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Growth and Development of *Rhizophora* spp. Seedlings on Different Substrates and Insertion Level in the Wouri Estuary Mangrove (Douala, Cameroon)

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ABSTRACT

Mangroves are threatened with extinction worldwide. Many mangrove reforestation projects have been developed, but very few have achieved their restoration objectives. With the ambitions to contribute for rehabilitation of mangrove ecosystems, the aim of this study was to assess the effects of substrate composition and level of insertion on the growth and development of *Rhizophora* seedlings in nurseries. The experiment was conducted in a semi-lighted nursery set up in situ. *Rhizophora* propagules were reared on three types of mangrove substrates (type 1: 75% of mud and 25% of sand; type 2: 50% of mud and 50% of sand, and type 3: 25% of mud and 75% of sand) with different levels of insertion (at ¼; ½ and ¾). Seedling heights and diameters were recorded, as well as the time of appearance of the first leaves and the variation of number of leaves. The greatest average height (40.2 ± 7.48 cm) and the highest mean diameter (1.2 ± 0.01 cm) were obtained for the substrate composed of 75% mud and 25% sand. The best growth and development of seedlings were recorded on the substrate type 1 with an insertion at ¼. Seedling production at ¼ insertion of substrate type 1 appeared the most effective combination. The production of *Rhizophora* seedlings on the substrate combining 25% Sand and 75% Mud with an insertion level at 1/4 seems to be the best method for the complete restoration and rehabilitation of the mangrove ecosystem of the Wouri estuary.

Keywords: Wouri estuary, propagules, rehabilitation, substrate, sand, mud.

INTRODUCTION

Numerous projects to restore severely degraded mangroves are being implemented around the world [Djamaluddin et al., 2019; Leal and Spalding, 2022]. These projects involve the reforestation of native species and the study of various techniques related to habitat recovery, such as propagule maintenance, seedling production, assessment of the optimum planting and seedling transplanting period, planting procedures, crop and plantation monitoring, assessment of environmental factors, in particular light, temperature and edaphic parameters [Ellison et al., 2020; Silva & Maia, 2019].

In Africa, some projects to restore damaged mangroves have been reported in several countries, including Benin, Cameroon, Côte d'Ivoire, Kenya, Mozambique, Nigeria, Senegal, and Tanzania [Bocquet, 2018; Egnankou, 2009; Kairo et al., 2001; Lopez-Portillo et al., 2017; Nakouzi et al., 2018; SOMN, 2022; Zabbey and Tanee, 2016].

Since some years now, mangroves in Cameroon have been the subject of several studies as: descriptive studies on biological and socio-economic potential [Nfotabong-Atheull et al., 2013]; highlight their ecological role in protecting the coast and synthesising approaches, results, challenges and constraints from many other studies [Ajonina et al., 2016]. A number of reforestation projects have been launched to restore mangroves in Cameroon, including CAMERR Project Restoration of mangrove ecosystems. Started on 2020, the project aims to restore 1000 hectares of mangroves and the objective is to support the restoration and sustainable management of the mangrove, the protection of biodiversity and the strengthening of local resilience around the coastline of Douala city to thus contribute to the resilience of ecosystems and residents of the mangrove periphery.

A decade before the above project, funded by the International Tropical Timber Organisation (ITTO), the Participatory Rehabilitation and Management of Mangroves and Watersheds in the Coastal Zone of the Douala-Edea National Park, abbreviated to the "Douala-Edea Mangroves Project", was submitted by the Government of Cameroon, initiated and implemented by the Cameroon Ecology (Cam-Eco) carried out between 2010–2013.

Despite a long tradition of mangrove restoration, it is still difficult to ensure the success of restitution. However, the natural development conditions are optimal, and seeds are abundant and available in all seasons [Rovai et al., 2012; Sarwom et al., 2022]. The failures observed in mangrove reforestation are linked with pre-planting, planting and post-planting procedures [Lewis et al., 2019; Mukherjee et al., 2015]. Among main failures, there are the poor use of ecological knowledge describing the conditions in which mangroves thrive and insufficient knowledge of the physical disturbances linked to the geomorphological processes of mangrove ecosystems [Balke et al., 2013; Elliott et al., 2016; Laurenda et al., 2022; Rovai et al., 2012], lack of involvement of local communities, inappropriate choice of species and plantation sites, and inadequate monitoring of plantations. Some projects fail to meet technical criteria such as substrate composition and the degree to which propagules of *Rhizophora* spp. are inserted when setting up nurseries.

Most projects involve planting *Rhizophora* spp. propagules in nurseries, but few of these projects have detailed monitoring and follow-up plans, and in most cases little documentation or recommendations for modifications to the original planting plan exist [Lee et al., 2019; Kodikara et al., 2017].

With the ambitions to contribute for restoration of mangrove ecosystems through reforestation, a preview work was carried out to evaluate the impact of light and conservation period of propagules on seedling growth of *Rhizophora* spp. [Boubakary et al., 2019]. This paper reports the follow of preview work and the aim is to examine the effect of substrate composition and the level of insertion on the growth and development of seedlings of the genus *Rhizophora*.

MATERIAL AND METHODS

Study sites

The work was carried out in the mangrove swamps of the Wouri river estuary, which form part of Cameroon's large mangrove swamp complex. The study sites were located in the Douala 1st district (Wouri bridge, 04°00'262 N 09°40'486 E), where the nurseries were set up, and Douala 3rd district (Cité Berge 03°70'955 N 009°53'257 E and Mbanga-Pongo 03°98'345 N 09°74'085 E), where the propagules were collected (Figure 1).

