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Fungal Rhizosphere Effects of Cassava (*Manihot esculenta*), Pawpaw (*Carica papaya* L.), Fluted Pumpkin (*Telfairia occidentalis*) and Scent Leaf (*Ocimum gratissimum*) Plants from Soils at Rumuola, Rivers State, Nigeria

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ABSTRACT: The presence of rhizosphere microorganisms are known to promote plants growth, therefore the objective of this paper was to assess the fungal rhizosphere effects of Cassava (*Manihot esculenta*), pawpaw (*Carica papaya* L.), fluted pumpkin (*Telfairia occidentalis*) and scent leaf (*Ocimum gratissimum*) plants from soils obtained at Rumuola, Obio-Akpor Local Government Area, Rivers State, Nigeria using appropriate standard techniques.Results obtained show that the range of mean rhizosphere count, non-rhizosphere count and rhizosphere effect are as follows; 1.5×10^7cfu/g to 7.35×10^7cfu/g, 1×10^8cfu/g to 6.60×10^8cfu/g, 0.137 to 11.136 for *Manihot esculenta*; 1.09×10^6 cfu/g to 8.5×10^6 cfu/g, 1.65×10^5 cfu/g to 8×10^6 cfu/g, 0.34 to 6.606 for *Carica papaya* L.; 3.25×10^6 cfu/g to 9.9×10^6 cfu/g, 1.3×10^6 cfu/g to 5.3×10^6 cfu/g, 1.2 to 2.5 for *Telfaria occidentalis*; 3.6×10^6 cfu/g to 7.9×10^6 cfu/g, 4.6×10^6 cfu/g to 8.4×10^6 cfu/g, 0.66 to 1.71 for *Ocimum gratissimum*. The fungi isolated from the four plants rhizosphere are *Saccharomyces cerevisiae*, *Candida* spp., *Aspergillus niger*, *Mucor* spp., *Fusarium* spp., *Penicillium* spp. with *Saccharomyces cerevisiae* and *Candida* spp. occurring in all the rhizosphere. The high rhizospheric effects agree with the presence of these diverse fungi in the rhizosphere, capable of serving economic importance as regards improved healthy plant growth.

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Cassava (*Manihot esculenta)* plant is an herbaceous perennial woody shrub that can grow up to three metres in tropical regions (Akinpelu*et al*., 2011). It is a dicotyledonous plant of the family Euphorbiaceae and growing in area between 30º N and 30º N from the equator, hence distribution is all Africa, Asia, Central and South America (Feyisa, 2021). It has leaves which are fingerlike, deeply indented, palmate 3-7 lobed, attached to a slender stem by long petioles; the flowers are small, greenish-yellow occurring in panicles while the seeds which are formed in capsules explode to distribute the contents upon ripening. The mature cassava plant (12 months old) contains 6%, 44% stem and 50% tuber; the tuber is long, tapered with a firm homogenous flash encased in detachable rind, about 1mm thick, rough and brown on the outside (Aye and Oo, 2010).*Carica papaya* (pawpaw), an evergreen, tree-like herb, 2-10 m tall, typically unbranched,

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although branched sometimes due to injury, containing white latex in all parts. It has cylindrical stem about 10-30 cm in diameter, hollow with prominent leaf scars and spongy-fibrous tissue. The root system is extensive, spirally arranged leaves and clustered near apex of trunk. Its flowers are tiny, yellowish, shaped like funnel, solitary or clustered in the leaf axils, of 3 types i.e. female, male and hermaphrodite flowers (Orwa *et al*., 2009).

Telfairia occiidentalis Hook. F. (fluted pumpkin) of the family Curcubitaceae which origin is probably the south eastern Nigeria, hence widely distributed among the Igbo tribe, principally around Imo State (Akoroda, 1990), where it has widespread varieties (variants in pod and seed colour, seed and plant vigour, leaf size and their succulence, anthocyanin content of leaves and petioles or shoots, monoecious or dioecious plants) (Chewya and Eyzaguirre, 1999). The leaves of fluted pumpkin are spirally arranged with length of 3-5.5 cm; its fruit is drooping, ellipsoid berry 20 - 50 cm by 40 - 95 cm weighing 10 kg with a compressed ovoid seed, 4.5 cm long either black or brownish red (Grubben and Denton, 2004).

