

RHIZOSPHERE AND NON-RHIZOSPHERE SOIL MYCOFLORA OF CORCHORUS OLITORIUS (JUTE)

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ABSTRACT

The physicochemical and microbial analyses of the rhizosphere and non-rhizosphere soils of *Corchorus olitorius* (Jute) were conducted. The soil samples were analyzed before planting of Jute seeds and the average values of the parameters were 11.24% (percentage moisture content), 0.29ml/g (water holding capacity), 1.36% (organic matter content) and 6.80 (soil pH). The textural class of the soil sample is sandy. *Aspergillus niger* and *Rhizopus stolonifer* occurred most followed by *Aspergillus flavus*, while *Penicillium chrysogenum*, *Saccharomyces cerevisiae* and *Neurospora crassa* occurred least in the rhizosphere and non – rhizosphere soil samples of Jute in this study. Occurrence of these fungi in both the rhizosphere and non-rhizosphere soils varied widely throughout the duration of this study. It can be concluded that the jute plants benefitted from the microorganisms (fungi) in their rhizosphere and non – rhizosphere zones since the plants showed healthy growth.

Keywords: *Corchorus olitorius*, Fungi, Non–rhizosphere soil, Rhizosphere soil, physicochemical

INTRODUCTION

Soil, a dynamic living matrix, is an essential part of the terrestrial ecosystem. In relation to plant growth, soil can be distinguished into two types, namely rhizosphere soil and non-rhizosphere soil. Rhizosphere is the narrow region of soil immediately surrounding the plant roots (Marschner *et al.*, 2004). It is the region where the soil and plant roots make contact, thus, characterized by increased microbial activities. The rhizosphere can also be described as a mixture of solid particles and active community of microorganisms, mostly bacteria (Haghighi *et al.*, 2011). The non-rhizosphere soil, also called the bulk soil is the soil free of plant roots and which is not part of any rhizosphere soil.

Fungi belonging to the following genera are common in the rhizosphere soil: *Aspergillus*, *Cladosporium*, *Cephalosporium*, *Botrytis*, *Chaetomium*, *Fusarium*, *Mucor*, *Penicillium*, *Verticillium*, *Trichoderma*, *Rhizopus*, *Gliocladium*, *Monilia*, *Alternaria*, *Pythium*, etc. (www.agriinfo.in).

Corchorus belongs to the Order Malvales, Family Malvaceae, Subfamily Grewioideae, Genus *Corchorus* and Species *olitorius*. The plant is an erect annual herb, height between 2 - 4m, usually strongly branched; stems reddish, fibrous and tough. Leaves are alternate, simple; stipules narrowly triangular with long point; petiole 0.5 – 7cm long; blade narrowly ovate, ovate or elliptical, almost glabrous, usually shiny dark green, 3–7 veined from the base (Velempini *et al.*, 2003).

It is propagated from seeds; well-dried seed keeps a high germination capacity for several years (Velempini *et al.*, 2003). Fresh and sometimes old seed shows dormancy caused by impermeability of the seed coat. To suppress the seed dormancy, it is recommended that the seed tied in a piece of cotton cloth be immersed for 5 seconds in almost-boiling water of about 85°C before sowing (Velempini, *et al.*, 2003). Another method is scarification with sandpaper.

The leaves are used fresh or dried. They can be stored after drying and used later on during periods of scarcity. It is widely consumed as a vegetable among rural communities in most part of Africa (Velempini *et al.*, 2003). Herbal tea is made from the dried leaves which are rich in beta-carotene, iron, calcium and vitamin C. The plant has an antioxidant activity with a significant α -tocopherol equivalent to vitamin E (Oyedele *et al.*, 2006). The leaves are used to produce soup by Yorubas as well as boiled and mixed with “Kuli-kuli” (groundnut cake) to form a dish known as “Kwado” in Hausa (Nath and Dantom, 1980).

Most of the previous studies on non- rhizosphere and rhizosphere soil bacteria and fungi have focused on other crops such as tobacco, okra and amaranthus (Oyeyiola, 2009; Sule and Oyeyiola, 2012); Olahan *et al.* 2015). Rhizosphere and non-rhizosphere mycoflora are known to protect their host plants from pathogenic organisms as well as promote growth and better yield of crop plants. This study will provide baseline of the fungi associated with the rhizosphere and non-rhizosphere soils of Jute (*Corchorus olitorius*). The objectives of this study are to isolate and identify fungi species in the rhizosphere and non – rhizosphere soils of *Corchorus olitorius* and to determine the physicochemical characteristics of the soil prior to planting.