The Douala 1st site was chosen for reasons of equipment safety and the Douala 3rd sites because the lush vegetation and the robustness of the trees that suggest a favorable environment for mangroves ecosystem health and the abundance of propagules. Propagules were collected under various trees. The climate of the region belongs to the equatorial domain of a particular type or Cameroonian. It is characterized by a long rainy season (March – November) with abundant rainfall (4000 mm per year) and a short dry season (December – February), high and stable temperatures (26.7 °C), and high humidity throughout the year with a maximum always close to 100% [Din et al., 2008]. The tides are semi-diurnal with amplitudes ranging from 1.35 to 3 m [Nfotabong-Atheull et al., 2013].

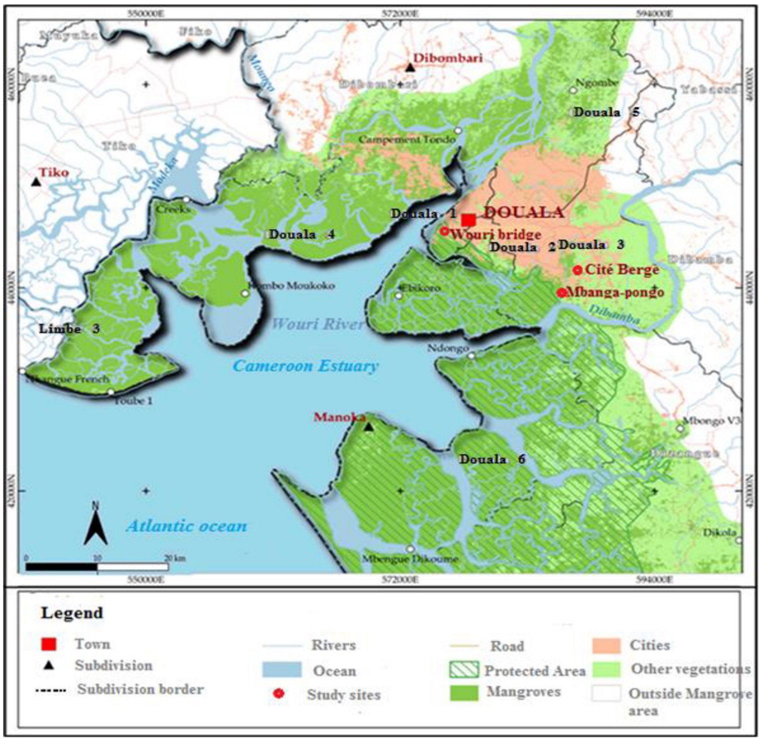

Figure 1. Location of the study sites

The soil of the region is characterised by a hydromorphic horizon that is generally sandy-muddy in texture and dark grey in colour [Balzer et al., 1995].

Data collection

A total of 360 *Rhizophora* propagules were harvested, sorted, and stored in situ for seven days. Simultaneously, a space was prepared, a nursery was built, and substrates were prepared during the low tide. Two batches of 180 healthy propagules measuring 25–40 cm in height and 1.1–1.2 cm in diameter were used in experiments 1 and 2.

Experience 1: Substrate preparation and seedlings raise

The substrates used were mangrove mud and fine sand extracted in situ [Costa et al., 2016]. The use process involved quantitative mix of mangrove sand and mud from three combinations:
- a substrate mix of 75% sand and 25% mud;
- a substrate mix of 50% sand and 50% mud;
- a substrate mix of 25% sand and 75% mud.

Each type of compound substrate was then placed in 60 plastic bags, each 25 cm wide and 35 cm high, and arranged in batches in a semi-illuminated nursery.

At the end of propagule conservation, three trials were carried out in the nursery depending on the composition of the substrate, 60 propagules per trial:
- the first trial consisted of transplanting the propagules into the bags containing the substrate composed of 75% mangrove mud and 25% fine beach sand extracted in situ;
- the second trial consisted of transplanting the propagules into bags containing a substrate composed of 50% mangrove mud and 50% fine beach sand;
- the third trial consisted of transplanting the propagules into bags containing a substrate composed of 25% mangrove mud and 75% fine beach sand extracted in situ.

Experience 2: Impact of insertion level

By referring to the optimal condition of propagules storage, the optimum light condition for seedlings in the nursery (semi-lighted) and on the best mineralogical substrate composition three (03) trials were used to determine the best level of insertion of propagules in the substrate [Boubakary et al., 2019].

A substrate mix composed of 25% sand and 75% mud was introduced into 180 bags (25 cm wide and 35 cm long each). The substrate-filled bags were arranged in three batches of 60. The propagules were inserted into the substrate at specific depths of 1/4, 1/2 and 3/4 of their height, respectively, within each group of pots. The arrangement of the seedlings in the nursery was the same as in the previous experiment.

The nursery site was located in the estuary, along a channel that was neither too high nor too low, ensuring that the plants were regularly submerged during high tide [Fontalvo-Herazo et al., 2011]. The dominant species was *Rhizophora racemosa* Meyer (Fig. 2). Biological growth parameters were recorded, including the appearance of the seedlings, the time taken for the first leaf to appear, the variation in the number of leaves with time. The height of seedlings using a tape measure, and both the base and mid-height diameters of the seedlings using a caliper. In addition, predator activity was also observed. These parameters were recorded weekly for a period of two months.

Data analysis

The "r commander" package of R software version 4.1.3 was used to perform descriptive statistic of data collected. The Student's parametric test was used to compare the average of heights and diameters among seedlings. Excel software 2013 was used for graphical representations.

RESULTS

Effect of substrate composition on the growth and development of *Rhizophora* spp. seedlings

Height growth of seedlings

Student's parametric test showed a significant difference between the mean heights of *Rhizophora* seedlings produced on the three types of substrates tested (P = 0.064). The mean heights of the seedlings were unevenly distributed over the three types of substrates as a function of time. The greatest average height (40.2 ± 7.48 cm) was obtained for the substrate composed of 75% mud and 25% sand and the lowest (38.76 ± 5.55 cm) for the substrate composed of 25% mud and 75% sand (Figure 3).

