IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 2 Ver. II (Mar-Apr. 2014), PP 18-22
www.iosrjournals.org

Fungi Colonization of the Rhizoplane of Okra (*Hibiscusesculentus*) Plant

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Abstract:*The frequency of occurrence of the colonizing fungal species in the rhizoplane soil of okra plant (Hibiscus esculentus) were studied at different stages of development. The most frequently isolated fungal species in this examined okra plant were; Schizosaccharomyces pombe, Aspergillus flavus and Aspergillus fumigatus, others were; Penicillium spinulosum, Mucos racemosus, Saccharomyces cerevisiae, Aspergillus nidulans, Penicillium digitatum, Aspergillus niger, Cladiosporium resinae, Monascus ruber and Alternaria tenius.Investigation was also carried out on how factors such as soil moisture content organic matter content and soil pH affect the relative abundance of the fungal with okra plant growth.*
Key Words: *Rhizoplane, Colonizing, Schizosaccharomyces, Relative abundance*

I. Introduction

Microorganisms found in the soil and its study can be equated with soil microbiology. Varieties of plant and animal found inhabiting in the soil are called soil organisms. Some of them are macro while some are micro, those classified as plants are called microflora, fungi is included (Brandy & Weil, 1999).The plant roots or tree roots are classified under soil macroorganism, the root system of plants is associated with inanimate environment composed of organic and inorganic substances also with a metabolically active community of microorganisms. The fungi found around cultivated soil is quite different from uncultivated soil community purposely because growing plant creates a unique habitat for microorganisms and are inturn affected by their population. Relationship that occurred amongmicroorganisms, soil and roots were observed by Foster and Marks, (1967) through transmission electron microscopy (TEM) of the soil-root interface.The rhizoplane is the root surface of the plant (Rovira, 1965) and different from the rhizosphere which is the zone (soil) surrounding the plant root (Rovira, 1965).The Rhizoplane which is the root surface of the growing plants in the soil provide a unique habitat, the rhizosphere, which is particularly favourable for the development of the microorganisms in the soil. However, the majority of the microorganisms stimulated by rhizoplane are usually harmless saprophytes which live mainly on dead, decomposing root tissues.The root excretions such as volatile and dissolved exudates affect microbial growth in the root surrounding soil, also most microbial activities in the soil is associated with the roots or fresh decomposing organic materials, therefore, soil microorganisms including "Fungi" depend on available organic compounds for survival while activity of microorganisms in non-cultivated soil will be dormant.It is claimed that the young root presents "a virgin niche" available for soil microorganism colonization and as the root grows through soil, the root exudates promote the fungal spores germination as a propagules and growth of fungal hyphae. The initial root microorganism colonizers disappeared as roots ages leaving a stable root surface microflora and microbial species eventually become dominant members of the final stable root surface population which depends on plants species and the soil (Peterson, 1958).The great variability that occurred in the microbial cover of the roots was reported by Bowen and Theodoros (1973) that the surface of the three weeks old plant root contains less than 10% microorganisms and increased to 37% in 90 days old plan root.The low surface occupancy by microorganisms in the root surface (rhizoplane) occurred as a result of relative few points of inoculation in the soil followed by little spread over the surface and also, soil parts of the root surface may not be conducive for microbial growth.Foster, (1962) observed that the relatively small number of microbial cover on the roots at the early age offers little protection against plant pathogens like *Phytophthoracinnamomi* and biological control of such organism occurred by attack on the soil propagule. The hypothesis that organic debris is a major source of inoculum for the plant root is consistent with Gray, et al; (1967) observation, that in a sand dune soil, organic matter particles provide less than 15% of the available solid surfaces.Microbial growth can be affected by volatile and dissolved exudates from the seeds and roots for relatively large distance for example, the germination of *Chlymadospores* of *Fusarium* is 10mm from the planted seeds as been reported by Stanghellini and Hancock, 1971. Plant root exudates can infect the susceptible plants and can as well inhibit spores germination of a resistant roots varieties as been reported by Buxton, (1957).Some saprophytic fungal initiated by the rhizoplane inhibiting rhizosphere were known to produce antibiotics which might possibly antagonize other organisms including plant pathogens.Some environmental factors like temperature, moisture content, soil pH etc. can influence

