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Growth Performance Evaluation of Leaf Characteristics of *Rhizophora Racemosa* Grown in amended Mangrove - Garden Soil

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ABSTRACT: The present study investigated the effect of soil type on leaf characteristics of the *Rhizophora racemosa*. Matured propagules were grown for 75days on mangrove and garden soil amended with Saw dust (SD), Rice husk (RH), NPK and RH+ SD+NPK combination treatments at the Botanical Garden of the University of Port Harcourt. Leaf properties such as leaf area, Leaf dry weight, specific leaf area and leaf dry matter content was determined 75days after planting. The leaf area of *Rhizophora racemosa* were not significantly different from one another at $p<0.05$ in the amended garden soils while in the amended mangrove soils there was significant difference. Result of leaf dry weight showed significant difference at $p<0.05$ in both amended soil types. Maximum Specific Leaf Area of 157 ± 31.7 cm^2 g^{-1} was observed in the garden soil amended with RH+ SD+NPK combination while the highest SLA of 143.7 ± 19.3cm^2 g^{-1} was observed in the mangrove soil amended with SD. Leaf Dry Matter Content varied from 0.224 ± 0.012g g-1 - 0.271 ± 0.013 g g-1 across the various amendment in the mangrove soil while it ranged from 0.256 ± 0.004 – 0.355 ± 0.041g g^{-1}) for the garden soil. Result indicated that *Rhizophora racemosa* can be successfully raised in amended garden and mangrove soil, and this may provide valuable information for mangrove forest restoration in the Niger delta region.

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Nigeria has the largest mangrove forest in Africa and the fifth largest in the world behind Indonesia, Australia, Brazil and Mexico (Daniel and Edward, 2013). The mangrove forest of Nigeria extends from Badagry in the West to Calabar in the East covering a total area of 10,000km2 along the coast. The forests comprises principally only three families and six species namely: *Rhizophoraceae* (the red mangrove) with three major species:- *Rhizophora racemos*a, *R. harrisonii* and *R. mangle*. The species *Rhizophora racemosa* is the most abundant taking about 90% of the mangrove forests and occurs at the outer body of water. *Avicenniaceae* – (white mangrove) *Avicennia Africana; Combretacea - Lagancularia racemosa* and *Conocarpus erectus*. Another exotic plant that is found associated with the mangrove forest of Niger delta is *Nypa fruticans* (Nypa Palm) (NDES, 2000).

Mangrove provides many ecosystem services, such as support for local livelihoods through the provision of fuel, food and construction materials. It host a wide variety of biodiversity, providing habitats for fauna including aquatic and terrestrial insects, fish, crustacean, mammalian, amphibian, reptilian and avian species, Hogarth (1999). Mangroves have been found to sequester high amount of carbon comparable only to those of the dense Amazon forest, (Donato *et al.,* 2011). This makes mangroves an important asset for carbon trading initiatives such as Reduced Emissions from Deforestation and Degradation (REDD+) in addition to their financial value in terms of the payments for ecosystem services (PES) that they support, (Lucas *et al* 2014.). *Rhizophara racemosa* is a major dominant species (NDES, 2005) and adorns the coastal areas of the Niger delta region (RPI, 1985) where it occurs in the intertidal zone with a wide range of hydrological conditions. Due to their unique location, mangroves are subject to fluctuations in freshwater as well as sea-level rise and salinity. Salinity has been held as a controlling factor that determines the development, growth and distribution of mangrove forests (Twilley and Chen, 1998). However, recent findings by Doyle (2003) have shown that their growth in the estuarine environment is due primarily to their adaptation traits and their poor competitive ability with other plants. Supporting the non-salinity precondition, (Doyle, 2003) has demonstrated that mangrove can survive, thrive and successfully regenerate given limited competition from other plants in freshwater systems thus concluding that it was actually the substrate and nutrient quality that play a

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much greater role than the factors of hydrology and salinity in determining mangrove growth and form.

