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Research Article

Ecophysiology and Seedlings Nutrient Contents of Forest Species *Ricinodendron heudelotii* (Mull. Arg.) and *Cola acuminata* (P. Beauv.) Influenced by Biofertilizer and Salinity

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Abstract

Ricinodendron heudelotii and *Cola acuminata,* are important plants species whose exploitation became abusive over the years due to the high utilization of their fruits as Non-Timber Forest Products (NTFP). They face to multiple challenges: a recalcitrance of seeds and salinity that limits regeneration. Therefore, regeneration seems an appropriate corridor for domestication with the optimization of plant mycorrhizal symbiosis otherwise called arbuscular mycorrhizal fungi (AMF). But alongside this domestication can be added constraints due to salinity of the soils in coastal region. This justify the aim of this work which was to study dynamics and evaluate the effect of salinity and mycorrhizal biofertilizers on the *Ricinodendron heudelotii* and *Cola acuminata* seedlings. To undergo this purpose, data were collected in two villages (Kendje and Njombeng) in Mungo division, and assay were conducted in greenhouse at the Faculty of Science, University of Douala-Cameroon. In the field, the identification of species was assessed over an area of 1600 m ²as well as the circumference of the trees, the individual number of *Ricinodendron heudelotii* and *Cola acuminata* among other species in order to assess their maturity and rarity in the forest. The second part was carried out in the greenhouse for the purpose of germination, obtaining seedlings and evaluating the effects of arbuscular mycorrhizal fungi (*Gisgaspora margarita*) as biofertilizers on the seedlings in saline conditions (0, 50, 100 and 200 mM of NaCl). Some parameters were determined on seedlings (plant growth, dry weight, distribution of ions in plant organs, chlorophyll and carotenoid content) over a period of twenty-six weeks. Globally *Cola acuminata* is more present in the forest (5.88%) than *Ricinodendron heudelotii* (1.47%) with average circumference of 105cm for both species. AMF-biofertilizer alleviates the deleterious effect of salt stress on plants growth parameters depending of concentration. Moreover, for those species, the distribution of Na^+ is more accumulated in the root's plants unlike K^+ and P which are more concentrated in the leaves.

Keywords

Ecophysiology, Mycorrhizal Biofertilizer, Salinity, NTFP

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1. Introduction

The world has a forest area of 4.06 billion hectares, which corresponds to 31% of total land area [10]. The Congo Basin represents approximately 70% of Central Africa's forests including Cameroon [2]. Since the 1990s, there is a renewed interest and awareness of the Congo Basin countries on the role played by Non-Timber Forest Products (NTFP) in the local economy of the Central African sub-region. Indeed, more than half of the rural population of this region participates in the harvest of NTFP to feed, care and pull revenues [22]. Non-woody forest products remain one of the main sources of products and equipment for food, health, habitat, and income for the population [3]. However in Cameroon, among the 3000 species estimated as useful, *Ricinodendron heudelotii* and *Cola acuminata* has long been contributed to food security, traditional pharmacopoeia and pharmaceutical industry. However, these two species are threatened by overexploitation [8]. To maintain the availability of those Non-Timber Forest Products, creation of large-scale nurseries for domestication are among other techniques scheduled. Domestication remains limited due to a problem of adaptation of nurseries and the weak development of their root system. Ferdermann [11] shown that rhizospheric microorganisms such as Arbuscular Mycorrhizal Fungi (AMF) stimulate overall plant growth when in symbiosis because they boost the development of their root systems. They play a key role in the production of phytohormones. The presence of AMF in the rhizosphere of forest plants species amplify their root system [5]. By obtaining all their carbon substances from their host plant, AM fungi help with their hyphal network, draw minerals and water from the soil, thus increasing their host's nutritional resources, mainly for phosphorus which also protects them against saline stress and several types of pests [4-14].

In Cameroon, halomorphic soils cover significant surfaces in the semi-rainwater areas and naturally along the atlantic coast; they impose the plants of severe salinity constraints creating particular ecological conditions at the roots level [21]. Sunderland [27] showed the difficulty of seed germination and phytosanitary constraints of *Diclidophlebia xuani* in experimental nursery in the forest on which salt concentration cause young leaves to curl and drop off from shoots. Thus, it would be important to define the level of tolerance of NaCl necessary to optimize the growth of young plants in the presence or not of the biofertilizer mycorrhizae. Thus, the overall objective of the study is to evaluate the dynamic and effect of AMF on the growth of seedlings of *Ricinodendron heudelotii* and *Cola acuminata* under saline conditions.

2. Materials and Methods

2.1. Study Sites

The work was conducted at the Kendje and Njoumbeng villages in the Littoral region of Cameroon. These villages are located in the fairly degraded forests that are subject to extensive human activities. The climate is equatorial, strongly influenced by the Guinean monsoon, and succession of four seasons in the year (two raining and two dry seasons). The average temperature in 27 ℃. The vegetation essentially includes large marshy areas consisting mainly of degraded mangrove. Then, the experiment was carried out on greenhouse at the research site located at the Faculty of Sciences of the Douala University (4°02'53N and 9°42'15E). It's tropical climate average temperature 26.2 ℃ and 19 m altitude.

2.2. Plant Material

The seeds of *Ricinodendron heudelotii* and *Cola acuminata* are local varieties harvest and stored at the Institute of Agronomic and Development Research (IRAD) of Nkolbisson Yaounde-Cameroon. Seeds before the cultivation were surface sterilized with 70% (v/v) ethanol solution for 15 minutes, then rinsed several times with distilled water. These seeds were left pregerminated beforehand (Figure 1).