Ocimum gratissimum (scent leaf), an herbaceous plant belonging to the family Labiatae and the genus *Ocimum* (Nadkarni, 1999), is indigenous to tropical areas, especially in West Africa and India. *Ocimum gratissimum*, known in different part of the world by various names, and particularly in Nigeria, the Yoruba called it “effinrin-nla”, Igbo tribe, “Ahuji” and the Hausas, “Daidoya” (Effaraim *et al*., 2013). An aromatic, perennial herb, 1-3 m tall, *Ocimum gratissimum* has an erect stem, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips (Bhavani *et al*., 2019). According to Lawrence (2017), the leaves of the plant measures up to 10 x 5 cm, ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at its base with a coarsely crenate, serrate margin, pubscent and with dots on both the sides.

Soil is an active living matrix and an important component of the terrestrial ecosystem; in reference to the growth of plant, it is divided into two types, namely rhizosphere soil and non-rhizosphere soil (Olahan *et al*., 2016). Rhizosphere can be defined as the region of soil surrounding plant roots (Ambreen *et al*., 2012). This definition encompasses not only the roots and region of nutrient uptake by the roots, but extends into soils by action of root products and the trophic interactions that are affected by these products or by roots (Mahamuni *et al*., 2012). The rhizosphere has associates with both pathogenic and symbiotic fungi (Sule and Oyeyiola, 2012). They average between 10^5 and 10^6 organisms per gramme of rhizosphere soil with species of *Saccharomyces*, *Verticillium*, *Rhizopus*, *Acremonium*, Monilia, *Geotrichum*, *Mucor*, *Penicillium*, *Gliocladium*, *Curvularia*, *Aspergillus*, *Trichoderma*, *Rhodotorula*, *Cladosporium*, *Cephalosporium*, *Pythium*, *Botryis*, *Chaetomium*, etc. as the common and important fungi (Sule and Oyeyiola, 2012; Olahan *et al*., 2016). The non-rhizosphere soil, also called the bulk soil is the soil free of plant roots and are not part of any rhizosphere soil (Olahan *et al*., 2016).

The microorganisms present in the rhizosphere play important roles by ensuring the ecological fitness of their plant host. This includes plant protection/growth promotion such as altering plant hormones, secretion of organic compounds, increasing abiotic stress tolerance in plants, rhizoremediation and enhancing nutrient availability and uptake and disease control, profound effects on seed germination and seed vigour (Raaijmakers, 2009; Mendes *et al*., 2013; David, 2014; Olahan *et al*., 2016), as well as the production of antibiotics, geochemical cycling of minerals and plant colonisation (Peer *et al*., 2012). Also, exudates from roots such as sugars, organic acid anions or amino acids in large proportion are easily degradable rhizosphere microbes which results in high microbial density and activity in the rhizosphere (Olahan *et al*., 2017). The objective of this paper is to assess the fungal rhizosphere effects of Cassava (*Manihot esculenta*), pawpaw (*Carica papaya* L.), fluted pumpkin (*Telfairia occidentalis*) and scent leaf (*Ocimum gratissimum*) plants from soils obtained at Rumuola, Obio-Akpor Local Government Area, Rivers State, Nigeria

MATERIALS AND METHODS

The study was carried out in Captain Elechi Amadi Polytechnic, Rumuola, Obio-AkporLocal Government Area, Rivers State, Nigeria.

Five rhizosphere soil samples each of cassava (*Manihot esculenta*), pawpaw (*Carica papaya* L.), fluted pumpkin (*Telfairia occidentalis*) and scent leaf (*Ocimum gratissimum*) were collected by gently uprooting the plants using sterile shovel. The plants were shaken to remove unwanted soil particles. The soil particles adhered to the roots was transferred to already labelled sterile polyethylene bags for each sample. Also, five soil samples adjacent few centimeters (5cm) away from each cassava, pawpaw, fluted pumpkin and scent leaf plant root was also collected, transferred to already labelled sterile polyethylene bags for each plant and was considered as non-rhizosphere soil, serving as controls. The samples were carried aseptically to the

Biology/Microbiology Laboratory 1, Science Laboratory Technology Department Polytechnic, Rumuolaand processed within 1 hour of collection.