MATERIALS AND METHODS

Description of the study site

The study site was a plot of land measuring 50 by 100ft. in the Biological garden situated on the main campus of University of Ilorin, Ilorin, Kwara State, Nigeria.

Procurement of jute seeds

The jute seeds used were purchased from an agricultural shop in Ilorin and stored in a sterile polythene bag prior to use. They were taken to the Herbarium section of the Department of Plant Biology, University of Ilorin for confirmation of their identity.

Determination of some physicochemical parameters of the soil samples

Organic matter content, texture and water holding capacity of soil samples from the study site were determined prior to planting using the methods of Pramer and Schmidt (1964), while the soil moisture content as well as pH were determined using the methods of Sule and Oyeyiola (2012) and Henry and Boyd (1998) respectively.

Planting of the jute seeds

The jute seeds were broadcasted on the seedbeds at the study site. The seedbeds were watered manually twice daily (morning and evening hours). The seeds which depended on the naturally - occurring organic matter of the soil in the seedbeds started germinating after one week. The study lasted for 8 weeks.

Collection of rhizosphere and non- rhizosphere soil samples

The soil samples were collected with the use of sterile hand trowel into two previously labeled sterile black polythene bags. The rhizosphere soil samples were collected by manually uprooting some *Corchorus olitorius* plants and shaking-off the adhering soil into a sterile polythene bags (Sule and Oyeyiola, 2012) while the non-rhizosphere soil samples were collected by taking soil about 5 meters away (horizontally) from the root of the plant. The soil samples were taken to the Laboratory for further analyses on weekly basis for 8 weeks consecutively.

Determination of population and occurrence of the fungal isolates

This was done by transferring 1.0 g of the non – rhizosphere soil into 9.0 ml of sterile distilled water and shaking the mixture to form 10^{-1} dilution. The dilution was done up to 10^{-2} . Then 0.1 ml of aliquot was plated in duplicate into set plates of Potato Dextrose Agar (PDA) and the inoculum spread by means of sterile L – shaped glass spreader. The plates were incubated at room temperature for 72 hours and the number of fungal colonies counted. The above procedures were repeated in order to determine the fungal population of the rhizosphere soil (Dubey and Maheshwari, 2005). The occurrence of each fungal species was noted visually and recorded.

Isolation, characterization and identification of the fungi isolates

Fungi were isolated from the rhizosphere soil and non-rhizosphere soils and then sub-cultured in sterile PDA plate until pure culture was obtained. The pure culture was stocked in sterile PDA slant (Fawole and Oso, 2007). The fungal isolates were characterized based on their macroscopic and microscopic characteristics (Fawole and Oso, 2007). They were then identified using standard Mycology textbooks (Onions *et al.*, 1981; Samson and Van Reenen – Hoekstra, 1988).

Determination of rhizosphere effect (R : S ratio)

Rhizosphere effect is an indication of the degree of stimulation of microorganisms (fungi) in the root region of a plant and is determined by dividing the population of fungi in cfu/g in the rhizosphere soil by that obtained in the non – rhizosphere soil (Dubey and Maheshwari, 2005).

$$R : S \text{ ratio} = \frac{\text{Fungal population in the rhizosphere zone}}{\text{Fungal population in the non-rhizosphere zone}}$$

RESULTS

Physicochemical analyses of the soil samples

Results of the physicochemical analyses of the soil samples prior to planting indicated that the soil is basically sandy with slightly acidic character (Table 1).

Table 1: Physicochemical characteristics of the experimental soil prior to sowing of jute seeds

Soil Characteristics	Values
Soil pH	6.80
Silt (%)	4.8
Clay (%)	3.8
Sand (%)	91.4
Textural Class	Sandy
Moisture Content (%)	11.24
Water Holding Capacity (ml/g)	0.29
Organic Matters (%)	36

Fungal population in the rhizosphere and non-rhizosphere soils of jute on plants age

Fungal population in the rhizosphere and non-rhizosphere soils of jute were 1.3×10^3 cfu/g and 5×10^2 cfu/g and respectively at the end of the first week after planting (Figure 1). At the end of 2nd week after planting, the fungal population increased in both soil samples 3.0×10^3 cfu/g for rhizosphere soil and 2.0×10^3 cfu/g for non-rhizosphere soil (Figure 1). At the end of the 8th week after planting, the fungal population in the rhizosphere soil was 2.0×10^3 cfu/g while that of the non-rhizosphere soil was 1.9×10^3 cfu/g (Figure 1). The peak of the fungal population was noticed in the 5th week while the lowest fungal population was noticed in the 1st week in the rhizosphere and non-rhizosphere soils.

