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Fungal Diversity in the Rhizosphere and Rhizoplane of Okra (*Abelmoschus esculentus* L.) Moench. in Nsukka, Enugu State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The growth and development of economically important crops are usually affected positively or negatively by the microbes present in the rhizosphere and rhizoplane. Based on this, the study was carried out to determine the fungal diversity in the rhizosphere and rhizoplane of okra plant.
Methods: Okra seeds were purchased from an agricultural shop in Nsukka main market and were planted at Botanic garden, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Physicochemical properties of the soil sample were evaluated prior to planting and as the plant aged. Rhizosphere and rhizoplane samples were collected at two weeks interval and dilution plate method was used in fungi isolation after which they were identified. The frequency of occurrence and the colony forming unit per gram of the sample (cfu/g) were evaluated.
Results: The Physicochemical properties of the soil samples fluctuated as plant aged at two weeks intervals. The pH was slightly acidic to neutral which is ideal for most plant to grow. The water retention capacity, moisture content and organic matter content increased from 11.47-27.90 ml/g, 5.03-21.07% and 2.35-3.68% respectively at two weeks interval but fluctuates at subsequent weeks. A total of eleven (11) fungi were isolated from the rhizosphere and were identified as, *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus bertholletius, Fusarium oxysporum, Galactomyces candidum, Helminthosporium solani, Rhizopus stolonifer, Mucor racemosus,*

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Penicillium citrinum and *Trichoderma viride*. All fungi were present in the rhizoplane except *Aspergillus bertholletius* and *Penicillium citrinum*. Rhizosphere had a greater number of fungi than the rhizoplane. *Aspergillus* species were predominant in both the rhizosphere and rhizoplane. *A. niger* had the highest frequency of occurrence of 58.67% on the 6th week and 65.79% on the 4th week in rhizosphere and rhizoplane respectively. The colony forming unit (cfu/g) of *Aspergillus niger* was significantly different from all other isolates at P≤0.001 followed by *Mucor racemosus* and *Fusarium oxysporum* with significant differences at P≤0.05 and P≤0.01 respectively.

Conclusion: The rhizosphere and rhizoplane of okra plants has been shown to be rich in fungal diversity and a greater number were obtained from the rhizosphere. The data obtained from this work could be exploited by microbial ecologist to ascertain ecological associations and biomass increase by the fungal communities which also forms part of ecosystem. The fungi had no pathological effect on the plant which suggest positive effect on the growth and development of okra plant as the plant aged.

Keywords: Rhizosphere; rhizoplane; isolate; physicochemical; fungi; frequency of occurrence.

1. INTRODUCTION

The root system of higher plants is not made up with only organic or inorganic substances but also with a vast community of microflora. Plant roots provides a unique habitat for microorganisms by secreting exudates such as simple sugars, amino acids and many other compounds around the roots [1]. The plant is in turn affected by the diversity and population of microflora that it has stimulated since plant root region is a site from which microorganisms obtain their nutrients and through which pathogenic ones penetrates. Consequently, the association of both the animate (microflora) and inanimate (organic and inorganic) objects with the plant roots is pivotal for soil fertility and crop production [2,3]. Berendsen et al. [4] also observed that soil microorganism in particular rhizosphere and rhizoplane microbes attract much attention, as these microbiomes play key roles in determining plant health and productivity. The rhizosphere is a microecological region in direct contact with plant roots. It is a highly dynamic zone of interactions between plant roots and microflora. Bonkowaski et al. [5] defined rhizosphere of plants as the soil that clings to the roots after being gently shaken off. The plant roots and associated microflora determines the actual extent of the rhizosphere. It is a more competitive and metabolically busier environment than the bulk soil. Hinsinger et al. [6] considered this area of soil to be the most biodiverse and dynamic habitat on earth.