Seedling growth is linked to substrate type. The relationship between growth in height, growth in diameter and substrate type is strong for all the combinations tested. For the substrate of 75% mud and 25% sand, the correlation coefficient (R^2) is 0.9406 and the linear equation is $y = 2.6513x + 29.417$. For the substrate composed of 50% mud and 50% sand, the correlation coefficient (R^2) is 0.9631 and the linear equation is $y = 1.9838x + 31.704$. For the substrate of 25% mud and 75% sand, the correlation coefficient (R^2) is 0.9653 and the linear equation is $y = 1.993x + 30.795$. The correlation coefficients are close to 1 in all three types, showing the strong link between seedling height and substrate type (Figure 4).

Analysis of variance showed that substrate type had a significant influence on height growth, diametric growth and leaf development in *Rhizophora* spp. seedlings (Table 1).

Seedling diameter growth

Student's parametric test showed a significant difference between the mean seedling diameters according to the substrate types (P= 0.064). The mean diameters of the seedlings were unequal on the three types of substrates tested. The highest mean diameter (1.2 ± 0.01 cm) was recorded on the substrate of 75% mud and 25% sand and the lowest mean diameter (1.18 ± 0.02 cm) on the substrate of 25% mud and 75% sand (Figure 5).

Figure 2. Seedlings in nursery in situ: (a) at low tide; (b) at high tide

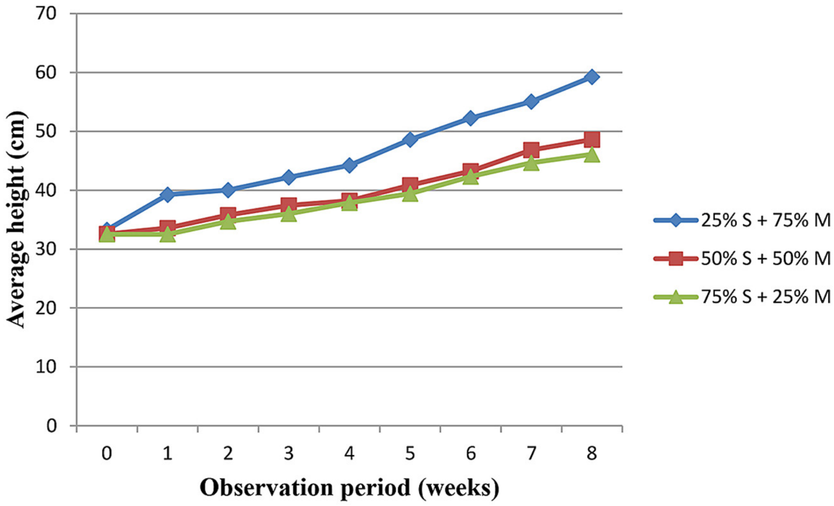

Figure 3. Variation in seedling heights according to the substrates: S – sand, M – mud

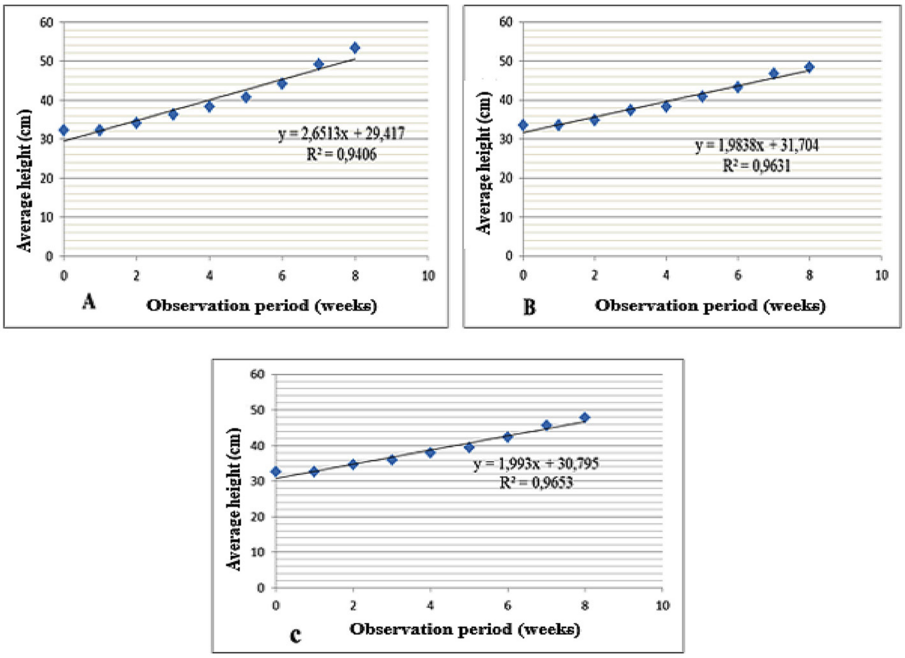

Figure 4. Relationship between seedling height growth and type of substrate: (a) 75% mud and 25% sand, (b) 50% mud and 50% sand, (c) 25% mud and 75% sand

Table 1. Analysis of variance of seedling growth on different types of substrates

Parameters	Means Sq	F-value	P-value
Height growth	141.186	3.604	0.04279
Diameter growth	0.00151481	4.1735	0.02783

The growth in diameter of the seedlings is linked to the type of substrate. For the substrate of 75% mud and 25% sand, the correlation coefficient (R^2) is 0.9449 and the linear equation is $y = 0.0067x + 1.1789$. For the substrate composed of 50% mud and 50% sand, the correlation coefficient (R^2) 0.6998 and the linear equation is $y = 0.0052x + 1.1682$. On the substrate composed of 25% mud and 75% sand, the correlation coefficient (R^2) 0.8963 and the linear equation is $y = 0.0073x + 1.1507$. In all, the analysis shows that diametric growth depends on the type of substrate on which the seedlings develop. The correlation is strongest for the substrate of 75%