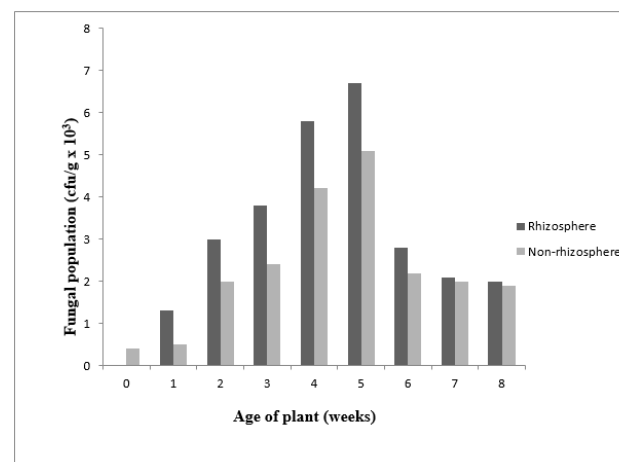


Figure 1: Fungi population in the non- rhizosphere and rhizosphere soils of Jute

Rhizosphere effect (R : S ratio) of fungi in the soils

The rhizosphere effect of fungi in the rhizosphere of jute plant ranged from 1.05 to 2.60 (Figure 2). The highest and lowest rhizosphere effect were obtained in the first and last week respectively (Figure2).

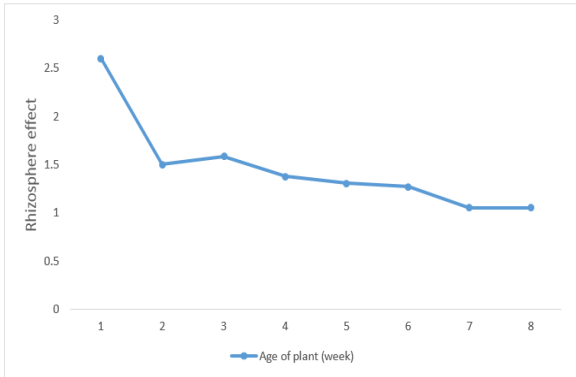


Figure 2: Rhizosphere effect of fungi on roots of Jute

Distribution of fungal isolates in the soil samples

Eight (8) fungal isolates were obtained from the rhizosphere and non-rhizosphere soil samples (Table 2). *Rhizopus stolonifer* and *Aspergillus niger* were present throughout the eight weeks in both non-rhizosphere and rhizosphere soils. *Penicillium chrysogenum*, *Saccharomyces cerevisiae*, and *Neurospora crassa* were present for the 1st, 2nd and 3rd weeks, but were absent for the rest of the weeks. On the 3rd, 7th, and 8th weeks *Aspergillus flavus* was present, but absent in the rest of the weeks. These organisms were absent in the 1st, 2nd and 3rd weeks, but present for the rest of the weeks for the non-rhizosphere soil. *Trichothecium roseum* was absent in the 1st, 2nd, 3rd, and 4th weeks, present on the 5th, 6th, 7th and 8th weeks, but were absent for these weeks in the non-rhizosphere. For the 1st, 2nd, 3rd, and 4th weeks *Alternaria alternata* was present but absent for the 5th, 6th, 7th and 8th weeks but the reverse was the case for the non-rhizosphere soil (Table 2).

Table 2: The distribution of the fungal isolates in the rhizosphere and non-rhizosphere soils of jute

Fungal isolates		Sampling period (Weeks)							
		1	2	3	4	5	6	7	8
<i>Rhizopus stolonifer</i>	(N)	+	+	+	+	+	+	+	+
	(R)	+	+	+	+	+	+	+	+
<i>Aspergillus flavus</i>	(N)	+	+	+	+	+	+	+	+
	(R)	+	+	+	+	+	+	+	+
<i>Penicillium chrysogenum</i>	(N)	+	+	+	+	+	+	+	+
	(R)	+	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	(N)	+	+	+	+	+	+	+	+
	(R)	+	+	+	+	+	+	+	+
<i>Trichothecium roseum</i>	(N)	-	-	-	-	+	+	+	+
	(R)	-	-	-	-	+	+	+	+
<i>Saccharomyces cerevisiae</i>	(N)	+	+	+	+	+	+	+	+
	(R)	+	+	+	+	+	+	+	+
<i>Alternaria alternata</i>	(N)	+	+	+	+	-	-	-	-
	(R)	+	+	+	+	-	-	-	-
<i>Neurospora crassa</i>	(N)	+	+	+	+	-	-	-	-
	(R)	+	+	+	+	-	-	-	-

+ = Isolated; - = Not isolated; N = Non – rhizosphere soil; R = Rhizosphere soil.

