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GERMINATION TRAITS OF *AVICENNIA OFFICINALIS* IN RESPONSE TO SALINITY

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Course No: FWT- 4114

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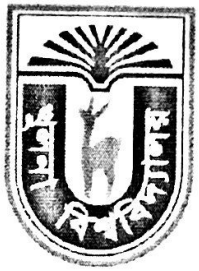

Project Thesis has been submitted to Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh for the partial fulfillment of the requirements for the Degree of Bachelor of Science (Hons.) in Forestry.

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Dedicated to….

My departed mother, as well as respected father and beloved sister

Declaration

I, Lulu Rayhan Khushi, declare that this thesis submitted for the Degree of Bachelor of Science (Honors) in Forestry to Forestry and Wood Technology Discipline, Khulna University, Khulna, is my own original research work and have not previously been submitted or it has not been accepted to any other institution.

I, hereby, give consent for my thesis, if accepted, to be available for any kind of photocopying and for inter-library loans.

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Approval

Project thesis has been submitted to Forestry and Wood Technology Discipline, Khulna University, Khulna, Bangladesh, for the partial fulfillment of the requirements for the 4-year professional B.Sc. (Hons) degree in Forestry. I have approved the style and format of the project thesis.

Hossain 4/5/16

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Lulu Rayhan Khushi

ABSTRACT

Avicennia officinalis is an exclusive pioneer mangrove tree species in the Sundarbans. It is the second principal species of coastal afforestation in Bangladesh. Salinity regulates distribution, germination, survival, growth, early development, dominance and zonation of mangroves. Seed viability was tested. Germination traits of *Avicennia officinalis* in response to salinity were studied. Cumulative Germination Percentage reached maximum (91.67%) at 0 ppt salinity level within 26 days after seed sowing, and decreased with increasing salinity. Final Germination Percentage, Germination Initiation Time, Mean Germination Time, Germination Index, Germination Value, and Coefficient of Uniformity of Germination varied from 91.67±2.85 to 39±4.17%, 6 to 17 days, 13 to 33 days, 7.41± 0.34 to 1.25± 0.14 seed day^{-1}, 19.36± 2.41 to 0.76 ±0.13 $\%^2$ day^{-2}, and 0.09± 0.02 to 0.02 ±0.00 day^{-2} respectively. Salinity induced physiological infliction upon the seeds of the species rendering them not readily able to germinate spontaneously and vigorously. Therefore, germination success of *Avicennia officinalis* decreased significantly ($p < 0.05$) with increasing level of salinity.

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CHAPTER ONE

INTRODUCTION

1.1 Background and Justification of the study:

Mangroves are the littoral plant communities in the tropical and subtropical coastal areas in the world. Mangrove forest bears unique ecological and economic significance in protecting coastal areas, sustaining biodiversity as well as providing wood (Hutchings and Saenger, 1987; Ahmed et al., 2012). Sundarbans is the single largest tract of mangrove forest in the world (Spalding et al 2010) which is located between 21 ° 30' and 22 ° 30' N latitudes and between 89 ° 00' and 89 ° 55' E longitudes.

The Sundarbans represents about 44% of the total government managed forest in Bangladesh (Rahman, 1998). There are 334 species of plants, 49 species of mammals, 315 species of birds, 400 species of fish, and 53 species of reptiles in the Sundarbans (Karim, 1995). It is a natural home for *Panthera tigris* and *Axis axis* (Moss, 1993). It has been declared as World's heritage site in 1997 (Basar, 2012). It protects the south western part of Bangladesh against frequently occurring tropical storms, tidal surges and also protects the coastal agricultural lands against salt intrusion from the sea. Moreover, it also provides employment opportunities for more than 600000 people in the country (ESCAP, 1987). It contributes 41% of the forest revenue and 45% of timber and fuel wood consumption of Bangladesh (FAO, 1995). Sundarbans bears outstanding biological interest, and provides ample opportunities for outdoor recreation, scientific research and conservation education (UNESCO, 1978). In view of its ecological, environmental, protective, economic, and recreational values, maintaining continuous vegetation cover in the Sundarbans is of paramount importance for Bangladesh.

Avicennia officinalis L. is an exclusive mangrove (Mahmood, 2015) and one of the pioneer tree species in the Sundarbans (Naskar and Bakshi, 1987; Siddiqi, 2001). This species naturally occurs in the Sundarbans from less saline to strong saline zones (ODA, 1985; Siddiqi, 2001; Mahmood, 2015). It is mostly planted on saline substrate in the coastal regions of Bangladesh (Das and Siddiqi, 1985; Siddiqi and Khan, 1990) and becoming the second principal species (20%) in the coastal afforestation programme (Das and Siddiqi, 1985). This species creates suitable condition for the species of the next seral stages like *Heritiera fomes, Excoecaria agallocha* (Naskar and Bakshi, 1987). Considering its ecological significance in the

Sundarbans, Bangladesh Forest Department started coastal afforestation programme with *S. apetala* and *A. officinalis* to provide protection to the coastal areas (Fig. 1) against cyclone damage and tidal surges (Siddiqi and Khan, 1997).

