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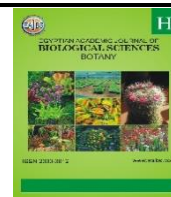
# EGYPTIAN ACADEMIC JOURNAL OF BIOLOGICAL SCIENCES BOTANY



ISSN 2090-3812

[www.eajbs.com](http://www.eajbs.com)

Vol. 16 No.1(2025)



## Concentration of Mycorrhizal at Different Soil Depth of *Pinus caribaea* Var. Hondurensis Plantation

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### ARTICLE INFO

Article History

Received:15/12/2024

Accepted:14/2/2025

Available:18/2/2025

### Keywords:

Aerobic microbial, Yield and mold, Mycelium, Hyphae, Fungi.

### ABSTRACT

Mycorrhizae being a symbiotic association between fungi and plants are more endemic in pine's plantation rhizosphere, but there is dearth of information on the level of endemism. The study therefore investigated availability of mycorrhizal at different levels of rhizosphere of *Pinus caribaea* plantation with a view to ascertaining the concentration of mycorrhizal at different soil depth.

Systematic cluster sampling technique was used for the laying of sample plots in an hectare of 25 years old plantation of *Pinus caribaea*. At the centre of the plantation, a 50 x 50 m main plot was laid and was further divided into temporary sample plots of 10 x 10 m. Five of such temporary plots were located at four corners and centre of the main plot. From each temporary sample plots, five (5) samples were randomly collected from different rhizosphere depths of 0 – 10 cm, >1 0 – 20 cm and > 20 – 30 cm for laboratory analysis of mycorrhizal fungi following standard method. Analysis of variance (ANOVA) was used to test for differences in microbial and fungi concentrations at different depths using Completely Randomized Design as experimental design. Treatments were three (3) rhizosphere depth (0 – 10 cm, >1 0 – 20 cm and > 20 – 30 cm.) with five (5) replicates. There were no significant differences ( $P > 0.05$ ) among treatments in Total Aerobic Microbial Count (TAMC) and Total yield and Mold Count (TYMC) of mycorrhizal in the soil samples. More mycorrhizal of TAMC that are too numerous to count were found at soil depth 0 – 10 cm and >1 0 – 20 cm while the highest TYMC was recorded for soil depth > 20 – 30 cm. At the rhizosphere of 25 years old plantation of *Pinus caribaea* in the FRIN arboretum, mycorrhizal mycelium were readily present at soil depth 0-30cm with more concentration at depth 0- 20cm.

### INTRODUCTION

Mycorrhizal fungi are important components of the soil microbial community and contribute to the maintenance of ecosystem productivity and diversity (Vander-Heijden *et*

*al.*, 2008). They allow plants to draw more nutrients and water from the soil. They also increase plant tolerance to different environmental stresses. Moreover, these fungi play a major role in soil aggregation process and stimulate microbial activities (Amir *et al.*, 2008). In a common mycorrhizal network, it is hard to tell where one mycorrhizal ends and another begins (Iwanski *et al.*, 2006). Because of this vast network, a single plant can be connected to a completely different species of plant halfway across a forest (Amir *et al.*, 2008). There are two main forms of mycorrhizal; (i) *ectomycorrhizae* which simply surrounds the outside of the roots and (ii) *endomycorrhizae*, that their hyphae grow in between the cell wall and the cell membranes of the roots (Iwanski *et al.*, 2006). Mycorrhizal forms symbiotic association with several species of plant in forest ecosystem of which pine plantation had been reported to be an important species which benefit from the symbiotic association (Marx, 1991).

The Forestry Research Institute of Nigeria (FRIN) arboretum is endowed with myriad of indigenous and exotic tree species. Among the plantation of various tree species in FRIN genetic repository is Pine plantation with dearth of information about its mycorrhizal composition. This study therefore investigated mycorrhizal concentration at different levels of rhizosphere of *pinus caribaea* plantation in the FRIN arboretum.

Little studies have been carried out on mycorrhizal associations with Pine plantation in Nigeria. The source, effectiveness and concentration of mycorrhizal that have been used for several studies in the nursery are unknown. It is therefore pertinent to carry out this study in order to ascertain the source and concentration of the mycorrhizal that will be used as growth enhancer.

An important factor in the performance of plantation of conifers is the association of plant roots with ectomycorrhizal (ECM) fungi (Castellano, 1996; Marx, 1991). ECM fungi are essential for nutrient acquisition and plant protection against root pathogens and drought stress (Smith and Read 1997). *Pinus* species are dependent on symbiosis to develop optimally under natural conditions. ECM fungi naturally established in nurseries are diverse, and their establishment depends on several factors, including host species relationships, silvicultural practices, and nursery conditions (Le Tacon, 1992). The study therefore looked into availability of mycorrhizal at different levels of rhizosphere of *pinus caribaea* plantation with a view to ascertaining the concentration of mycorrhizal at different soil depth.