A B

Figure 1. *Pre-germination of Ricinodendron heudelotii (A) and Cola acuminata (B).*

2.3. Interaction Between *Ricinodendron heudelotii*, *Cola acuminata* and Their Environment

A florist statements in the degraded forest of the localities of Njoumbeng and Kendje Bwapaki was made. A total of 10 quadrats with surface plots 10m × 10m = 100 m^2 (S1) are measured using a decameter and delimited by stakes and rally rods on the sites. The number of *Ricinodendron heudelotii* and *Cola acuminata* trees and all other forest species are identified and inventoried using the application plant net as well as simple observation. Then, circumferences of each tree were measured. The previous surface was doubled to obtain a surface S2 (100 x 2 = 200 m^2). Then it was doubled to obtain a surface S3 (400 m^2). The process thus repeated until the minimum area is reached on an area of 1600 m^2which is the surface inside which the number of species is not increasing any more.

2.4. Trial Device

Seeds of *Ricinodendron heudelotii* and *Cola acuminata* were surface sterilized with 70% (v/v) ethanol solution for 15 minutes, then rinsed several times with deionized water, then put into germinative container filled with decontaminated sand. After three weeks, when primordial leaves of plants were fully established. Pregerminated seedlings were transferred to 7 L plastic pots (Teku Container MCC 31; Germany) filling with 5 kg of a 3:1 (w/v) mixture of heat pasteurized (70 ℃ for 24 h) dry soil substrate and sterilized sand. They were planted at 5 cm depth one per hole. The experiment was carried out in two blocks with randomized complete design with height treatments and four replicates for a total of 32 pots per block. All plants were fertilized with a nutrient standard solution containing 0,005% of NO_3; 0,002% of Cl; 0,005% of CO_2; 0,001% of Pb; 0,02% of Na; 0,002% of Mg; 0,002% of K; 0,0005% of Fe; 0,01% of (H_2SO_4); 0,8% of $CaSO_4$ (Wacquant, 1974).

2.5. Fertilization

Strains of mycorrhizal fungi come from the regional laboratory for applied biological and microbiological control of IRAD located in Yaounde-Cameroon. The inoculum consisted of propagules of *Gisgaspora margarita.* In planting hole, 100g of inoculum was added [20] of each seedling singly or in combination with NaCl. One plant was grown in the middle of each pot. Three different concentrations (50, 100, 200 mM) of NaCl were supplied. Untreated plants (free of biofertilizer and NaCl) were used as control. Plants were watered with 50 ml of nutrient standard solution every morning. The daily quantity of solution added to each pot were the same for all treatments. The mycorrhizal inoculum were applied only once at the beginning of the experiment while each week, a quantity of 50ml of different concentrations of saline solutions was brought to the corresponding pots.

2.5.1. Plant Growth Parameters Determination

Two weeks after transplantation and during the vegetative stage, number of leaves and plant height were assessed at regular intervals of 14 days. The leaf area was measured at the end of study (this was the average of all plant leaves areas). This leaf area (S in cm^2) was calculated as followed: $S = 0.8 \times L \times l$, in which L and l are respectively the length and width of each leaf.

2.5.2. Assessment of Chlorophyll and Carotenoids Contents in Leaves

The chlorophyll and carotenoids contents were determined according to the Lichtentaler method. This determination is based on the principle that acetone is a solvent that will extract chlorophyll pigments from plant cells. The concentrations estimated in µg/ml of pigments are calculated according to the method described by Lichtentaler (1987).

$$\text{Chlorophyll a} = 12.25\ (\text{OD Chlb}) - 2.79\ (\text{OD Chla})$$

$$\text{Chlorophyll b} = 21.5\ (\text{OD Chla}) - 5.1\ (\text{OD Chlb})$$

$$\text{Chlorophyll a + chlorophyll b} = 7.15\ (\text{OD Chlb}) + 18.71\ (\text{OD Chla})$$

$$\text{Carotenoids} = ((1000 \times \text{OD Carotenoids}) - ((1.82 \times \text{Chla}) + (85.02 \times \text{Chlb}))) / 198$$

2.5.3. Biomass and Nutrients Determination

Plants were harvested 26 weeks after planting. Data on weight fresh leaves, stems and roots were recorded. Leaves, stems and roots were separately dried at 45 ℃ for 72 h and their dry weights determinated. These different parts of plant were isolated to analyse N, P, K, Na and total polyphenols contents. N is determined by mineralization by acid attack of 0.1g of sample, distillation by steam distillation and dosage with 0.01N sulfuric acid (Kjedahl method, standard NF ISO 11261). P by colorimetry with ammonium phosphomolybdate yellow after calcination of 1g of dry matter at 450 ℃ for four hours and extraction with 1N nitric acid. Reading at 430 nm wavelength. (Standard NF EN 14672). K and Na are determined by flame emission spectrometry by direct reading in the extract digested with 1N nitric acid (AFNOR Standard NF T 90-019). The total polyphenols are measured by the method of RIBEREAU-GAYON (1968). It is done using the reagent Folin-Ciocalteu (FC).

2.6. Statistical Analysis

Statistical analysis were performed using R version 2020 software. The collected data were subjected to an analysis of variance. The comparison of the means, in the event of sig-

nificant results, was made by Duncan's test at 5% level. Multifactorial ANOVA was used to estimate whether different concentration of NaCl and mycorrhizal inoculation, single or in combination had a significant influence on the measured parameters.