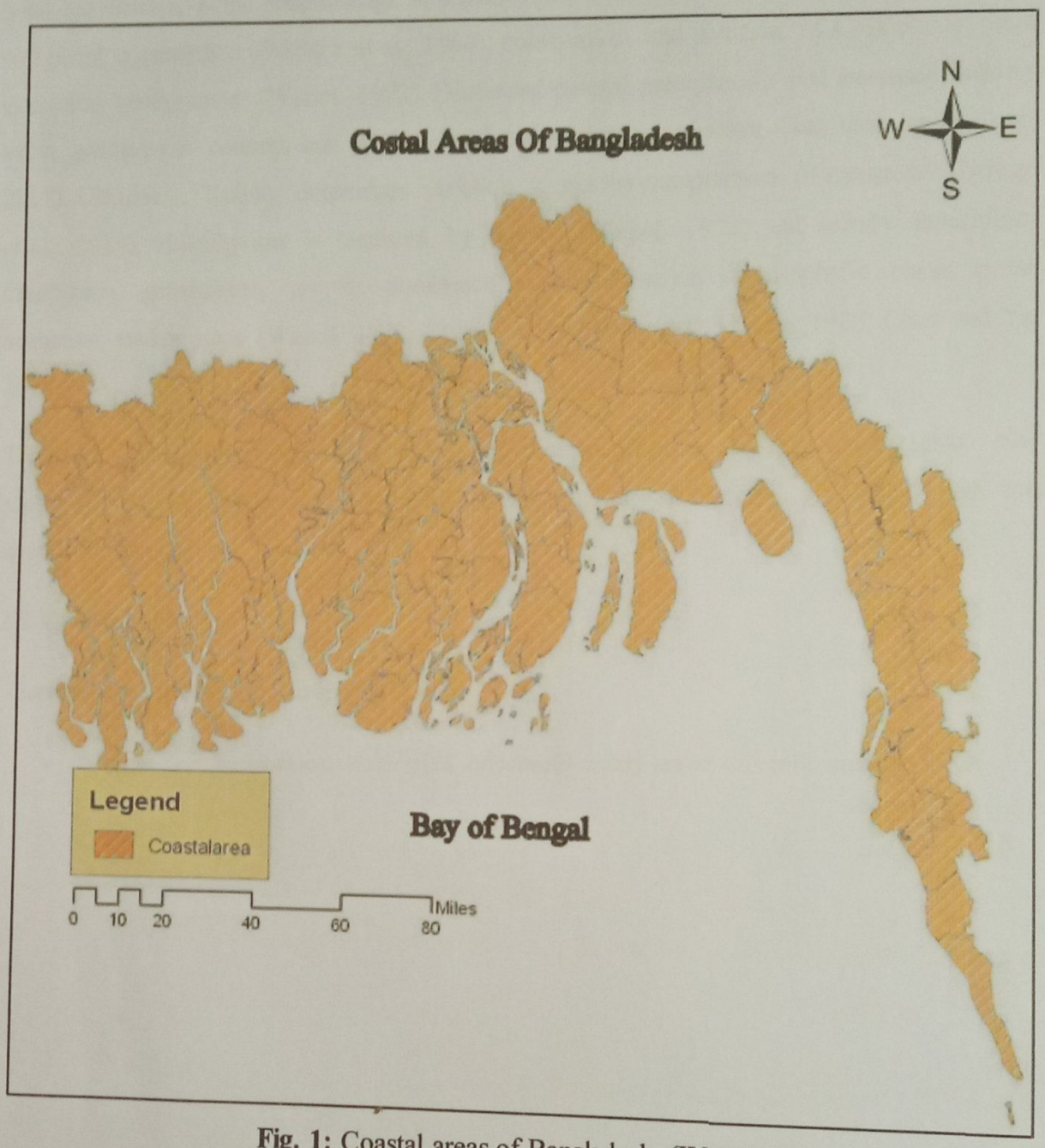

Fig. 1: Coastal areas of Bangladesh (IUCN)

Approximately, two lac hectare of newly accreted coastal land has so far been brought under plantations with mangrove species. Approximately 52% of *Sonneratia apetala* in the coastal plantations has been affected by stem borer *Zeozera conferata* (Islam et, al., 1989) To solve this problem, *S. apetala* has been planted in mixture with *A. officinalis* (Zabala, 1990). So, *A. officinalis* can be an important species for mangrove plantations in Bangladesh. The species

plays an important role in coastal ecosystem by stabilizing the shores and by preventing excessive shifting of coastline and soil erosion resulting from tidal current (Das et al., 2014).

Seed germination is the critical stage in a plant's life cycle (Waisel, 1972) which determines successful regeneration (Melana et al., 1980), colonization and zonation of a halophytic plant in a saline environment (Waisel, 1972). Decreased coastal precipitation and increased salinity affect germination patterns and tidal wetland vegetation composition (Janousek and Folger, 2013). Ultimately, Salinity determines variation in species composition of mangrove (Urrego et al., 2014). Halophytism is regulated by salinity (Waisel, 1972) and salinity determines distribution, germination, growth, dominance and reproduction of halophytic plants in the mangrove environment (Waisel, 1972; Chapman, 1976; Das and Siddiqi, 1985; Chen and Ye, 2014).

Therefore, the germination traits of *A. officinalis* in different salinity levels might play significant role for mangrove restoration, nursery raising and coastal plantations with this species in Bangladesh.

1.2 General objective:

General objective of this study was to

- explore the germination traits of *A. officinalis* seeds under different salinity levels.

CHAPTER TWO
REVIEW OF LITERATURE

A comprehensive review of available literature on *Avicennia officinalis* has been presented. Moreover, literature on influences of salinity on germination of halophytes have been reviewed.

2.1 *Avicennia officinalis*

2.1.1 Systematics of *Avicennia officinalis* Linn.

Kingdom: Plantae

Division: Spermatophyta

Sub division: Angiospermae

Class: Dicotyledonae

Sub class: Gamopetalae

Order: Labiales

Family: Avicenniaceae

Genus: *Avicennia*

Species: *Avicennia officinalis* Linn.

2.1.2 Global distribution of *Avicennia officinalis*

Avicennia officinalis is distributed from south Indo-Malaya to New Guinea and eastern Australia (Tomlinson, 1994; Fig. 2). This species is found to occur in the mangrove forests of Bangladesh, India, Pakistan, Indonesia, Malaysia, Mayanmar, Andaman Island, Philippines, and New Guinea. In Bangladesh, this species naturally grows in the Sundarbans, Chakaria Sundarbans and is also planted in the coastal areas of Bangladesh (Mahmood, 2015 Fig. 3).

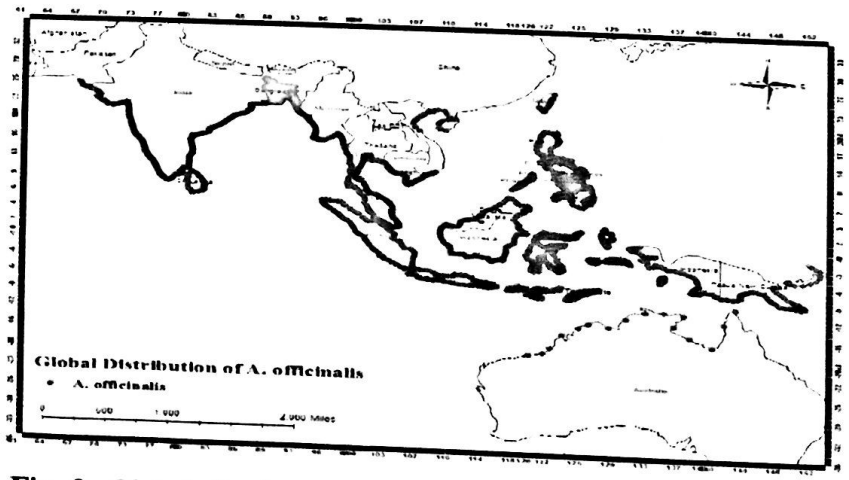

Fig. 2: Global distribution of *Avicennia officinalis* (Walsh, 1974).

Avicennia officinalis, *Avicennia marina*, and *Avicennia alba* belong to the family Avicenniaceae. They appear as pioneer tree species in mangrove succession, often occur in association with *Porteresia coarctata* on newly accreted substrate alongside the rivers, channels and creeks (Chaudhuri and Chaudhuri, 1994). Among these, *A. officinalis* is found to occur most frequently on the land ward border. Growth of *A. officinalis* is higher than that of *A. marina* and *A. Alba* (Siddiqi, 2001).

