Effect of different salt concentrations on the growth and biomass of *Rhizophora apiculata* seedlings

M Basyuni$^{1,2,*}$, D A Keliat$^1$, B Utomo$^1$ and R Amelia$^1$

$^1$Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, Indonesia.
$^2$Center of Excellence for Mangrove, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: *m.basyuni@usu.ac.id

**Abstract.** *Rhizophora apiculata* Blume is very easy species to find and is widely distributed in mangroves. This study was purposed to determine the optimum salinity concentration for mangrove seedling growth as a reference when rehabilitating mangrove forests. The effect of salinity on the growth and development of *R. apiculata* mangrove seedling roots was carried out in the greenhouse, Universitas Sumatera Utara. This study used five levels of salinity concentration of 0%, 0.5%, 1.5%, 2%, and 3% with 10 replications. Parameter observations comprised of height, diameter, number of leaves, leaf thickness, leaf area, number of main roots, number of lateral roots, main root length, lateral root length, main root diameter, lateral root diameter, canopy water content, root moisture content, and canopy and root ratio. The most parameter of *R. apiculata* seedlings was at the salinity concentration level of 0.5%.

1. Introduction

Mangroves are areas that are formed as a result of the continuous formation of mud and land by plants so that it gradually turns into semi-land [1]. The ability of mangroves to grow in saltwater is due to the ability of plant roots to excrete or secrete salt, that species of the genera Rhizophora have roots that can separate salt [2,3]. Salt separation occurs during the evaporation or transpiration process in the leaves. This leaf evaporation caused negative pressure to cause the water in the root system is attracted to the xylem and this event also occurs the separation of fresh and marine water in the root membrane [4].

The selection of Rhizophora apiculata in this study is a good step considering that this species is easy to grow and their propagules are easy to find compared to other species [5], besides that this type of propagule is also available in large numbers. Knowledge of the best salinity concentration for the growth and development of the roots of *R. apiculata* seedlings is required for the rehabilitation program for forest areas and non-forest areas. This work aimed to determine the level of the best salinity concentration on the growth and improvement of the roots of the mangrove seedlings of *R. apiculata*.

2. Materials and methods

2.1. Plant materials

*Rhizophora apiculata* Blume (Rhizophoraceae) propagules were sampled from Pulau Sembilan, Langkat, North Sumatra, Indonesia. Planting of the *R. apiculata* propagules with various salt contents for five months was performed in the greenhouse of the Faculty of Agriculture, Universitas Sumatra Utara.
2.2. Propagule selection and planting

*R. apiculata* propagules were used from mother trees aged 5 years or more. The accepted propagules were ecologically mature and healthy with a brownish-green color hypocotyl, not attacked by diseases and pests. Ten *R. apiculata* propagules for each salinity concentrations of 0%, 0.5%, 1.5%, 2%, and 3% were planted in plastic bottles on January-May, then filled with sterilized sand media. In this work, salt concentration is the ratio of the mass of salt powder to the mass of the solution [6]. A commercial salt powders (Marine Salt, Singapore) were used and applied by dissolving 5.66 g, 17 g, 22.6 g, and 34 g powder of salt for 1 liter of tap water for 0%, 0.5%, 1.5%, 2% and 3% salinity as previously described [6].

2.3. Observed parameters

The observed parameters were (1) Seedling survival percentage, (2) Measurement of seedling height is measured using the rule. Measurements were made at the base of the *R. apiculata* seedlings to the point where the seedling growth. Height measurements were performed at the end of harvesting. (3) Measurement of seedling diameter was done using calipers. Measurements were made at the end of the observation after harvesting. The diameter was measured at a length of 1.5 cm from the base of the *R. apiculata* seedlings [6]. (3) Calculation of the number of leaves was performed at harvesting. (4) Leaf thickness measurements were carried out at the end of the observation together with seedling height data collection using a scrub micrometer. (5) Calculation of the number of roots was performed manually using a counter after harvesting of *R. apiculata* seedlings at 5 months. The number of roots was calculated based on the position of the roots in the root system (level of branching) according to the classification of [7,8], which consisted of the main and lateral root. (6) Diameter measurements were taken after harvesting *R. apiculata* seedlings at 5 months. Root diameter provided important information related to soil pore size and root penetration potential [7,8]. Root diameter measurements were performed on each branching type using calipers [7,8].