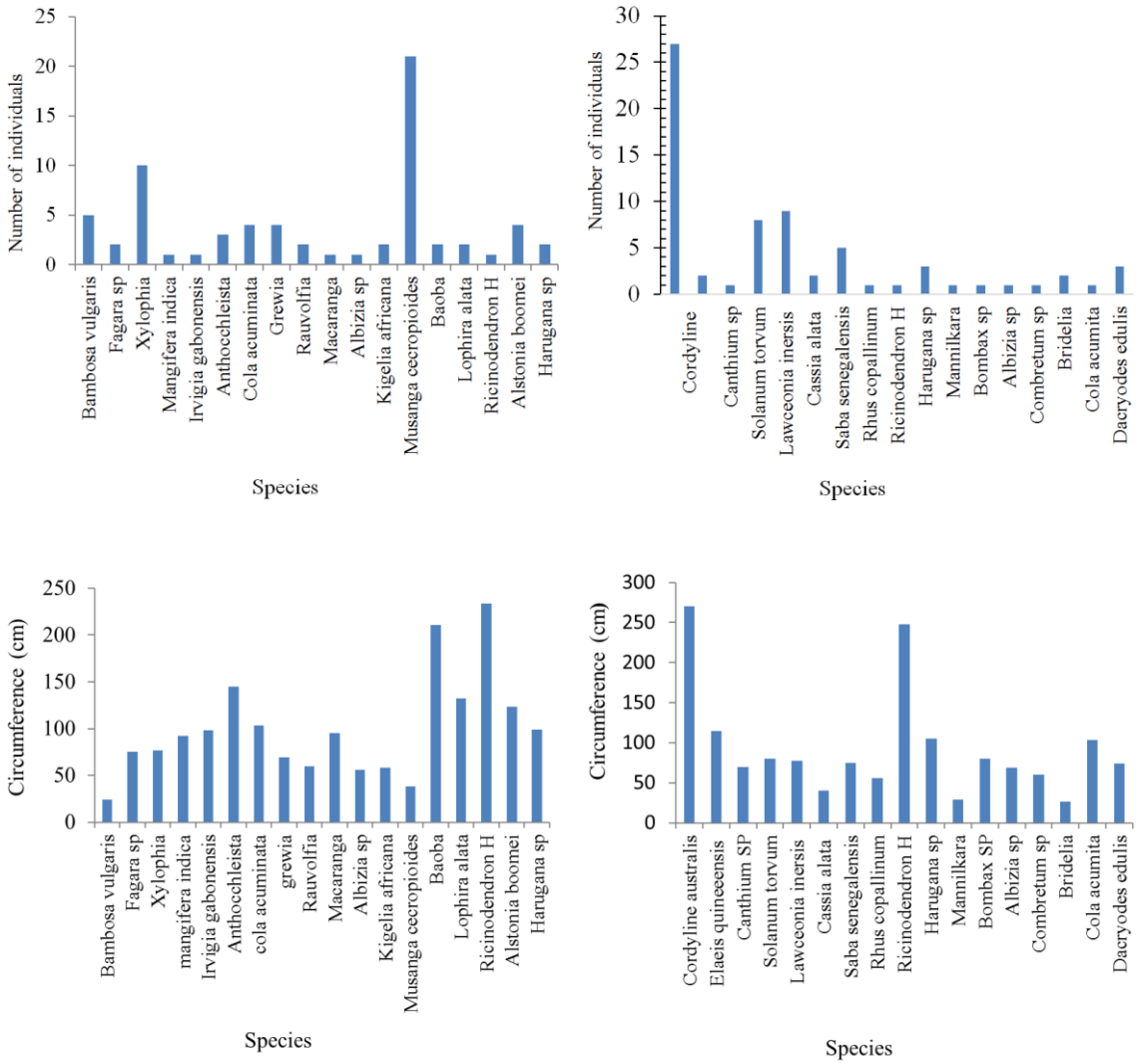


Figure 2. *Number of individuals and their circumferences (A: Kendje; B: Njoumbeng).*

3. Results

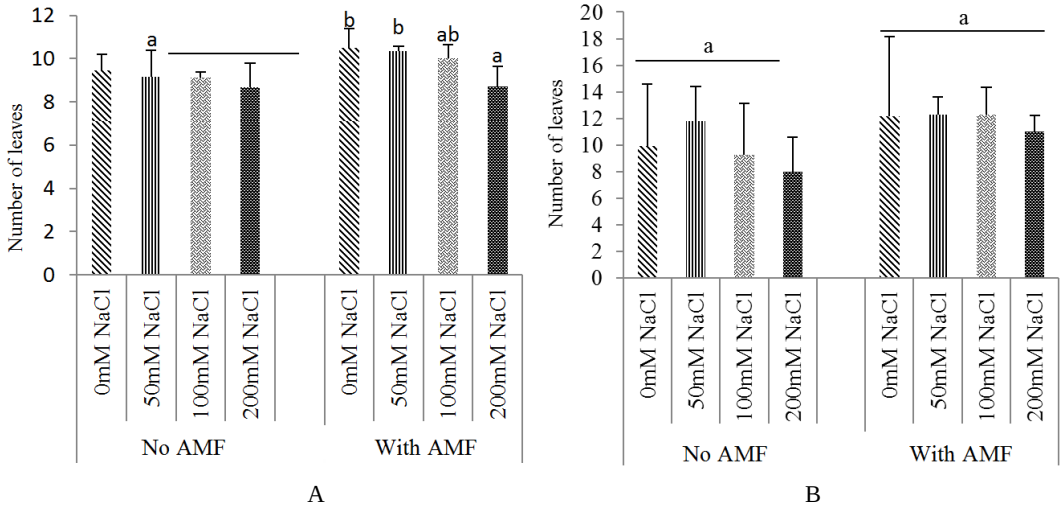
3.1. Identification and Interaction of Plant Species in the Two Sites

Many plant species interact with *Riciniodendron heudelotii* and *cola acuminata*, like: mosses, algae, lichens and ferns. Mosses and ferns of *Microsorum punctatum* genus lives on the trunk of *Ricinodendron heudelotii*. Figure 2 presents the number of individuals according to species and their circumference in the two villages. At Kendje, species such as *Bambosa vulgaris*, *Xylophia* sp, *Musanga cecropioides* and *Cola acuminata* are the most present than *Ricinodendron heudelotii*. The species like *Lophira alata*, *Harugana* sp, *Macaranga*, *Anthocehleista*, *Ricinodendron heudelotii* have a greater circumference at breast height in the site. Similarly, at the Njoumbeng site, *Cordyline australis* and *Lawceonia inersis* are more represented than *Ricinodendron heudelotii* and *Cola acuminata* in the forest. The species *Cordyline australis*, *Elais guineensis*, *Ricinodendron heudelotii*, *Harugana* sp. and *Cola acuminata* have a greater circumference at breast height.

