

**SALINITY AND PARENT-OF-ORIGINS AFFECT LEAF NEOGENESIS OF *Avicennia officinalis* L. IN THE SUNDARBANS, BANGLADESH****Md. Rabiul Alam\* and Mahmood Hossain***Forestry and Wood Technology Discipline, Khulna University, Khulna-9208, Bangladesh*

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**Abstract**

Photosynthetic efficiency as well as growth of mangroves are closely associated with its new leaf production and leaf area. *Avicennia officinalis* L. is one of the exclusive and ecologically significant mangrove pioneer tree species in the Bangladesh portion of the Sundarbans. The effect of salinity and parent-of-origins on the new leaf production and leaf area of the mangrove *A. officinalis* in the less salinity zone (LSZ), moderate salinity zone (MSZ) and high salinity zone (HSZ) of the Sundarbans were studied under experimental conditions in a randomized block design. The results of the experiment highlighted that the number of new leaf production and leaf area of *A. officinalis* from the less, moderate and high salinity zones were reduced significantly ( $p < 0.05$ ) with the increase in salinities. Yet, new leaf numbers as well as leaf area of this species did not differ significantly ( $p < 0.05$ ) between and among the three salinity zones from 0 to 20 ppt salinities. However, from 25 ppt and above salinities, the new leaf numbers and leaf area of the moderate and high salinity zones were significantly higher ( $p < 0.05$ ) compared to less salinity zone. Hence, saline environments and parental origins affected leaf neogenesis, and thereby influence the growth of this species. Therefore, the seedlings of *A. officinalis* of moderate and strong salinity zones origin can sustain in the increasing saline environments, and thereby contribute to the sustainable vegetation cover of the Sundarbans. Further, *A. officinalis* seedlings generating from the MSZ and HSZ can be used for mangrove plantation establishment in the high salinity areas of Bangladesh.

**Keywords:** Growth, Mangrove, Leaf neogenesis, Parent-of-origin, Salinity, Sundarbans**Introduction**

Salinity is the most critical ecological stressor for plant growth in the mangrove environments (Waisel, 1972; Tomlinson, 2016). It affects germination, propagule growth initiation and seedlings' growth (Alam et al., 2018a, b; Nasrin et al., 2020; Nasrin et al., 2021), physiology (Waisel, 1972; Alam et al., 2019), morphology (Alam et al., 2018a, b) as well as establishment and further regeneration of mangroves (Chapman, 1976). However, mangroves can tolerate salt that keeps them free from the competition with terrestrial plants (Krauss & Ball, 2013). Therefore, salinity controls halophytism of many mangrove plants species growing in the intertidal zones (Waisel, 1972; Tomlinson, 2016). The halophytic attributes of mangroves are favored by its salt resistance and salt tolerance (Chapman, 1976). Whilst mangroves tolerate salt, these attributes of salt tolerances differ considerably among different mangrove plants species. For example, in the Sundarbans of Bangladesh (Minar et al., 2013), one of the most extensive natural mangrove vegetation covers in the world (Abdullah et al., 2019; Nasrin et al., 2022), mangrove species' distribution varies noteworthy (Acharya & Kamal, 1994). The mangrove species, such as *Heritiera fomes*, *Excoecaria agallocha* and *Cerriops decandra* are the most abundant and dominant species in less, moderate and high salinity zones, respectively in the Sundarbans (Siddiqi, 2001). Salinity regulates the growth of *Sonneratia apetala* in the Sundarbans (Rahman et al., 2020; Nasrin et al., 2021). Therefore, salinity implicates in the growth and

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dominance of mangroves (Nasrin et al., 2016; Abdullah et al., 2019) as well as mangroves' distribution and zonation (Waisel, 1972; Tomlinson, 1986; Tomlinson, 2016).

Apart from the salinity, the maternal origins and environments also influence the physiological, morphological, and various growth attributes of mangroves (Nasrin et al., 2021). The seeds and propagules of mangroves produced from a particular physical environment may germinate and grow more satisfactorily in the similar type of environment (Waisel, 1972; Alam et al., 2018a, b). *Rhizophora mangle* L. seedlings of various parental origins exhibit remarkable variations in their growth characteristics (Proffitt & Travis, 2010). They observed a visible impact of parental genotypes on the growth of *R. mangle* seedlings. Alam et al. (2018a) and Nasrin et al. (2021) found that the chlorophyll content and growth attributes of *A. officinalis* and *S. apetala* seedlings originating from the moderate and high salinity zones are higher in high saline conditions than those of less salinity zone. Mangrove propagules developing in the high saline environments experience the exposure to high salinities while attached with their parental trees, and thus they are already adapted with high saline conditions (Zheng et al., 1999; Alam et al., 2018a, b). Therefore, the parents of origins affect the physiology and the growth performances of mangrove plants species growing under different salinities.

