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**Effect of salinity on the germination of *Excoecaria agallocha*
(Lin.)**

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**FORESTRY AND WOOD TECHNOLOGY DISCIPLINE
KHULNA UNIVERSITY
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Effect of salinity on the germination of *Excoecaria agallocha*
(Lin.)



Course Title: Project Thesis

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This dissertation has been prepared and submitted to the Forestry and Wood Technology Discipline, Khulna University, Khulna-9280, Bangladesh for the partial fulfillment of the four years professional B.Sc. (Hon's) degree in Forestry

FORESTRY AND WOOD TECHNOLOGY DISCIPLINE
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2018

Dedicated
To
My Beloved Parents

Abstract

Excoecaria agallocha (Lin.) is a mangrove species found in Sundarbans mangrove forest. In this study, the effects of salinity and zonal variation on the germination of this species were investigated at different salinity levels. The study was conducted in hydroponic culture. Seed germination were found 77% for LSZ and 33% for MSZ up to 5 ppt. Comparatively higher value was found at 0 ppt and . Comparatively lower value was found at 25 ppt. It was found that there was no significant difference in germination between two contrasted salinity zones.

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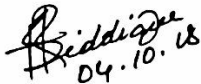
I also provide my thanks to our nursery staffs for their labor in completing my work.

Finally, I would like to express my appreciation and gratitude to my beloved parents who always inspired me and sacrificed their happiness for my education and happiness.

Md. Towhidur Rahman

APPROVAL

Project thesis submitted to the Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh, for the partial fulfillment of the four years professional B.Sc. (Hon's) degree in Forestry. I have approved the style and format of the project thesis.


04.10.18

Mohammad Raqibul Hasan Siddique

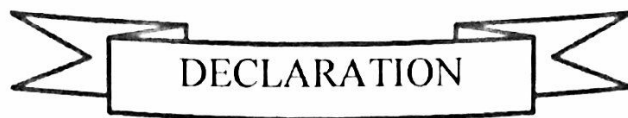
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DECLARATION

I, Md. Towhidur Rahman, hereby declare that the project thesis is based on my original research work except for quotations and citations, has been carried out under the direct supervision of Mohammad Raqibul Hasan Siddique, Associate Professor, Forestry and Wood Technology Discipline, Khulna University, Khulna which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted or accepted for any other degree at Khulna University or any other institutions.

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Chapter: One

Introduction

Background / Justification of the Study

Mangroves are characterized by evergreen woody plant species with some physiological and structural adaptations to a habitat influenced by saline and tidal inundation in the tropical and subtropical sheltered coastline (Saenger et al., 1983; FAO, 1994; Field, 1995). They can tolerate saline and to some extent of waterlogged condition and play a vital role in supporting coastal food webs and nutrient cycles in the adjacent coastal ecosystems (Alongi, et al., 2000; Machiwa and Hallberg, 2002; Mumby et al., 2004; Mahmood et al., 2008). Salinity as a specific and dominant factor determines the reproduction of halophytes. Mangroves seem to have undergone modifications with respect to phenology and mode of germination as a means of overcoming toxicity of chlorides on seed germination (Bhosale and Sinde, 1983).

Salinity affects plants through several ways: drought stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization and reduction of cell division and expansion (Hasegawa *et al.* 2000; Munns 2002; Muscolo *et al.* 2007, 2013; Zhu 2007; Sidari *et al.* 2008). Consequently, the germination, survival and growth are reduced (Muscolo et al. 2011; Schleiff and Muscolo 2011). Recently, Alam et al. (2018) found a significant impact of salinity on the size and weight of germinations of *A. Officinalis*. Salinity creates two major stresses: osmotic and ionic stresses.

The adaptations for germination of mangrove seeds proceed along different lines in different species (Henckel, 1963). Variously they exhibit vivipary, crypto-vivipary or no vivipary. Salt tolerance increases with the age of the seedlings in case of both viviparous and crypto-viviparous germination (Joshi et al. 1972). Vivipary could be an adaptive feature for the plant to overcome the harsh tidal environment for seedling establishment (Bhosale and Sinde, 1983). Most of the mangrove species shed their fruits during the rainy season to increase the chances of survival at lower saline condition of the ecosystem (Bunt et al. 1982).