3.2. Effects of Biofertilizer and Salt Stress on Growth Parameters of the Two Species

Figure 3 presents the effect of salinity and Arbuscular

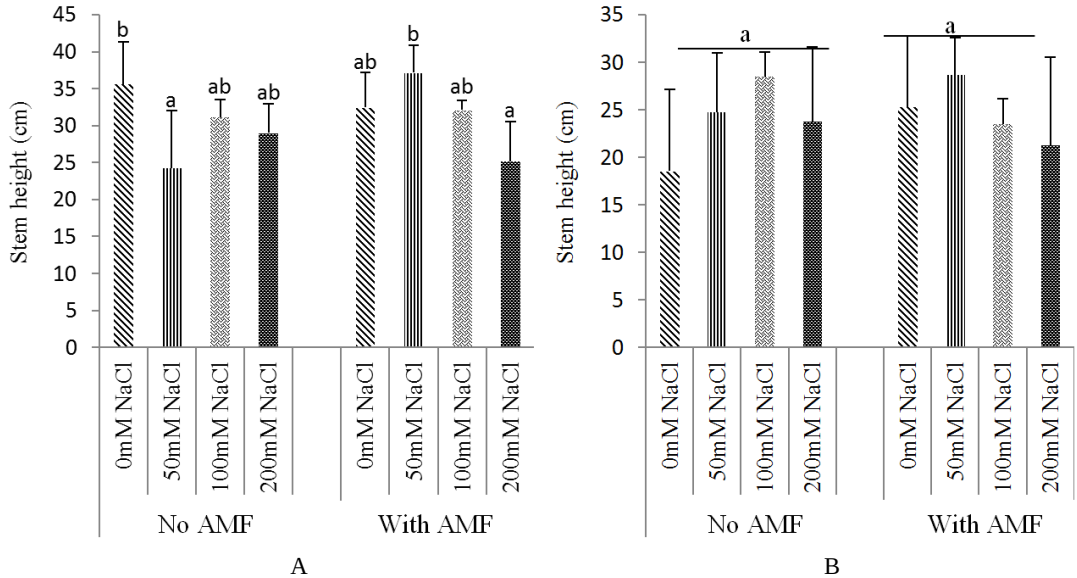
Mycorrhizal Fungi on the variation in the average number of leaves of the species. Overall, *Ricinodendron heudelotii* and *Cola acuminata* plants inoculated with biofertilizer show a higher average of leaves 10.50 ± 0.51 and 12.13 ± 3.49 respectively, compared to uninoculated at 50 mM, 100 mM, 200 mM NaCl. However, these differences are not significant ($p>0.05$) for these two species of non-timber forest products.



AMF= Arbuscular Mycorhizal Fungi. Means with the same letters are not significantly different at 5%

Figure 3. *Effect of mycorrhizae inoculation on number of leaves: Ricinodendron heudelotii (A) and Cola acuminata (B) under salt stress.*

The plants height varied according to salinity and biofertilizer (Figure 4). The inoculation of early-stage of *R. heudelotii* plants increased their height at 50 mM of NaCl with a drop in growth when the concentrations increase. Nevertheless, there were some significant differences between the treatments (P $p<0.05$). Similarly, inoculated seedlings of *Cola acuminata* performed well in height (25.55 ± 4.35 cm) without NaCl supply. This growth increase at 50 mM (28.66 ± 2.29 cm) and started to decline with increasing salinity.

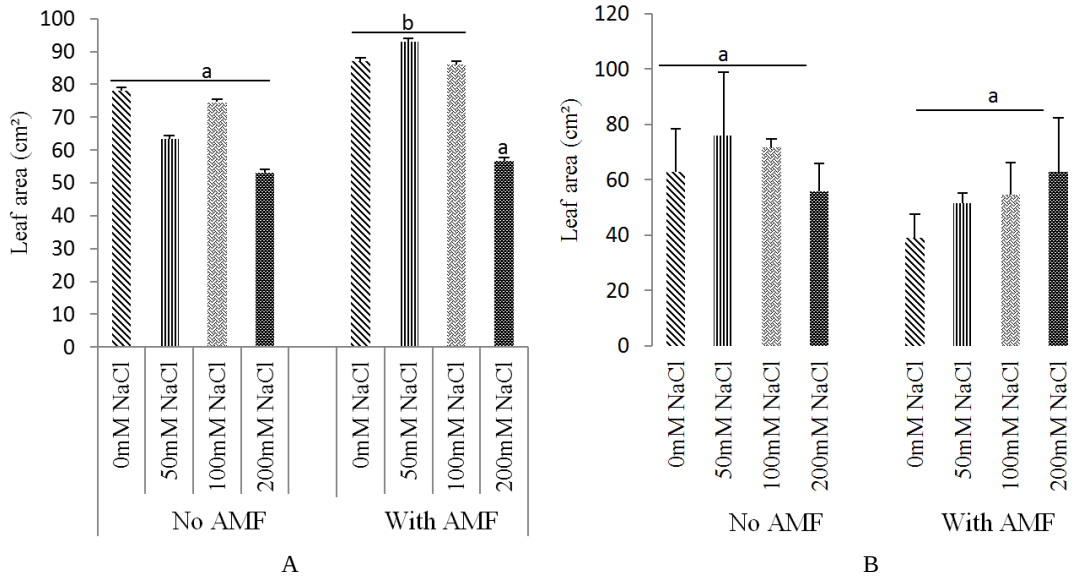


AMF= Arbuscular Mycorrhizal Fungi. Means with the same letters are not significantly different at 5%

