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# Studies on Rhizosphere and Rhizoplane Microflora of Tomato (Lycopersicon esculentum Mill) Seedlings

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## Abstract

Investigations were carried out on the rhizosphere and rhizoplane microflora of tomato *(Lycopersicon esculentum)* seedlings planted in Amugwu village of Obige Obukpa in Nsukka Local Government Area of Enugu State, Nigeria. The rhizosphere and rhizoplane microflora of tomato seedlings were isolated and identified. Isolated bacteria were *Bacillus subtillis, Proteus vulgaris, Citrobacter sp. and Erwinia sp.* The fungi isolated from the rhizosphere were *Trichoderma lignorum, Rhizopus stolonifer, Aspergillus niger, Penicillium oxalicum, Sclerotium rolfsii* while *R. stolonifer, S. rolfsii, T. lignorum, A. niger* and *Fusarium solani.* Some of these organisms were more predominant in the rhizosphere while some were more predominant in the rhizoplane. The rhizosphere soil contained a great spectrum of bacteria whereas there were no bacteria isolated from the rhizoplane. Also, fungi were more abundant in the rhizosphere than in the rhizoplane. The rhizosphere and rhizoplane microflora increased with plant age but declined after about twelve weeks.

**Keywords**: Rhizosphere, Rhizoplane, Microflora, tomato, seedlings.

## Introduction

The root system of higher plants is associated not only with an inanimate environment composed of organic and inorganic substances but also with a vast community of metabolically active microorganisms. The microflora that responds to the presence of living roots is distinctly different from the characteristic soil community. The plant roots create a unique subterranean habitat for microorganisms by secreting peculiar exudates. The plant, in turn is markedly affected by the populations it has stimulated since the root zone is a site from which inorganic nutrients are obtained and through which pathogen may penetrate. Consequently, interactions between the macro- and micro-organisms in this locale can have a considerable significance for crop production and soil fertility. These microbial populations around the root region make use of these organic substances for their energy requirements. In plants, exudates can be a healing and defensive response to repel insect attack, or it can be an offensive habit to repel other incompatible or competitive plants *(*Ford *et al., (2009).*

The root surface and its adhering soil are termed the rhizoplane. According to Sylvia (2005) and Singer (2006), the rhizoplane is the root epidermis and outer cortex where soil particles, bacterial and fungal hyphae adhere. In more practical terms, this refers to the remaining microorganisms and soil particles after the roots have been shaken vigorously in water. Several microorganisms have been reported on this region of the root. There are more microbes in the rhizoplane than in the more loosely assoicated rhizosphere. This is determined by counting the number of colony forming units (CFUs) which are determined by spreading extracted soil microorganisms across an agar and counting the number of independent clusters of microorganisms. The unique environment immediately surrounding the roots is called the rhizosphere. It is the region around plant roots where simple sugars, amino acids and many other compounds are exuded by plants and made available to the microorganisms (Campbell, 1989). Bacteria and fungi that live within the cells of the root are not considered a part of the rhizoplane, but instead called endophytes (Sylvia, 2005).

Tomato (*Lycopersicon esculentum*) is an important vegetable crop of the family Solonaceae grown and consumed worldwide. As a fruit it can be eaten raw but is used mainly for the preparation of sauce. Tomato contains many nutrients

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such as minerals and vitamins and can be processed and canned as juice, pulp, paste or even into powder. Odunfa (1979) has reported that the rhizosphere and rhizoplane microflora affect the growth of nearby plants.

Based on the need for adequate information on the natural microflora around the root regions of culrivated crops, this study is being carried out to investigate the rhizosphere and rhizoplane microflora of tomato plants. This will inform on the type of agricultural management system that will enhance the production of this important vegetable crop.

## Materials and methods

**Study site, soil properties and collection of Tomato seeds**

This study was carried out at Amugwu village of Obige Obukpa in Nsukka Local Government Area of Enugu State of Nigeria. The study period covered May to July, 2013. The tomato seeds used for this study were treated seeds obtained from Crop Science Dept of the University of Nigeria, Nsukka, Nigeria.

Physical and chemical analyses of the soil sample employed for this study were carried out in the Soil Science Departmental Laboratory of University of Nigeria, Nsukka, Nigeria. The analyses carried out covered soil texture, moisture content, organic matter content and pH (Dongmo and Oyeyiola, 2006; Oyeyiola, 2009). Each determination was replicated three times.