One gramme (1g) from the each soil sample (rhizosphere and non-rhizosphere) was weighed into a test tube containing 9ml of sterile distilled water (10^{-1}), from which a ten-fold serial dilution was carried out by pipetting 1ml into several test tubes containing 9ml of sterile distilled water up to 10^{-5}. From 10^{-5} dilution, an aliquot of 0.1ml was plated onto a freshly prepared Potato Dextrose Agar plates. The plates were inoculated in duplicates employing spread plate method using sterile gas rod. The inoculated plates were incubated at 37°C for 3 -5 days. Discrete colonies that developed (30-300) were counted and recorded as fungi and estimated using equation 1:

$$cfu/g = \frac{\text{Average number of colonies x dilution factor}}{\text{Volume plated}} \quad (1)$$

The quantitative rhizosphere effect of the plants was calculated using the equation 2 according to Subabarao (2000):

$$\mathrm{R/S} = \frac{No.MCR}{\mathrm{No.\,MCN - R\ soil}} \quad (2)$$

Where No. MCR = number of microorganisms per gramme of rizosphere soil; No. MCN-R soil = number of microorganisms per gramme of non- rizosphere soil

Fungi isolates obtained from pure cultures (after subculturing) were identified on the basics of their cultural, morphological and physiological characteristics; this include colour, texture and colonial characteristics on the medium. The isolates were also subjected microscopic examination using wet mount method described by Ogbulie *et al*. (2001) and key to identification of specific species was treated according to Samson *et al*. (1981).

RESULTS AND DISCUSSION

Fungal counts obtained from *Manihot esculenta* and *Carica papaya* L. rhizosphere soil, non-rhizosphere soil and rhizosphere effects are presented in Table 1.The fungal counts of *Manihot esculenta* (cassava) rhizosphere are in the following order: 7.35x10^7 cfu/g (sample D) > 5.05x10^7 cfu/g (Sample A) > 3.8x10^7cfu/g (sample C) > 1,7x10^7 cfu/g (Sample B) > 1.5x10^7 cfu/g (Sample E); the non-rhizosphere fungi counts are in the following order: 6.60x10^8 cfu/g (Sample D) is > 1.85x10^7 cfu/g (Sample A) is > 1.5 x 10^7 cfu/g (Sample B) is > 1.08x10^8 cfu/g (Sample E) is > 1x10^8 cfu/g (Sample C) while the rhizosphere effects values are in the following order: 11.136 (Sample D) > 2.279 (Sample A) > 1.133 (Sample B) > 0.38 (Sample C) > 0.137 (Sample E). The *Carica papaya* L. (pawpaw) rhizosphere fungal count is in the following order: 8.5 x 10^6 cfu/g (Sample A) > 1.7 x 10^6 cfu/g (Sample C) > 1.2 x 10^6 cfu/g (Sample B) > 1.12 x 10^6 cfu/g (Sample D) > 1.09 x 10^6 cfu/g (Sample E); the non-rhizosphere fungal count is in the following order: 8 x 10^6 cfu/g (Sample A) > 5 x 10^6 cfu/g (Sample C) > 2.2 x 10^6 cfu/g (Sample D) > 1.5 x 10^6 cfu/g (Sample B) > 1.65 x 10^5 cfu/g (Sample E) while the rhizosphere effects are in the following order: 6.606 (Sample E) > 5.09 (Sample D) > 1.0625 (Sample A) > 0.6 (Sample B) > 0.34 (Sample C)

Table 1:Fungal counts obtained from *Manihot esculenta* (Cassava) and *Carica papaya* L. (Pawpaw) Rhizosphere Soil, Non-Rhizosphere Soil and Rhizosphere Effects