Figure 4. *Effect of mycorrhizae inoculation on height of the plants under salt stress: Ricinodendron heudelotii (A) Cola acuminata (B).*

Mycorrhizal biofertilizer and salinity significantly influenced leaf area of *Ricinodendron heudelotii*. This is how the inoculum induced a significant increase ($p<0.05$) of leaf area of the plants with increasing concentration of salinity except

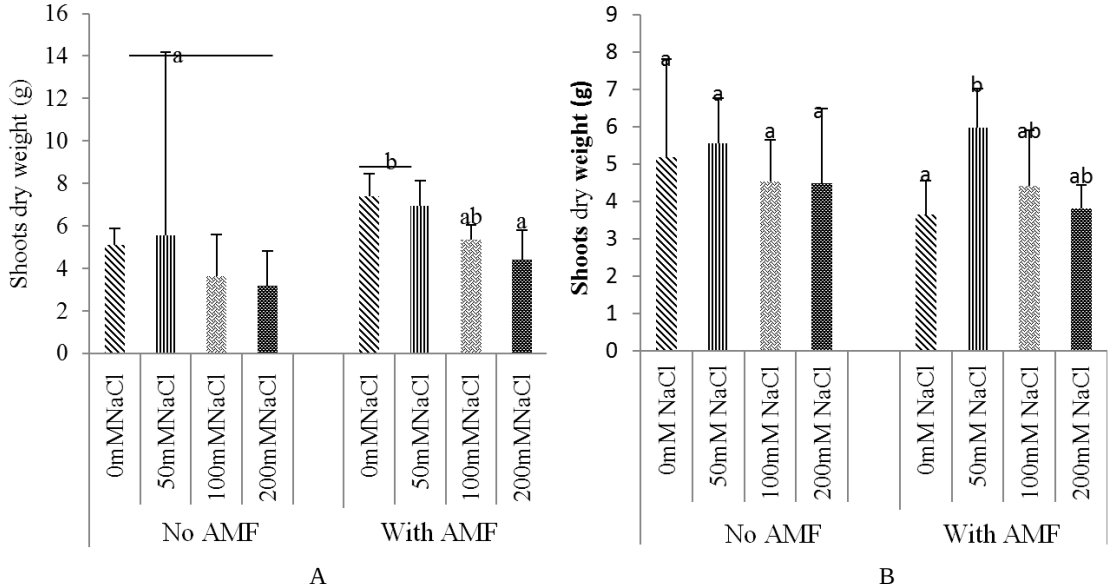
at 200mM NaCl. Concerning *Cola acuminata*, non-inoculated plants as well as those inoculated with increasing salt stress did not show a significant difference (p>0.05) on the leaf area.



AMF= Arbuscular Mycorhizal Fungi. Means with the same letters are not significantly different at 5%

Figure 5. *Effect of mycorrhizae inoculation on the leaf area as influenced by salinity: Ricinodendron heudelotii (A) and Cola acuminata (B).*

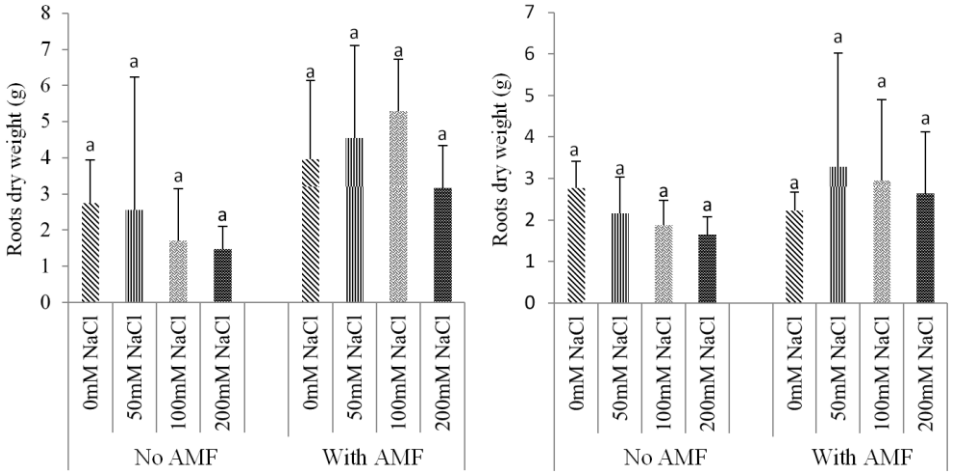
Figure 6 shows the variation of dry biomass in the aerial part of the two species according to different concentrations of NaCl and AMF. The shoots dry weight (SDW) was influenced by salinity singly or in combination with the mycorrhizal inoculum. Overall, the increase of salt stress leads to a drop of dry mass of plants. Ricinodendron plants that received 50, 100, 200 mM concentrations of NaCl with biofertilizer had an average of 6.93 ±0.68 g, 5.33 ±0.42 g, 4.39 ±0.81 g of stem dry weight, respectively. Similarly, Cola plants treated with 50, 100, 200 mM concentration of salt with the inoculum have respectively an average of 5.98±0.60 g, 4.4±0.86 g, 3.97±0.42 g of SDW.



AMF= Arbuscular Mycorrhizal Fungi. Means with the same letters are not significantly different at 5%

Figure 6. *Effect of NaCl and biofertilizer on SDW: Ricinodendron heulotii (A) and cola acuminata (B).*

Application of NaCl singly or in combination with biofertilizer led to a significant increase in roots dry weight (RDW). The root dry weight of *Ricinodendron heudelotii* and *Cola acuminata* was positively influenced by treatements. However, the plants were subjected to salt stress, a decrease of their root dry weight was observed. In all cases, there was no significant difference ($p>0.05$) between the treatments applied (Figure 7).