The root surface is termed the rhizoplane [7]. Sylvia et al. [8] and Singer and Donald [9] defined rhizoplane as the root epidermis and outer cortex where soil particles, bacteria and fungi hyphae adhere. It is practically defined as the remaining microorganisms and soil particle after the roots have been shaken vigorously in water. Several microorganisms have also been reported on this region of the root.

Okra is an annual herbaceous hairy plant in Malvaceae family known for its edible seed pods. It is sometimes referred to as 'lady's finger'. Okra comes in two colours; red and green. Biologically classified as a fruit but generally utilized like a vegetable in cooking. Okra may be used in developing countries to mitigate malnutrition and alleviate food insecurity [10]. Millions of Nigerians consume okra as soup [11]. It is an excellent source of vitamins C and K. Its low in calories and has a highly dietary fiber content. Based on the afore mentioned economic importance of this crop, it is necessary to obtain adequate information on the diversity of mycoflora of the crop, since it affects plant health positively and negatively. Hence, the present research was carried out to determine the fungal diversity in the rhizosphere and rhizoplane of okra plant.

2. MATERIALS AND METHODS

2.1 Study Site and Location

The study site was located at the Botanical garden, Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Enugu State, Nigeria.

2.2 Procurement of Okra Seeds

The okra seeds used were purchased from an agricultural shop in Nsukka main market. They were collected aseptically in a sterile polythene bag properly tied and taken to the laboratory

prior to use. The okra seeds were kept in a locker at room temperature of about 28 ±2°C when not in use.

2.3 Land Preparation and Seed Planting

The experimental site was cleared manually and the soil sample was collected using wheel barrow and shovel. The soil samples were poured into forty (40) sterile polyethene bags about half filled and the bags were kept five (5) in rows and eight (8) in column. Four seeds were sown 2-5 cm deep per hole in each bag [7].

2.4 Physicochemical Analyses of the Soil Samples

Physicochemical analyses of the soil samples of the study site was carried out before the sowing of okra seeds and also at certain stages of development (two weeks interval) of the okra. The physicochemical properties of the soil carried out were soil texture, water retention capacity (WRC), pH, moisture content and organic matter content of the soil. This was carried out in the Department of Soil Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

2.5 Collection of Rhizosphere and Rhizoplane Soil Samples

The method of Oyeyiola [11] and Vasanthakumari and Shivinna [12] were used for collection of rhizosphere and rhizoplane samples respectively. The rhizosphere soil samples were collected by carefully uprooting test plants and shaking-off the adhering soil into sterile polythene bags while the root samples (rhizoplane) were washed under running water for about 20 mins, and then in two changes of sterile distilled water. Excess water was blotted off with sterile blotter discs. The roots were cut into bits of about 1-2 cm into a different sterile polyethylene bags and both samples were taken to the laboratory for isolation on two weeks interval for 12 weeks consecutively. Five test plants were uprooted per treatment.

2.6 Isolation of Rhizosphere Fungi

Potato Dextrose Agar (PDA) was used for both culturing and sub-culturing of the fungi to obtain pure cultures. Ten gram of rhizosphere soil sample were poured into 90 ml of sterile distilled water and was shaken vigorously to homogenize the content. Serial dilutions were made from the stock solution and 0.2 ml aliquot was inoculated into the set PDA plates and incubated at laboratory temperature of 28±2°C. The fungal colonies were counted after 4-7 days of incubation and expressed in colony forming unit per gram of the soil (cfu¯g). The number of colonies for each fungus in each plate was multiplied by the dilution factor to obtain the number per gram in the original soil sample, and then the average per 3 plates was calculated.

2.7 Isolation of Rhizoplane Fungi

Abdel-Hafez et al. [13] method of isolation with a little modification was used. The root (1cm) segments (5 per plate) were plated in PDA plates at room temperature for 4-7 days and the fungal colonies were counted and expressed in colony forming unit per 5 segments of the roots.