	Fungal Counts (cfu/g)					
	Rhizosphere		**Non- Rhizosphere**		**Rhizosphere Effects**	
	Cassava	**Pawpaw**	**Cassava**	**Pawpaw**	**Cassava**	**Pawpaw**
A	5.05 x10^7	8.5 x 10^6	1.85 x 10^7	8 x 10^6	2.279	1.0625
B	1.7 x10^7	1.2 x 10^6	1.5 x10^7	1.5x 10^6	1.133	0.6
C	3.8 x 10^7	1.7 x 10^6	1 x 10^8	5 x 10^6	0.38	0.34
D	7.35 x 10^7	1.12 x 10^6	6.60x10^7	2.2 x 10^6	11.136	5.09
E	1.5 x 10^7	1.09 x 10^6	1.08 x 10^8	1.65 x 10^5	0.137	6.606

Key Cfu/g =Colony forming unit per gramme Rhizosphere effect

The fungal counts obtained from *Telfariaoccidentalis* and *Ocimumgratissimum* rhizosphere soil, non-rhizosphere soil and rhizosphere effect are presented in Table 2.The *Telfariaoccidentalis* rhizosphere fungal counts are in the following order: 9.9 x 10^6 cfu/g (Sample E) > 5.85 x10^6 cfu/g (Sample B) > 4.5 x 10^6 cfu/g (Sample D) > 3.55 x 10^6 cfu/g (Sample C) >3.25 x10^6 cfu/g (Sample A). The non-rhizosphere fungal counts are in the following order: 5.3 x 10^6 cfu/g (Sample E) > 3.9 x10^6 cfu/g (Sample B) > 3.5x10^6 cfu/g (Sample D) > 1.9 x 10^6 cfu/g (Sample C) > 1.3 x 10^6 cfu/g (Sample A). The rhizosphere effect values are in the following order: 2.5(Sample A) > 1.8 (Sample C, E) > 1.5 (Sample B) > 1.2 (Sample D) The rhizosphere fungal counts from *Ocimumgratissimum* are in the following order: 7.9 x 10^6 cfu/g (Sample D) > 7.8 x 10^6 cfu/g (Sample C) > 5.6 x 10^6 cfu/g (Sample A) > 5.0 x 10^6 cfu/g (Sample E) > 3.6 x 10^6 cfu/g (Sample B). The non-rhizosphere fungal counts are in the following order: 8.4 x 10^6 cfu/g (Sample A) > 7.5

x 10^6 cfu/g (Sample D) > 4.85 x 10^6 cfu/g (Sample C) > 4.45 x 10^6 cfu/g (Sample B) > 4.6 x 10^6 cfu/g (Sample D). The rhizosphere effect values are in the following order: 1.71 (Sample D) > 1.60 (Sample C) > 0.80 (Sample B) > 0.67 (Sample D) > 0.66 (Sample A).

Table 2: Fungal counts obtained from *Telfariaoccidentalis*(Fluted Pumpkin) and *Ocimumgratissimum* (Scent Leaf) Rhizosphere Soil, Non-Rhizosphere Soil and Rhizosphere Effects

	Fungal Counts (cfu/g)					
	Rhizosphere		**Non- Rhizosphere**		**Rhizosphere Effects**	
	F. Pumpkin	**Scent Leaf**	**F. Pumpkin**	**Scent Leaf**	**F. Pumpkin**	**Scent Leaf**
A	$3.25 x10^6$	$5.6\ x\ 10^6$	$1.3 x 10^6$	$8.4 x 10^6$	2.5	0.66
B	$5.85 x10^6$	$3.6\ x\ 10^6$	$3.9 x10^6$	$4.45 x 10^6$	0.16	0.80
C	$3.55 x 10^6$	$7.8 x 10^6$	$1.9 x 10^6$	$4.85 x 10^6$	1.8	1.60
D	$4.5 x 10^6$	$7.9 x 10^6$	$3.5x10^6$	$4.6 x 10^6$	1.2	1.71
E	$9.9 x 10^6$	$5.0 x 10^6$	$5.3 x 10^6$	$7.5 x 10^6$	1.8	0.67

Key: Cfu/g =Colony forming unit per gramme Rhizosphere effect; F. Pumpkin =Fluted Pumpkin