Mangroves' growth is intricately linked with and dependent on its photosynthetic efficiency in the saline environments (Tomlinson, 2016). The decreasing photosynthetic efficiency of mangroves is associated with the reduced numbers of new leaves production and narrower leaf area under increasing salinity (Tomlinson, 1986). The new leaf production of *X. granatum* significantly decreased at higher level of salinities (Siddique et al., 2017). The decrease in leaf neogenesis threatens the survival of mangroves under high salinities (Ball & Pidsley, 1995). Suarez and Medina (2006) studied that the new leaf production and leaf area of *A. germinans* significantly decreased with increasing salinity. Therefore, it is evident that salinity strongly influences the new leaf production as well as leaf area of the mangroves, and hence the survival and growth of many mangroves are severely hindered by salinity and parent-of-origins.

Among the mangroves, *Avicennia officinalis* L. is one of the important exclusive mangrove pioneer species that grows from the less to the high saline areas of the Sundarbans (Siddiqi, 2001; Giri et al., 2010; Mahmood, 2015). Specifically, *A. officinalis* grows and scatters on newly formed mud flats near river mouths. It grows with *Sonneratia apetala* Buch.-Ham and *Aegiceras corniculatum* L. and occupy the newly formed lands of the Sundarbans (Naskar & Bakshi, 1987; Siddiqi, 2001). Further, this species is adapted to grow on low lying clay soils which are often flooded by high tides (Tomlinson, 1986). In addition, the species is planted in the saline substrates in the coastal areas of Bangladesh (Mahmood, 2015). It shows different physical and mechanical adaptations to survive in harsh saline conditions (Tomlinson, 1986; Alongi et al., 1992). Being a pioneer species, *A. officinalis* generates favorable natural habitats for many other mangroves, thereby maintaining ecological succession and sustainability of mangrove forests (Alam et al., 2018b) The survival and growth of mangroves are dependent on its photosynthetic efficiency under saline environments.

The primary functions of leaf are photosynthesis and transpiration (Fathima et al., 2005). New leaf production is the principal component of plant species which is necessary for the respiration and photosynthesis because it determines the efficiency of the photosynthetic conversion of available light to assimilate carbon dioxide, and thereby influences the growth of plants (Cai et al., 2014). The leaf area (LA) has been an important parameter which is necessary for scientific study on ecophysiology regarding light interception, photosynthesis, evapotranspiration as well as plants' nutrition (Blanco & Folegatti, 2005). Despite being ecologically significant species, there is not much information on the impact of salinity and parent-of-origin on the new leaf production of *A. officinalis* in the Sundarbans. Therefore, this investigation was executed to find out the influences of salinity and parent-of-origin on the new leaf production (leaf neogenesis) and leaf area of *A. officinalis* from less to high salinity zones of the Sundarbans.

## Materials and Methods

### Sources of experimental material

The study sites from where the propagules of *A. officinalis* were collected, were the same as Alam et al. (2018b). The trees of *A. officinalis* experience low salinity (0.5-5 ppt) stress in less salinity zone (LSZ), moderate salinity (5-18 ppt) stress in moderate salinity zone (MSZ) and strong salinity (18-30 ppt) stress in high salinity zone (HSZ) of the Sundarbans of Bangladesh (Siddiqi, 2001; Alam et al., 2018a). The mother trees of mangroves accumulate salt in their propagules in order to train them up to cope with salinity in the physical environments in which the seedling initiate their growth (Alam et al., 2018b; Tomlinson, 2016). Therefore, in order to examine the parental influences

on the new leaf production of *A. officinalis*, the mature propagules of *A. officinalis* from three salinity zones, namely, LSZ (N 22° 22' 23.0" - E 89° 44' 33.7"), MSZ (N 22° 28' 05.1" - E 89° 30' 53.2") and HSZ (N 22° 12' 15.6" - E 89° 11' 41.5") were collected from the Sundarbans (Fig. 1). The propagule length of this species from LSZ, MSZ and HSZ were  $41.7 \pm 1.7$  mm,  $34.0 \pm 1.5$  mm and  $27.6 \pm 1.1$  mm, respectively and propagule width were  $31.2 \pm 1.3$  mm,  $25.1 \pm 1.2$  mm, and  $19.9 \pm 1.2$  mm, respectively (Alam et al., 2018a). The seedlings of *A. officinalis* were salinity zone-wise grown up in the three nursery beds in Khulna University campus in 2015.