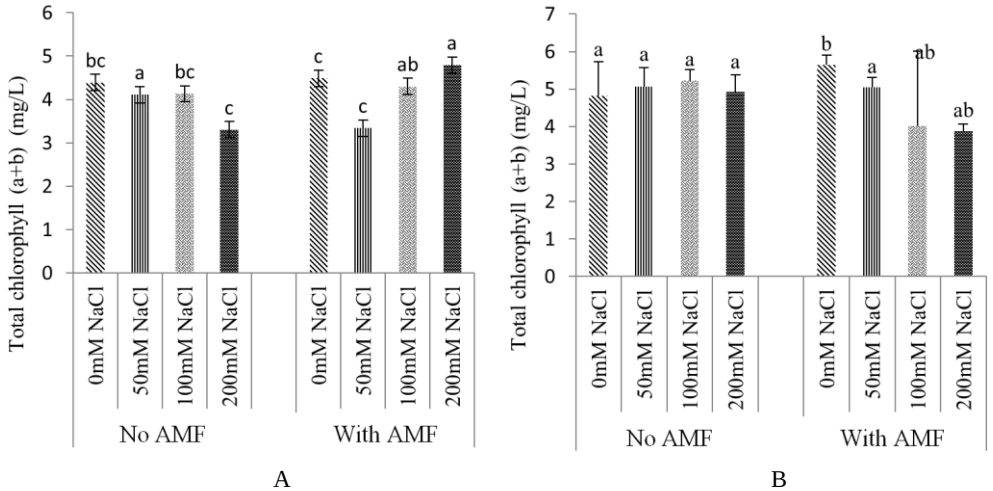


AMF= Arbuscular Mycorrhizal Fungi. Means with the same letters are not significantly different at 5%.

Figure 7. *Effect of NaCl and biofertilizer on RDW: Ricinodendron heudelotii (A) and Cola acuminata (B).*

3.3. Effect of Biofertilizer and Salt Stress on Total Chlorophyll and Carotenoids Contents

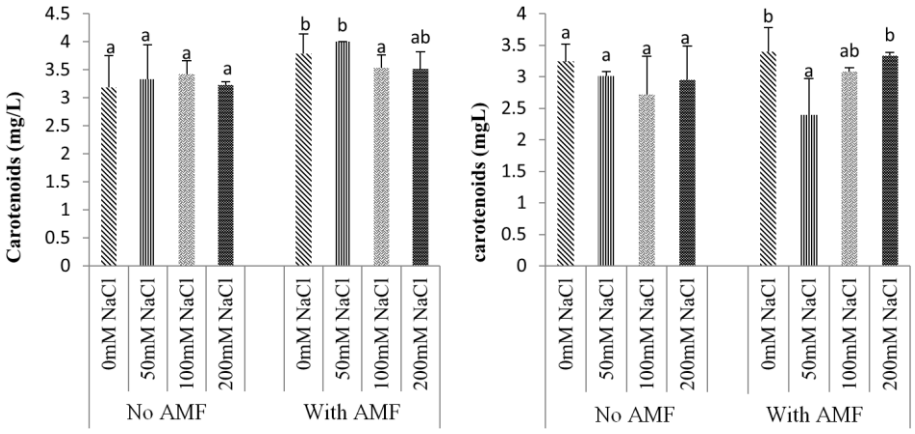
Overall, salinity has significant influence on total chlorophyll rate of *Ricinodendron heudelotii* seedlings despite inoculation or not (Figure 8A). As for *Cola acuminata* AMF inoculated plants has significant total chlorophyll content ($p<0.05$). The rate decreased with encreasein salinity (5.816 ±0.06 mg/L at 0mM of NaCl to 3.606 ± 0.04mg/L at 200mM of NaCl (Figure 8B). Otherwise, the greatest total chlorophyll content are obtained on unstressed saline plants (0mM of NaCl).



AMF= Arbuscular Mycorrhizal Fungi. Means with the same letters are not significantly different at 5%.

Figure 8. *Effect of mycorrhizae on total chlorophyll of Ricinodendron heudelotii (A) and Cola acuminata (B) under salt stress.*

Figure 9 shows the carotenoids content of *R. heudelotii* and *C. acuminata* plants under inoculation and salt constraint. For non-inoculated seedlings, salinity has no significant difference on carotenoids pigments ($p>0.05$). There is relationship between inoculated treatments for the two species. Thus, AMF significantly influence carotenoids content. At 0mM NaCl, carotenoids values were higher with 3.39mg/L and 3.44mg/L for *R. heudelotii* and *C. acuminata* respectively.



AMF= Arbuscular Mycorrhizal Fungi. Means with the same letters are not significantly different at 5%.

Figure 9. *Effect of mycorrhizae on carotenoids content of Ricinodendron heudelotii (A) and Cola acuminata (B).*

3.4. Effect of NaCl and Biofertilizer on Some Physiological Parameters of *Ricinodendron heudelotii* and *Cola acuminata*

3.4.1. Nutrients Content in Different Parts of *Ricinodendron heudelotii* Seedlings

Table 1. *Effect of salinity on non-inoculated Ricinodendron heudelotii plants content.*

Amount (µg/gMS)	Organs	NaCl (mM)				
		0	50	100	200	p
N	Leaves	1339.67 ±51.67a	3889.00±326.025c	4196.00±0.00c	3094.00±66.0b	p<0.05
	Leaves	410.81±12.88a	302.05±20.47a	283.92±0.00a	309.52±5.43a	p>0.05
P	Stems	233.41±7.32b	161.50±10.94a	166.49±.00a	160.55±2.82a	p>0.05
	Roots	238.44±7.49b	184.42±12.49a	194.90±.00a	190.83±3.35a	p>0.05
	Leaves	2052.15±63.93b	1652.80±113.02a	2627.48±0.00c	2143.52±36.87b	p<0.05
K^+	Stems	1919.73±59.80b	2091.83±143.04b	1668.62±0.00a	1816.58±31.24ab	p>0.05
	Roots	1855.23±57.79b	1652.80±113.02b	1549.91±0.00a	1405.85±24.18a	p>0.05
	Leaves	227.50 ±8.75a	197.75 ±16.57a	297.55 ±0.00b	267.12 ±5.68b	p<0.05
Na^+	Stems	249.46 ±9.60a	238.38 ±19.99a	249.47 ±0.00a	244.27 ±5.20a	p>0.05
	Roots	272.80±10.49a	309.29 ±25.93ab	351.20 ±0.00b	343.88 ±7.31b	p<0.05

Means followed by different letter are significantly different by Duncan's test ($p < 0.05$).