2.8 Identification of the Fungal Isolates

The isolates were identified based on their morphological and microscopic features. The morphological identification was based on the observed culture growth patterns, texture and mycelia colour. Microscopic identification was done by teasing a small portion of the fungal mycelial in a lactophenol cotton blue on clean slides, covered with clean cover slips. The prepared slide was examined under the microscope at x400 magnification. Fungal identification was confirmed with the aid of some mycological books by Barnett and Hunter [14], Agrios [15] and Ellis et al. [16].

2.9 Determination of Percentage Frequency of Occurrence of Fungal Isolates

The number of times of occurrence for each species was recorded and calculated as a ratio of the total number of fungal species isolated and was expressed as a percentage (%).

The calculation was done using the formula:

$$Percentage\ frequency\ of\ occurrence\ (\%) = x/n \times 100/1$$

Where,

X= number of times of occurrence of each fungal species
N= total number of occurrences of all the fungal species

3. RESULTS AND DISCUSSION

The soil pH prior to planting was 6.4 but increased to 6.8 after sowing and germination of seeds at two weeks interval and later, 7.2 at 12 weeks. The pH which was slightly acidic to neutral would be favourable for the growth of okra plant (Table 1). Various studies have shown that slightly acidic to neutral soil is ideal for most plant to grow because most of the compounds containing plant nutrients solubilize in this state. This result is in line with the work of Eze and Amadi [7], Oyeyiola [11]. The water retention capacity, moisture content and organic matter content increased from 11.47-27.90 ml/g, 5.03-21.07% and 2.35-3.68% respectively at two weeks interval but fluctuates at subsequent weeks. Vasanthakumari and Shivinna [12] showed that microbial oxidation of soil organic matter is greater in soils with fluctuating moisture than in soil with constant wet or dry conditions. Morgan et al [2005] in their research found that fungi can efficiently contribute to the nutrient mobilization in the rhizosphere and are able to produce enzymes involved in the hydrolysis of nitrogen and phosphorus compounds from the organic matter. The texture of the soil was sandy loam as shown in Table 1. The texture, which is the proportion of sand, silt and clay varies with plant age. This may be attributed to the varying content of the exudates released by the roots and also the particular mycoflora present at that stage of plant development. This result is in line with that obtained by Huang et al. [18]; Olanrewaju et al. [19]; Olahan et al. [20]. Liu et al. [21] also observed that root exudates have a greater effect on rhizosphere microbes in sandy loamy soil. The texture determines the drainage, aeration and the amount of water the soil can hold. Therefore, sandy loam soil is categorized as nutritionally rich, aerated and permeable.

A total of 11 fungi were isolated from the rhizosphere of okra and were identified as, (Table 2). Many researchers have isolated diverse population of fungi from the rhizosphere and rhizoplane of various plants. Arotupin and Kinyosoye [22] isolated seven fungi in their work with cassava plant. Sule and Oyeyiola [23] and Olahan et al. [20] isolated 25 and 4 fungi in their work with cassava cultivar TMS 30572 and tobacco (*Nicotiana tabacum*) respectively. Some of the fungi encountered in this research were also isolated by them. Oyeyiola [11] isolated *Rhizopus stolonifer, Aspergillus niger, Aspergillus fumigatus* and *Mucor racemosus* in his research on the rhizosphere mycoflora of okra (*Hibiscus esculentus*) which is in line with this work. This suggest that okra plants release common unique exudates that attracts these specific fungi irrespective of the geographical region where it grows. Abdel-Hafez et al. [13] reported that most of the rhizosphere and rhizoplane fungi of wheat plant were members of the genera, *Aspergillus*, *Helminthosporium* and *Fusarium* spp while Eze and Amadi [7] isolated *Trichoderma, Rhizopus, Aspergillus, Penicillium, Mucor* and *Fusarium* spp among others, from the rhizosphere and rhizoplane of tomato seedlings. It was observed that *Aspergillus niger, Rhizopus stolonifer, Mucor racemosus* and *Trichoderma viride* were present in all the weeks in the rhizosphere while the only isolate found to be present throughout the isolation periods in the rhizoplane was *Aspergillus niger*. *Penicillium citrinum* and *Aspergillus flavus* were present only on 2nd and 6th week respectively. The presence of *Penicillium citrinum* only on the 2nd week in the rhizosphere suggest inability of the fungus to survive competition for nutrient and space. This

