Associating *Azadirachta indica* A. Juss and *Eucalyptus camaldulensis* Dehnh Shelterbelt on the Growth yardsticks, Microbial Population at Kiyawa, Dutse, Jigawa State



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Abstract

This study was carried out in the year 2021 at Kiyawa community which aimed to investigate growth attributes, identified the fungi, bacteria and determined the microbial load in the neem and eucalyptus shelterbelts. 1 kilometer line transect was laid with four sample plots established $(30 \times 30m)$ which were used for collecting data of the tree with an interval of 100m from each plot. Also, 1×1m mini-plots were used for measuring quantity of litters. Diameter at breast height and height were accessed with the aid of meter rule and Haga altimeter while volume and Basal area were calculated. Potato Dextrose Agar (P.D.A) media used for the isolation of fungi while Nutrient Agar (N.A) medium was used to isolate bacteria sterilized in autoclave for 15 minutes at 121°C. Fungi Morphology was studied with aid of microscope by observing colony features. The data collected was analyzed using descriptive statistic (table) and inferential statistic: t-test was employed. The results showed that Azadirachta indica plots had the highest Dbh, Basal area, Height, and volume of 82.25cm±1.23, 0.90m²±0.15, 15.90m±0.24, 970.78 m³±4.27m³followed by the Eucalyptus camaldulensis hotspot which had the least value of 62.50cm±0.93, 0.463m²±3.43, 3.75m±0.03 and 347.85±2.43m³ respectively. The findings showed the presence of three (3) species of bacteria in each study site. Eucalyptus shelterbelt has Staph avenus and Bacillus cereus while Bacillus subilis and E. coli were presence in Neem hotspot. Pseudomonas spp can be traced to both hotspots. Fungi traced to the both study sites were Aspergillus niger and Penicillum species. However, Aspergillus flaming occurred in eucalyptus hotspot while Fusarium oxysporum present in Neem shelterbelt. Result revealed that grand mean microbial load of fungi (Eucalyptus 1.45x 10⁶; Neem1.50x10⁶) is higher than bacteria (Eucalyptus 1.24 x 10⁶; Neem1.3 x10⁶). There is significant difference between the bacterial microbial loads between the study sites at ($p \le 0.05$). The hotspots support better growth attributes of neem tree and fungi microbial load. Therefore, neem tree is recommended for economic and ecological reasons and adaptive arable crops should also intercrop with tree to supply improve economic value of shelterbelt and land user.

Keywords: Comparative, Growth yardsticks, Kiyawa, Microbial population and Shelterbelts.

INTRODUCTION

Forest inventory has been defined by Husch *et al.*, (1972) as the procedure for obtaining information on the quantity and quality of the forest resource and many of the characteristics of the land area on which the tree is growing. A complete forest inventory for timber resources evaluation provides the following basic information: a description of the forested area including ownership and accessibility, estimates of timber quality and quantities and estimates of growth and drain. Non-timber information may also be included on wildlife, areas of recreational and touristic interest, soil and land use capabilities on water shed values. Forest biodiversity protection relies on the ability to assess hot spots, quantify and predict spatial and temporal trends of key species which maintain a natural disturbance regime and limit harmful human activities (Stohlgren *et al.*, 1999). Protected areas made to known by the International Union for Conservation of Nature (IUCN) as an area of land and/or sea especially dedicated to the protection and maintenance of biological diversity, and of natural and associated cultural resources, and managed through legal or other effective means (IUCN, 1994). Forest protected areas help conserve ecosystems that provide habitat, shelter, food, raw materials, genetic materials, a barrier against disasters, a stable source of resources and many other ecosystem goods and services and thus can have an important role in helping species, people and countries adapt to climate change. They can thus continue to serve as a natural storehouse of genetic material into the future. They help in the conservation of indigenous species that are resistant to pests, diseases and pathogens, environmental stresses and nutrient loss.

Soil is a complex and dynamic ecosystem where substantial physical, chemical, and biological processes take place (Jelena *et al.*, 2018). According to Nannipieri *et al.* (2003), the most important biological processes in soil (80-90%) occur due to microbial enzyme systems reactions. Rousk *et al.* (2008), stated that soil chemical and physical characteristics are major factors of soil microbial community structure. The physico-chemical properties of soil are ultimately related to soil fertility which affects the