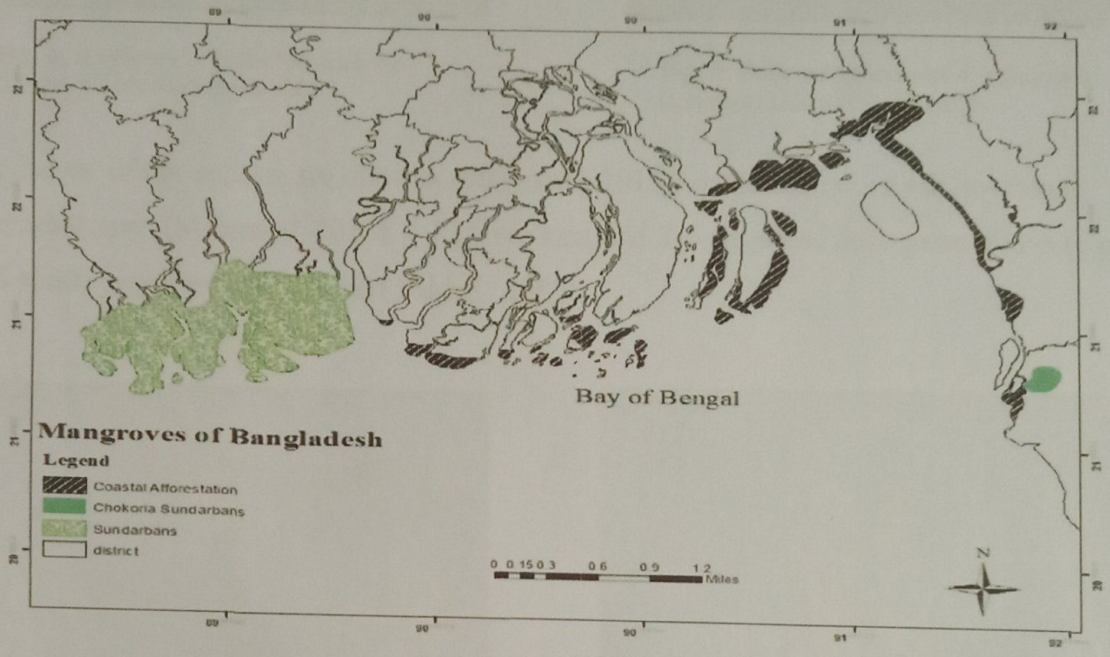

Fig. 3: Distribution of *Avicennia officinalis* in Bangladesh

2.1.3 Morphology of *Avicennia officinalis*

Avicennia officinalis is a small to medium sized tree (Fig. 4), usually 10 to 13 m in height but may attain height up to 20 m in suitable places. Main stem often becomes crocked (Das and Siddiqi, 1985; Siddiqi, 2001). Older trees usually form spreading crown. The stem becomes hollow and rotten as the tree grows older (Das and Siddiqi, 1985). Bark appears smooth, lenticellate, and lighter - grey in color (Mahmood 2015). The pneumatophores are thin and finger like appearance (Fig. 5) and numerous lenticels appear over the surface of pneumatophores (Das and Siddiqi, 1985). Usually, pneumatophore develops within two years of the anchorage of the seedlings (Siddiqi, 2001). Short aerial roots might be projected from the trunk (Das and Siddiqi, 1985; Tomlinson, 1987).

Fig. 4: *A.officinalis* in the Sundarbans
(Courtesy: Lulu Rayhan Khushi)

Fig. 5: Pneumatophores of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

The leaves of this species are obovate (Mahmood 2015), ovate-elliptic or elliptic-oblong, with a rounded apex (Mahmood 2015), 4-12.5 cm long and 2-6 cm wide, dark green above (Fig. 6), and bluish grey beneath (Das and Siddiqi, 1985)

Fig. 6: Leaves of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

Fig. 7: Inflorescence of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

2.1.4 Phenology of *Avicennia officinalis*

Budding commences in late March – late May in the Sundarbans. The inflorescences of *A. officinalis* are head-like (Fig. 7) with 2-12 small yellow flowers congested towards a head. Flowering of this species commences in April- August and seeds ripen in July-October in the Sundarbans. Flowers are yellow in color (Fig. 8). Fruits are broadly ovate (Fig. 9) about 3 cm long with short apical break (Tomlinson, 1987; Mahmood 2015). This species shows periodicity in seed production (Zabala, 1990). Seed producing ability of this species is much more than many other true mangroves (Naskar et al., 1999).

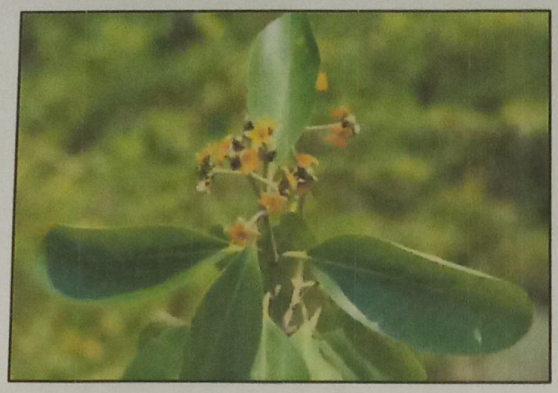

Fig. 8: Flowers of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

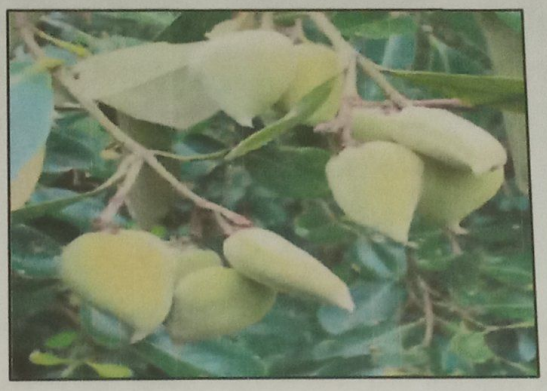

Fig.9: Fruits of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

2.1.5 Adaptations of *Avicennia officinalis*

The seeds are buoyant (Siddiqi, 2001), crypto viviparous (Fig. 10), germinate immediately after falling from the tree, or while attached with the mother tree (Joshi et al., 1972; Naskar and Mandal, 1999). Germination is epigeal (Fig. 11) (Zabala, 1990). The propagules usually loss viability within few days when kept in air (Siddiqi, 2001). The species produces pneumatophores and aerial roots. There are numerous lenticles over the surfaces of pneumatophores, aerial roots and stem to trap oxygen from the atmosphere for root's respiration. This species is a salt accumulator, and the leaves of it contain salt glands (Tomlinson, 1987).

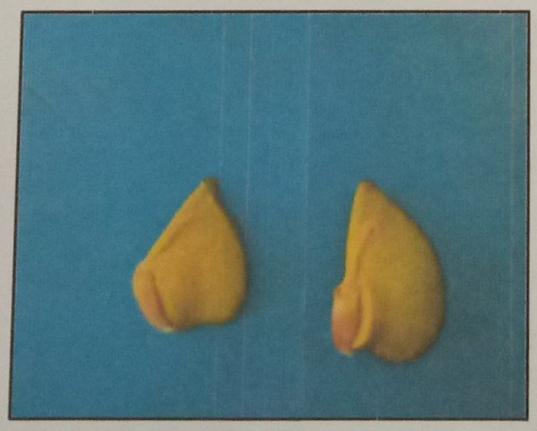

Fig. 10: Cryptovivipary of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

Fig. 11: Germination of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

2.1.6 Habitat of *Avicennia officinalis*

Avicennia officinalis is found to occur in the intermediate estuarine zone of lower intertidal region. It is a light demanding species and can tolerate water salinity up to 63 ppt (Tomlinson 1987; Robertson and Alongi, 1992; Mahmood 2015). This species is able to tolerate fluctuating

salinities (MacMillan, 1974). This species grows on soft, recently consolidated mud banks. It prefers the temperature ranging from 20° to 30°C, absolute humidity ranging from 70% to 90% and mean annual rainfall 2540 mm, mostly moonsonic (Zabala, 1990).

2.1.7 Regeneration of *Avicennia officinalis*

2.1.7.1 Natural regeneration

The fruit of *A.officinalis* is dicotyledonous and single seeded. Mature seeds shed from the mother trees during July to October (Mahmood, 2015) .The seeds are buoyant and are able to spread by tidal water (Siddiqi, 2001). Seeds germinate immediately after falling, or even in the trees and shows cryptoviviparity (Joshi et al., 1972; Naskar and Mandal, 1999). It coppices well (Siddiqi, 2001). The species naturally occurs along the riverbanks (Fig. 12).