Table 1. The physicochemical characteristics of the soil prior to planting and the rhizosphere soil samples at two weeks interval

Soil sample characteristics	0	2	4	6	8	10	12
	Two weeks interval						
pH	6.4	6.8	6.8	6.8	6.9	6.9	7.2
WRC ml/g	11.47	27.9	30.12	29.79	30.97	24.12	22.09
Moisture (%)	5.03	21.07	15.34	22.73	22.39	17.92	22.10
Organic matter content	2.35	3.68	3.01	3.03	2.50	3.62	2.34
Coarse sand (%)	49	54	51	50	47	39	52
Clay (%)	11	12	14	14	14	14	14
Silt (%)	19	13	11	11	11	11	11
Fine sand (%)	21	21	24	24	28	28	28
Soil texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam

Table 2. Isolates present in the rhizosphere and rhizoplane of okra plants

Fungal Strains	Rhizosphere (Age of plant in weeks)						Rhizoplane (Age of plant in weeks)					
	2	4	6	8	10	12	2	4	6	8	10	12
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus fumigatus	-	+	+	-	-	-	-	-	+	-	-	-
Aspergillus flavus	-	-	+	-	-	-	-	-	+	-	-	-
Aspergillus bertholletius	-	-	-	+	-	+	-	-	-	-	-	-
Fusarium oxysporum	+	+	+	+	+	-	+	+	-	-	+	-
Galactomyces candidum	+	+	+	-	+	+	+	-	-	-	-	-
Helminthosporium solani	-	-	+	-	+	+	-	-	+	-	+	+
Rhizopus stolonifer	+	+	+	+	+	+	+	-	+	+	+	+
Mucor racemosus	+	+	+	+	+	+	+	+	+	+	-	-
Penicillium citrinum	+	-	-	-	-	-	-	-	-	-	-	-
Trichoderma viride	+	+	+	+	+	+	-	+	+	+	+	+

Key = + Present; - Not present

result is slightly different from the report of Olanrewaju et al. [19] whose *Penicillium* spp isolation was from the rhizoplane on the 1st week and thereafter was absent. The appearance of *Aspergillus flavus* only on the 6th week on both the rhizosphere and rhizoplane may be attributed to the release of certain exudates by the root which supported the growth at that particular age of the plant but did not occur in other weeks. Huang et al. [18] and Neumann et al. [24] showed that a particular component of root exudates can trigger the growth of an organism. Huang et al. [18] explained further that plant roots release a broad variety of chemical compounds to attract and select microorganisms in the rhizosphere. A study by Neumann et al. [24] with lettuce showed that there was quantitative difference in the components of root exudates, particularly sugars and amino acids which may imply preference in the microbial association. *Aspergillus flavus, A. fumigatus* and *Galactomyces candidum* occurred once during the 6th and 2nd week respectively while others fluctuate at different intervals (Table 2).