floristic composition of forest. There is a mutual connection between the soil microflora and the vegetation of an ecosystem. Microorganisms help in mineralization and decomposition of plant materials to a form that can be absorbable by plants (Pietikainen, 1999). Sigstad et al., (2002) also pin pointed that bacterium as the most occurrence and it is through their metabolic activity that minerals and soil organic matter are transformed in a way that important nutrients such as N, P, and S are simultaneously converted into useable forms for plant and other micro-organisms. Eucalyptus camadulensis tree has phyto-chemicals that are known to possess antitermic repellent activities (Jibo et al., 2021; Geoff, 2007) and releases compounds which inhibit the germination or growth of other potential competitor's plants. Outside their natural ranges, eucalyptus is both lauded for their beneficial economic impact on poor populations (Luzar, 2007). According to "Merriam Webster" Shelterbelt are barrier of trees and shrubs that provide protection (as for crops) from wind and storm and lessens erosion. A shelter belt is a planting usually made up of one or more rows of trees or shrubs planted in such a manner as to provide shelter from wind and to protect soil from erosion. Shelter belts can also be known as windbreak because they are commonly planted in hedge rows around the edges of field on farms. It's uses cannot be unnoticed and it plays a vital role in our daily lives and specially as farmers. Some of the uses are to: Providing habitat for wildlife and serve as woods if the trees are harvested, Windbreaks within one's environment reduces the cost of heating and cooling and saves energy. This research seeks effort to generate information on the essence of utilizing shelter belts. Therefore, the aim of the study is to access the growth variables and microbial population of Katika Shelterbelt, along Kiyawa-Jahun road, Jigawa State, Nigeria with the view of providing better management and conservation strategies for the shelterbelts.

Materials and method

The study area

The two protected areas i.e shelter belts are located in Katika along Kiyawa and Jahun road. The shelter belt was established in 1989 by the Department of Forestry, Ministry of Environment, Kano State. Kiyawa is located in the southern region of the state. The region is about 500-600m above the sea level Maryam *et al.*, (2019). Jigawa and Kano are ruled under the same government and later fall under Dutse Emirates. It covered an area up to 3 hectares. The Shelterbelts comprises of Neem and Eucalyptus tree species which are planted in rows (JARDA, 2016). The Shelterbelt is located at the coordinate of Latitude 11°47′05″N, 09°36′30″E and Longitude 11°78°472°N, 9°08°33°E. The annual mean temperature is about 25°C but the mean monthly value ranges between 21°C in the coolest month and 31oc in the hottest month (Azare *et al.*, 2019) and also soil type is sandy (Salami and Lawal, 2018); Jibo *et al.*, 2021)



Figure 1: Showing map of Kiyawa Source: Cartography and GIS Lab, Bayero University, Kano (2020)



Source: Field source (2021) **Plate 1:** Shelter belt of *Azadirachta indica* (Neem)



Source: Field source (2021) **Plate 2**: Shelter belt of *Eucalyptus camaldulensis*

Data collection

Sampling Layout and procedure

Systematic sampling design (systematic line transects) was used in laying out of the plot. A line transects of 1km with four samples of size $30m \times 30m$ was laid in each shelterbelt. Eight sample plots were assessed during experiment for both studies. All woody plants within transects were enumerated while 1m x 1m sample plot was laid within each of the main sample plot for soil collection (Aminu, 2021; Salami, 2017)



Figure 2: Plot layout for the methodology

Tree enumeration

All Woody plants with DBH of 10 cm was enumerated. Tree growth variables such as the diameter at the base (Db), Diameter at breast height (DBH), diameter at the middle (Dm), Diameter at the top (Dt) and height was measured with Spigeal relascope. Basal area and volume was evaluated with appropriate formula with equation 1 and 2.

Soil collection

The sample plots laid in the shelter belts, thus, was used for soil collection. Soil samples were collected at three depths along the diagonal for each of the sample plot with the aid of a soil auger. The soil sample was collected at soil depth of 0-15cm only, because the number of count of bacteria and fungi always decrease with the depth of soil sample (Lawal *et al.*, 2018)

Identification of Micro-Organisms

Fungi Morphology was studied with aid of microscope by observing colony features (Colour and texture) and by staining with lacto phenol cotton blue and observed under compound microscope for the conidia, conidiophores and arrangements of spores (Ameba, 2001). Gram's staining was carried out on the growth culture plate to differentiate gram's negative organism from gram's positive organism. Biochemical test was carried out base on Gram's result.

Isolation of Micro-organisms

Potato Dextrose Agar (P.D.A) media was used for the isolation of fungi, the plate was kept at room temperature for 7 days. Dilution was prepared and used for the isolation of Bacteria. One (1g) of soil sample was taken and serial dilution was carried out in distilled water. Nutrient Agar (N.A) medium was used to isolate bacteria sterilized in autoclave for 15 minutes at 121°C. After 2 hours of incubation at 37°C. Streaking plate method was used to get single colonies of the culture, (Shanmugam *et al.*, 2013).