microorganisms in the rhizoplane of a plant in two ways. The effect may be directly by affecting the growth rate and the multiplication of the organism or indirectly by controlling plant growth thereby affecting the nature and amount of exudates from the root as been reported by Rovalt *et. al*; (1963).Nithmetra and Kakaka (1972) reported that low pH factors fungal growth in their habitat.Some fungi can be predominate over the others in rhizoplane, Oyeyiola and Hussain (1992) reported *Aspergillus* and *Penicillium* species predominating the rhizosphere of wheat plant. Generally, the number of organism in the rhizoplane and rhizosphere had been found to increase with the age of the plant.Biotic and Abiotic factors greatly alter the survival of fungi n the root surface of growing plant.Okra (*Hibiscusesculentus*) is one of the most popular vegetables of the tropics. The plant belongs to the family *Malvaceae* and originated from Africa.The fruit are mostly borne on the top of the stem and above the foliage with few fruit per plant. These are two varieties, the short stemmed and the long stemmed varieties (Greensill, 1964).The Okra plant is an adaptable plant that grows on any type of soil. The plant thrives in full sunlight and can also tolerate moderate shade, is drought resistant and responds to organic manure (Olanrewaju, 2011). This paper provide information on fungal and colonization of the rhizoplane of Okra plant (*Hibiscusesculentus*). This research was of interest because fungi participate actively in degrading debris in the soil thereby providing nutrient needed for plant growth to increase the yield of the plant.

II. Methodology

Physiochemical Analysis of the Soil

The physical and chemical analysis of the experimental soil were carried out prior to sowing of the Okra plants and also after seven days interval of the Okra plants aged as follows;

Soil pH Determination

The pH of the experimental soil was determined using Blank (1975) method.

Soil Moisture Content Determination

The moisture content of the experimental soil was determined prior to the sowing of okra seed and also at seven days interval as the plant aged using primer and Schmidt (1964) method.

The moisture content is expressed as:

$$\frac{\text{Loss in weight of the soil sample} \times 100}{\text{Initial weight of the soil sample}}$$

Water Holding Capacity

The water holding capacity of the experimental soil was determined once by a modification of the Pramer and Schimidt (1964) method. This was calculated as below;

$$\frac{\text{Amount of water retained by the soil (ml)}}{\text{Weight of the soil used (g)}}$$

The unit is ml/g

Soil Organic Matter Content Determination

The soil organic matter of the experimental soil was determined prior the planting of Okra seeds and after planting of the seeds weekly as the okra pant aged. The method of Walkley-Black wet oxidation was used.

The calculation of percentage organic matter content was done using the formula below;

$$\%\ \text{organic carbon} = \frac{\text{milliequivalent } FeSO_4 \text{ for blank} - \text{milliequivalent } FeSO_4 \text{ for sample} \times 0.003 \times 100(f)}{\text{Weight of air-dry soil}}$$

Where;

F = Correction factor which is 1.33

Milliequivalent = Normality of solution x ml of solution used

.'. % organic matter in soil = % organic carbon x 1.72g

Soil Texture Determination

The soil texture of the experimental soil was determined once using the soil hydrometer method and the texture was identified with reference to the textural triangle of Pramer and Schimidt, (1964).

Media Used

Potato dextrose ager (PDA) was used for the isolation of fungi in the sampled soil examined.

Isolation of Fungi from Okra Seeds

Both surface and non-surface sterilized seed were used. Both seed types were crushed in sterile mortal and pestle.Serial dilution was done up to 10^{-3}. 0.1ml was taken from $10^{-1}$and 10^{-2} dilution from both

suspensionprepared and were spread onto PDA plate separatelyin aseptic condition. The plates were incubated at room temperature for 24 hours. The different growth observed were counted and were sub-cultured to obtain pure fungal isolates.

Raising of Okra Plants

The experimental site was located at Osin farm along Ajaba road, Ila-Oragun, Osun State, Nigeria. Two to three viable seeds of Okra were planted in a heap at a depth of about 5cm and spacing is about 50-75cm. Isolation of fungi was done weekly after planting of Okra seeds from rhizosplane (root) samples as the plants aged.Each sample collected weekly was also labeled appropriately (Odunfa and Oso, 1979). All sample collected were used for isolation of fungi.

Isolation of Fungi from the Rhizoplane

The root surface with its adhering soil particles after shaking to remove surrounding soil called "rhizosphere soil" made up the rhizoplane "root surface"

The roots were aseptically cut into between 0.5 and 1.0 cm length using sterile scalpel. At the young age between seven days to twenty-one days of planting when the roots are still tiny, 0.1g of the cut pieces was weighed; these were introduced into the dilution tubes containing 0.9g and 9ml of the sterile distilled water respectively. These were shaken for some minutes and each suspension represented10^{-1} dilution. From 10^{-1} dilution, 10^{-3}dilution was prepared aseptically.