Leaf weight and Leaf area basically are important factors in most ecophysiological studies in terrestrial ecosystems regarding their roles as light interceptors, sites of evapotranspiration and photosynthesis. Both are useful in quantifying primary production and competition among species respectively by physiologists and ecologist (Pandey and Singh, 2011). Also specific leaf area (SLA) and leaf dry matter content (LDMC) are both important indicators in plant ecology because they are connected to the various critical aspects of plant growth and survival (Akonye and Nwauzoma, 2003; Garnier *et al*.; 2001). Several studies have revealed their critical roles in explaining variations in potential growth rate and the ecological behaviour of plants (Diaz *et al*, 2004).
Given this, the present study focused on the possibility of raising *R.racemosa* propagule on other soil type outside the normal mangrove soil using different leaf characteristics as indicator of performance. It is expected that the result will open up other media of raising mangrove propagule for the revegetation of the mangrove ecosystem of the Niger delta which is fast disappearing due to several anthropogenic factors.

MATERIALS AND METHOD

Site description: The study was carried out at the Botanical Garden of the University of Port Harcourt. The area lies within the tropical rain forest ecological zone in southern region of Nigeria popularly referred to as the Niger delta. Uko and Tamunobereton-Ari (2013) has reported the meteorological characteristics of the study area. Monthly temperature during the dry season (November – February) ranged between 31.13 – 33.08^{o}C with a mean of 31.97^{o}C while during the rainy season (March – October), the temperature ranged 25.71 – 32.48^{o}C with a mean of 29.095^{o}C. The area records a 2400mm mean annual rainfall (Dike and Nwachukwu, 2003) which peaks during the months of July and September.

Experimental design: The experiment consists of two treatments [garden soil (GS) and mangrove soil (MS)] and each was subdivided into five subsets. The five subsets were numbered 1 to 5 for each treatment. Subset number 1 had no amendment and served as control GS or MS, number 2 was amended with Saw dust (SD), number 3 was amended with Rice husk (RH), number 4 amended with NPK (10:10:10) and lastly number 5 was amended with a combination of SD+RH+NPK . Each subset of a particular treatment was replicated six times and was made by filling 2.0kg of mangrove and garden soil respectively in a polyethylene bag (28cm x 20cm). Mangrove soil were collected from the intertidal zone of Ogbogoro estuarine waters (535525.548N, 270244.003E and 535498.008N, 270306.874E) where *R. racemosa* grows using hand trowel while garden soil was collected from botanical garden at approximately 5cm deep. Debris and partially decomposed materials were carefully removed before soil collection. The soils were homogenized separately and potted into garden and mangrove soil respectively. Each of the polythene bag containing the prepared soil was amended with its assigned amendment. This was done by weighing 15g of the relevant amendment in a balance (Labtech digital Balance BL20001) and subsequently mixed with the soil in the first 10cm of the prepared soil types while in the combination amendment 5g each of SD, RH and NPK was first weighed in a balance and mixed together first before applying onto the soil. The polythene bags containing the amended soil were laid out in a partially shaded plot, allowing for natural sunlight and rain to reach it and left for four days to allow for decomposition and thorough mixing with the soil material. Nevertheless, the polythene bags with the soil were watered once every two days with fresh tap water throughout the duration of the experiment ensuring that the soil remains wet. Matured propagules were assessed using colouration as described by Duke and Allen, (2006) and by touch (Cavalcanti, 2007). The propagules had a mean fresh weight of 25.884±1.42g and a height of 33.345±1.48cm. On day five (5) post soil amendment, propagules of *R. racemosa* were planted in the prepared soil at 5cm depth. Soil samples for physicochemical analysis were collected to a 5-cm depth from each polythene bag using a spartula. The samples were air-dried and passed through a 2-mm sieve. Soil pH and electrical conductivity (EC) were measured in a soil-water suspension (1:1 and 1:5 soil to water ratio, respectively electrometrically) using OAKTON

PH/Conductivity/TDS/Salinity/Temperature Multi parameter testr35 35425-00. Total Organic Matter was determined by loss on ignition method while Total organic carbon (TOC) was determined by calculation using total organic matter result applying the 'Van Bemmelen' factor (Nelson and Sommers, 1996). Total N was measured by the Kjeldahl (Wright and Westoby, 2003).