Fig. 12: Natural growth of *A. officinalis* in the Sundarbans
(Courtesy: Lulu Rayhan Khushi)

2.1.8.2 Artificial regeneration

Seedlings of *A. officinalis* were planted in the vacant areas of the Sundarbans and offshore islands. However, survival of the planted seedlings could not be ensured due to its high palatability to deer (Siddiqi, 1996).The species was planted in the Chokoria Sundarbans for reforesting the area. However, it was unsuccessful because of human and other biotic interferences.

Mature fruits of *A. officinalis* were collected from Sundarbans to raise coastal plantations. Germination started within three days and 90% germination success was found within 10 days after sowing. Plantations were raised either by seeds or seedlings. Seeds are sown by dibbling or broadcasting. Dibbling process is preferable where seeds are sown by 1 m x 1m spacing (Saenger and Siddiqi, 1993). Pretreated fruits can also be used to decrease establishment time (Siddiqi, 2001). Vacancy filling with seedlings is preferable to sowing (Das and Siddiqi, 1985).

2.1.9 Uses of *Avicennia officinalis*

Avicennia officinalis is mainly used as fuel wood and anchor logs part. Wood is also used for furniture, house posts, boat building and general construction. Leaves are good fodders, different parts of plant have medicinal value (Siddiqi, 2001; Zabala, 1990).

2.2 Salinity and seed germination

Sea water characteristically contains high proportion of sodium chloride (NaCl). Salinity is the specific dominant factor which regulates halophytism. Salinity exerts both osmotic and ionic effects on germination. Chloride salts are most toxic for seed germination and the degree of imbibition, delay, or inhibition of germination is proportional to an increase in external osmotic potential (Waisel, 1972). Saline environment can affect and inhibit germination in two ways: 1) by preventing uptake of water by the embryo due to the presence of high osmotic potential in the germination medium and 2) by poisoning the embryo due to the toxic effect of Na^+ (Waisel, 1972). Germination success of different mangrove species (Sonneratia *apetala, Heritiera fomes, Xylocarpus mekongensis, Xylocarpus granatum, Amoora cucullata)* remarkably decrease with increasing salinity (Siddiqi et al., 1989; Hoque et al., 1999 Mahmood 2015). *Sonneratia lanceolata* seeds germinate better in salinities ranging from fresh water to 5% sea water (Ball and Pidsley, 1995). Joshi, et al. (1972) reported that *Acanthus illicifolius* germinated on saline substrate only when salinity was remarkably lowered during monsoon. Most of the salt marches germinate at low levels of salinity (Ball and Pidsley, 1995)

Higher concentration of salt adversely affects almost all the physiological process of mangroves (Waisel, 1972; Chapman, 1976; Ball and Pidsley, 1995). Salinity affects photosynthesis, morphology and growth of mangroves. The photosynthetic efficiency of *Aegiceras corniculatum* and *Avicennia marina* decreases with increasing salinity (Ball and Farquhar, 1984). Both constant and fluctuating salinity significantly affect photosynthesis and growth, thereby affecting morphological and physiological characteristics of mangroves (Lin and Sternberg, 1993). Mangrove species often show stimulated growth at low salinity (Downtown, 1982; Mahmood 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the study area

Sundarbans is located between 21 ° 30' and 22 ° 30' N latitudes and between 89 ° 00' and 89 ° 55' E longitudes. Based on the level of water salinity, Sundarbans has been divided into less saline (LS), moderate saline (MS) and strong saline (SS) zones having salinity ranging from 0.5-5 ppt, 5-18 ppt and 18-30 ppt respectively (Siddiqi, 2001). Rainfall is strongly seasonal (from May to October) with 87 % of the mean annual rainfall (1500 mm). Temperature ranges from 18.50 to 35.20 °C in summer and from 12.20 to 28.80 °C in winter. Soil is silty to sandy clay loam, as well as bulk density, particle density and porosity vary from 1.18 to1.27 g/cc, 2.31 to 2.52 g/cc and 46 to 52%, respectively. Soil pH is 7.8 (Siddiqi, 2001). *Heritiera fomes* is dominant followed by *Xylocarpus mekongensis, Bruguiera gymnorrhiza,* and *Excoecaria agallocha* in LS zone while *Excoecaria agallocha* dominates, and is often mixed with *Heritiera fomes* and *Ceriops decandra* in MS zone. Moreover, *Ceriops decandra* mostly forms closed understorey with *Excoecaria agallocha* and *Xylocarpus mekongensis* as overstorey in the SS zone. However, *Avicennia officinalis* forms pure patches in the three saline zones of the Sundarbans (ODA, 1985; Ali, 1998; Moss, 1993; Siddiqi, 2001).

3.2 Seed collection and processing.

Pure stands of *A. officinalis* were identified from the Overseas Development Agency (ODA) vegetation map (ODA, 1985) and salinity map of the Sundarbans. Mature Seeds were collected from randomly selected mother trees of *A. officinalis* from Amoorbunia (Fig. 13) which is within freshwater zone of the Sundarbans and lies between N 22 ° 22 ' 25.0 " and E 89 44 ° 35 ' 7 ". Fruits were collected by climbing on mother trees (Fig. 14) during August, 2015.

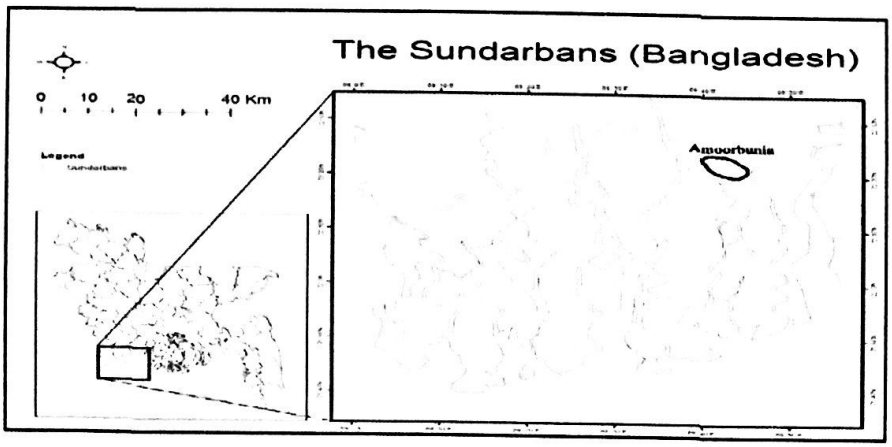

Fig.13: Location of the seed collection area

Collected seeds were brought to forest nursery at Khulna University (fig. 15). Collected seeds were sorted manually. Insect attacked, oversized, and under sized seeds were discarded.