E. agallocha is the 2nd most important timber resource in terms of abundance, distribution and commercial value and among the few non-viviparous mangrove species of the

Sundarbans. It is a small to medium sized tree (Siddiqi 2001) and found to dominate at the south-eastern part of moderate salinity areas of the Sundarbans (Ellison et al. 2000). This species was used as a raw material for newsprint and fuel wood. The density of *E. agallocha* with diameter at breast height (DBH) of 10 and 15 cm has been decreased to 35 and 44 % during the period of 1985 and 1996 respectively (Hussain and Ahmed 1994). This scenario indicates over-exploitation attitudes of *E. agallocha*.

Moreover, salinity is increasing in the coastal regions due to reduced fresh water flow from upstream (Islam & Wahab, 2005; Gopal & Chauhan, 2006; Basar, 2012). Consequently, it poses a threat to the vegetation of the Sundarbans (Minar et al., 2013) as well as the vegetation of the coastal regions. Moreover, increasing salinity may create a challenge to select suitable mangrove species for coastal afforestation. Hence, identifying the salt adapted phenotypes of *E. agallocha* is of paramount importance for the sustainable ecosystem management of the Sundarbans and for successful coastal reforestation in the high saline substrates.

It is important to know the regeneration ecology of mangrove species (Pascual, 2016) because successful regeneration determines the establishment of a wetland mangrove plant community (Wall & Stevens, 2015). However, several researchers studied the effect of salinity on mangroves and found varied results eg. Mahmood et al. 2014 studied *Heritiera fomes* Buch.-Ham., *Xylocarpus mekongensis* Pierre, *Xylocarpus granatum* K. D. Koenig, and *Amoracucullata* Roxb in the Sundarbans of Bangladesh, *Aegiceras corniculatum*, *A. marina* from Hong Kong (Ye et al. 2005); *A. schaueriana* and *Laguncularia racemosa* from Southeast Brazil (Cavalcanti et al. 2007); *Ceriopstagal* from India (Patel et al. 2010); *C. roxburghina* from India (Elumalai & Manikandan 2013). Even the germination varies for same species if collected from two distinct salinity zones (Alam et al, 2017).

Despite having typical ecological significance and adaptability of *E. agallocha* in a wider range of salinity, the variations in characteristics within this species due to salinity are still unknown. However, this knowledge would be of particular importance for identifying and selecting more salt-adapted phenotype(s) of *E. agallocha* for coastal afforestation programs in Bangladesh. However, studies on the effect of salinity and zone, on the germination of seed and seed viability of the important tree species like *Excoecaria agallocha* of the Sundarbans and somewhere else is scarce.

1.2 Objectives of the Study

Therefore, the intent of my research is to find out

- ✓ The effect of salinity on the germination of *Excoecaria agallocha*.
- ✓ Does the germination vary due to salinity in between two contrasted salinity zone?

Chapter: Two

Materials and Methods

2.1 Seed collection

The dominant patches of *Excoecaria agallocha* were identified with the help of vegetation map (ODA, 1985) and salinity map to collect the seeds of this species from two different maternal origins, viz., LSZ and MSZ in the Sundarbans. From each saline zone, seeds were collected from a minimum of 50 mother trees during the month of July, 2017. The collected seeds were then sorted manually to get the healthy ones considering the visual appearance and kept zone wise separately in the laboratory.

2.2 Materials

The experiment was carried out in hydroponic culture in glasshouse at Khulna University nursery as it permits efficient control on level of salinity and ease of management.

The following materials were used to perform the experiment.

- ❖ Plastic bowl
- ❖ Tap water
- ❖ Course sand
- ❖ Salt (NaCl)
- ❖ Salinity Refractometer ATAGO, S/MILL-Japan.
- ❖ Plastic tub

2.3 Seed Germination

The experiment was conducted through use of a randomized block design in a glass house in the forest nursery of Khulna University to study. Twenty seven bowls (75 cm × 75 cm × 12 cm of each) filled with a 3 cm thick layer of coarse sand were prepared. From the zone-wise previous collection, 100 germinations were sown in each of the 27 trays prepared for each of the two saline zones. Distilled water was used for 0 ppt treatment level. The other eight levels of salt solution (5, 10, 15, 20, 25, 30, 35 and 40 ppt) were prepared by using crude sea salt (unrefined sea salt) containing all the chemical constituents of sea water. Salt solution of respective was added to the bowl in such way that a thin layer of solution can be seen on the layer of coarse sand. Frequently the solution layer and their salinity level were checked and

maintained. Total number of germinated seeds were counted and recorded on daily basis for almost 10 weeks.