Measurement of leaf characteristics: After seventy five (75) post planting day, the plants were harvested from the potted bags and excess soil segments washed off from the roots with fresh tap water. Care was taken not to damage the roots of the plants. The harvested plants were separated into roots, stem and leaves. Leaf samples were immediately placed between wet papers, sealed in a plastic bag, labeled and placed in a dark

container with ice. Transporting of samples to the laboratory was within 30minutes and the samples were subsequently placed in water in the dark at 5°C for 12 h after which the petiole was removed under water Garnier *et al*., (2001). This procedure ensured that the leaf samples fully rehydrated. After 12h leaves were then dried with tissue paper to remove surface water and leaf area estimated. Leaf area was estimated by laying a sample of the rehydrated leaf to be measured on a 1-cm grid graph paper and the outline traced and counted (Pandey and Singh, 2011). The number of square centimeters completely covered by the trace was counted and this gives the area of the leaf. There after the leaves of each plant were immediately weighed to determine their saturated fresh weight. Also the stem and roots of each plant were weighed separately on a digital Labtech digital balance, BL20001. The entire plant sample components were thereafter oven-dried at 60°C using Memmert oven for (48) hours until constant weight was maintained. Values of LDMC were calculated as the ratio between leaf dry mass and saturated fresh mass (g g^{-1}), and SLA was expressed as the ratio of leaf area to leaf dry mass (cm^2 g^{-1}).

Data Analysis: Data were analyzed by one-way analysis of variance (ANOVA) followed by Scheffe's test for comparisons of all treatments against the control. The value of $P< 0.05$ was selected as a limit of statistical significance.

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RESULTS AND DISCUSSION

Soil Characteristics: Results of analysis of the soil revealed that the means of pH, Organic Carbon, Total Nitrogen, Electrical Conductivity, Salinity, and OM varied significantly at ($P<0.05$) between mangrove soil and garden soil before amendment (Table 1).

Leaf Area and Leaf dry Weight of R. racemosa grown in amended mangrove and garden soil: The leaf area of *R. racemosa* grown in garden soil had a maximum mean value of 14.3 ± 0.8cm^2 in the bag amended with RH while the lowest mean value of 9.8 ± 0.8cm^2 was recorded on the control bag, (Figure 1). In the amended mangrove soil, the maximum leaf area of 17.1 ± 0.9cm^2 was recorded in the combination amendment SD+RH+NPK while the minimum value of 10.2 ± 0.9cm2 was recorded in the control bag, (figure 1). Within group significant difference was observed between MS and MS+SD; MS and MS+SD+RH+NPK; MS + RH and MS+SD+RH+NPK; MS +NPK and MS+SD+RH+ NPK.

S=Soil, S + SD = Soil + Saw Dust, S + RH = Soil + Rice Husk. S + SD + RH + NPK = Soil + Saw dust + Rice husk + NPK

Table 1: Physicochemical Properties of the different soils before planting

Parameter		Mangrove soil	Garden soil
Soil texture		Silty Loam	Clayey
Particle size analysis - Sand		15.54	27.83
	Silt	84.80	11.63
	Clay	0.0	60.53
pH		3.58	6.22
Conductivity µS/cm		1685	20.9
Salinity (ppm)		909	19.5
Organic matter content (%)		29.79	3.27
Moisture content (%)		23.53	14.40
Total N (ppm)		1.80	0.41
Total P (ppm)		0.68	2.54
Total Organic Carbon (%)		17.3	1.89

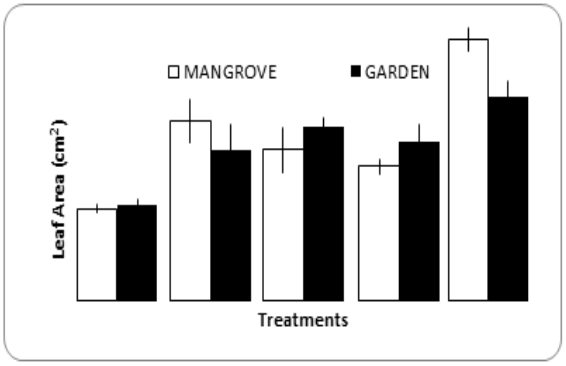

Fig 1: Leaf area of R. racemose grown in amended mangrove and garden soil

The leaf dry weight (LDW) of *Rhizophora racemosa* recorded a maximum value of 0.181 ± 0.02g in the garden soil treatment amended with NPK while the lowest value of 0.103 ± 0.008g was recorded in the control (GS), (figure 2). There was a significant difference at p=0.05 of leaf dry weight within the garden soils treatment. In the amended mangrove soils, maximum LDW value of 0.222 ± 0.015g was recorded in the combination treatment (SD+RH+NPK), While the minimum value of 0.092 ± 0.004g was recorded in control (MS). Specifically, significant difference was observed within the following amendment, MS and MS + NPK; MS and MS+SD+RH+NPK; MS+SD and MS+RH and MS+SD+RH+NPK respectively.

Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC) for Rhizophora racemosa grown in amended mangrove and garden soil: Mean specific leaf area (SLA) and leaf dry matter content (LDMC) were not significantly different (p=0.05) between the mangrove plants grown in the two types of soil (Figure 3 and 4). Maximum SLA in the mangrove soil amended was observed in the set amended with SD (143.7 ± 19.3cm^2 g^{-1}) while in the garden soil the maximum value of 150.56 ± 14.96cm^2 g^{-1} was observed in the set amended with GS+SD+RH+NPK. The lowest SLA values for both soil types was 75.5 ± 5.39cm^2 g^{-1} and

80.5 ± 17.7 cm^2 $g^{-1}$for the mangrove and garden soil respectively and was observed in the set amended with NPK respectively; (figure 3).

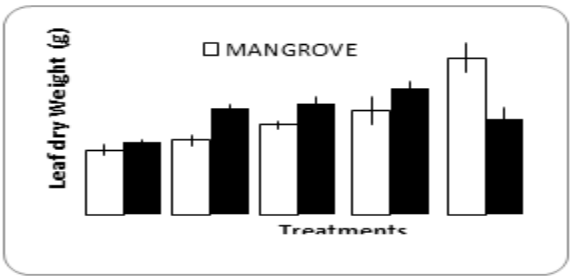

Fig 2: Leaf dry weight of R. racemosa grown in amended mangrove and garden soil

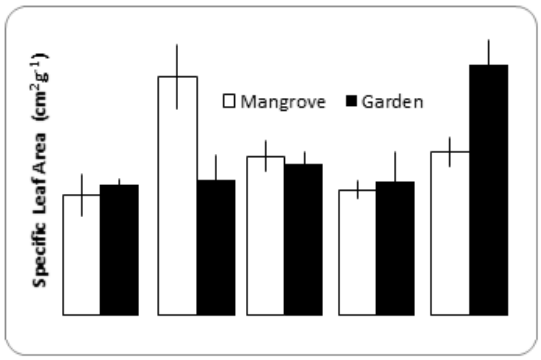

Fig 3.0: Mean SLA of *R. racemosa* grown on amended mangrove and garden soil

LDMC varied from 0.224± 0.012g g^{-1} (MS + SD) to 0.271 ±0.013 g g^{-1} (S + SD +RH + NPK). Within the amended mangrove soils there was no significant difference in LDMC values at P<0.05 for the various amendments. In the garden soil, the minimum LDMC value of 0.256 ±0.004 g g^{-1} was recorded in control (GS) while the maximum value of 0.355 ±0.041 g g^{-1} was observed in the garden soil amended with NPK (Figure 4). The uniqueness of mangroves habitat makes salinity an important factor restraining propagule germination, its growth and reproduction. Many studies have highlighted the negative effects of salinity on mangroves establishment, growth and development. Such negative relationship as seedling emergence rate and salt content of *Avicennia marina* (Patel *et al*., 2010) delay in root initiation of *Acanthus ilicifolius* (Chen and Ye, 2014) and reduction in final seedling establishment rates of *Aegiceras corniculatum* besides, (Medina and Francisco, 1997) who have reported considerable decreases in leaf number and area of *Avicennia. germinans* with increase soil salinity. This study showed that the leaf area of *Rhizophara racemosa* in the amended garden soil treatments showed no significant differences. However, in the amended mangrove soil, the highest mean value of 17.1 ± 0.9cm^2 was recorded in the SD+RH+NPK plot and this was significantly different from the mean value of the control plot. The low leaf area as recorded in the control set relative to other amendments of the mangrove soil is an indication of salinity stress. Parida and Das, (2005) have reported a reduction in leaf size of *Rhizophora mucronata, Rhizophora apiculata* grown in medium saline soil with consequent production of smaller leaves as an adaptation to reduce transpirational loss.