The test tomato seeds were washed thoroughly under tap and then rinsed with distilled water before surface sterilizing them with calcium hypochlorite (*chlorox*) solution. Ten sterile polyethylene bags were filled with sterilized top soil obtained from the study site. Fifteen seeds were sown per bag and germination monitored. Seedlings were watered regularly for two weeks. Two weeks after germination, the seedlings were tested for the presence of rhizosphere and rhizoplane micro-organisms.

**Isolation of Rhizosphere Microflora**

The method of Mansour and Hamdi (1983) was used. Some tomato roots were carefully uprooted from the polyethylene bags and taken to the laboratory in sterile containers. The roots were manually shaken to remove loose soil particles. The roots were cut into about 2mm segments and 10g representative samples shaken into 90ml of sterile distilled water. Serial dilutions were made from this stock and plated for isolation of bacteria and fungi. Nutrient Agar (Oxoid) was employed for the isolation of bacteria. Inoculated plates were incubated at 37$^{o}$C and observed frequently for colonies. Colony counts were taken after 24 hrs and 48hrs. Potato Dextrose Agar (PDA) was employed for the isolation of rhizosphere fungi and incubation was at laboratory temperature of 28-30 ± 2$^{o}$C. Counts were taken after 3 and 4 days. Pure cultures of associated bacteria and fungi were obtained through several transfers. Pure isolates were identified using standard bacteriological and mycological methods

**Isolation of *Rhizoplane microflora***

Root samples were collected from the tomato plants in polyethylene bags and taken to the laboratory in a sterile bag. After washing under tap, the roots were cut into 2mm segments and isolations made following the method of Eze and Amadi (2013) with slight modifications.

**Statistical analysis**

The mean percentage frequency of occurrence of the isolated microflora organisms was obtained and the data analyzed using one way analysis of variance (ANOVA). The means were separated using Duncan's Multiple Range Test (DMRT**).**

## Results

Results of this study show that both bacteria and fungi were isolated from the rhizosphere and rhizoplane of tomato plants. Isolated fungi were *Rhizopus stolonifer, Trichoderma lignorum, Aspergillus niger, Sclerotium rolfsii, Penicillium oxalicum, Alternaria alternata, Curvularia lunata, Cladosporium fulvum, Geotrichom albidum, Mucor circinoides* and *Thielaviopsis basicola*. These organisms were abundant in the rhizosphere soil in this study **(**Table 1). It was observed that some of the organisms became less abundant as the seedlings aged. The bacterial species consistently isolated from the rhizosphere in this study included *Bacillus subtilis, Citrobacter* sp., *Erwinia* sp. and *Proteus* sp. (Table 2). An unidentified species of *Bacillus* was also isolated. While *Bacillus subtilis* appeared as flat smooth colonies with restricted tendency to spread on the nutrient agar (NA) plates the unidentified *Bacillus* sp. appeared as flat, sticky wrinkled colonies. *Citrobacter, Erwinia* and *Proteus* spp. appeared with variable colours ranging from whitish to grayish-yellow on the agar plates. No bacterium was isolated from the rhizoplane of any of the tomato seedlings in the present study.

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Results of soil analysis showed that the pH was 6.5 showing slight acidity (Table 3). The texture of the soil was found to be sandy-loam and the organic matter content was quite high at 6.8%**.**

With regards to the rhizoplane, the predominant fungal species were *S.rolfsii, R.stolonifer, T.lignorum, A niger and Fusarium solani* were most predominant (Table 4). *Penicillium oxalicum, F. oxysporium, Alternaria alternata* and *Rhizoctonia solani* were moderately present while. *Pythium splendens* which was not isolated in the rhizosphere soil occurred in the rhizoplane from the $4^{th}$-$8^{th}$ week. Mycofloral organisms like *C. lunata*, *C. fulvum*, *T. basicola*, and *G. albidum* which were present in the rhizosphere were not found on the root surfaces (rhizoplane) of the tomato seedlings in this study.

The results of this study also showed that the number of both rhizosphere and rhizoplane microflora increased with the age of the seedlings but with maximum increase between the $8^{th}$ and $10^{th}$ week (Fig. 1). In all, there were more organisms isolated from the rhizosphere than from the rhizoplane of tomato seedlings all through the duration of this study.

