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# Rhizosphere Mycoflora of Okro (*Hibiscus esculentus*)

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**Abstract:** Fungi present in the rhizosphere and rhizoplane of Okro (*Hibiscus esculentus*) were isolated and identified. The fungi were *Penicillium frequetans*, *Penicillium oxalicum*, *Penicillium palitans*, *Rhizopus stolonifer*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus clavatus*, *Mucor hiemalis*, *Mucor racemosus*, *Alternaria herbarum* and *Alternaria triticina*. *R. stolonifer*, *A. niger* and *A. clavatus* were predominant in both the rhizosphere soil and the rhizoplane, while *P. oxalicum* and *A. herbarum* were predominant in the rhizosphere soil only. *Mucor hiemalis*, *Penicillium frequetans*, *P. oxalicum*, *A. clavatus*, *P. palitans* and *A. triticina* were present in the rhizosphere soil and/or the rhizoplane, but they were absent from the non-rhizosphere soil. The rhizosphere soil contained a greater spectrum of fungal species than either the rhizoplane or the non-rhizosphere soil. The experimental soil was sandy loam in texture. The rhizosphere effect increased progressively with increase in plant age until the 6th week after seed sowing and then declined.

**Key words:** Rhizoplane, soil, plant, predominant

## INTRODUCTION

The rhizosphere is a region around plant roots where simple sugars, amino acids and many other compounds are exuded by the plant and are available to the microorganisms (Campbell, 1989; Singleton and Sainsbury, 1991; Klein, 1992). Although the rhizoplane is the root surface (Alexander, 1977; Manners, 1993), it is often regarded as a part of the rhizosphere (Alexander, 1977; Lawrence, 2000). Microbial proliferation in the rhizosphere and the rhizoplane occurs in response to the input of organic compounds exuded by the roots (Liljeroth and Baath, 1988). However soil factors, such as moisture influence the amount of exudation and hence the colonization of the roots (Whipps and Lynch, 1986). Microorganisms growing on the plant roots can influence plant growth positively or negatively (Liljeroth and Baath, 1988). The influence of exudates upon rhizosphere microorganisms varies with plant age as well as plant type (Abdel-Rahim *et al*., 1983).

The rhizosphere mycoflora affect the health of plants in many ways (Odunfa, 1979). Therefore it becomes necessary that there is adequate information on the rhizosphere mycoflora of crops. Hence, the present study was carried out to determine the rhizosphere mycoflora of Okro, a crop whose fruits and leaves are consumed as soups by millions of Nigerians.

## MATERIALS AND METHODS

### Description of the Study Site and Procurement of Okro Seeds

The study site was situated in the Biological Garden of University of Ilorin, Kwara State, Nigeria. This study was carried out from May to September 2007.

The Okro seeds used for this study were bought from an agricultural seed seller in Ilorin. The seeds were collected in a sterile polythene bag (to prevent the introduction of foreign microorganisms onto the seeds), properly tied and then taken to the laboratory. The seeds were kept in a locker at room temperature, (25±3°C) when not in use.

**Physical and Chemical Analysis of Soil Samples**

Physical and chemical analysis of the soil of the study site were carried out prior to sowing of the Okro seeds. The physical and chemical analysis carried out were determination of soil texture, water holding capacity, moisture content, organic matter content and pH. The methods of Dongmo and Oyeyiola (2006) were used for all the determinations. Each determination was carried out in duplicate.

**Collection of Soil and Plant Root Samples**

Soil and plant root samples were collected using the methods of Dongmo and Oyeyiola (2006).

Each rhizosphere soil sample was collected by carefully uprooting a plant and shaking the soil adhering to the roots into a sterile polythene bag. Two plants were sampled and the two subsamples of rhizosphere soil were collected together in the same polythene bag. Each non-rhizosphere soil sample was collected with a sterile hand trowel into a sterile polythene bag. The sample of the soil of the study site prior to sowing of the okro seeds was collected in the same way as a non-rhizosphere soil sample. Each plant root sample was collected by using a sterile scalpel to cut off the roots at the base of the stem into a sterile polythene bag. Two plants were sampled for their roots and the two subsamples of roots were collected together in the same polythene bag. The samples and plant root samples were collected at 1 week intervals.

**Isolation, Preservation and Identification of Fungi**

The isolation of fungi were carried out using the methods of Dongmo and Oyeyiola (2006). The preservation of pure cultures of fungal isolates was done on potato dextrose agar slants in McCartney bottles as stock cultures in the refrigerator at 5-7°C.

The identification was done by characterizing each fungus in pure culture and making reference by Onions *et al*. (1981) and Samson and Van Reenen-Hoekstra (1988) to get its name.

**Determination of Rhizosphere Effect**

The rhizosphere effect was determined as described by Dongmo and Oyeyiola (2006).