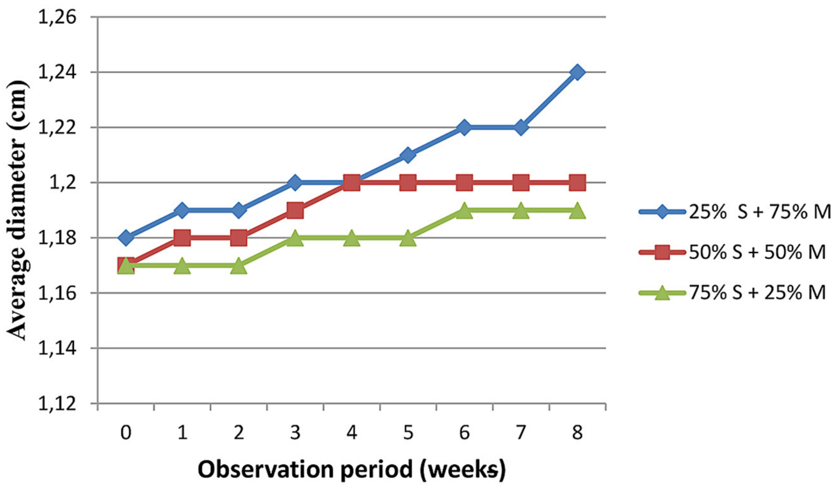

Figure 5. Mean variation of seedling diameters according to substrate type and time: S – sand, M – mud

mud and 25% sand, with an R^2 value of 0.9449, which is closest to 1 (Fig. 6).

Leaf development according to substrate type

The appearance of leaves in the seedlings was uneven across the different substrates and observation time. In all batches of propagules that were harvested, collected and transplanted without prior preservation, no propagule showed a leaf after two weeks on the three substrate combinations. However, the appearance of leaves was recorded on all batches from the third week onwards, with a higher rate (40%) for the substrate of 75% mud and 25% sand and a lower rate (20%) for the substrate of 25% mud and 75% sand. In the fourth week, 53% of the seedlings for the substrate of 25% mud and 75% sand had 4 leaves, 61.66% of the seedlings on the substrate of 50% mud and 50% sand had 4 leaves and 55% of the seedlings for the substrate of 25% mud and 75% sand bore 4 leaves. In the fifth week, 93.33% of the seedlings on the 25% mud and 75% sand substrate had at least 4 leaves, 90% of the seedlings for the 50% mud and 50% sand substrate had at least 4 leaves and 98.33% of the seedlings on the 75% mud and 25% sand substrate also had at least 4 leaves. By the sixth week, all seedlings were bearing at least 4 leaves. By the seventh week, 11% of seedlings on the 25% mud and 75% sand substrate had 6 leaves, 5% on the 50% mud and 50% sand substrate and 20% on the 75% mud and 25% sand

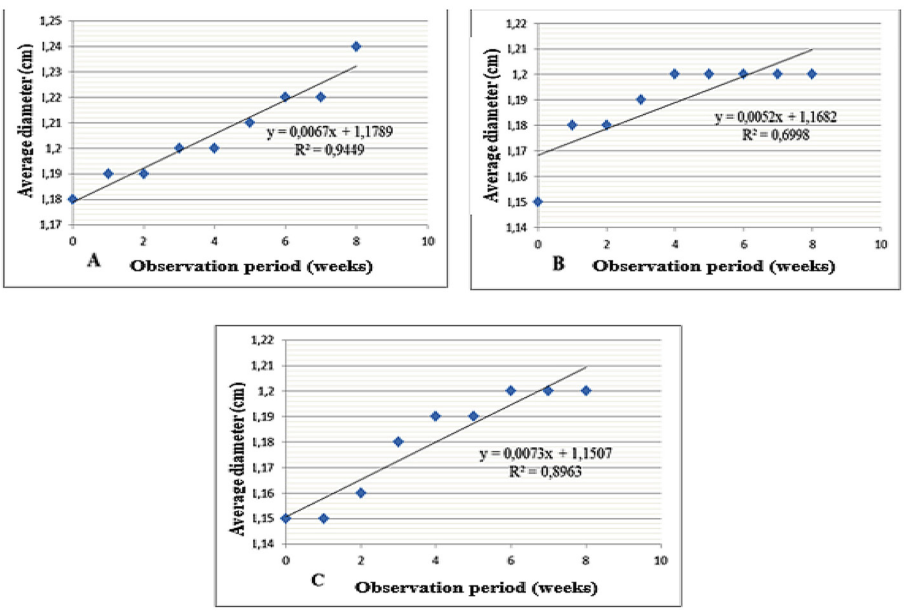

Figure 6. Relationship between seedling diameter growth and substrate type: (a) 75% mud and 25% sand, (b) 50% mud and 50% sand, (c) 25% mud and 75% sand

substrate. By the eighth week, 66.66% of seedlings for the 25% mud and 75% sand substrate had 6 leaves, 70% on the 50% mud and 50% sand substrate and 91.66% for the 75% mud and 25% sand substrate (Table 2).

Among the three types of substrates analysed, the substrate of 75% mud and 25% sand was more favourable to leaf development.

Relationship between diameter and height growth of seedlings and substrate type

For the three substrates tested, diametric growth is linked to the seedling growth in height. The correlation coefficient R^2 = 0.9709867 is stronger on the substrate of 75% Mud and 25% Sand and is closest to 1. The characteristics of the substrate therefore determine the life of the mangrove community (Table 3).