## MATERIALS AND METHODS

### Study Area:

The study was carried out at arboretum of Forestry Research Institute of Nigeria (FRIN) Jericho Hill Ibadan, Oyo State. The FRIN is situated at Jericho hill in Ibadan North West Local Government area of Oyo state. The area lies between the longitude 07° 23' 18" N to 07° 23' 43" N and latitude 03° 51' 20" E to 03° 51' 43" E. The climatic condition of the area is tropically dominated by rainfall pattern from 1400mm-1500mm. The average temperature is about 31.2°C. The area experiences two distinct seasons which are dry season and rainy season usually commenced from November to March, while the rainy start April to October (FRIN Meteorological station, 2017).

### Data Collection Procedure:

In an hectare of 25 years old plantation of *Pinus caribaea*, systematic cluster sampling technique was used for sample plots location. At the centre of the plantation, a 50 x 50 m main plot was laid and was further divided into temporary sample plots of 10 x 10 m. Five of such temporary plots were located at four corners and centre of the main plot. (Adekunle *et al.*, 2013; Adekunle and Olagoke, 2008, Adekunle, 2006).

## Mycorrhizal at Different Soil Depth

From each temporary sample plots of 10 x 10 m, five (5) samples were randomly collected from different rhizosphere depths of : 0 – 10 cm, >1 0 – 20 cm and : > 20 – 30 cm. Samples from each depth and five temporary sample plot were pooled together to obtain Compost samples. Therefore, there were 5 soil samples for each of 0 – 10 cm, >1 0 – 20 cm and : > 20 – 30 cm making 15 samples for laboratory analysis

### Laboratory Analysis Of Mycorrhizal Samples:

The soil samples were air dried and sieved to pass through 2 mm sieve. The concentrations of mycorrhizal at different depths were analyzed at FRIN Central Laboratory for mycorrhizal fungi composition following standard method of sprout counting.

### Data Analysis:

Analysis of variance (ANOVA) was used to test for differences in microbial and fungi concentrations at different depths using Completely Randomized Design as experimental design.

Treatments were three (3) rhizosphere depth (0 – 10 cm, >1 0 – 20 cm and : > 20 – 30 cm.) with five (5) replicates.

## RESULTS AND DISCUSSION

The analysis of variance (ANOVA) of Total Aerobic Microbial Count (TAMC) and Total yield and Mold Count (TYMC) of mycorrhizal in the soil samples collected from the three soil depths were not significantly different ( $P > 0.05$ ) among soil depths (Tables 1 and 2). It was found that the most prominent microbes was Arrbscular mycorrhizal that formed relationship with plant roots mainly within the root cell as endophyte. The pH of the experimental soil is slightly acidic (6.69) and it is loamy sand in textures. The organic carbon content and available phosphorus of the soil were higher ( $17.9\text{gkg}^{-1}$  and  $51\text{gkg}^{-1}$ ) respectively when compared with the critical value of  $15\text{mgkg}^{-1}$  Sand  $>20\text{mgkg}^{-1}$  respectively. The concentration of Fe Mn and Zn fall within the critical value range of 5-200 $\text{mgkg}^{-1}$ , 1.5, 100 $\text{mgkg}^{-1}$  and 1-5 $\text{mgkg}^{-1}$  respectively which Cu is lower than the critical value of 1.2-2 $\text{mgkg}^{-1}$ . Therefore, the soil is said to be low in major nutrient with the exception of p which is higher than the critical values. The results established the assertion of Simard *et al.* (2012) that Mycorrhizal networks extend over extensive underground connections and establish physical links between plants of the same or different species. It has been found that every kind of mycorrhizal has evolutionary backgrounds, anatomical structures, and ecological functions to transcend deep into the soil (Mao *et al.*, 2019). Consequently, they exert different influences on plant protection, nutrient acquisition, and the cycling of carbon and nutrients in the soil (Malewski *et al.*, 2022). The results suggest that mycorrhizal with fungal diversity have potentials to colonize the soil effectively in a conducive edaphic factor (Neuenkamp *et al.*, 2018). This can shape plant populations and communities and determine the coexistence and diversity of plants at the local ecosystem (Mao *et al.*, 2019). More mycorrhizal of TAMC that are too numerous to count were found at soil depth 0 – 10 cm and >1 0 – 20 cm while the highest TYMC was recorded for soil depth > 20 – 30 cm (Table 3). This is an indication that there is more mycorrhizal mycelium at the top rhizosphere of the 25 years old pine plantation. According to Pachlewski and Pachlewska (1994), Pinus species are highly dependent on the presence of compatible mycorrhizal fungi. Mycorrhizal moulds were found grown in the three soil depth investigated (Plates 1, 2 and 3). The presence of mycorrhizal communities in the soil, which are characteristic of this species, provides the trees with access to nutrients in the soil and creates favorable conditions for their growth and development (Pachlewski and Pachlewska, 1994). Several studies have shown that Pines are known to host diverse belowground ectomycorrhizal (ECM) communities (Van Der Heijden *et al.*,