## RESULTS AND DISCUSSION

The pH of the soil was 6.9, which was slightly acid and very close to neutrality. This pH would be favourable for the growth of okro (Table 1). Soil pH is one of the most important factors affecting soil fertility (Foth and Ellis, 1988) and the ideal soil for most plants is slightly acid to neutral, since in this state most of the compounds containing the plant nutrients have their most ideal solubility (Kellogg, 1998). The texture of the soil of the study area was found to be sandy loam, which would also be favourable for the growth of Okro. Hartman *et al*. (1988) reported that okro is adapted to wide variety of soils, but a deep fertile sandy loam with good drainage is optimum.

Table 1: Physical and chemical characteristics of the soil of the study area

| Characteristics | Results |
|---|---|
| pH | 6.9 |
| Moisture content (%) | 4.8 |
| Water holding capacity (mL $g^{-1}$) | 0.3 |
| Organic matter content (%) | 4.3 |
| **Mineral fraction** | |
| Sand | 83.0% |
| Silt | 6.0% |
| Clay | 11.0% |
| Soil texture | Sandy loam |

Table 2: Percentage frequencies of occurrence of fungi in the rhizosphere soil and rhizoplane of Okro and the non-rhizosphere soil

| | Plant age (weeks) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Isolates | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Treatment mean |
| **From rhizosphere soil** | | | | | | | | | |
| *Rhizopus stolonifer* | 17.5 | 18.7 | 14.3 | 15.1 | 18.2 | 13.6 | 12.8 | 14.4 | 15.6b |
| *Aspergillus niger* | 18.0 | 20.0 | 26.4 | 27.0 | 29.0 | 30.0 | 33.0 | 33.8 | 27.2a |
| *Aspergillus clavatus* | 16.0 | 18.5 | 19.1 | 16.7 | 16.0 | 14.0 | 14.6 | 13.0 | 16.0b |
| *Penicillium oxalicum* | 16.4 | 16.0 | 14.8 | 14.8 | 14.6 | 16.2 | 11.8 | 11.9 | 14.6c |
| *Alternaria herbarum* | 15.0 | 17.1 | 16.0 | 14.0 | 11.3 | 12.0 | 12.9 | 12.3 | 13.9c |
| *Penicillium palitans* | 6.8 | 4.7 | 5.6 | 5.1 | 3.4 | 3.4 | 4.8 | 4.8 | 4.8d |
| *Alternaria triticina* | 7.7 | 5.0 | 3.8 | 4.5 | 4.2 | 5.2 | 4.4 | 3.8 | 4.8d |
| *Aspergillus japonicus* | 2.6 | -- | -- | -- | -- | -- | -- | -- | 2.6d |
| *Rhizopus oryzae* | -- | -- | -- | -- | -- | 1.8 | 2.8 | 2.9 | 2.5d |
| *Mucor racemosus* | -- | -- | -- | 2.8 | 3.3 | 3.8 | 2.9 | 3.1 | 3.2d |
| **From rhizoplane** | | | | | | | | | |
| *Rhizopus stolonifer* | 30.0 | 32.0 | 22.8 | 24.0 | 26.0 | 24.2 | 20.0 | 20.0 | 24.9b |
| *Aspergillus niger* | 34.1 | 36.0 | 39.8 | 35.2 | 30.1 | 30.2 | 35.4 | 36.0 | 33.4a |
| *Aspergillus clavatus* | 28.4 | 22.6 | 24.0 | 27.3 | 30.7 | 29.0 | 24.3 | 24.2 | 26.3b |
| *Penicillium frequetans* | 7.5 | 9.4 | 9.0 | 8.6 | 6.0 | 6.5 | 9.3 | 9.0 | 8.2c |
| *Mucor hiemalis* | -- | -- | 2.0 | 2.1 | 4.3 | 4.3 | 4.5 | 5.1 | 3.7c |
| *Rhizopus oligosporus* | -- | -- | 2.4 | 2.8 | 2.9 | 3.1 | 4.1 | 3.6 | 3.2c |
| *Rhizopus oryzae* | -- | -- | -- | -- | -- | 2.7 | 2.4 | 2.1 | 2.4c |
| **From non-rhizosphere soil** | | | | | | | | | |
| *Rhizopus stolonifer* | 50.0 | 52.0 | 48.4 | 30.4 | 24.3 | 17.4 | 13.7 | 18.3 | 31.8a |
| *Aspergillus fumigatus* | 30.0 | 23.4 | 21.6 | 20.6 | 28.5 | 20.3 | 16.7 | 16.7 | 22.2a |
| *Mucor racemosus* | -- | -- | -- | 10.4 | 10.4 | 17.0 | 20.0 | 15.2 | 14.6b |
| *Aspergillus japonicus* | -- | -- | -- | 18.6 | 20.6 | 20.5 | 22.4 | 23.0 | 21.0b |
| *Rhizopus oligosporus* | 20.0 | 24.6 | 30.0 | 20.0 | 16.2 | 18.3 | 20.0 | 17.8 | 20.9b |
| *Rhizopus oryzae* | -- | -- | -- | -- | -- | 6.5 | 7.2 | 9.0 | 7.6c |

Means followed by the same letter(s) in the same column for the same sample are not significantly different at 5% level (p = 0.05) based on Duncan's Multiple Range Test (DMRT)