Effect of insertion level in the substrate on the growth and development of *Rhizophora* spp. seedlings

Analysis of variance shows that the level of insertion of the propagules in the substrate has a very significant influence on the growth in height of the seedlings; the same observation is pertinent for growth in diameter and leaf development (Table 4).

Mortality

The mortality rate of seedlings over eight weeks was observed as follows:

- 6.66% in the group of propagules with ¼ of their height inserted in the substrate;
- 15% in the group of propagules with ½ of their height inserted in the substrate;
- 33.33% in the group of propagules with ¾ of their height inserted into the substrate.

Height variation of seedling according to propagule insertion level

The growth of the seedlings was influenced by the depth of insertion of propagules in the substrate. In eight weeks, the seedlings showed different heights depending on the propagule's insertion level in the substrate. The highest mean height (44.43 ± 6.59 cm) was recorded in the batch of propagules inserted at ¼ into the substrate; the lowest mean height (20.19 ± 8.30 cm) in the batch of propagules inserted at ¾ into the

Table 2. Leaves variation according to time and substrate composition

Observation period (weeks)	Maximum number of leaves	Percentage of seedlings with leaves		
		25% M and 75% S	50% S and 50% M	75% M and 25% S
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	2	20	31.66	40
4	4	53	61.66	55
5	4	93.33	90	98.33
6	4	100	100	100
7	6	11.66	5	20
8	6	66.66	70	91.66

Table 3. Correlation between diameter and height of seedlings on different substrates

Substrate composition	p-value Mean diameter	p-value Mean height	Correlation coefficients
25% S and 75% M	5.921^{e-16}	2.292^{e-6}	0.9709867
50% S and 50% M	2.864^{e-16}	2.323^{e-7}	0.7272307
75% S and 25% M	1.86^{e-15}	2.884^{e-7}	0.8967169

Table 4. Analysis of variance of the growth of seedlings at different level

Parameters	Mean Sq	F-value	P-value
Height growth	1289.61	41.232	2.036^{e-09}
Diameter growth	0.207251	553.65	2.2^{e-16}

substrate. For the batch of propagules inserted at ½ the average height recorded over eight weeks is (35.58 ± 6.70) (Figure 7).

Plantlet height growth is related to the insertion level of propagules into the substrate. Propagules inserted at ¼ grow faster (Figure 8A). The linear equation for growth is $y = 2.532x + 34.639$ and the correlation coefficient $R^2 = 0.9455$. Propagules inserted at ½ grow normally, the linear equation for growth is: $y = 2.3467x + 26.196$ and the correlation coefficient is $R^2 = 0.9173$. Propagules inserted at ¾ on the other hand, grow slowly. The linear equation for growth is $y = 1.1945x + 8.8853$ and the correlation coefficient $R^2 = 0.9633$ (Figure 8C). In all cases, growth in height is strongly linked to the level of propagules in the substrate with correlation coefficients close to 1.

Evolution of seedling diameters according to insertion level propagule

As with height growth, the diametric growth of seedlings is unevenly distributed as a according to time and insertion level of propagules in the substrate. Deeper the propagule is slower it grows. The largest average diameter (1.13 ± 0.06 cm) was obtained in the batch of propagules inserted at ¼.

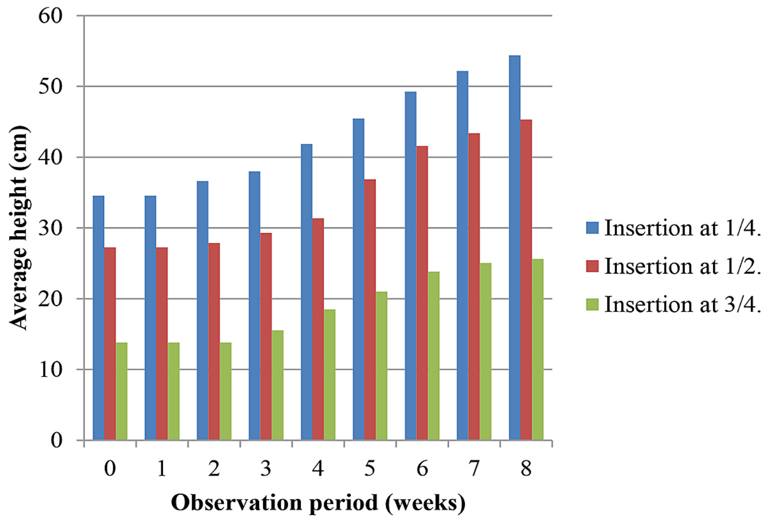

Figure 7. Variation in mean plantlet height seedlings according to insertion levels