Table 2. *Effect of salinity on inoculated Ricinodendron heudelotii plants content.*

Amount (µg/gMS)	Organs	NaCl (mM)				
		0	50	100	200	p
N	Leaves	1508.00±184.0b	3972.00±.00b	4279.67±164.83ab	4139.67±170.60ab	p<0.05
	Leaves	346.66±1413.3b	324.00±.000b	340.66±1311.45b	259.6±1069.51a	p>0.05
P	Stems	610.00±5903.0c	677.00±0.0b	859.33±521.63a	555.66±432.3c	p<0.05
	Roots	1015.6±2390.7a	2408.0±2.92.3b	1704.66±327.66b	1478.0±721.0a	p<0.05
	Leaves	944.58±222.31b	1757.60±59.68c	1513.87±29.11b	703.54±73.34a	p<0.05
K^+	Stems	1022.02±240.5ab	1297.93 ±44.07a	1145.08 ±22.02a	1081.12±52.73b	p>0.05
	Roots	944.58±222.31a	1519.58±51.60b	1145.08 ±22.02a	832.87±50.38a	p>0.05
	Leaves	153.64±36.16b	236.98 ±9.47b	231.97±4.46b	186.80 ±13.99a	p<0.05
Na^+	Stems	153.64±36.16b	265.06 ±9.00a	278.15 ±5.34bc	312.80 ±15.25c	p<0.05
	Roots	153.64 ±36.16a	336.77 ±6.55c	330.02 ±6.34ab	206.80 ±13.99ab	p<0.05

Means followed by different letter are significantly different by Duncan's test ($p < 0.05$).

Nitrogen (N) content in *R. heudelotii* leaves inoculated or not is higher in the treatments with 50, 100, 200 mM NaCl. The phosphate content is higher in the leaves than the stems and roots plants without biofertilizer, in addition *R. heudelotii* with inoculated seedlings showed a higher phosphate content in the leaves compared to stems and roots. Potassium was more concentrated in the leaves too, unlike sodium, with more concentration in roots, (Tables 1 and 2).

3.4.2. Nutrients Content in Different Parts of *Cola acuminata* Seedlings

Salt stress significantly reduced nitrogen content in leaves of non-inoculated plants. The control recorded the highest value. Phosphorus, potassium and sodium values increase with different saline solution until 200 mM where values drop in leaves, stems and roots. Analysis of variance did not reveal any significant difference between the nutrient's contents by Duncan's test at those NaCl concentrations (table 3).

Inoculated plants with AMF show high N contents in leaves at 50, 100 and 200 mM NaCl compared to control. Phosphorus is more accumulated in the roots of *Cola acuminata* plants compared to the leaves and stem, especially at 100 mM NaCl. Potassium is more concentrated in leaves. On other hand, sodium is more present in the roots. Globally, there were no significant difference between the nutrient's contents by Duncan's test at those NaCl concentrations (table 4).

Table 3. *Effect of salinity on non-inoculated Cola acumminata plants content.*

Amount (µg/gMS)	Organs	NaCl (mM)				
		0	50	100	200	p
N	Leaves	2464.00±658.27a	2149.00±622.56a	1923.00±481.00a	1848.67±288.702a	p>0.05
	Leaves	117.48±34.03a	155.18±24.24a	273.22±72.98a	141.78±35.44a	p>0.05
P	Stems	117.48±34.03a	124.23±19.40ab	218.98±58.50b	134.20±33.55ab	p>0.05
	Roots	111.21±32.22a	130.42±20.37a	258.40 ±69.03a	142.79±35.70a	p>0.05
	Leaves	830.05±240.44a	1298.0 ±202.75a	1940.76±518.44a	1144.16±286.04a	p>0.05
K^+	Stems	359.02±104.00a	1054.15±164.66ab	1283.45±342.85b	1017.84±254.46ab	p>0.05
	Roots	975.09±282.45a	1054.15±164.66a	1507.71±402.76a	822.71±205.67a	p>0.05
Na^+	Leaves	133.01±38.52a	202.89±31.69a	273.22±72.98a	238.04±59.51a	p>0.05

Amount (µg/gMS)	Organs	NaCl (mM) 0	50	100	200	p
	Stems	108.81±31.52a	244.57±38.20ab	354.50±94.70b	280.96±70.24ab	p>0.05
	Roots	193.23±55.97a	244.57±38.20a	354.50±94.70a	238.04±59.51a	p>0.05

Means followed by different letter are significantly different by Duncan's test ($p < 0.05$).

Table 4. *Effect of salinity on inoculated Cola acumminata plants content.*

Amount (µg/gMS)	Organs	NaCl (mM) 0	50	100	200	p
N	Leaves	1795 ±422.54a	2613.33 ±88.62b	2151.33 ±41.33ab	2350.67±114.66a	p>0.05
	Leaves	138.13 ±32.51a	116.59 ±11.43a	169.75±3.26a	106.93±9.60a	p>0.05
P	Stems	91.74 ±21.59a	138.66±4.70b	167.82±3.22b	155.00±7.59b	p<0.05
	Roots	101.57 ±23.90a	160.70±25.32b	170.40 ±3.27b	147.82±7.21b	p>0.05
	Leaves	944.58±222.31a	1757.60±59.68b	1513.87±29.11b	1503.54±73.34b	p<0.05
K^+	Stems	1022.02±240.54a	1297.93±44.07a	1145.08±22.02a	1081.12±52.73a	p>0.05
	Roots	944.58±222.31a	1519.58±51.60a	1145.08±22.02a	1032.87±50.38a	p>0.05
	Leaves	153.64 ±36.16a	236.98 ±9.47b	231.97±4.46b	286.80±13.99b	p<0.05
Na^+	Stems	153.64 ±36.16a	265.06 ±9.00b	278.15±5.34b	212.80±15.25b	p<0.05
	Roots	153.64 ±36.16a	336.77 ±6.55b	330.02 ±6.34b	286.80 ±13.99b	p<0.05

Means followed by different letter are significantly different by Duncan's test ($p < 0.05$).