One ml of each 10^{-3}dilution was taken with a sterile 2ml syringe and transferred unto a sterile set PDA plate.

The inoculums was then spread on a plate with the aid of a glass spread which was sterilized by dripping in75% alcohol and pass over flame and allow to cool. The plate was left for some minutes for the inoculums for diffuse into the medium. The pate were incubated at room temperature for 24 – 72 hours. Different fungi grows observed were sub-cultured using stab method and incubated and observed until pure fungi isolate were noticed. Confirmed pure fungi isolate were inoculated unto PDA agar slant and incubated at room temperature until sufficient growth was obtained and stored as stock cultures in the refrigerator for preservation.

Characterization and Identification of Fungi Isolated

The colonial morphology of the pure culture of each fungus was determined.Microscopic examination of each pure fungi isolate was also made. The colonial characteristics determined are; colour of the colony on the surface of agar, colour and the colour changesof the reverse of the colony and the rate of the growth on the medium. For the microscopic examination, two clean microscopic slides and cover slips were used. The various fungal isolate were identified by reference to onions *et al*., (1981), Robert and Ellens (1988) and Harrow (1968).

III. Result and Discussion

Physiochemical Characteristic of Experimental Soil

The result of the physiochemical analysis of the experimental soil before seed planting are shown in table 1.

The texture of the experimental soil was loamy sand.

TABLE 1: Physical and Chemical Characteristics of Experimental Soil Prior to Seed Plant

Experimental Soil	Experimental Physiochemical characteristics						
	pH	Water holding capacity (ml/g)	Moisture content (%)	Organic matter (%)	Mineral Fraction (%)		
					Silt	Clay	Sand
Prior to seed planting	7.2	0.48	17.0	4.7	12.0	4.0	8.40

TABLE 2: Frequency of Occurrence of Fungi Isolate in the Rhizoplane of Okra Plant with Increasing Age

Isolates	Time of Sampling (Days)							
	7	14	21	28	35	42	49	56
Schizosaccharomyces pombe	+	+	-	+	+	-	+	-
Penicillium spinulosum	+	-	-	-	-	-	-	-
Mucor racemosus	+	-	-	-	-	-	-	-
Aspergillus flavus	+	+	+	+	-	+	-	-
Saccharomyces cerevisiea	-	-	-	-	+	-	+	-
Aspergillus nidulans	-	-	+	+	-	-	-	-
Penicilium digitatum	-	-	+	-	-	-	-	-
Aspergillus niger	-	-	+	-	-	-	-	-
Cladiosporum resinae	-	-	+	-	-	-	-	-
Monascus ruber	-	-	-	-	+	-	-	-
Aspergillus fumigatus	-	-	-	+	+	+	-	+
Alternaria tenius	-	-	-	-	-	+	-	-

TABLE 3: Percentage Frequency of Occurrence of Fungal Isolates in Rhizoplane of Okra Plant with Increasing Age

Isolates	Time of Sampling (Days)							
	7	14	21	28	35	42	49	56
Schizosaccharomyces pombe	70.0	18.2	-	48.0	21.7	-	50.3	-
Penicillium spinulosum	17.7	-	-	-	-	-	-	-
Mucor racemosus	6.0	-	-	-	-	-	-	-
Aspergillus flavus	6.9	27.3	18.2	7..4	-	36.7	-	-
Saccharomyces cerevisiea	-	-	-	-	16.7	-	49.7	-
Aspergillus nidulans	-	-	27.3	3.6	-	-	-	-
Penicilium digitatum	-	54.5	18.2	3.7	-	-	-	-
Aspergillus niger	-	-	9.1	-	-	-	-	-
Cladiosporum resinae	-	-	27.3	-	-	-	-	-
Monascus ruber	-	-	-	-	28.3	-	-	-
Aspergillus fumigatus	-	-	-	37.3	33.3	51.1	-	100
Alternaria tenius	-	-	-	-	-	12.2	-	-

TABLE 4: Frequency of Occurrence and Percentage Frequency of Occurrence of Isolated Fungi from Surface Sterilized and Non-Surface Sterilized Seeds of Okra (*Hibiscusesculentus*)

Fungi isolates	Non-Surface Sterilized Seeds		Surface Sterilized Seeds	
	Occurrence	% occurrence	Occurrence	% occurrence
Schizosaccharomyces pombe	+	91.6	+	98.1
Aspergillusflavus	+	6.9	+	1.9
Saccharomycescerevisiae	+	1.5	-	-

Key: + = Present
- = Absent

Isolation of Fungi from Okra Seed

A total of three fungi were isolated from surface sterilized and non-surface sterilized seed of Okra (*Hibiscusesculentus*) (Table 4). These were *schizosaccharomycespombe, Aspergillusflavus* and *saccharomycescerevisiae* on the surface sterilized seeds, *Schizosaccharomycespombe* was the dominant fungi found growing all over the seeds surfaces while *Aspergillusflavus* growth was relatively low.