DISCUSSION

The physicochemical characteristics of the soil of the study area prior to planting of Jute seeds were in line with the optimum requirements of *Corchorus olitorius*. Amount of moisture in the soil depends on the amount of precipitation, time interval between rainfall and soil sample collection, soil type, season of the year, soil temperature, vegetation cover and organic content (Baker, 2006). The rhizosphere and non - rhizosphere soils studied were slightly acidic (pH 6.80 for the rhizosphere soil and pH 6.92 for the non – rhizosphere soil). These acidity levels favour microbial colonization.

The fungi isolated from the rhizosphere and non-rhizosphere soils in this study namely *Aspergillus flavus*, *Aspergillus niger*,

Alternaria alternata and *Saccharomyces cerevisiae*, *Rhizopus stolonifer*, *Penicillium chrysogenum*, *Trichothecium roseum* and *Neurospora crassa* corroborated the findings of a number of researchers such as Arotupin and Akinyosoye (2006), Oyeyiola (2009), Sule and Oyeyiola (2012) as well as Olahan *et al.* (2015). Arotupin and Akinyosoye (2006) worked on the microbiological and physicochemical characteristics of cassava cultivated soils and isolated *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. repens*, *Botrytis cinerea*, *Neuspora sitophila*, *Varicosporium elodea* from the soil samples. Oyeyiola (2009) worked on the rhizosphere mycoflora of Okro (*Hibiscus esculentus*) and isolated the following fungi species: *Rhizopus stolonifer*, *R. oligosporus*, *R. oryzae*, *Aspergillus niger*, *A. fumigatus*, *A. japonicas*, *A. clavatus*, *Mucor hiemalis*, *M. racemosus*, *Alternaria herbarum* and *A. triticina*.

Sule and Oyeyiola (2012) isolated fungi in the following genera: *Aspergillus*, *Saccharomyces*, *Doratomyces*, *Rhizopus*, *Penicillium*, *Ustilago*, *Trichoderma*, *Humicola*, *Cladosporium*, *Papulospora*, *Mucor*, *Moniliella*, *Monascus*, *Neurospora*, *Brettanomyces*, *Botrytis*, *Byssoschamys*, *Geotrichum*, *Gliocladium*, *Acremonium*, *Piricularia*, *Papulospora*, *Oidiodendron*, *Rhodotorula* and *Trichophyton* from the rhizosphere soil and rhizoplane of cassava cultivar TMS 30572. Olahan *et al.* (2015) isolated *Aspergillus niger*, *A. terreus*, *A. oryzae* and *Schizosaccharomyces pombe* from the rhizosphere soil of tobacco (*Nicotiana tabacum*). In this same study, *Aspergillus oryzae* was absent from the rhizoplane soil of the same plant while the other fungi were present.

The variation in the species composition of the microbes isolated from the soil samples as the age of the jute plant increased is also in line with the observations of Andreote (2010) who reported that species composition of microbes in the rhizosphere and non-rhizosphere soils of plants fluctuates qualitatively and quantitatively with the growth stage of the plants. Also, the higher number and occurrence of more microflora in the rhizosphere soil compared to those of the non-rhizosphere soil of Jute in this study agreed with the submission of Bopaiah and Shetty (1991) who reported that the rhizosphere soils of Pepper, Cacao, Pineapple and Coconut had greater numbers of microflora than the non – rhizosphere soils.

The values of the rhizosphere effect (R : S ratio) of fungi on roots of Jute in this study were greater than 1, indicating selective stimulation in the rhizosphere zone of roots of the Jute plant (Robert, 1995).

Conclusion

The results from this study showed that the rhizosphere soil is colonized by different species, population and diversity of fungi compared to the non – rhizosphere soil. Hence, the Jute plant would benefit from the rhizosphere effect. The significance of this study is that it provides a baseline of fungi that could be found in the rhizosphere and non-rhizosphere soils of jute

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