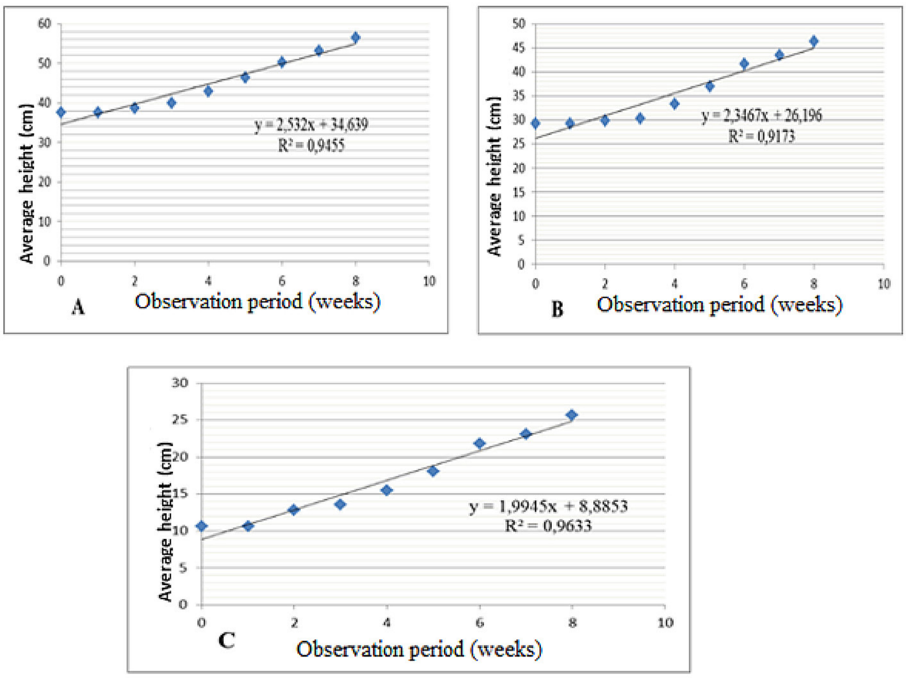

Figure 8. Relationship between average seedling height and propagule insertion levels: A – propagules inserted at ¼; B – propagules inserted at ½; C – propagules inserted at ¾

The lowest average diameter (0.77 ± 0.01 cm), is obtained in the batch of propagules inserted at ¾. For the batch of propagules inserted at ½ in the substrate, the average diameter obtained in eight weeks is (0.95 ± 0.02 cm) (Figure 9).

The diametric growth of seedlings is related to the level of insertion of the propagule in the substrate. For propagules inserted at ¼ in the substrate, the correlation coefficient $R^2 = 0.6157$ and the linear equation $y = 0.0098x + 1.0851$; growth was rapid for the first four weeks, then a slowdown is observed for three weeks and an acceleration from the seventh week onwards (Figure 10A), whereas in propagules inserted at ½ in the substrate, diametric growth was rapid for the first three weeks and slowed for a fortnight before continuing. The linear equation is $y = 0.0092x + 0.9189$ and the correlation coefficient ($R^2 = 0.9298$) indicates that the relationship is very strong (Figure 10B). For the propagules inserted at ¾ in the substrate, diametric growth is rapid for the first two weeks then slows from the fifth week before resuming at the seventh week. The linear equation is $y = 0.0053x + 0.7487$ and the correlation coefficient ($R^2 = 0.8533$) indicates that the relationship is strong (Figure 10C).

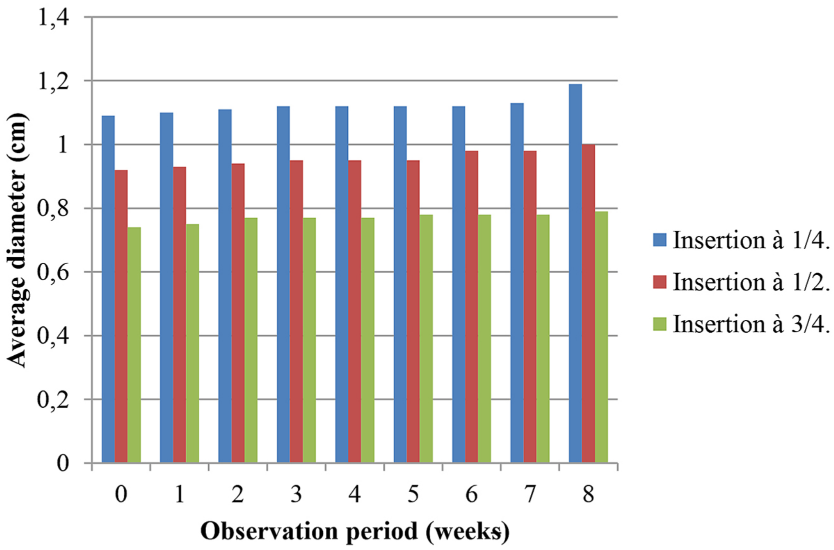

Figure 9. Variation of seedling diameters depending to insertion levels and time

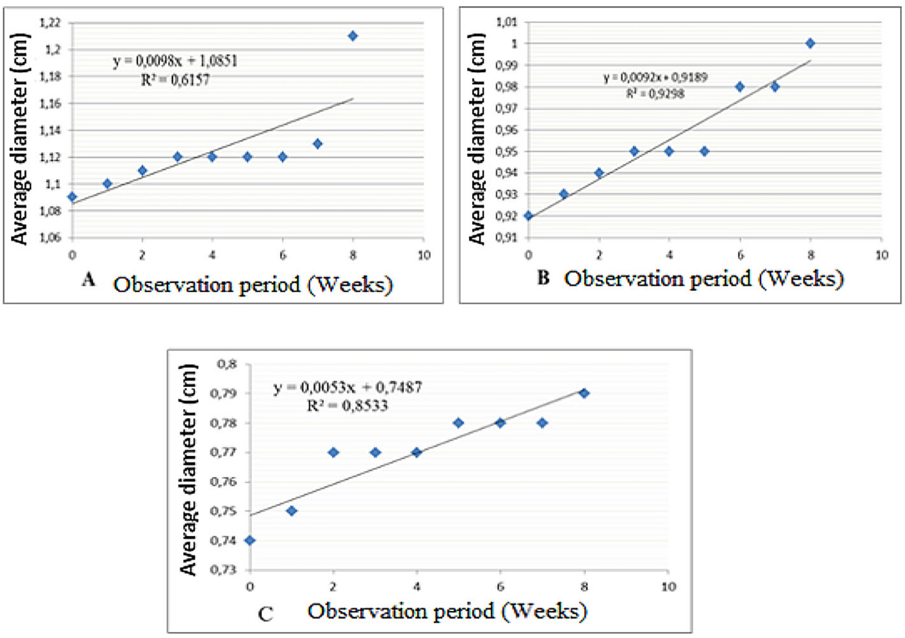

Figure 10. Relationship between diameter and insertion level of propagules in the substrate: A – propagules inserted ¼, B – propagules inserted ½, C – propagules inserted ¾

Relationship between diameter growth and height growth of seedlings at different insertion levels

Diametric and height growth of seedlings are linked to the insertion levels of propagules into the substrate. The strongest correlation was observed for propagules inserted at ½ into the substrate, followed by propagules inserted at ¾ and the lowest for propagules inserted at ¼ (Table 5).