Fungi isolated from non-surface sterilized Okra seeds were identified as the same with thosefrom surface sterilized seeds in addition to *Saccharomycescerevisiae* (Table 4). *S. cerevisiae* also occurred relatively low on the seeds of non-surface sterilized Okra.

Schizosaccharomycespombe was the dominant fungal isolate from both surface sterilized and non-surface sterilized Okra seeds (Table 4).

Isolation of Fungi from Rhizoplane of Okra Seed

A total of twelve fungi were isolated from the rhizoplane of Okra plant (Table 2).

The isolated fungi include; *Schizosaccharomyces pombe, Penicillium spinulosum, Mucor racemosus, Aspergillus flavus, Saccharomyces cerevisiae, Aspergillus nidulans, Penicillium digitatum, Aspergillus nigar, Cladosporum resinae, Monascus ruber, Aspergillus fumigatus,* and *Alternaria tenus. S. pombe, A. flavus, P. digitatum*and *A. fumigatus* occurred at middle age till maturity. Other colonizer of the rhozoplane are not persistently occurring (table 2 and 3).

Description of Okra Plant Growth

Planted Okra seeds emerged seedling four days after the plantingof its seeds.

Okra plant began flowering at forty-two days after seed planting. First, Okra fruits emerged forty-four days after seed planting and the fruit matured at fifty-six days after planting.

The rhizoplane fungi isolated at seeding stage are more than those isolated at the period near maturity that is between (42-56) days, but those few species growing in this period, their number increase quantitatively (Table 3). This observation is in line with what was observed by Odunfa, (1980), Oyeyiola and Hussain, (1992). All the fungi general isolated in the rhizoplane of Okra plant had been found to colonized the rhizosphere and non-rhizosphere soil of Okra plant as reported by Olanrewaju, (2011). The presence of this fungi general in these zones may be as a result of those fungal general been the normal soil mycoflora of that particular experimental soil. This common fungi general include; yeast, *Aspergilli, Peniccilli* and *monascus.*

The isolated fungi from rhizoplane of Okra might have been introduced by Okra seeds (Table 4). Okra seeds harboured few surfacesmycoflora (Table 4) which got introduced into the soil during and subsequently colonized plant root and later diffuse into the root surrounding soil by root exudation. The microflora of the root

zone is truly derived from both the seed and the soil, but due to the relatively low numbers on the seed, it might be justifiable to conclude that most of these root zone microorganisms originatedfrom the soil (Peterson, 1958).
Fewer species of fungi were reported by Ugoji, (1993), Oyeyiola and Hussain, (1992) in the rhizoplane of two varieties of pigeon pea (*Cajanscajan (*L) Millop), wheat, Okra and cowpea respectively, than in the rhizosphere this must be attributed to the presence of greater amount of organic nutrient in the rhizosphere compare to the rhizoplane and lower competition in the rhizosphere when also compare to the rhizoplane.

The study of fungal species colonizing the rhizoplane of Okra plant revealed that yeast and *Aspergillus* were the dominant genera in the rhizoplane and were associated with the root throughout the life of the Okra plant. The predominance of *Aspergillus* may be attached to their highsporulating ability. The success of yeast can also be attached to its reproduction method which is budding or fission.

Some of the fungi isolated in the temperate zone from the root region of other plants e.g. wheat in Kano area as been reported by Oyeyiola and Hussain, (1992) were absent from Okra roots in this work, such fungi include; *Aspergillus japanicum, Epicoccum nigrum, Penicillium sclerotium* and *Talaromyces trachyepermus.*
Some soil pathogenic fungi such as *Fusaria, Penicillia* and *Aspergilli* have been reported as pathogens of many plants. However, since the development of the Okra plants used in this work was not adversely affected, it means that the *Fusarium oxysporum* and other potentially pathogenic fungi were not present in the experimental soil.

This study shows that, the rhizoplane mycoflora shared benefit from their association with the Okra plant roots.
Further studies are therefore encouraged to ascertain the specific role played by those isolated fungi that contributed to the well growth and perhaps to improve the yield of Okra plants.

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