The germination growth initiation traits were calculated as follows:

$$\text{PGIT (day)} = \text{Day of first growth initiation} - \text{Day of germination sowing} \dots \dots \dots (1)$$

$$\text{MGT (day)} = \frac{\sum n_i d_i}{\sum n_i} \dots \dots \dots (2)$$

Where n_i is the number of growth initiated germinations in d_i ; d_i is the number of days after sowing (Orchard, 1977)

$$\text{PGIP} = \frac{\text{Number of growth initiated seeds at the end}}{\text{Total number of sown seeds}} \times 100 \dots \dots \dots (3)$$

2.4 Preparation of Salt solution:

A stock of salt solution of 100 ppt was prepared by adding normal salt (NaCl) in water, which was checked with hand salinity Refractometer ATAGO, S/MILL-Japan. Using the stock solution, solutions of various salinity levels were prepared using the following formula:

$$V_1 S_1 = V_2 S_2$$

Here, V_1 = Volume of solution in cubic centimetre

S_1 = Final concentration of solution in ppt

S_2 = Known concentration of stock solution in ppt (100 ppt)

V_2 = Determining volume of stock solution in ppt

2.5 Statistical analysis

PGIT, MGT and PGIP of *Excoecaria agallocha* of three saline zones under different salinity treatments were analyzed by two-way ANOVA and correlation to find out the impact of the increasing salinity on the growth initiation traits and their variations between the saline zones. LSD followed by Bonferroni adjustment (sig.^b) at the 0.05 significance level were performed as post-ANOVA tests for pair wise comparisons in respect of all the studied parameters between saline zones and salinity zone (origin) interactions at the respective salinity levels. These analyses were performed to find out the variations in the impact of salinity gradient on the germination morphology, and germination growth initiation traits of *Excoecaria agallocha* in the two distinct saline zones of the Sundarbans.

Chapter: Three

Results

Seedlings growth initiation traits of *Excoecaria agallocha*

Seedlings growth initiation time (PGIT) of *Excoecaria agallocha* did not vary significantly ($p=0.7387$, $p>0.05$) with the increasing saline treatments for less saline zone but for moderate saline zone it varied significantly (MSZ: $p= 1.29e-05$, $p<0.05$) (Fig. 3a). After sowing, the seedlings of *Excoecaria agallocha* originating from LSZ and MSZ, initiated their growth after 4 and 7 days respectively at 0 ppt and 11 and 17 days, respectively at 20 ppt salinity level.

PGIT showed positive correlation (LSZ: $r = 0.067308$ and MSZ: $r= 0.883$, Table 2) with increasing salinity. On the other hand, there was no significant difference ($p\text{-value} = 0.5185$) in PGIT values between less and moderate saline zones with the increasing salinity treatments.

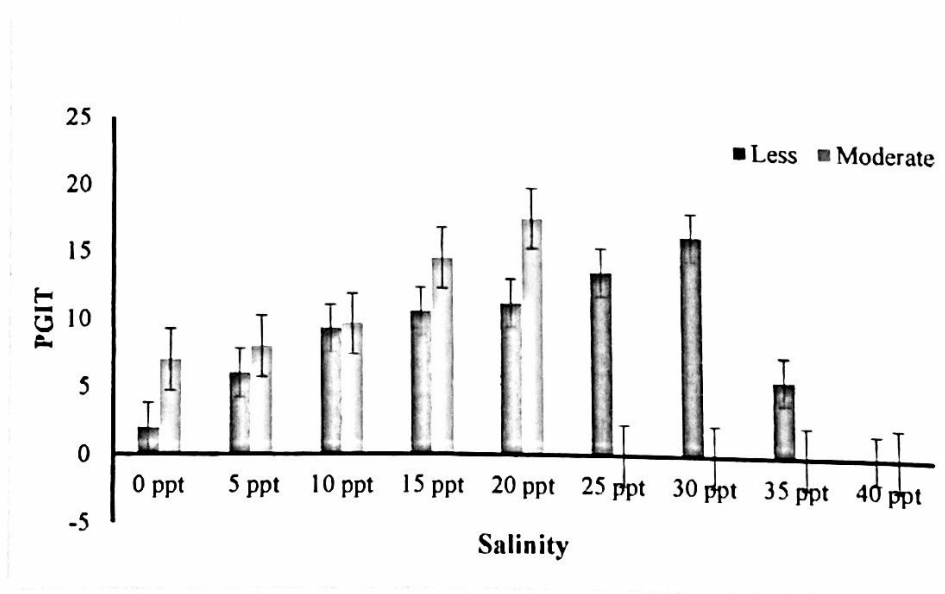


Fig-3.1: Effect of salinity level on PGIT

Bonferroni adjustment showed no significant difference in PGIT values for less saline zone. However, for moderate saline zones, significant difference was found for salinity level from