2.4. Statistical analysis

Data were analyzed using the Dunnett test to compare all (0 percent) controls and treatments using ANOVA on a single-way basis. The data is shown as ± SD mean (n= 10). The P values < 0.05 were selected as the statistical limit. All statistical analyzes were developed using SAS 9.4 (SAS Institute) software.

3. Results and discussion

The ability of mangroves to grow and be able to survive in high and low salinity areas is indicated by the results of a 5-month study which showed that there was no difference in the percentage of survival and mortality of *R. apiculata* seedlings at different salinity levels, this was due to *R. apiculata* seedlings. able to survive in conditions of high and low salinity although not able to grow well at salinity concentrations that support the growth of the mangrove seedlings *R. apiculata*. This result was supported by the statement [9] which states that almost all types of mangroves are salt-tolerant species, but are not types that require salt for their life (salt demanding) [3,9].

Table 2 showed the results of research conducted for 5 months, the maximum growth of seedlings was shown at 0% salinity (control), namely 28 cm, and the lowest at 2% and 3% salinity, namely 18.86 cm. Salinity did not have a statistically significant effect of 0% salinity (control) at P < 0.05 for a Dunnet measure, while salinity was at 1,55 mm for the maximum lateral root diameter of 3 percent, and salinity was at 2 percent, i.e. 1.27 cm (Tables 3). In contrast with 0 percent salinity (control) at P<0.05 in the test by Dunnet, the use of salinity was not statistically important.

The growth in height and diameter of the *R. apiculata* seedlings is decreasing at a higher level of salinity, the seedlings will grow but in abnormal (depressed) conditions. This finding was supported by the statement of [6,10] which states that the growth rate, photosynthesis rate and water use of *R. apiculata* and *R. stylosa* seedlings grown in relatively lower salinity conditions are higher than seedlings planted in low salinity conditions. This phenomenon is also reinforced by the research of [11] that *R.
mucronata seedlings planted in salinity conditions of about 1.0% showed a higher increment in diameter and stem height than seedlings planted in conditions of about 2.8% salinity. Increasing the concentration of salinity will inhibit the growth of mangrove seedlings, to reduce the occurrence of salt stress, there are secondary metabolites in mangrove plants according to research conducted by [12] regarding the addition of synthetic mRNA terpenoids to see their effect on salt stress in Kandelia candet and Bruguiera gymnorhiza, a study that terpenoids can help plants withstand to stress on salt.

Table 1. Percentage of survival of R. apiculata seedlings.

| No | Parameter | Survival percentage (%) |
|----|-----------|--------------------------|
| 1  | Salinity 0% | 100±0.0                 |
| 2  | Salinity 0.5% | 100±0.0                |
| 3  | Salinity 1.5% | 100±0.0                |
| 4  | Salinity 2.0% | 100±0.0                |
| 5  | Salinity 3.0% | 100±0.0                |

Data are represented as mean ± SD (n= 10)

Table 2. Growth parameters of R. apiculata seedlings.

| Salinity | Height (cm) | Diameter (mm) | Thick leaves (mm) | Number of leaves |
|----------|-------------|---------------|-------------------|-----------------|
| 0%       | 29.15±7.19  | 4.52±0.40     | 0.47±0.02         | 6.40±0.94       |
| 0.5%     | 28.45±8.69  | 4.85±0.58     | 0.50±0.04         | 6.90±1.20       |
| 1.5%     | 25.21±5.67  | 4.79±0.38     | 0.53±0.07         | 7.10±1.91       |
| 2.0%     | 18.86±2.84* | 4.01±0.41*    | 0.40±0.06         | 5.90±0.32       |
| 3.0%     | 18.86±4.45* | 3.50±0.22*    | 0.40±0.05         | 2.90±0.63*      |

Data are shown as mean ± SD (n= 10). * indicates statistically significant control (0%) at P <0.05 with the Dunnet's test.

Salinity administration had a statistically significant effect on P<0.05 with Dunnet 's test on 3 percent salinity study, associated to 0 percent salinity (control). The highest leaf thickness of R was shown by Table 2. Apiculata seedlings had a salinity of 1.5%, i.e. 0.53 mm and the lower of 0.395 mm, at 3% salinity. In contrast to 0 percent salinity (control) at P<0.05 by Dunnet test the administration of salinity was not statistically relevant. Table 2 revealed R. apiculata had the highest leaf area, at 0.5% salinity, i.e. 24.53 cm2 and the lowest of 3% salinity, i.e. 3.40 cm2. The salinity administration had a statistically significant impact compared to 0% of salinity (control) at P<0.05 by Dunnet's test 2 % and 3% (height and diameter).