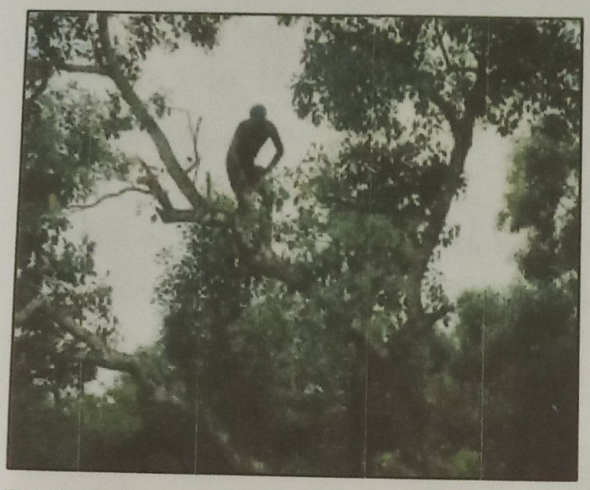

Fig. 14: Collection of *A. officinalis* seeds
(Courtesy: Lulu Rayhan Khushi)

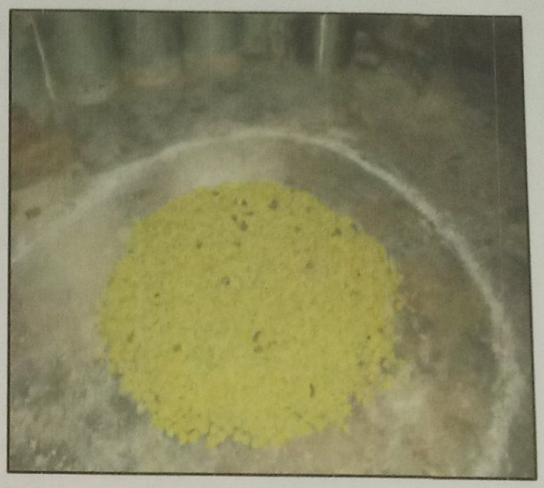

Fig. 15: Seeds of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

3.3 Preparation of stock solution from crude sea salt

Crude sea salt was used for this experiment in order to maintain an ideal condition of sea water as much as possible. Stock solution of 80 ppt was prepared by adding tap water in sea brine and concentration of solution was checked with a hand held salinity Refractometer (Master Refractometer Manual, Qtago, Tokyo, Japan)

3.4 Preparation of salt solution of different concentrations

Using the stock solution, solutions of different salinities (0, 5, 10, 15, 20, 25, 30, and 35 ppt) were prepared by using the following formula:

$$V_1S_1=V_2S_2$$

Here,

V_1 = volume of solution in cubic centimeter (cc).

S_1 = final concentration of solution in ppt.

V_2 = determining volume of stock solution in ppt.

S_2= known concentration of stock solution in ppt.

3.5 Experiment setup

Completely Randomized Design was adopted for this experiment. Twenty four germination trays (75 cm×75 cm×6 cm) were prepared. The trays were filled with 3 cm thick layer of coarse sand. 100 seeds were sown on each tray (Fig. 16). Eight levels of salinity prepared with crude sea salt (0, 5, 10, 15, 20, 25, 30, and 35 ppt) were applied randomly with three replications to the germination trays. Salinity level in each tray was checked and corrected at every 24 hours interval. Average temperature and relative humidity during the experimental period were recorded as 34.60 °C and 65.50% respectively. Initiation of root and shoot (Fig. 17) was considered as germination. Number of germinated seeds were counted and recorded at 24 hours interval for 49 days.

Fig. 16: Seeds on the germination tray
(Courtesy: Lulu Rayhan Khushi)

Fig.17: Germinated seeds of *A. officinalis*
(Courtesy: Lulu Rayhan Khushi)

3.6 Germination traits

Cumulative Germination Percentage (CGP), Final Germination Percentage (FGP), Germination Initiation Time (GIT), Mean Germination Time (MGT), Germination Index (GI), Germination Value (GV), and Coefficient of Uniformity of Germination (CUG) were calculated for all the replications at all salinity levels.

3.6.1 Cumulative Germination Percentage (CGP)

Cumulative Germination Percentage indicates germination patterns over time at different saline treatment levels. CGP was calculated as:

$$\text{CGP}\ (\%) = \frac{\text{germinated seeds (first day)}}{\text{Total number of sown seeds}} \times 100 + \frac{\text{germinated seeds (second day)}}{\text{Total number of sown seeds}} \times 100 + \ldots\ldots$$

3.6.2 Final Germination Percentage (FGP)

Final Germination Percentage indicates germination success at the end of the experiment. Formula for calculating Final Germination Percentage (Ellis & Roberts, 1981) was as:

$$FGP = \frac{Total\ Number\ of\ germinated\ seeds}{Total\ number\ of\ sown\ seeds} \times 100$$

3.6.3 Germination Initiation Time (GIT)

GIT indicates rapidity or delay of initiation of germination. Germination Initiation Time was calculated as:

GIT (day) = Day of first count – Day of seed sowing.

3.6.4 Mean Germination Time (MGT)

Mean Germination Time indicates time requirements for concentrated germination. MGT represents the mean time that maximum germination occurs around this time. Formula for calculating mean time germination (Orchard, 1977) was as:

$$MTG = \frac{\sum n_i d_i}{\sum n_i}$$

Where n_i = number of germinated seeds in d_i; d_i= days taken after sowing and $\sum n_i$ = total number of germinated seeds at the end of experiment.

3.6.5 Germination Index (GI)

Germination Index is calculated for finding out the seed vigor. It is a good indicator of dormancy decay and how vigorously seeds are germinating in the germination medium. High value indicates good quality. It is considered as the best predictor of dormancy depth. Germination Index was calculated as:

$$GI = \sum \frac{n}{d}$$

Here n =number of seedlings emerging on day'd' and d = day after planting (Karaguzel et al., 2004).

3.6.6 Germination Value (GV)

Germination Value indicates speed of germination. Germination value (GV) is the Combination of both germination speed and total germination (Hossain et al., 2005). It

indicates the influence of treatment on germination of seed. In the interpretation of Brown & Mayer (1988), this index is an expression of speed and totality of germination, and their interaction. In addition, inclusion of peak value (PV) makes it different from GI. Germination value of treated seeds was measured by using the following equation.

GV = (final) MDG × PV

Here (MDG) is the Mean Daily Germination, calculated as the cumulative percentage of full seed germination at the end of the test, divided by the number of days from sowing to the end of the test. Speed of germination is expressed as Peak Value (PV), which is the maximum mean daily germination (cumulative percentage of full seed germination divided by number of days elapsed since sowing date) reached at any time during the period of the test (Czabator, 1962).

3.6.7 Coefficient of Uniformity of Germination (CUG)

High value of Coefficient of Uniformity of Germination indicates concentrated germination in time. Coefficient of Uniformity of Germination (CUG) measures the variability among seeds in relation to the mean germination time of the sample (Heydecker, 1973, Bewley & Black, 1994). The following formula was used to calculate CUG.

$$CUG = \sum_{i=1}^{k} n_i / \sum_{i=1}^{k} (\bar{D} - D_i)^2 n_i ;$$

$$\bar{D} = 100 / CRG; CRG = (\sum_{i=1}^{k} n_i / \sum_{i=1}^{k} D_i n_i)100$$

Where n_i : number of seeds germinated on the ith day and D_i : number of days counted from the day of sowing to the collection of the data. This coefficient can be applied only if the germination frequencies have normal distribution (Heydecker 1973, Bewley & Black 1994).

Table 1. Limits and units of different germination parameters. *k*: Last day of germination; *n*= total number of seeds in germination condition.

Parameter	Limit	Unit
Final germination percentage	$0 \leq G \leq 100$	%
Mean time germination	$0 < MTG \leq k$	day
Germination index	$0 < GI \leq n$	Seed/day
Germination value	$0 \leq GV \leq 10,000$	$\%^2$ / day
Coefficient of uniformity of germination	$0 < CUG < \infty$	Day^{-2}

3.7 Statistical analysis

Final germination percentage (FGP) and Germination value (GV) are expressed in percentage. FGP and GV were transformed to ArcSine in MS Excel. FGP, GIT, MGT, GI, GV, CUG were analyzed separately by one way Analysis of Variance followed by Ducan's Multiple Range Test (DMRT), and correlation using SAS 6.12 statistical software.