Collar height

Average collar heights were constant throughout the period during which the seedlings were observed in the nursery. No changes were observed (Figure 11).

Leaf development according to insertion level of propagules

The appearance of leaves was recorded from the third week in the three different batches of seedlings. The leaf appearance rate was 40%, 31.66% and 20% respectively for propagules inserted at ¼, ½ and ¾. At the fifth week, seedlings in all batches had at least 2 leaves and 4 leaves at the sixth and seventh week. At the eighth week, 61.66% of the seedlings transplanted at ¼ had 6 leaves, 6.66% of the seedlings inserted at ½ in the substrate had 6 leaves and none of the seedlings in the batches of seedlings inserted at ¾ had 6 leaves (Table 6). Inserting seedlings at ¼ in the substrate is more favourable for leaf development.

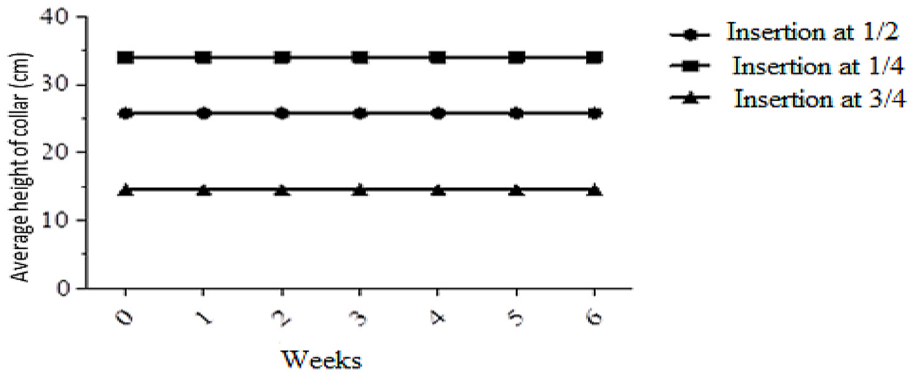

Figure 11. Average crown height of seedlings in nurseries

Table 5. Diameter and height of seedlings at different insertion levels

Level of insertion in the substrate	p-value Mean diameter	p-value mean height	Correlation coefficients Mean diameter/Mean height
¼	1.044^{e-11}	3.732^{e-7}	0.686032
½	5.17^{e-14}	2.44^{e-6}	0.9491166
¾	5.389^{e-15}	8.405^{e-4}	0.8752866

Table 6. Average percentage of seedlings with leaves as a function of time and level of insertion of seedlings in the substrate

Observation period (weeks)	Maximum number of leaves	Average percentage of seedlings with leaves		
		¼	½	¾
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	2	40	31,66	20
4	2	80	68.33	55
5	2	100	100	100
6	4	35	28,33	10
7	4	85	63,33	18.33
8	6	61.66	6.66	0

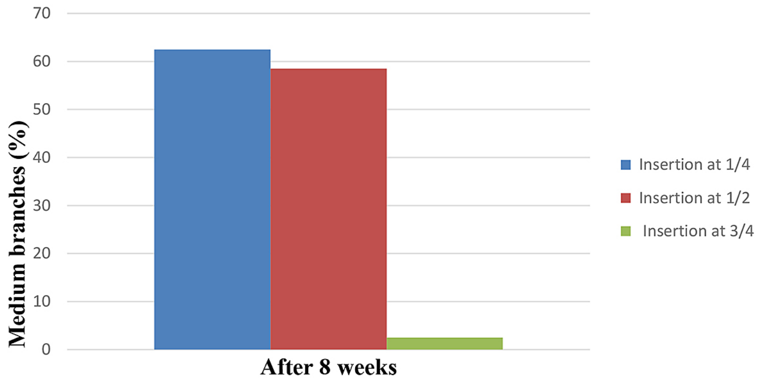

Figure 12. Percentage of branching of seedlings at different heights of propagules insertion

Development of branches in seedlings

Branching development in seedlings was influenced by the different depths of propagules insertion in the substrate. The highest percentage of branching in seedlings (62.5%) was obtained after eight weeks of observation for propagules at ¼ in the substrate. For propagules inserted at ½ the percentage of branching was 58.82% and the lowest percentage (2.5%) was recorded for propagules inserted at ¾ (Figure 12). The production of *Rhizophora* seedlings by insertion of propagules at ¼ is more favorable to branching development.

DISCUSSION

Rhizophora spp. seedlings on different type of substrates and insertion level showed significant difference. Jumawan et al. [2015] showed that the soil's quality affects largely the diversity of mangroves. The ability of soils to retain C, water and nutrient ions are strongly influenced by the soil texture [Havlin et al., 2014]. Basically, soil texture is determined through the particle size percentage of clay, silt and sand [Ashman and Puri, 2002]. Mangrove plants may grow in different types of soil reason why Jorquia [2022] recommends more essential that planters consider the type of substrate in planting mangroves.