Table 2 depicted the number of leaves, leaf thickness and leaf area decreased drastically at high salinity concentrations. It can be seen that salinity affects the morphology of the structure of a plant, both the relatively smaller leaf size, the small number of leaves, and the thick leaves of the mangrove seedlings, this will have an impact on the absorption of nutrients and reduced water at high salinity levels to inhibit the rate of photosynthesis, in the end, these plants will experience abnormal growth. These findings followed research[6], which indicate that the salinity level would influence the amount of leaves produced in the marina seedlings Rhizophora stylosa and Avicennia marina, where the higher the salinity, the less the amount of leaves produced.

Table 2 depicts that the highest diameter of R. apiculata seedlings was shown at 2% salinity, namely 4.43 mm, and the lowest at 3% salinity, namely 2.81 mm. Giving salinity did not have a statistically significant effect related to 0% salinity (control) at P <0.05 with the Dunnet's test, while for the highest lateral root diameter at the 3% salinity level was 1.55 mm and the lowest was at 2% salinity, namely 1.27 cm (Table 3). The administration of salinity was not statistically significant compared to 0% salinity (control) at P <0.05 by Dunnet's test.
Table 3. Root number and root diameter.

| Salinity | Root number | Root diameter |
|----------|-------------|---------------|
|          | Main Root (cm) | Lateral root (cm) | Main Root (cm) | Lateral root (cm) |
| 0%       | 1.10±0.32    | 33.90±5.04    | 3.34±1.07     | 1.44±0.17     |
| 0.5%     | 1.80±0.79    | 46.60±9.26*   | 4.43±1.35     | 1.35±0.38     |
| 1.5%     | 2.30±1.25    | 56.10±10.15*  | 3.62±1.27     | 1.27±0.22     |
| 2.0%     | 2.30±1.06    | 37.80±6.34*   | 2.96±0.44     | 1.40±0.16     |
| 3.0%     | 1.20±0.42    | 18.90±5.51*   | 2.81±0.72     | 1.55±0.33     |

Data are shown as mean ± SD (n= 10). The sign (*) indicates statistically significant control (0%) at P <0.05 with the Dunnett's test.

Based on the parameters of the number of roots, root length, and root diameter, salinity did not have a significant effect. Good root growth will illustrate good plant growth as well. Roots are part of plants that have a very important function for plant growth, especially for canopy growth, where the roots will transfer the absorbed ions from being channeled to the plant canopy, this is confirmed by the statement [13] which states that the root is an organ that is in direct contact with the saline environment, therefore the root is a structure that functions to regulate ion uptake and transport. The roots are the main barrier against the movement of the solution into the plant and as a result, the ion concentration delivered to the shoot is very different from the ion concentration in the external medium [14].

Table 4. Summary of parameters observed in R. apiculata seedlings.

| Parameters                  | Salinity         |
|-----------------------------|------------------|
| Height                      | 0%               |
| Diameter                    | 0.5%             |
| Number of leaves            | 0.5% dan 1.5%    |
| Thick leaf                  | 1.5%             |
| Number of the main root     | 0.5%, 1.5% dan 2%|
| Number of lateral roots     | 1.5%             |
| Length of the main root     | 0.5%             |
| Length of lateral root      | 2%               |
| The diameter of main root   | 0.5%             |
| The diameter of the lateral root | 3%           |
| Canopy content              | 2%               |
| Root water content          | 2%               |
| Canopy root ratio           | 0%               |
| Leaf area                   | 0.5%             |

The number of roots of R. apiculata seedlings was inversely proportional to the diameter of the roots at high levels of salt concentration. The fewer the number of roots, the greater the root diameter, the high level of salinity concentration which causes salt stress which has an impact on the roots of the mangrove species R. apiculata. The environment is the main factor in determining the success of a plant to grow. [15] reports that the number of mangrove roots is strongly influenced by the location where it grows and can be an indication of the suitability of mangroves to where they grow.