***Table 1: Frequency of Occurrence (%) of Fungi in Rhizosphere Soil of Tomato (Lycopersicon esculentum) Seedlings***

| | Plants Age in weeks | | | | | | |
|---|---|---|---|---|---|---|---|
| Rhizosphere Fungal isolate | 2 | 4 | 6 | 8 | 10 | 12 | *Mean |
| *Rhizopus stolonifer* | 12.63 | 15.48 | 20.35 | 23.49 | 24.36 | 25.62 | $20.32^{a}$ |
| *Trichoderma lignorum* | 12.38 | 13.11 | 14.31 | 17.08 | 18.37 | 20.13 | $15.80^{b}$ |
| Aspergillus niger | 10.17 | 11.33 | 13.59 | 16.32 | 17.13 | 19.27 | $14.64^{b}$ |
| *Sclerotium rolfsii* | 6.48 | 6.31 | 6.37 | 7.27 | 8.45 | 12.14 | $7.84^{c}$ |
| *Penicillium oxalicum* | 9.18 | 5.22 | 6.53 | 7.23 | 8.65 | 7.26 | $7.35^{c}$ |
| *Alternaria alternata* | 8.76 | 10.46 | 6.66 | 5.25 | 6.38 | 5.43 | $7.16^{c}$ |
| *Curvularia lunata* | 6.27 | 4.34 | 3.36 | 1.52 | 0.00 | 0.00 | $2.58^{d}$ |
| *Cladosporum fulvum* | 4.73 | 4.18 | 2.74 | 1.43 | 1.82 | 1.47 | $2.73^{d}$ |
| *Rhizoctonia solani* | 5.40 | 6.32 | 4.64 | 4.74 | 4.63 | 2.17 | $4.65^{cd}$ |
| *Thielaviopsis basicola* | 3.25 | 2.05 | 2.54 | 1.32 | 0.00 | 0.00 | $1.53^{d}$ |
| *Geotrichum albidum* | 2.65 | 2.48 | 2.46 | 1.37 | 0.00 | 0.00 | $1.49^{d}$ |
| *Botrytis cinera* | 4.22 | 3.77 | 3.62 | 2.30 | 0.00 | 0.00 | $2.32^{d}$ |
| *Mucor circinoides* | 4.27 | 3.49 | 2.67 | 2.38 | 2.73 | 1.26 | $2.80^{d}$ |
| *Fusarium solani* | 5.28 | 6.83 | 5.78 | 5.89 | 6.77 | 2.95 | $5.58^{c}$ |
| *Fusarium oxysporium* | 3.70 | 4.63 | 4.38 | 2.41 | 1.71 | 2.29 | $3.19^{cd}$ |

*Means followed by a common letter are not significantly different (P≤0.05) Duncan's Multiple Range Test (DMRT).

***Table 2: Frequency of Occurrence (%) of Bacteria in Rhizosphere Soil of Tomato (Lycopersicon esculentum) Seedlings***

| | Plant Age (wk) | | | | | | |
|---|---|---|---|---|---|---|---|
| Rhizosphere Bacterial Isolate | 2 | 4 | 6 | 8 | 10 | 12 | *Mean |
| *Bacillus subtilis* | 12.92 | 28.67 | 34.59 | 33.89 | 26.70 | 23.44 | $26.70^{a}$ |
| *Bacillus* sp. | 29.59 | 14.33 | 16.35 | 18.34 | 15.05 | 17.82 | $18.58^{bc}$ |
| *Citrobacter* sp. | 18.20 | 22.12 | 17.62 | 17.22 | 26.21 | 18.90 | $20.05^{bc}$ |
| *Erwinia* sp. | 19.56 | 17.38 | 14.47 | 14.44 | 13.90 | 20.55 | $16.72^{c}$ |
| *Proteus* sp. | 20.13 | 17.50 | 16.97 | 19.11 | 16.14 | 19.29 | $1819^{bc}$ |

*Means followed by a common letter are not significantly different (p≤ 0.05) Duncan's Multiple range Test (DMRT).

