fixing bacteria; and their present in the rhizosphere soils of growing plum and cherry tomatoes could be as a result of plant-microbe symbiosis, or benefits from plant root exudates. These plant-microbe interactions generally affect the rhizosphere microbial community and plant productivity generally. The presence of *Saccharomyces* sp all through the five-weeks of the study; both in the bare soil, and the rhizosphere soils could also be attributed to availability of nutrients from the root exudates (soluble sugars) and its physiological attributes.

The population study of the bacterial isolates for five-weeks is in agreement with reports from a scholar that bacteria population in the rhizosphere soil ranges from 10^8 to 10^9 CFU/g of soil [15]. The total heterotrophic bacterial count (THBC) ranged from 1.0×10^6 to 4.5×10^9 CFU/g of soil, for the bare soil and rhizosphere soils of cherry and plum tomatoes. There were fluctuations in the THBC for bare soil for the five-week period (Table 2.0). There was however, a progressive increase in THBC for the rhizosphere soils of cherry and plum tomatoes, for the five-week period [12]. There were fluctuations in the Total rhizobial counts (TRC) for bare soil and the rhizosphere soils studied (Table 4.0); and also, the total fungal counts (TFC) of the soil samples studied (Table 3.0). The THBC and TRC from this study were higher than TFC, and this helps buttress the point and report from previous studies that amongst rhizosphere microorganisms, bacterial influence plant growth more than others.

5. CONCLUSION

In conclusion, our result suggested that the interactions in the rhizosphere between plant roots, soil and microorganisms are necessary for plant health and productivity. The study revealed the presence of notable plant

growth promoting rhizobacteria (*Bacillus* and *Enterococcus* sp), as well as nitrogen fixing bacteria (*Rhizobium* sp). This is desirable, considering the effect it will have on the growth of tomato, a highly nutritious and economic crop; they can be applied as biofertilizers, to encourage sustainable growth of tomato plants. Further investigations like efficiency test under greenhouse and field conditions are needed to evaluate the role of the isolated PGPR.

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