Based on Table 3 and Figure 4, it can be seen that from the observation parameters, namely diameter, number of leaves, leaf area, number of main roots, length of main roots, and diameter of main roots, it can be seen that the mangrove species R. apiculata can grow well at the concentration level. the salinity of 0.5%, at higher and lower concentration levels this species will experience growth disturbances. The results obtained in this study are supported by research by [6] which states that R. stylosa has decreased...
growth at a salinity concentration level of more than 0.5% but the *A. marina* species can experience a growth at a concentration level of 2% and if more than 2% it will decrease.

4. Conclusions

*R. apiculata* experienced well growth at a salinity level of 0.5% based on parameters of diameter, number of leaves, leaf area, number of main roots, length of main roots, and diameter of main roots. The higher the salinity level, the more disturbed plant growth were recoded.

References

[1] Brunier G, Anthony EJ, Gratiot N, Gardel A 2019 Exceptional rates and mechanisms of muddy shoreline retreat following mangrove removal *Earth Surf Processes Landf* 44 pp 1559–71
[2] Parida AK, Jha B 2010 Salt tolerance mechanisms in mangroves: a review *Trees* 24 pp 199–217
[3] Flowers TJ, Colmer T D 2015 Plant salt tolerance: adaptations in halophytes *Ann. Bot.* 115 pp 327–31
[4] Pittermann J 2010 The evolution of water transport in plants: an integrated approach *Geobiology* 8 pp 112–39
[5] Asaeda T, Kalibbala M 2009 Modelling growth and primary production of the marine mangrove (*Rhizophora apiculata* BL): a dynamic approach *J. Exp. Mar. Biol. Ecol.* 371 pp 103–11
[6] Basyuni M, Putri LAP, Nainggolan B, Sihaloho PE 2014 Growth and biomass in response to salinity and subsequent fresh water in mangrove seedlings *Avicennia marina* and *Rhizophora stylosa* *J Manaj Hut Trop* 20 pp 17–25
[7] Pi N, Tam NFY, Wu Y, Wong MH 2009 Root anatomy and spatial pattern of radial oxygen loss of eight true mangrove species *Aquat Bot* 90 pp 222–30
[8] Basyuni M, Keliat DA, Lubis MU, Manalu NB, Syuhada A, Wati R, Yunasfi 2018 Growth and root development of four mangrove seedlings under varying salinity *IOP Conf Ser: Earth Environ Sci* 130 012027 pp 1-6
[9] Medina E 1999 Mangrove physiology: the challenge of salt, heat, and light stress under recurrent flooding in: *A Yanez-Arancibia y A L Lara-Dominguez* (eds.) *Ecosistemas de manglar en América tropical* USA: Instituto de Ecología A.C. México, UICN/ORMA, Costa Rica, NOAA/NMFS Silver Spring MD 380 p
[10] Kodikara KAS, Jayatissa LP, Huxham M, Dahdouh-Guebas F, Koedam N 2018 The effects of salinity on growth and survival of mangrove seedlings changes with age *Acta Bot Bras* 32 pp 37-46
[11] Basyuni M, Telaumbanua TFC, Wati R, Sulistyono N, Putri LAP 2018 Evaluation of *Rhizophora mucronata* growth at first-year mangrove restoration at abandoned ponds Langkat North Sumatera *IOP Conf. Ser.: Earth Environ Sci* 126 012118 pp 1-5
[12] Basyuni M, Baba S, Kinjo Y, Putri LA, Hakim L, Oku H 2012 Salt-dependent increase in triterpenoids is reversible upon transfer to fresh water in mangrove plants *Kandelia candel* and *Bruguiera gymnorrhiza* *J Plant Physiol* 169 pp 1903–08
[13] Shabala S, Mackay A 2011 Ion transport in halophytes *Adv Bot Res* 57 pp 151–19
[14] Colmer TD, Greenway H 2011 Ion transport in seminal and adventitious roots of cereals during o2 deficiency *J Exp Bot* 62 pp 39–57
[15] Duke NC 2016 Oil spill impacts on mangroves: recommendations for operational planning and action based on a global review *Mar Pollut Bull* 109 pp 700–15

Acknowledgements

This work was in part funded by *Program Pengembangan Desa Mitra* (PPDM) 2019 from the Directorate for Research and Community Service, Ministry of Research, Technology, Republic of Indonesia.