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***Table 3: Characteristic Properties of Soil Employed For Study***

| Soil characteristics | Values |
|---|---|
| pH | 6.5 |
| Moisture content (%) | 5.3 |
| Water Holding capacity ($m^1/g$) | 0.5 |
| Organic matter content (%) | 6.8 |
| Sand | 78.6% |
| Silt | 17.1% |
| Clay | 4.3% |
| Soil texture | Sandy clay |

***Table 4: Frequency of Occurrence (%) of Fungi on Root Surface (Rhizoplane) of Tomato (Lycopersicon esculentum) Seedlings***

| Root surface | Plant Age (wk) | | | | | | |
|---|---|---|---|---|---|---|---|
| (Rhizoplane) Fungal Isolate | 2 | 4 | 6 | 8 | 10 | 12 | *Mean |
| *Rhizospus stolonifer* | 13.78 | 14.65 | 15.24 | 17.21 | 20.35 | 20.07 | 16.88[a] |
| *Sclerotium rolfsii* | 23.11 | 16.37 | 18.13 | 20.11 | 15.21 | 16.76 | 18.28[a] |
| *Aspergillus niger* | 8.30 | 13.27 | 14.57 | 15.35 | 14.34 | 16.61 | 13.74[c] |
| *Fusarium solani* | 14.36 | 9.56 | 7.22 | 6.14 | 8.18 | 7.42 | 8.81[d] |
| *Penicillium oxalicum* | 7.44 | 7.14 | 8.21 | 10.27 | 6.32 | 5.23 | 7.44[d] |
| *Trichoderma lignorum* | 12.00 | 14.17 | 14.15 | 16.16 | 18.34 | 20.18 | 15.83[ab] |
| *Fusarium oxysporium* | 5.68 | 7.48 | 4.00 | 5.20 | 5.39 | 3.78 | 5.26[e] |
| *Alternaria alternata* | 5.26 | 6.12 | 7.32 | 3.14 | 6.38 | 5.43 | 5.61[e] |
| *Rhizoctonia solani* | 4.20 | 5.53 | 6.47 | 3.23 | 4.33 | 4.52 | 4.71[e] |
| *Mucor circinoides* | 5.87 | 4.17 | 3.56 | 2.12 | 1.16 | 0.00 | 2.81[f] |
| *Pythium sp* | 0.00 | 1.54 | 1.13 | 1.07 | 0.00 | 0.00 | 0.62[g] |

* Means followed by a common letter are not significantly different (P≤0.05) Duncan's Multiple Range Test (DMRT).

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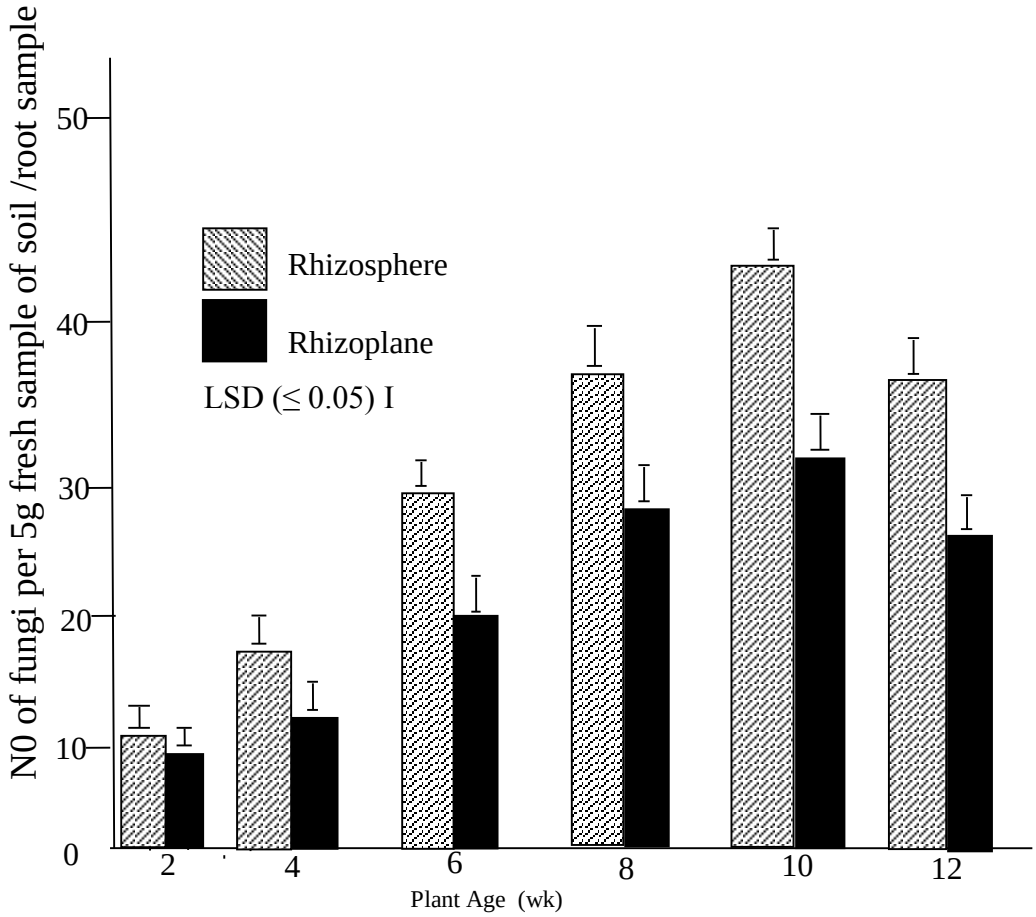