Data analysis

The data collected was analyzed using descriptive statistics such as tables while inferential such as T- test was employed to compare fungi, bacterial and microbial population in the study sites.

Stem Volume estimation

Volume of individual trees encountered in the plots. Mean volume for sample plots were calculated by dividing the total plot by the number of sample plots. Volume per hectare was obtained by multiplying mean volume per plot (VP) with the number of 50×50 m plots.

The volume of all trees in the sample plots was calculated using this formula:

Basal area calculation

The basal area of all trees in the sample plots was calculated using this formula: $-n^2$

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BA = \frac{\pi D^2}{4}
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The total basal area for each of the sample plot was obtained by adding the BA of all trees in the plot while mean BA for the plot (BAp) was obtained by dividing the total BA by the number of sample plots. Basal area per hectare was obtained by multiplying mean basal per plot with the number of 30 x 30m plots in a hectare (4).

Where ha BA = Basal area per hectare. Where BA = Basal area (m²),

D = Diameter at breast height (cm) and Pie (3.142).

Results

Parameters	Study site	Min	Max	Total	Mean	Stand Error
DBH(cm)	Neem	19.5	145	8441.75	82.25	±1.23
	Eucalyptus	18.0	107	6562.25	62.50	±0.93
Basal area (m²)	Neem	0.15	1.65	92.70	0.90	±0.15
~ /	Eucalyptus	0.025	0.90	48.56	0.463	±3.43
Height (m)	Neem	2.90	13.00	818.85	15.90	±0.24
inigit (iii)	Eucalyptus	1.20	6.30	393.75	3.75	±0.03
Volume (m ³)	Neem	56.55	1885	99,989.8	970.78	±4.27
volume (III)	Eucalyptus	21.60	674.1	36,524.25	347.85	±2.43

Table 1: Growth	Variables of Neem and	d Eucalyptus Species in the Shelterbelts	

Table 2: Occurrence of Soil bacteria in Eucalyptus and Neem Shelterbelt

SN	Species	Eucalyptus hotspot	Neem hotspot
1	Staph averus	V	
2	Bacillus cereus	V	
3	Pseudomonas spp	V	V
4	Bacillus subilis		v
5	E.coli		V
Total		3	3

Note: V= present

Table 3: Occurrence of Soil Fungi in Eucalyptus and Neem Shelterbelt

S/N	Species	Eucalyptus hotspot	Neem hotspot
1	Aspergillus niger	V	V
2	Aspergillus flamings	V	
3	Penicillum spp	V	V
4	Fusarium oxysporun		V
Total		3	3

Note: V= present

 SN	Species	Eucalyptus hotspot (CFU/g)	Neem hotspot (CFU/g)
1	Staph averus	1.32×10^{6}	
2	Bacillus cereus	9.2 x10 ⁵	
3	Pseudomonas spp	1.48 x10 ⁶	2.18 x10 ⁶
4	Bacillus subilis		$1.46 \text{ x} 10^6$
5	E.coli		1.56 x10 ⁶
 Grand m	ean	1.24 x 10⁶	1.3 X10 ⁶

Table 4: Mean microbial population of soil bacteria in Eucalyptus and Neem shelterbelt

Note: CFU/g is colony forming unit

Table 5: Mean microbial	population of soil	l fungi in Eucalyptus an	d Neem shelterbelt

SN	Species	Eucalyptus hotspot(CFU/g)	Neem hotspot(CFU/g)
1	Aspergillus niger	1.30x10 ⁶	1.88 x10 ⁶
2	Aspergillus flamings	1.01 x10 ⁶	Nil
3	Penicillum spp	2.04 x10 ⁶	1.38 x10 ⁶
4	Fusarium oxysporun	Nil	1.23 x10 ⁶
	Grand mean 1.45 x	10 ⁶ 1.50 x10 ⁶	

Note: CFU/g is colony forming unit

Discussion

Growth parameter indices

The results showed that *Azadirachta indica* plots had the highest Dbh, Basal area, Height, and volume of 82.25cm ± 1.23 , 0.90m² ± 0.15 , 15.90m ± 0.24 , 970.78m³ ± 4.27 m³ followed by the *Eucalyptus camaldulensis* hotspot which had the least value of 62.50cm ± 0.93 , 0.463m² ± 3.43 , 3.75m ± 0.03 and 347.85 ± 2.43 m³ respectively. However, the results were obtained from two different studies sites. It showed that *Azadirachta indica* had the greatest volume not only even volume even in terms of richness, height and growth compared to *Eucalyptus camaldulensis*. Mean basal area obtained was 0.90 ± 0.15 m² from Neem shelterbelt which implied that the study area had a high values of trees density and values that can be useful when properly managed and harvested for human purposes such as the construction of furniture's, electric poles, foal fuel, charcoal production followed by 0.463 ± 3.43 m². The inventory count of different species in the study area was fifty (50) in number. The result disagrees with the finding of Salami *et al.* (2021), whose reported higher mean volume and Basal area (14126.59m³; 339998m²) at Warwade plantation in the area. No documentation on the growth assessment of shelterbelt in the past years.