4. Discussion

4.1. Identification of Species

In Kendje and Njoumbeng villages, many species have well-developed circumference amount the Non-Timber Forest Products (NTFP) such as *Ricinodendron heudelotii*, *Mangifera indica*, *Cola acuminata*. However, these species were not numerous in the site due to their overexploitation and the other hand to the destruction for agricultural or firewood purpose. This result confirm that land use changes in the Congo Basin and could be the major factors for degradation and deforestation [26-30].

In both sites, the presence of several species was noted but their number per unit area was few. This would be due to their overexploitation by local population. *Musanga cecropioides* and *Cordyline australis* are numerous because they are species with less socio-economic interest as show by the previous study [9-24]. This leads to a real problem of conservation and sustainable management of NTFP and rational use of land [25].

4.2. Growth Parameters and Photosynthetic Pigments

Plants growth was effective with salinity up to certain concentration which caused the drop of growth parameters. Also, plants inoculated has a positive effect on seedlings by inhibition NaCl action. These results corroborate of those of authors who have claimed that growth and plant production are improved by the mycorrhizal symbiosis even when these plants grow on relatively mineral or saline constraints [7, 13-16]. Similary, previous study mentioned that the contribution of mycorrhizal fungi make easy to facilitate the increased tolerance of plants under environmental drought stress [19].

Shoot dry weight (SDW) was influenced by the application of NaCl alone or in combination with AMF-inoculated up to certain threshold. Overall, the increase of saline concentration beyond 50 mM leads to drop of dry biomass of the plants [18]. These authors reported that high salinity in soil negativily affects most morphological parameters of *L. scindicus*. The addition of AMF allowed the improvement of those parame-

ters. AM symbiosis-induced plant development has been linked in part to increase P feeding, enhanced water uptake from the soil, and increased osmotic potential of soil mediated by mycorrhizal fungi. The roots dry weight of *R. heudelotii* and *C. acuminata* seedlings was positively influenced by the presence of the mycorrhizae which means that they promote root expansion but this growth begins to be slowed down when salt stress increases from 50mM [16]. These results are consistent with those obtained by [6] who showed that the length and density of absorbent hairs decrease significantly depending on the dose of salt at *Vicia faba*. As well as those of [23], who explained that the osmotic effects of salt stress can also limit the growth of roots, which limit the possibilities of absorption of the nutrients of the soil.

Many studies have reported a significant drop in chlorophyll and carotenoids concentrations when plants are exposed to salt, and varied perspectives on the impact of salinity on these photosynthetic pigment contents have been reported [28]. The loss of chlorophyll in salt-stressed plants has long been assumed to be a marker of oxidative stress that occurs during chlorophyll production. The salt stress damages chloroplasts and increases the activity of chlorophyll-degrading enzymes such as chlorophyllase [17]. In this study, the chlorophyll content in leaves, was significantly influenced by both salinity and AMF inoculation [32]. There was a reduction in leaf total chlorophyll content due to the increasing salt levels, possibly as the result of the repression of specific enzymes of the photosynthesis system as well as the reduced uptake of nutrients such as nitrogen (N) and magnesium (Mg) for chlorophyll biosynthesis as reported in a study on *Linum usitatissimum* plants [16].

4.3. Nutrients Content in Different Parts of Seedlings

Leaf nitrogen content of inoculated and non-inoculated *R. heudelotii* and *C. acuminata* plants increases globally with salinity. The rate begins to drop up to a certain concentration of NaCl. These results corroborate with those obtained by [33] which reported a decrease in metabolic activities caused by the oxidative stress generated by the excessive salinity of the environment. In most cases, nutrients analysed are more concentrated in the leaves than other parts of the two plants species despite some exceptions, especially when the plants have been inoculated. This could be attributed to the ability of mycorrhizae to supply nutrients throughout hyphae network draw minerals and water from the soil, thus increasing their host's nutritional resources, mainly for phosphorus which also protects them against saline stress and several pests [1-15].

The accumulation of sodium ion in the various organs of plants according to growing doses of NaCl particularly in the roots suggests that arbuscular mycorrhizal fungi would promote the retention of Na^+ at the root levels as showed by [12-29].

5. Conclusion

The study aimed to assess interactions between *Ricinodendron heudelotii* and *Cola acuminata* with their environment, then evaluate the effect of saline stress and mycorrhizal biofertilizers on some parameters of seedlings. Both species are in symbiosis with epiphytes and interact ~~ion~~ with other local trees. Increasing doses of NaCl alone or in combination with arbuscular mycorrhizal fungi had a positive effect on shoot length, number of leaves, dry weights, leaf area of seedlings up to a tolerance threshold as well as photosynthetic pigments. Specifically, mycorrhizal association improved the salt tolerance of the plants by enhancing their nutrient contents of their leaves, but extend in shoots and roots at moderate salt concentrations. Inoculation with AMF therefore is an important practice, it may protect plants against salinity by alleviating the salt-induced oxidative stress.

Abbreviations

NTFP: Non-Timber Forest Products
AMF: Arbuscular Mycorrhizal Fungi

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Conflicts of Interest

The authors declared no conflict of interest.

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