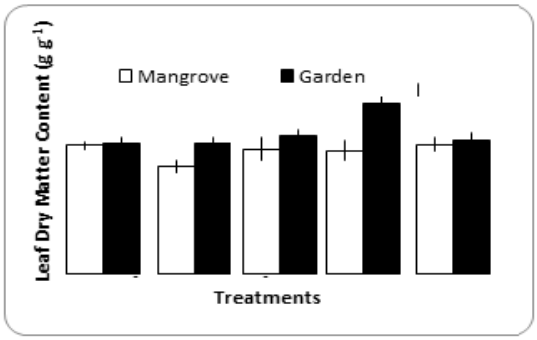

Fig 4: Mean LDMC of *R. racemosa* grown on amended mangrove and garden soil

Mean values of Leaf dry weight in both the garden and mangrove soil treatment were higher in the amended sets relative to the control. Responses to soil salinity during the early growth of mangrove seedlings have been reported as an important determinant of survival. Specifically, *Bruguiera. sexangula* exhibited a 45% reduction of its dry mass under medium saline soil when compared with low salinity conditions after three months of growth (Chen and Ye, 2014) and the report further suggested that growth and development may also be likely impaired at the exhaustion of the initial food reserves in the propagule. The high Leaf dry weight observed in amended mangrove and garden soil may be attributed to the influence of the nutrients in the amendment. Reports of Chen and Ye, (2014) attest to increase in biomass (leaf, shoot, branch growth) of several species of mangrove as *Rhizophora mangle Avicennia. germinans Ceriops tagal*et on nutrient addition. This is in agreement with the report of (Bhardwaj, 2014) that a good growing media would provide excellent anchorage, serve as nutrient and water repository among others with a consequential positive influence on seedling re-establishment in the field and productivity.

Various reports as Shipley and Vu (2002) have revealed that SLA is a reflection of previously stored resources and hence plant species with high SLA displays high productivity, and as well do better in resource-rich environments while plant species with low SLA do better in resource-poor environments

where retention of captured resources is a higher priority. Specific leaf area being a characteristic of the leafiness of a plant recorded an overall highest value in the garden soil amended with SD+RH+NPK combined. This high Specific leaf area may be attributed to the optimal growth condition in terms of nutrient and very low salinity in the amended garden soil as against the high saline soil of the mangrove soil. Evidence have shown that mangroves develops luxuriantly in lower salinities (Tahira *et al.,* 2015) as mangroves in high salinity environment spend more energy to maintain water balance and ion concentration rather than for primary production and growth (Clough, l984). This is in agreement with the findings of the present study. The differences in the SLA of within garden soil amended soil may be attributed to rate and availability of nutrients in the amendment. In the amended mangrove soil the highest SLA value was observed in the plot amended with SD, however this may have resulted from the liming effect of the organic residue of saw dust as (Haynes and Mokolobate, 2001) has observed liming effect of organic manure such as sawdust. The highest value for LMDC of *Rhizophora racemosa* was recorded in the amended garden soil when compared to amended mangrove soil. This corroborates the findings , of (Yong *et al.,* 2005) that high salinities reduces biomass, denaturing of terminal buds in *Rhizophora mangle* seedlings and as well the report of (Medina and Francisco, 1997) that elevated salinities causes reduction in leaf area and decrease in the total Nitrogen, Potassium and Phosphorus minerals of mangrove species. The high saline mangrove soil used in our research may be responsible for the low values of leaf characteristics of the propagules as its early growth and development were hampered by the salinity stress.

Conclusion: The study has demonstrated that the effects of salinity besides other environmental stressors on the mangrove plants specifically in the Niger delta region which is plagued with increasing sea rise level and the consequent high salinity content. Thus the amendment of garden soil with rice husk, saw dust and NPK as lone treatment or in combination may result in growth performance of the mangrove *Rhizophora racemosa;* it is thus recommended that the mangrove species can be successfully established and raised in amended garden soil for restoration purposes.

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