In this work, the seedlings of *Rhizophora* spp. showed good growth and development on all the types of substrates tested in nursery, with the best results on the substrate composed of 75% Mud and 25% Sand. The combination of 75% Mud and 25% Sand forms an excellent substrate. The results obtained are similar to those of Costa et al. [2016] who examined the growth and survival of *Rhizophora* spp., *Avicennia* and *Laguncularia* seedlings on three different types of substrates in nursery on the coast of Brazil. Dewiyanti et al. [2023] obtained the different results in the station where the substrate was composed of: 84.6 % Sand, 10.2 % Dust and 5.1 % Clay. The research was carried out at mangrove rehabilitation. The highest value of stem height and stem diameter measured was at station 1, where the average value of stem height reached 1.22 cm per week and stem diameter was 0.25 mm per week. The lowest values for measuring stem height, and stem diameter were at stations 3 with a stem height value of 0.57 cm per week, and stem diameter 0.12 mm per week. Bocquet [2018] investigated on the significant impact of muddy substrate on seedling growth and development. According to these authors, the physical and chemical characteristics of muddy substrates allow the retention of moisture and nutrients required by seedlings. The soil pores are different for clay, silt and sand. For clay, the soil pores are the smallest when compared to silt and sand. Hence, this type of soil has a higher ability to retain water and nutrients [Ashman and Puri, 2002].

In mangrove forest, the nutrients are supplied by the litter fall from trees and suspended materials from surface runoff above ground, fauna activities and microorganisms' decomposition mechanism [Cannicci et al., 2008]. The litter fall or surface materials were utilized by macro invertebrates such as *Scylla serrata* and that is broken down into smaller sizes. Then, the process was taken over by microorganisms until all the materials deposit into the soil permanently.

The substrate factor also has a significant effect on the number of leaves, depending on the different substrates tested. There was a significant effect of composite substrates on the parameters measured. The number of leaves at the end of observation of Dewiyanti et al. [2023] from the station 1, 2, and 3 were 21, 25, and 5 strands. In all study stations, the average addition of leave was one strands, Delvian et al. [2019], reported that at twelve weeks of observation, the highest number of leaves was 7 strands. Parameters of water physics, water chemistry, and substrate are environmental factors that affect mangrove growth [Wantasen, 2014]. Nursinar et al. [2023] showed that the number of *Rhizophora apiculata* leaves that were sown every month increased and the number of leaves increased by one leaf every month.

However, it is important to remember that *Rhizophora* spp. propagules are able to develop and grow independently of the substrates tested because according to Windusari et al. [2014], mangroves develop in mud clay (silt) mixed with organic matter, but they are also found in areas with peat or substrate with a high sand content. An adequate insertion height of propagules in substrate of precise composition is essential for the rapid growth and development of seedlings of the genus *Rhizophora*. The production of seedlings in a nursery by insertion at ¼ in the substrate would seem to be a better approach. These results are in line with those of Poveda and Guiraud [2014] who proved during reforestation that, propagules should be planted neither deep nor too much on the surface and oppose those of Nakouzi et al. [2018] in the Practical guide to the production and planting of mangrove species in Benin (Cotonou) who found that propagules should be sunk $^1/_3$ straight into the mud for best results. The difference in results could be linked to the edaphic condition of the experimental environment in which this work was carried out, opposite to the edaphic condition of the natural environment.

Mortality of species of the genus *Rhizophora* observed in mangroves is due to several factors, including intra and interspecific competition, diseases, herbivores, predation and the natural senescence of trees. It should be noted that all stages of development are affected, including propagules, young trees and adult trees. However, mangroves in the early stages of development experience higher mortality, and small seedlings are more at risk because of competition with larger trees for light and/or nutrients.

Mortality of mangrove seedlings is influenced by several factors, including intra and interspecific competition, diseases, herbivores, predation and the natural senescence of trees propagules, young trees and adult trees and level of insertion into the substrate; according to Jimenez and Lugo [1984]. The observations of these authors are similar to those made in the context of this work for batch of seedlings resulting from the insertion of propagules at ¾ in the substrate. The high mortality rate observed in the range of propagules inserted deep into the substrate reflects the poor adaptability of the seedlings at this level of insertion [Poveda and Guiraud, 2014]. In the group of seedlings inserted slightly into the substrate the mortality rate was low, reflecting the favourable condition for the survival of seedlings of the genus *Rhizophora*.

The viability of reforestation depends on duration and ecological conditions. Plant growth and development parameters are not constant over time. In the work carried out in the mangroves of the Wouri estuary, the growth in diameter and height of the seedlings, the development of primary and secondary branches and stilt roots were faster for the batch of seedlings resulting from the insertion of propagules at ¼ in the substrate and very slow for the batch of propagules inserted deep in the substrate. The observed variability could be explained by the individual or combined effects of environmental parameters such as propagule retention time, the effect of light, the effect of substrate composition, and the level of insertion of propagules into the substrate, the salinity of the environment [Jorquia, 2022]. The results obtained as part of the work carried out in the mangroves of the Wouri estuary are similar with those of Bocquet [2018] obtained at the end of the ecological assessment of reforested mangroves in the Saloum delta in Senegal.

CONCLUSIONS

The type of substrate use and the insertion level of propagule affect seedling germination and growth of *Rhizophora* spp. These are therefore important to consider in the ecological restoration of mangroves, which are essential ecosystems for climate regulation, coastal protection and biodiversity. Ideal substrate and insertion depth are two important factors that affect the survival and growth of mangrove seedlings or propagules in restoration projects. The ideal substrate is usually soft, muddy, and rich in organic matter, while the insertion depth is the depth at which the seedling or propagule is planted in the substrate.

Practical limitations related to the ideal substrate and insertion depth may include the availability and accessibility of suitable sites for mangrove restoration, which may be constrained by land use, ownership, and governance issues. Furthermore, the variability and uncertainty of environmental conditions, such as salinity, tidal inundation, and storm events, that may affect the suitability of the substrate and the survival of the seedlings or propagules. It's also important to indicate the trade-offs and synergies between different restoration objectives, such as carbon sequestration, coastal protection, and biodiversity conservation, that may require different substrate types and planting densities. We can also add the costs and benefits of different restoration methods, such as direct planting, natural regeneration, or assisted natural regeneration, that may have different impacts on the substrate and the insertion depth.

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