2015; Tedersoo *et al.*, 2020) although there are typically fewer ECM species associated with Pine compared to Oak species (Van Der Heijden *et al.*, 2015). Invariably, mixtures of both host species are expected to harbor higher richness of ECM fungal taxa and more host-specialist fungi because of increased habitat heterogeneity, modified soil physical conditions and resource niche partitioning that allow the co-occurrence of many species (Neuenkamp *et al.*, 2018). However, changes in host preference composition can influence fungal and plant community dynamics, age, as well as the structure and functioning of common mycorrhizal networks, with consequences for ecosystem condition and resilience (Van der Heijden *et al.*, 2015).

**Table 1:** Analysis of Variance (ANOVA) TAMC of the Total Aerobic Microbial Count (TAMC) of mycorrhizal in the soil samples collected from the three soil depths of the study area.

SV	df	SS	MS	F-cal	P-Value
Treatments	2	107953.733	53976.867	0.675	0.528ns
Errors	12	960198.000	80016.500		
Total	14	1068151.733			

ns= nssignificant at  $P > 0.05$

**Table 2:** Analysis of Variance (ANOVA) of the Total yield and Mold Count (TYMC) of mycorrhizal in the soil samples collected from the three soil depths of the study area.

SV	df	SS	MS	F-cal	P-Value
Treatments	2	380000000	190000000	5.23	0.605ns
Errors	12	435400000	36283333.3		
Total	14	473400000			

ns= nssignificant at  $P > 0.05$

**Table 3:** Total Aerobic Microbial Count (TAMC) and Total yield and Mold Count (TYMC) of mycorrhizal in the soil samples collected from the three soil depths of the study area.

Soil depths (cm)	Replicates	TAMC (cfu/g)	TYMC (cfu/g)
0 - 10	1	$1.88 \times 10^6$	$1.5 \times 10^5$
	2	TNC	$6.40 \times 10^5$
	3	$2.36 \times 10^7$	$6.00 \times 10^5$
	4	$7.50 \times 10^5$	$5.50 \times 10^5$
	5	$1.39 \times 10^7$	0
>10 - 20	1	TNC	$2.80 \times 10^6$
	2	$2.05 \times 10^7$	$1.35 \times 10^5$
	3	$2.40 \times 10^6$	$0.50 \times 10^5$
	4	$1.34 \times 10^7$	$3.00 \times 10^5$
	5	$2.11 \times 10^7$	$1.00 \times 10^6$
>20 - 30	1	$2.23 \times 10^7$	$7.10 \times 10^6$
	2	$4.35 \times 10^6$	$0.50 \times 10^5$
	3	$1.39 \times 10^7$	$0.50 \times 10^5$
	4	$2.10 \times 10^7$	$2.00 \times 10^5$
	5	$1.57 \times 10^7$	$6.50 \times 10^5$



### Mycorrhizal at Different Soil Depth



**Plate 1:** Germination of mycelium of mycorrhizal fungi in the soil sample collected at 0 – 10 cm soil depth



**Plate 2:** Germination of mycelium of mycorrhizal fungi in the soil sample collected at >10 – 20 cm soil depth.



**Plate 3:** Germination of mycelium of mycorrhizal fungi in the soil sample collected at > 20 – 30 cm soil depth.

## Conclusion

At the rhizosphere of 25 years old plantation of *Pinus caribaea* in the FRIN arboretum, mycorrhizal mycelium were readily present at soil depth 0-30cm with more concentration at depth 0-20cm. The topsoil from the plantation floor can be a good source of mycorrhiza for enhancement plant growth.

### Declarations:

**Ethical Approval:** Based on the fact that artificial models and other related subjects were not used in the study, ethical approvals are not required.

**Conflict of interest:** There is no interest conflict at all.

**Authors Contributions:** Every author has contributed significantly to the research proposal and the entire study. Everyone has painstakingly read the manuscript, verified the authenticity and correctness of the data and its interpretation, and has given their approval for its submission.

**Funding:** The author(s) received no specific funding for this work.

**Availability of Data and Materials:** All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

**Acknowledgements:** Not applicable.

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### Mycorrhizal at Different Soil Depth

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