CHAPTER FOUR

RESULTS

4.1 Cumulative Germination Percentage (CGP)

Cumulative Germination Percentage was the highest at 0 ppt salinity level throughout the experimental period, attained its maximum (91.67%) within 26 days, and decreased with increasing levels of salinity, CGP was the lowest (9.33%) at 35 ppt salinity level within 26 days after seed sowing (Fig. 18).

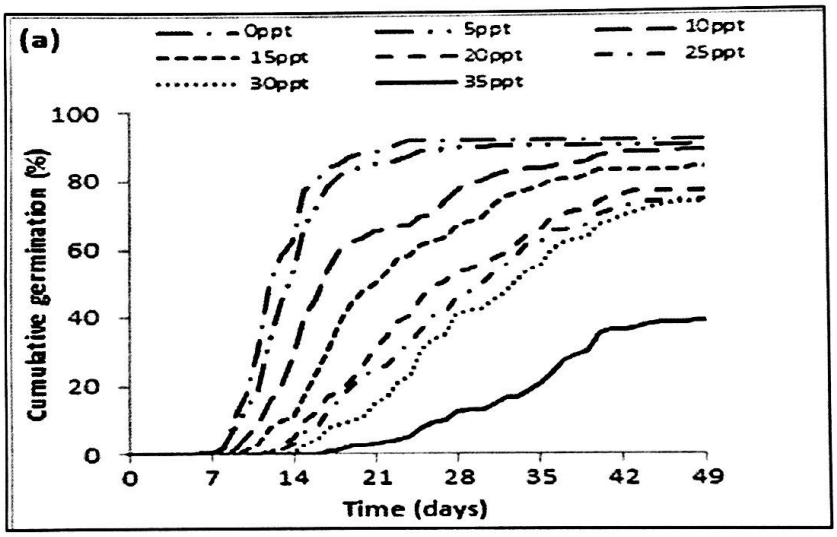

Fig. 18: Cumulative Germination Percentage of *A. officinalis* over time.

Table 2. Correlation between salinity levels (SL) and FGP, GIT, MGT, GI, GV, and CUG of *Avicennia officinalis* (all the correlations are significant, $p < 0.05$).

SL vs FGP	SL vs GIT	SL vs MGT	SL vs GI	SL vs GV	SL vs CUG
-0.84	0.96	0.95	-0.95	-0.93	-0.70

4.2 Final Germination Percentage (FGP)

Final Germination Percentage was the highest (91.67±2.85%) at 0 ppt salinity level and lowest (39±4.17%) at 35 ppt salinity level (Fig. 19). FGP of *A. officinalis* varied significantly ($F=14.63$, $p < 0.05$, Appendix 1) among different saline treatments. Strong negative correlation ($r = -0.84$, $p < 0.05$, Table 2) was observed between salinity levels and FGP. However, FGP showed similar values from 0 ppt to 15 ppt; 10 ppt to 20 ppt; and from 15 ppt to 30 ppt salinity levels (Fig.19, Appendix 1).

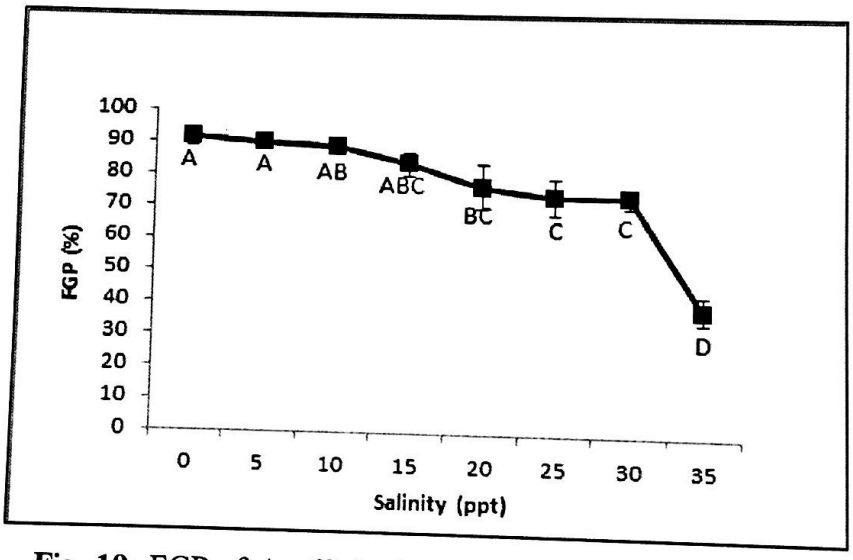

Fig. 19: FGP of *A. officinalis* at different salinity levels.

4.3 Germination Initiation Time (GIT)

Germination Initiation Time of *A. officinalis* was the lowest (6 days) at 0 ppt salinity level and highest (17 days) at 35 ppt salinity level (Fig. 20). GIT of *A. officinalis* varied significantly ($F=46.36$, $p<0.05$, Appendix 2) among saline treatments. Strong positive correlation ($r = 0.96$, $p<0.05$, Table 2) was found between salinity levels and GIT. Increasing salinity delayed the initiation of germination. However, GIT showed similar values from 0 ppt to 5 ppt, 10 ppt to 15 ppt, 20 ppt to 25 ppt, and from 25 ppt to 30 ppt salinity levels (Fig. 20, Appendix 2).

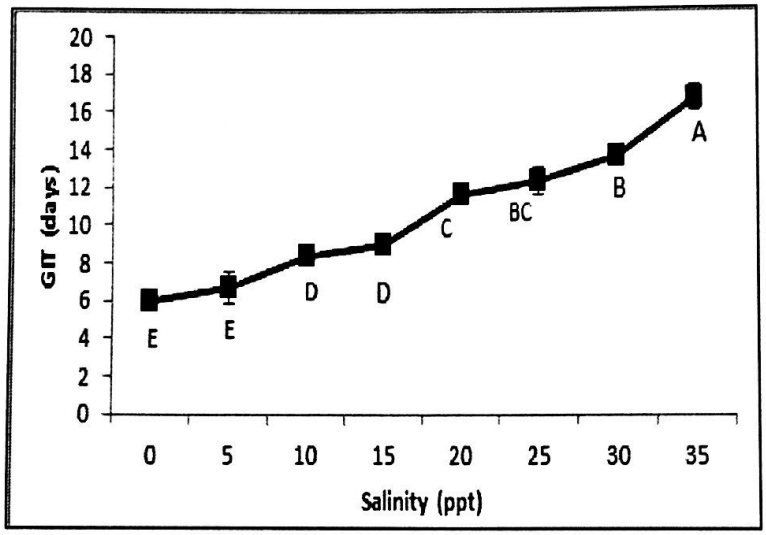

Fig. 20: GIT of *A. officinalis* at different salinity levels

4.4 Mean Germination Time (MGT)

Mean Germination Time was the lowest (14 days) at 0 ppt salinity level and highest (33 days) at 35 ppt salinity level (Fig. 21). MGT of *A. officinalis* varied significantly ($F= 27.82$, $p<0.05$, Appendix 3) among different saline treatments. Strong positive correlation ($r = 0.95$, $p<0.05$, Table 2) was observed between salinity levels and MGT. Increasing salinity delayed MGT. However, MGT showed similar values from 0 ppt to 5 ppt, 10 ppt to 15 ppt, 15 ppt to 20 ppt, 20 ppt to 25 ppt, and from 25 ppt to 30 ppt salinity levels (Fig. 21, Appendix 3).