15 ppt ($\text{sig}^b < 0.05$). However, after 20 ppt salinity levels no germination in MSZ was observed. Therefore, the Seedlings of *Excoecaria agallocha* originating from LSZ initiated their growth faster (lower PGIT values) than those originating from MSZ (higher PGIT values).

Pair wise comparisons using t tests with pooled SD for MSZ (Moderate Saline Zone)

Data: PGIT and Salinity

	0	5	10	15
5	1.0000	-	-	-
10	1.0000	1.0000	-	-
15	0.0183	0.0445	0.2088	-
20	0.0016	0.0035	0.0137	1.0000

Since PGIT increased with the increasing salinity, it affected MGT correspondingly (strong positive correlation between PGIT and MGT (LSZ: 0.778 and MSZ: $r = 0.905$; Table 2).

MGT of *Excoecaria agallocha* showed no significant variation in less saline zone (LSZ= 0.0966). However, significant variation was found for moderate saline zones (MSZ= 6.79×10^{-6} *** , $\text{sig}^b p < 0.0001$) with increasing salinity.

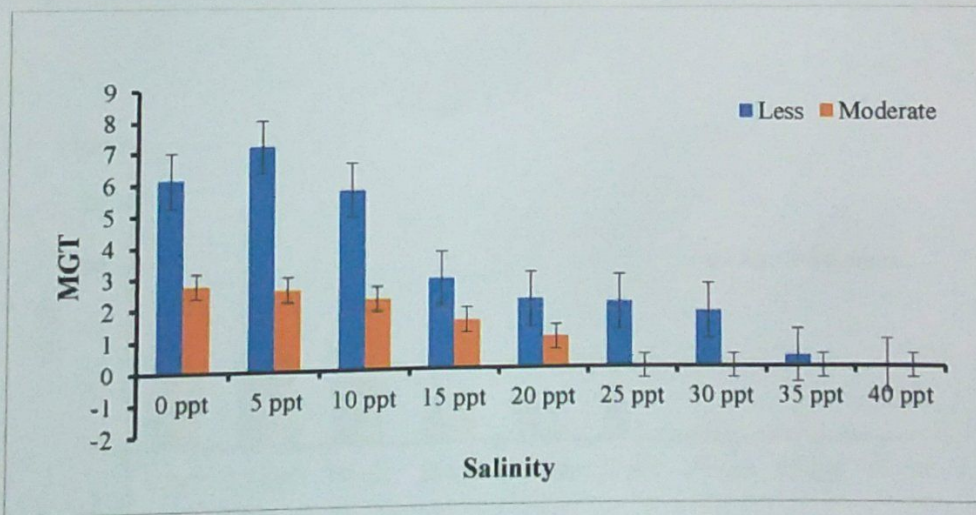


Fig-3.2: Effect of salinity level on MGT

Bonferroni adjustment (Table 3) showed significant difference in MGT of moderate saline zones was observed from salinity 15 ppt and above ($\text{sig}^b < 0.05$). On the other hand, no

significant difference in MGT values was observed for less saline zone. However, there was no significant difference (p-value = 0.0966) in MGT between less and moderate saline zones with the increasing salinity treatments.

Pair wise comparisons using t tests with pooled SD for MSZ (Moderate Saline Zone)

Data: MGT and Salinity

	0	5	10	15
5	0.52832	-	-	-
10	1.00000	1.00000	-	-
15	0.00249	0.07478	0.03489	-
20	0.00018	0.00290	0.00154	0.63641

A significant variation ($p < 0.0001$) in PGIP of *Excoecaria agallocha* was observed with the increasing saline treatments for both less and moderate saline zones (Low: $p = 3.316e-11$ *** and Moderate: $p = 1.971e-06$ ***) and PGIP was negatively correlated ($r = -0.91266$; Table) with the increasing salinity. PGIP of the seedlings of *Excoecaria agallocha* originating from LSZ was found from $83.33 \pm 2.2\%$ to $0.33 \pm 0.27\%$ across the salinity gradient (from 0 to 35 ppt). However, for MSZ, PGIP varied from, $42.66 \pm 6.27\%$ to 0% respectively across the salinity gradient (from 0 to 20 ppt). The growth initiation success (%) of the seedlings originating from LSZ was higher at high salinities than those from MSZ.