The rhizosphere soil contained a greater spectrum of fungal species than either the rhizoplane or the non-rhizosphere soil (Table 2). Abdel-Hafez (1982) also found greater spectrum of fungi to occur in the rhizosphere than in the rhizoplane and attributed this to the rhizoplane being a more selective substratum for fungi than the rhizosphere. The result obtained in this study is the same as was obtained for *Sorghum bicolor* (Odunfa, 1979), cowpea (Odunfa and Oso, 1979), sugar cane (Abdel-Rahim *et al*., 1983) and *Amaranthus hybridus* (Oyeyiola, 2002). *Rhizopus stolonifer*, *Aspergillus niger* and *Aspergillus clavatus* were predominant in both the rhizosphere soil and the rhizoplane, while *Penicillium oxalicum* and *Alternaria herbarum* were predominant in the rhizosphere soil only (Table 2). The fungal flora associated with plant roots is subject, either directly or indirectly, to the influence of a number of factors such as soil type and pH and the fungal flora of the root zone changes as the plant grows with certain fungi assuming predominance (Peterson, 1961). The predominance of fungi in the rhizosphere and rhizoplane had been observed by earlier researchers (Odunfa, 1979; Odunfa and Oso, 1979; Odunfa, 1980; Abdel-Hafez, 1982). A No. of fungi were present in the rhizosphere soil and rhizoplane but were absent from the non-rhizosphere soil. Such fungi could have come from the Okro seeds which were not introduced into the non-rhizosphere soil. Rhizosphere soil had the highest number of fungi each week followed by the rhizoplane and lastly by the non-rhizosphere soil (Fig. 1). These results are slightly different from what Abdel-Rahim *et al*. (1983) obtained for sugar cane plants in which the highest No. of fungi occurred in rhizosphere followed by the non-rhizosphere and lastly by the rhizoplane. The occurrence of greater number of fungi in rhizosphere than the non-rhizosphere soil is attributable to the presence of organic substances which came from the root exudation and dead root cells (Rovira, 1965; Richards, 1987).

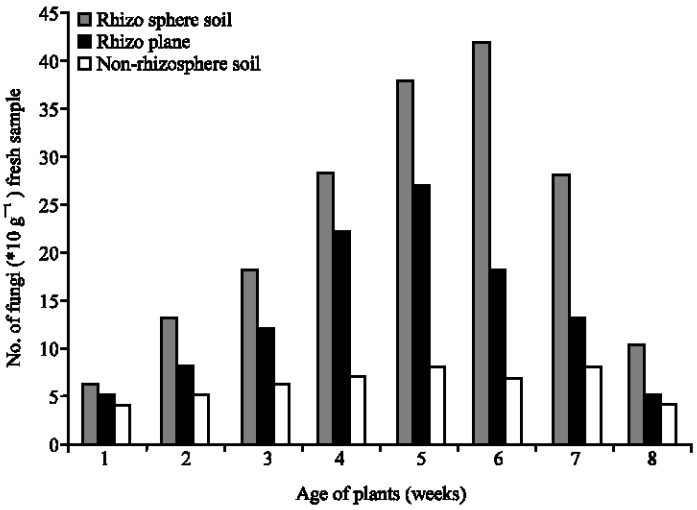


Fig. 1: The No. of fungi isolated from rhizosphere soil, rhizoplane and non-rhizosphere soil as the Okro plants aged


Fig. 2: Ratio of the No. of fungi isolated from the rhizosphere soil and non-rhizosphere soil (R/S ratio) as the Okro plants aged

The No. of fungi in the rhizosphere soil and rhizoplane followed the same pattern of progressive increase from the first week after seed sowing up to the 6th week and subsequent decline up to the 8th week. No clear pattern occurred in the No. of fungi in the non-rhizosphere soil as the plants aged. It is of common occurrence for numbers of microorganisms in the rhizosphere and rhizoplane to increase with increase in plant age (Rovira, 1965; Abdel-Rahim *et al*., 1983; Oyeyiola, 2002), which will be due to the progressive interaction between the roots and the microorganisms accompanied by continuous availability of nutrients for the growth of the microorganisms. Such interaction will be absent in the non-rhizosphere soil, and thus, there will not be any factor which cancause a specific

pattern to occur in the growth of the microorganisms. The decline in the numbers of fungi in the rhizosphere soil and rhizoplane after the 6th week could be due to reduction in the rate of growth of the Okro plants which would have resulted in a reduction in the rate of root exudation. The rhizosphere effect increased progressively with increase in plant age until the 6th week after seed sowing and then declined (Fig. 2). Similar pattern had been reported previously for other plants (Oyeyiola and Hussain, 1991; Oyeyiola, 2002). It would logically be a reflection of the enhancement of microbial growth in the rhizosphere soil as compared to the non-rhizosphere soil in which plant roots were absent. However different patterns have been reported by Odunfa (1979) and Odunfa and Oso (1979) in which the rhizosphere effect decreased during the early stages of growth of plants.

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