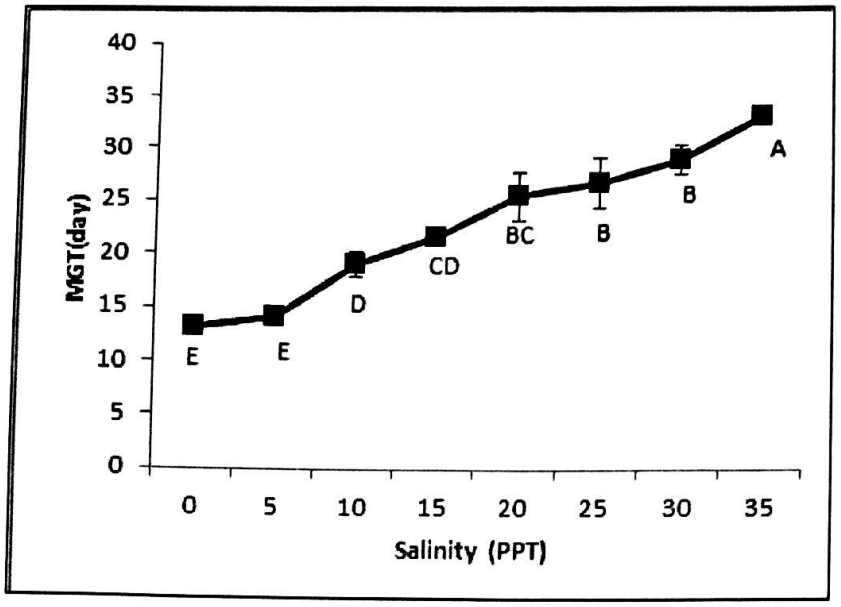

Fig. 21: MGT of *A. officinalis* at different salinity levels

4.5 Germination Index (GI)

Germination Index was the highest (7.40±0.34 seed/day) at 0 ppt salinity level and lowest (1.25±0.14 seed/day) at 35 ppt salinity level (Fig. 22). GI of *A. officinalis* varied significantly ($F = 35.09, p < 0.05$, Appendix 4) among different saline treatments. Strong negative correlation ($r = -0.95, p < 0.05$, Table 2) was observed between salinity levels and GI. GI decreased with increasing salinity. However, it showed similar values from 0 ppt to 5 ppt, 10 ppt to 15 ppt, 15 ppt to 20 ppt, 20 ppt to 25 ppt, and from 25 ppt to 30 ppt salinity levels (Fig. 22, Appendix 4).

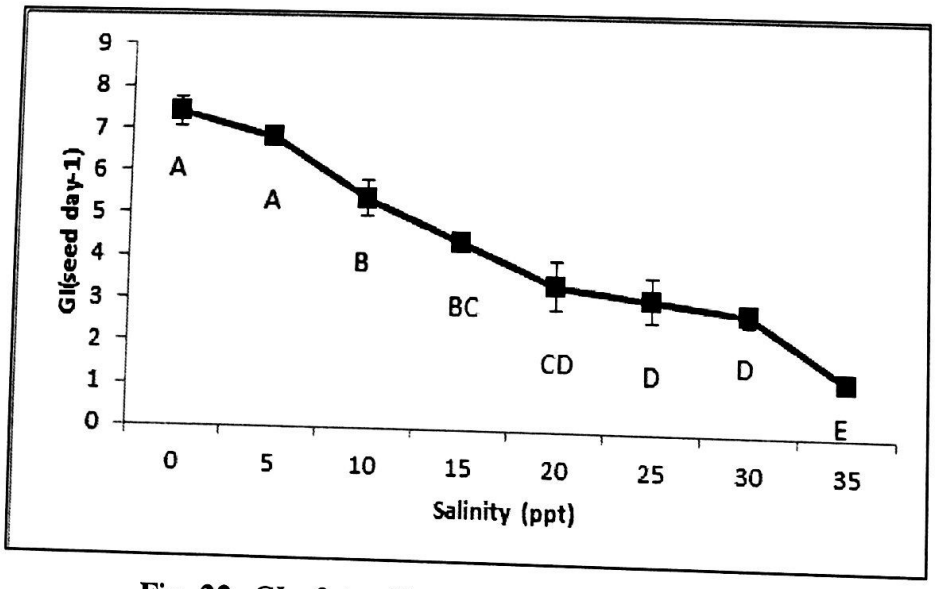

Fig. 22: GI of *A. officinalis* at different salinity levels

4.6 Germination Value (GV)

Germination Value was the highest (19.36±2.41%^{2} /day^2) at 0 ppt salinity level and lowest (0.77±0.13 %^{2}/day^2) at 35 ppt salinity level (Fig. 23). GV of *A. officinalis* varied significantly ($F = 34.89, p < 0.05$, Appendix 5) among different saline treatments. Strong negative correlation ($r = -0.93, p < 0.05$, Table 2) was observed between salinity levels and GV. GV decreased with increasing salinity. However, GV showed similar values from 0 ppt to 5 ppt, 10 ppt to 15 ppt, 15 ppt to 30 ppt, and from 30 ppt to 35 ppt salinity levels (Fig. 23, Appendix 5).

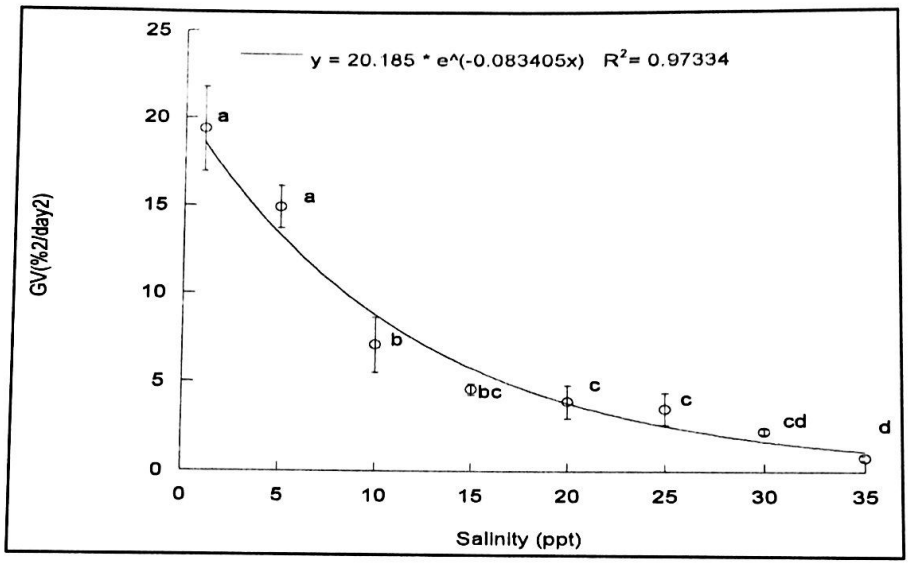

Fig. 23: GV of *A. officinalis* at different salinity levels

4.7 Coefficient of Uniformity of Germination (CUG)

Coefficient of Uniformity of Germination was the highest (0.09±0.01 day^2) at 0 ppt salinity level and lowest (0.02±0.00 day^2) at 35 ppt salinity level (Fig. 24). CUG of *A. officinalis* varied significantly ($F = 16.51, p < 0.05$, Appendix 6) among different saline treatments. Negative correlation ($r = -0.70, p < 0.05$, Table 2) was observed between salinity levels and CUG. CUG decreased with increasing salinity. However, it showed similar values from 10 ppt to 35 ppt salinity levels (Fig. 24, Appendix 6).