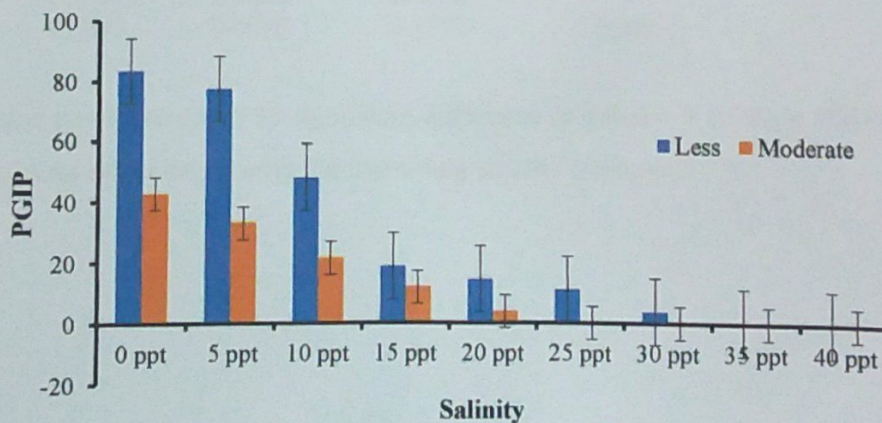


Fig-3.3: Effect of salinity level on PGIP

Bonferroni adjustment showed significant difference in PGIP in less saline zone from salinity 0 to 25 ppt but no significant difference in was observed from 25 ppt and above.

Pair wise comparisons using t tests with pooled SD for LSZ (Less Saline Zone)

Data: PGIP and Salinity

	0	5	10	15	20	25	30	35
5	1.0000	-	-	-	-	-	-	-
10	1.4e-06	2.0e-05	-	-	-	-	-	-
15	8.1e-11	4.2e-10	2.0e-05	-	-	-	-	-
20	2.7e-11	1.3e-10	2.8e-06	1.0000	-	-	-	-
25	1.2e-11	5.3e-11	6.8e-07	1.0000	1.0000	-	-	-
30	2.3e-12	8.8e-12	4.1e-08	0.0414	0.4803	1.0000	-	-
35	1.1e-12	4.1e-12	1.3e-08	0.0062	0.0735	0.4803	1.0000	-
40	1.0e-12	3.8e-12	1.1e-08	0.0051	0.0607	0.3998	1.0000	1.0000

Bonferroni adjustment showed significant differences in PGIP in moderate saline zone were observed from 15 ppt and above.

Pair wise comparisons using t tests with pooled SD for MSZ (Moderate Saline Zone)

Data: PGIP and Salinity

	0	5	10	15
5	1.0000	-	-	-
10	0.0632	0.8929	-	-
15	0.0054	0.0632	1.0000	-
20	0.0009	0.0080	0.1727	1.0000

On the other hand, there was no significant difference (p-value = 0.1978) in PGIP between less and moderate saline zones with the increasing salinity treatments.

Chapter: Four

Discussion

Effect of salinity on the seed germination of *Excoecaria agallocha*

Significant impact of salinity on the germination initiation traits of *Excoecaria agallocha* was observed. We found that the increasing salinity remarkably delayed the germinations growth initiation (higher PGIT values with the increasing salinity). Waisel (1972) and Kim et al. (2013) reported that high salt concentration in the medium induces high osmotic potential, ion toxicity and restricts water availability to germinations which, in turn, affect imbibitions, enzyme activity and cell division, thereby inhibiting and delaying growth initiation. This delaying effect on germination growth initiation of *Excoecaria agallocha* was not significantly different among the saline zones up to 10 ppt salinity levels. Therefore, the germinations initiated their growth almost equally salinity levels irrespective of their maternal origins.