The characterisation and identification of fungi isolated from the *Manihot esculenta* (cassava) rhizosphere are *Saccharomyces cerevisiae, Candida* spp. and *Aspergillus niger*; *Carica papaya* L. (pawpaw) are *Candida* spp., *Saccharomycescerevisiae, Mucor* spp., *Fusarium* spp. and *Penicillium* spp.; from *Telfariaoccidentalis* (fluted pumpkin) are *Saccharomycescerevisiae, Candida* spp., *Fusarium* spp., *Mucor* spp. and *Penicillium* spp while from *Ocimumgratissimum* (scent leaf), *Saccharomyces cerevisiae, Fusarium* spp., *Aspergillus niger* and *Candida* spp. were isolated.

The high fungal counts in the rhizosphere soil may be due to the adequate nutritional status of the soils which are available to the microorganisms; in addition, are the favourable physical chemical properties of the soils (Arotupin and Akinyosoye, 2006). Generally the difference in fungal population between the field from which rhizosphere and non rhizosphere soils were obtained in this study could have been due to variations in plants, age in fields, temperature and pH of the soil (Musyimi *et al*., 2018). Fungal activity increases with plant age and declines towards, maturity probably due to the plant secreting exudates in reduced quality and quantity that may contain antimicrobial metabolities (Makut and Owolewa, 2011). Also, the ability of fungi to utilize organic constituents from roots as source of energy may contribute to the disparity in population of the two regions (Gaddeya *et al.*, 2012).

The rhizosphere effect obtained in this study are generally high ranging from 0.16 to 11.136; the greater the rhizospheric effect the higher will be the microorganisms. According to Robert (1995) the rhizosphere effect greater than 1 indicate selective stimulation in the rhizosphere zone of root of plant. The varying types and quantities of rhizodeposits have been postulated to act as key factors influencing the density and diversity of the rhizospheric micro-organisms (Grayston and Campball, 1996).

The fungal species identified in this study are significant members of rhizosphere mycoflora of popular crops such as cassava, exhibiting great beneficial rhizosphere effects on them (Sule and Oyeyiola, 2012). Olahan *et al.* (2016) reported *Aspergillus niger* and *Saccharomyces cerersine* from the rhizosphere of cassava. Ahmed *et al*. (2022) had reported *Penicillium* and *Fusarium* in the rhizosphere soil of papaya. Tamarasi *et al*. (2008) had earlier isolated *Aspergillus* and *Fusarium* in the rhizosphere soil of *Ocimiumbasilicium* while Koricha *et al*. (2019) reported that *Candida* and *Saccharomyces* species are among the dominant yeast isolated from rhizosphere soil. Al-Abasi *et al*. (2021) reported that yeast could be significant in the rhizosphere of plant especially when the study reveals that *Saccharomyces cerevisiae* and *Candida* spp. occurred in all the rhizosphere soil collected. Different plant species host specific microbial communities (Alwathani *et al*., 2012) and that diversity and composition of the microbial taxa in the rhizosphere can be affected by plant species (Cappuccino and Shenma, 2008), pH and temperature might also have influenced the diversity in microorganisms (Musyimi *et al*., 2018).

Ahmed *et al*. (2022) reported that soil microflora despite causative of diseases in the crop field, plays an important role in decomposition and contribute to biogeochemical cycling. A broad range of microorganisms in the rhizosphere are capable of promoting plant growth, made possible by their ability to communicate with plants using complex, chemical signals; these chemicals signal compounds include auxins, gibberellins, glycolipids, and cytokinins which are beginning to be fully appreciated with regards to their biotechnological potentials (Obire and Abuda, 2010).

*Conclusion:*The study has confirmed that there is high population of fungi in the rhizosphere soil samples of cassava, pawpaw, fluted pumpkin and scent leaf plants as well as their ability to host numerous and diverse fungi. The presence of these microorganisms known to be involved in geochemical transformation can be developed to serve as bio-fertilisers as well as improve healthy plant growth.

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Data Availability Statement: Data are available upon request from the corresponding author.

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