The rhizosphere soil had a greater number of fungi in this study and this is attributed to the concentration of nutrients around the root region. Fungi requires nutrients for their growth and proliferation therefore, are host selective. This was also observed by earlier researchers [25, 11,7,26,27]. *A. niger* had frequency of occurrence of 58.67% on the 6th week and 65.79% on the 4th week which was significantly higher than every other isolate in both the rhizosphere and rhizoplane respectively. This could be as a result of high sporulation of the organism and production of antibiotics (Table 3). Dawar et al. [27] also obtained frequency of occurrence of 59.09% for *A. niger* in the rhizosphere of *Amaranthus viridis*. Some of the fungi species occurred once or twice and varies in their composition on the rhizosphere and rhizoplane as the plant aged. The variation in the species composition of the fungi isolated from the soil samples as the age of the plant increased is in line with the observations of Andreote et al. [28] who reported that species composition of microbes in the rhizosphere soils of plants fluctuates qualitatively and quantitatively with the growth stages of the plants. *Aspergillus fumigatus* was only present on the 4th and 6th weeks on the rhizosphere and was only present on the 6th week on the rhizoplane. *A. fumigatus* is mostly air-borne pathogen which could account for its low presence in the rhizosphere and rhizoplane. It was also observed that most of the isolates had the same frequency of occurrence on the 6th week especially in the rhizoplane. This suggests reduced or depleted organic nutrient at this particular age of the plant and under nutrient deficiency conditions, plants may preferentially select root microbiota to assist host nutrient uptake [29]. Oyeyiola [11] also reported decline in the frequency of occurrence of the rhizosphere and rhizoplane fungi on the 6th week while Eze and Amadi [7] reported decline on 10th week. *Galactomyces candidum* occurred in all the weeks in the rhizosphere except on the 10th week but was only present on the 2nd week in the rhizoplane. This result suggests inability of these fungi to withstand competition for nutrient and space. It has earlier been reported that fungi in the root region compete for nutrient and space which may also lead to microbial antagonism [30]. Most of these fungi strains have been reported to be pathogenic (*Aspergillus*,

Galactomyces, and *Helminthosporium* spp), saprophytic (*Penicillium*, *Mucor, Rhizopus* and *Fusarium* species) and antagonistic (*Trichoderma* species) but it depends on how and where the fungi occur [31,32,33]. Saprophytic fungi help in the decomposition of dead organic matter which is very important in soil mineralization processes. *Aspergillus niger* is the most common species implicated to be pathogenic on fresh fruits and vegetables and mostly found in decaying vegetation and has also been reported to be a source of bio-active compounds in the soil [34]. *Aspergillus, Penicillium* and *Fusarium* species have been shown to play a worthy role in increasing the bioavailability and utilization of residual Phosphorous in the rhizosphere [2,3]. This could be another approach to reduce the high cost of manufacturing Phosphate fertilizer in industry and also reduces environment pollution posed by chemical use [35]. Yehuda et al. [36] has reported *Rhizopus* spp to produce rhizoferrin which was found to be an efficient carrier of iron to plants with an efficiency that was comparable to that of synthetic chelates. *Galactomyces* and *Helminthosporium* spp are pathogenic and also saprophytic which suggest their co-existence in the rhizosphere and rhizoplane. *Trichoderma* species has long been recognized as biocontrol agents for the control of plant diseases and for their ability to enhance root growth and development, and crop productivity Kumar et al. [33]. *Trichoderma viride* isolated from the rhizosphere soils of Tea gardens of Assam, north eastern state of India showed substantial antifungal activity against five standard phytopathogenic fungi in vitro [Naglot et al. [37].

The colony forming unit (cfu/g) of *Aspergillus niger* was significantly different from all other isolates at P≤0.001 followed by *Mucor racemosus* and *Fusarium oxysporum* with significant differences at P≤0.05 and P≤0.01 respectively as shown in Table 4. The significant increase in the cfu of these aforementioned fungi may be due to their ability to establish faster interaction with the root of the plant than the other isolates. This is in line with the report of Abdel-Hafez et al. [9] in their research with lentil and sesame plants. Vasanthakumari and Shivanna [8] also obtained significant differences of P=0.01 and P=0.001 of rhizosphere and rhizoplane fungi of grasses of subfamily Panicoideae.