However, growth initiation of germinations originating from LSZ was significantly faster and more vigorous (lower PGIT and MGT values) than those originating from MSZ (higher PGIT and MGT values ;). Quick growth initiation of the germinations of this species is extremely important because Siddiqi (2001) reported that the germinations of *Excoecaria agallocha* lose their viability rapidly. We found that PGIP of *Excoecaria agallocha* decreased significantly with the increasing salinity treatments. Similar results were observed by Bytnerowicz & Carruthers (2014) and Freitas & Costa (2014), Hoque et al. (1999) for *S. apetala* and Mahmood et al. (2014) for *H. fomes*, *X. mekongensis*, *X. granatum* and *A. cucullata* at high salinities.

However, our study found insignificant salinity effect on PGIP was irrespective of germinations maternal origins. The highest PGIP were observed at 0 ppt treatment level irrespective of zones. This indicates that *Excoecaria agallocha* has facultative halophytic characteristics at this life stage. This might happen for the germinations of *Excoecaria agallocha* due to their maternal exposure to high salt conditions in MSZ. Waisel (1972) reported that such variations in germinations growth initiation are possible when germinations are collected from different saline conditions. He also reported that germinations originating in a saline environment would grow better in that environment.

Seeds of *Excoecaria agallocha* showed clear preference for low salinities from 0 to 5 ppt and salinity beyond 15 ppt significantly delayed root initiation and seedling establishment. Seed germination is extensively reported to be reduced, retarded (Patel et al. 2010, Abari et al 2011, Khan et al, 2003) or completely inhibited (Benabderrahim et al. 2011) at high salinities. High salinities could inhibit seed germination either by impeding water uptake (Werner et al 1995) or through facilitating the intake of toxic ions (Rehman et al 1997, Kaveh et al 2011).

Germinations growth initiation potential of *Excoecaria agallocha* was found to be restrictive to salinity. For example, PGIP of germinations originating from LSZ and MSZ was only 14.33% and 3.33% respectively at 20 salinity ppt. From 20 ppt upward, germination growth initiation was only observed for LSZ zone. Janousek & Folger (2013) reported that germination sources have implications on salt adaptability of mangroves during germination.

Though mangrove plants are well adapted to salinity (Tomlinson 1986), they are more sensitive to higher salinity during seed germination (Ungar 1996, Khan & Abdullah 2003, Debez et al. 2004). Seed germination of mangrove species may depend on the critical level of salinity which is species and site specific (Ye et al. 2005, Liu et al. 2006, Cavalcanti et al. 2007). *Excoecaria agallocha* are found to occur at the less saline areas and moderate areas of the Sundarbans (Karim 1988, Siddiqi 2001). The dominance characteristics of a mangrove species at a particular site may be related to its salt tolerance at germination stage (Harradine 1982).

We also found differences in the influence of salinity stress of the germinations on their growth initiation traits. Compared with MSZ, the germinations originating from LSZ initiated their growth quickly and vigorously with greater growth initiation success (%) even at high salinities. These results support our hypothesis that *Excoecaria agallocha* which grows in high saline conditions is the most salt-adapted phenotype of this species. The ability of the germinations originating from LSZ to initiate their growth faster and vigorously in high saline conditions could be a mechanism to colonize themselves in fragile mangrove environments. By doing so, *Excoecaria agallocha* continued its habitat-creating role in the high saline environments in the Sundarbans.

Chapter: Five

Conclusion

Excoecaria agallocha is highly sensitive to salinity, especially at early developmental stages. The favorable salinity range for seed germination is below 5 ppt, and salinity of 15 ppt decreased seedling establishment rate to 60%, which partly explained why few seedlings can be found in the mangrove reserve which has soil salinity slightly higher than 15 ppt. The adverse saline condition in the field might act as a primary obstacle for natural regeneration of this species. Thus, artificial breeding and culture should be adopted to ensure higher survival rate of *E. agallocha* seedlings.

Chapter: Six

Recommendation

It is predicted that more salt-adapted phenotype of *Excoecaria agallocha* will be able to negotiate high salinity regeneration niches in the Sundarbans, thereby continuing its ecological role even in high saline mangrove habitats. We suggest some experiments from the High saline zone and carry further studies on *E. agallocha* so that we can suggest *Excoecaria agallocha* of the Sundarbans be strategically selected for coastal afforestation on potentially high saline substrates in the coastal regions of Bangladesh in the future with confidence.

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