Presence of fungi and bacterial

Table 2 and 3 revealed the presence of soil bacteria and fungi in the study sites. The findings showed the presence of three (3) species of bacteria in the study site. Eucalyptus shelter belt has *Staph avenus* and *Bacillus cereus* while *Bacillus subilis* and *E. coli* were presence in Neem hotspot. *Pseudomonas spp* can be traced to both hotspots. This implies that this species can thrive and found in many habitats despite nature habitat. The presence of fungi: *Aspergillus niger* and *Penicillum species* were present in both study sites. However, *Aspergillus flaming* occurred in Eucalyptus hotspot while *Fusarium oxysporum* present in Neem shelterbelt. Four different species of fungi present in both sites. Two (2) was common to both while a species found in each of the sites respectively.

Relationship between Microbial populations of the study sites

The findings of the comparative analysis of the microbial load of both fungi and bacteria were influenced by physical features of the soil. This agrees with finding of (Ateh *et al.*, 2020) who reported that the texture of the soil determine the nature of microbes present. Microbial organism plays importance roles in the decomposition of organic matter, nitrogen fixation and nutrient cycling (Lawal *et al.*, 2018; Ateh *et al.*, 2019). The effects of the soil microbes are influenced by their population Classen, (2015). Microbial population in forest soils are determined by both chemical and physical properties of the soil (Seeley, 1981). The results from table 4 and 5 showed the relationship between microbial loads of bacteria and fungi found in the study site. The study revealed that five (5) species of bacterial were found which are *Staph averus, Bacillus cereus, Pseudomonas spp, Bacillus subilis and E. coli*. Microbial load of 1.48 x10⁶. The grand mean of Neem hotspot was recorded to be 1.3 X 10⁶ which is higher than Eucalyptus hotspot. There is significant difference between the bacterial microbial loads between the study sites at ($p \le 0.05$).

Furthermore, there is similarity in the microbial load values of fungi recoded in the two study sites with values of 1.45×10^{6} and 1.50×10^{6} respectively. *Aspergillus niger* is higher in Neem than Eucalyptus shelterbelt with the microbial load of 1.88×10^{6} and 1.3×10^{6} respectively while for *Penicillum spp*, the microbial load is higher in Eucalyptus (2.06×10^{6}) than Neem shelterbelt (1.38×10^{6}). The weight of the fungi is lower in Eucalyptus hotspot due to the effect of allele-chemicals which is higher in eucalytus than neem hot. The results from table 4 and 5 revealed that fungi load was higher than bacteria load at both study sites. This is in accordance to (Barbour *et al*, 1987; Zhou *et al*, 2018) who revealed that nature of physical properties of the forest soil determines the population and microbes in the soils. The dominant and structural organization of the sand textural class in the study provided a spatially heterogeneous habitat for fungal community because of smaller size fraction (silt and clay) host higher bacterial community than larger size particle (size). Atch *et al.*, (2019) supported the study carried that microbial load of fungi was higher than bacteria with the value of 4.49×10^{5} and 3.43×10^{5} respectively at the same soil profile level (0-15cm) in Girea soil of Adamawa. This study also agrees with Nkereuwem *et al.*, (2020) who reported that fungi did better than bacteria in adapting to drying rewetting stress across the different soil locations during the drying rewetting cycle. However, Adekunle *et al.* (2005), disagreed with the finding and reported that the amount of bacterial microbial load is higher than fungi microbial load in Akure Forest Reserve in southwestern, Nigeria with the range of (26.14×10^{6} , 360×10^{6} MPNg⁻¹) and (2.50×10^{6} to 23.34×10^{6} MPNg⁻¹) respectively.

Conclusion and recommendations

The hotspots support better growth attributes of neem tree and fungi microbial load. Bacteria microbial load is not prominent unlike fungi. Therefore, neem tree is recommended for economic and ecological reasons; adaptive arable crops should also intercrop with tree crops for improve economic value of shelterbelt and land user. Therefore, this study gives a basis for further research especially on degree of allelo-chemicals characteristics on the growth of arable crops/plants between neem and eucalyptus shelterbelt since there is no documentation at Kiyawa Shelter belt.

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