Table 3. Percentage frequencies of occurrence of the isolates from the Rhizosphere and Rhizoplane sample

Fungal strains	Age of plant (in two weeks interval)						
rhizosphere soil sample	2	4	6	8	10	12	Mean
Aspergillus niger	26.17	34.19	58.67	23.68	51.56	45.90	40.03a
Aspergillus fumigatus	-	18.80	14.67	-	-	-	16.74d
Aspergillus flavus	-	-	4.00	-	-	-	4.00e
Aspergillus bertholletius	-	-	-	3.95	-	6.56	5.26e
Fusarium oxysporum	14.02	29.91	34.67	28.95	12.50	-	24.01c
Galactomyces candidum	15.89	0.85	5.33	-	1.56	3.28	5.39e
Helminthosporium solani	-	-	4.00	-	4.69	9.84	6.18e
Rhizopus stolonifer	2.80	5.98	4.00	10.53	10.94	14.75	8.17e
Mucor racemosus	7.48	8.55	13.33	10.53	4.69	8.20	8.80e
Penicillium citrinum	30.84	-	-	-	-	-	30.84b
Trichoderma viride	2.80	1.71	5.33	22.37	14.06	11.48	9.63e
Rhizoplane sample							
Aspergillus niger	10.00	65.79	31.82	20.00	13.04	29.17	28.30a
Aspergillus fumigatus	-	-	2.27	-	-	-	2.27d
Aspergillus flavus	-	-	2.27	-	-	-	2.27d
Fusarium oxysporum	20.00	5.26	-	-	30.43	-	18.56b
Galactomyces candidum	10.00	-	-	-	-	-	10.00c
Helminthosporium solani	-	-	25.00	-	8.70	20.83	18.17a
Rhizopus stolonifer	20.00	-	25.00	20.00	21.74	33.33	24.01a
Mucor racemosus	40.00	10.53	6.82	40.00	-	-	24.34a
Trichoderma viride	-	18.42	6.82	15.00	26.09	16.67	16.40b

Means followed by the same alphabet(s) are not significantly different at P≤0.05 based on Duncan's Multiple Range Test (DMRT)

Table 4. Colony forming units of the isolates from the rhizosphere and rhizoplane of okra plant

Fungal strains	Rhizosphere	Rhizoplane	T-test value
Aspergillus niger	31833.33	9166.67	4.29***
Aspergillus fumigatus	5500.00	166.67	1.42
Aspergillus flavus	500.00	166.67	0.63
Aspergillus bertholletius	1166.67	0.00	1.56
Fusarium oxysporum	17666.67	1833.33	3.00**
Galactomyces candidum	1500.00	1666.67	-0.09
Helminthosporium solani	2000.00	3000.00	-0.49
Rhizopus stolonifera	5716.67	5000.00	0.34
Mucor racemosus	7333.33	3166.67	2.49*
Penicillium citrinum	5500.00	0.00	1.00
Trichoderma viride	7000.00	3833.33	1.28

= Significance at P≤0.05; **= Significance at P≤0.01; *= Significance at P≤0.001*

4. CONCLUSION

The rhizosphere and rhizoplane of okra plant has been shown to be rich in fungal diversity. The rhizosphere had a greater number of fungi which could be attributed to the release of exudates by the roots and concentration of nutrients on the root. The isolates have been reported by earlier researchers to be pathogenic on post-harvest crops but their interaction in the rhizosphere and rhizoplane of okra plant was beneficial since the plant grew buoyantly without any sign of defect. The data obtained from this work can be exploited by microbial ecologist to ascertain ecological associations and biomass increased by the fungal communities which also forms part of ecosystem. Also, the rhizosphere and rhizoplane fungi, with the host plant indicated that the functions of microbial communities should be integrated into soil fertility for sustainable food production. Some of the fungi like *Aspergillus niger* and *Trichoderma viride* could be harnessed for the production of extracellular enzymes and for biological control respectively. It would be necessary to carry out further research on the role, the specific content and pattern of root exudation during plant development which may have played pivotal role in the varying degrees in the frequency of occurrences of the fungi in the rhizosphere and rhizoplane of okra plant.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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