***Figure 1: Fungi Isolated from Rhizosphere and Rhizoplane of Tomato (Lycopersicon esculentum) Roots***

## Discussion

Various studies have shown that soil pH, soil fertility and texture affect the growth of crops. Soil pH is one of the most important factors affecting soil fertility (Forth and Ellis, 1988). Results of the soil analysis in this study showed that the soil was slightly acidic conforming with the earlier report of Kellogg (1998) to the effect that the ideal soil for most plants is slightly acidic to neutral.

The results of this study have shown that numerous microfloral organisms abound both in the rhizosphere and rhizoplane of roots growing in the soil with greater spectrum of microbial species in the rhizosphere than in the rhizoplane. Abdel–Hafez (1982) has reported greater spectrum of fungal mycoflora in the rhizosphere than in the rhizoplane and attributed this to the fact that the rhizoplane is a more selective substratum for micro-organisms than the rhizosphere. Bacterial microflora was not observed in the rhizoplane (root surface) of the tomato seedlings in this study. Many fungi observed in the rhizosphere but not in the rhizoplane in this study include *C. lunata, T. basicola, G. albidum and B. cinera.* The microfloral organisms associated with plant roots are affected either directly or indirectly by a number of factors such as soil type and pH. Peterson (1971) has reported that the microflora of the root zone changes as the plant grows with some organisms assuming predominance.

Various studies have shown the effect of plant root exudates on the germination and growth of fungal propagules (Eze, 2010). While some of these root exudates have stimulatory or promoting effects on the germination and growth of some fungi, some are known to exhibit inhibitory effects. Rovira (1965) reported that root exudates possess commonly occurring substances like amino acids, inorganic acids and sugars which differ greatly among species of plants and these compounds can lead to stimulatory or inhibitory effects on the microbial populations in the rhizosphere and rhizoplane. The more abundance of fungi in the rhizosphere

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than in the rhizoplane has been severally reported (Odunfa, 1979, Odunfa and Oso, 1979, Oyeyiola, 2009). Odunfa (1981) observed that some fungi require specific nutrient substances for growth and therefore are host specific. Oyeyiola (2009) had identified several fungal mycoflora populations in the rhizosphere and rhizoplane of Okro *(Abelmoschus esculentus*).

There was progressive increase in the number of microbial organisms in the rhizosphere soil and the rhizoplane as the seedling aged from the 2nd to the 10th week but subsequently declined after the 10th week. It has earlier been reported that there was increase in the number of microbial organisms in the rhizosphere and rhizoplane with increase in plant age of the root (Rovira, 1965), Abdel-Rahim *et al*., 1983; Oyeyiola, 2002). This may be due to progressive interaction between the roots and the microorganisms accompanied by continuous availability of nutrients for the growth of the microfloral organisms. Microbial proliferation in the rhizosphere and rhizoplane occur in response to the input of organic compounds exuded by the roots (Liljeroth and Baath, 1985). Soil factors such as moisture influence the amount of exudation and hence the colonization of the roots (Whipps and Lynch, 1986). Micro-organisms growing on the plant roots can have beneficial or detrimental influence on plant growth. Eze (2010) also showed that the age of plant has serious effects on the production of root exudates.

In conclusion, it is therefore, necessary to conduct more studies on the role of plant root exudates with a view to identifying those exudates that inhibit or stimulate the growth activities of the surrounding organisms. Findings can be exploited either way in the interest of soil-inhabiting micro-organisms in promoting growth of beneficial organisms or in controlling soil pathogens.

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