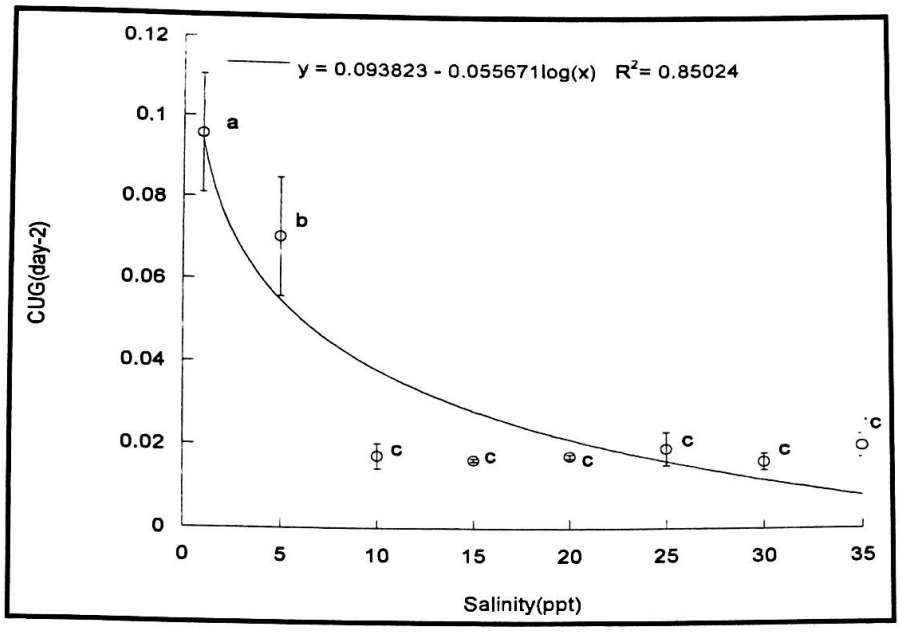

Fig. 24: CUG of *A. officinalis* at different salinity levels

CHAPTER FIVE

Discussion

Increasing salinity creates high osmotic potential in the germination medium, affects imbibition, and induces Na^+ toxicity on seeds, thereby delaying the initiation of germination (Waisel, 1972; Kim et al., 2013). It reduces water availability to the seeds which in turn influences enzyme activity and cell division (Clough, 1984). Salinity has inhibitory effect on seed germination (Hoque et al., 1999). Seed germination of true mangroves decreases with increasing salinity (Hootsmans, 1987; Hoque et al., 1999; Gul et al. 2013; Janousek and Folger, 2013; Kim et al., 2013; Bytnerowicz and Carruthers, 2014; Freitas and Costa, 2014). Increasing GIT and MGT as well as decreasing GI, GV and CUG with increasing salinity would be because of the inhibitory effect of salinity which is ultimately responsible for decreasing patterns of CGP and FGP of *A. officinalis* seeds. Since GIT and MGT increased with increasing salinity, seeds could not germinate vigorously, thereby reducing the speed of germination. Therefore, GI, GV and CUG decreased with increasing salinity. Germination behavior of *A. officinalis* indicates the characteristics of true halophytes.

CHAPTER SIX
CONCLUSION

GIT and MGT increased significantly with increasing salinity. But, FGP, GI, GV, and CUG decreased significantly with increasing salinity. Salinity induced physiological infliction upon the seeds of the species rendering them not readily be able to germination spontaneously and vigorously. New Scientific knowledge generated from this study might be helpful for biodiversity conservation, coastal afforestation, and for sustainable mangrove ecosystem management in Bangladesh.

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APPENDIX – A

Table 1: Analysis of variance for salinity treatment on final germination percentage (FGP)

Source	DF	Sum of Squares	F Value	Pr>F
Model	7	2637.35	14.63	0.0001
Error	16	412.094		
Corrected Total	23	3049.45		

Duncan's multiple range test for FGP at different salinity levels.

Ducan Grouping	Mean	N	Salinity
A	73.644	3	0
A	71.675	3	5
B A	70.504	3	10
B A C	66.477	3	15
B C	61.549	3	20
C	59.616	3	25
C	59.215	3	30
D	38.603	3	35

Table 2: Analysis of variance for salinity treatment on germination initiation time (GIT)

Source	DF	Sum of Squares	F Value	Pr>F
Model	7	283.95	46.36	0.0001
Error	16	14.00		
Corrected Total	23	297.95		

Duncan's Multiple Range Test for GIT at different salinity levels

Ducan Grouping	Mean	N	Salinity
A	16.66	3	35
B	13.66	3	30
C B	12.33	3	25
C	11.66	3	20
D	9.00	3	15
D	8.33	3	10
E	6.66	3	5
E	6.00	3	0

Table 3: Analysis of variance for salinity treatment on mean germination time (MGT)

Source	DF	Sum of Squares	F Value	Pr>F
Model	7	1050.13	27.82	0.0001
Error	16	86.28		
Corrected Total	23	1136.41		

Duncan's Multiple Range Test for MGT at different salinity levels

Ducan Grouping	Mean	N	Salinity
A	33.06	3	35
B	28.98	3	30
B	26.78	3	25
C B	25.47	3	20
C D	21.63	3	15
D	19.04	3	10
E	14.16	3	5
E	13.12	3	0

Table 4: Analysis of variance for salinity treatment on Germination Index (GI)

Source	DF	Sum of Squares	F Value	Pr>F
Model	7	92.49	35.09	0.0001
Error	16	6.03		
Corrected Total	23	98.51		

Duncan's Multiple Range Test for GI at different salinity levels

Ducan Grouping	Mean	N	Salinity
A	7.41	3	0
A	6.81	3	5
B	5.36	3	10
C B	4.35	3	15
C D	3.41	3	20
D	3.12	3	25
D	2.78	3	30
E	1.25	3	35

Table 5: Analysis of variance for salinity treatment on germination value (GV)

Source	DF	Sum of Squares	F Value	Pr>F
Model	7	1065.19	34.89	0.0001
Error	16	69.78		
Corrected Total	23	1134.98		

Duncan's Multiple Range Test for GV at different salinity levels

Ducan Grouping	Mean	N	Salinity
A	26.018	3	0
A	22.691	3	5
B	15.289	3	10
C B	12.352	3	15
C	11.188	3	20
C	10.549	3	25
C D	8.554	3	30
D	4.985	3	35

Table 6: Analysis of variance for salinity treatment on coefficient of uniformity of germination (CUG)

Source	DF	Sum of Squares	F Value	Pr>F
Model	7	0.02	16.51	0.0001
Error	16	0.01		
Corrected Total	23	0.03		

Duncan's Multiple Range Test for GV at different salinity levels

Ducan Grouping	Mean	N	Salinity
A	0.09	3	0
B	0.07	3	5
C	0.01	3	10
C	0.01	3	15
C	0.01	3	20
C	0.01	3	25
C	0.01	3	30
C	0.01	3	35