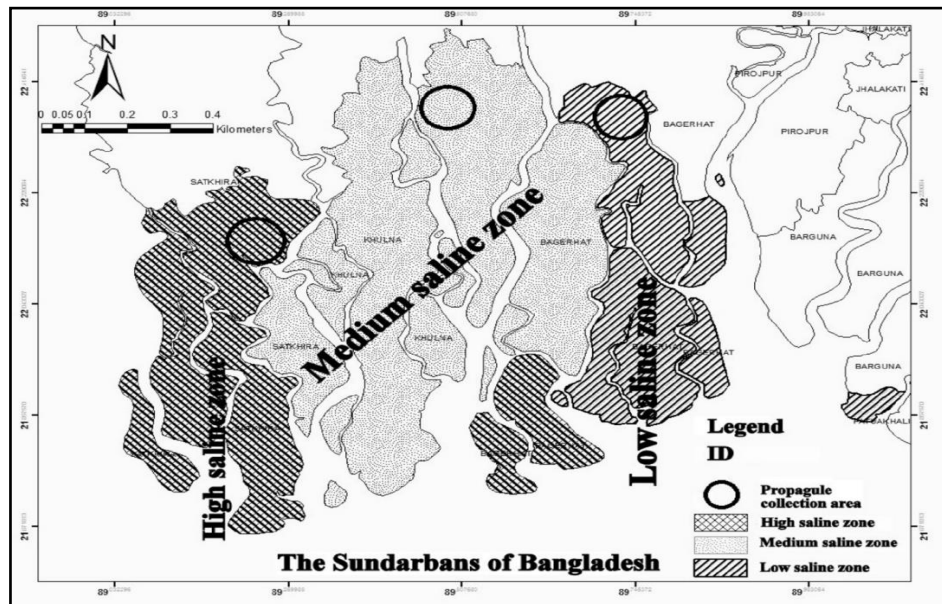


Figure 1. *A. officinalis* propagules collection sites in Sundarbans (Alam et al., 2018a; copyright license no. 5595450253452)

### Experimental design

The experiment was established in the forest nursery of Khulna University. Experimental design adopted for this study was similar to Alam et al. (2018b). A randomized block design (RBD) was maintained for this experiment (Fig. 2). The height of all the seedlings used for the experiment was one foot. Each seedling was transplanted in one plastic pot filled up with coarse sand. Then five seedlings were kept in a plastic box of 50 cm×25 cm×15 cm size each. Each plastic box containing five seedlings was considered as one replication. The number of leaves of the 5 seedlings in each box were counted and recorded replication-wise. 24 such plastic boxes, each contained five seedlings, were prepared with the seedlings of each salinity zone. Therefore, 72 plastic boxes were prepared for the three salinity zones. Salinity levels ranging from 0 (control) to 35 ppt at 5 ppt interval (8 levels) with three replications of each salinity level were applied randomly to the experimental plastic boxes containing the seedlings of *A. officinalis* of LSZ, MSZ and HSZ origins. Crude sea salt having all the chemical components seawater (Na, Mg, Ca, K, Sr, Cl, SO<sub>4</sub>, HCO<sub>3</sub>, Br, BO<sub>3</sub>, and F) was utilized for this experiment to retain the salinity condition that the seedlings of mangroves naturally experience in the mangrove habitats. Hoagland (modified) solution (full strength) (Mahmood et al., 2014) was administered to nourish the seedlings, and was changed weekly. This experiment was continued from August 2015 to July 2016.

Zone Salinity(ppt)	LSZ			MSZ			HSZ		
0	R1	R2	R3	R1	R2	R3	R1	R2	R3
5	R1	R2	R3	R1	R2	R3	R1	R2	R3
10	R1	R2	R3	R1	R2	R3	R1	R2	R3
15	R1	R2	R3	R1	R2	R3	R1	R2	R3
20	R1	R2	R3	R1	R2	R3	R1	R2	R3
25	R1	R2	R3	R1	R2	R3	R1	R2	R3
30	R1	R2	R3	R1	R2	R3	R1	R2	R3
35	R1	R2	R3	R1	R2	R3	R1	R2	R3

Figure 2. Experimental design of leaf neogenesis study of *A. officinalis*. Each replication contained 5 seedlings.

#### Data collection

Leaf shedding was counted and recorded replication-wise daily throughout the experimental period. After the end of the experimental period, total leaves number was counted replication-wise. Previously fallen leaves were computed replication-wise with the final leaf numbers. All of the leaves were scanned by CanoScan LiDE 120. Then the area of each leaf in each replication was measured in cm<sup>2</sup> by using image j software (1.5.3 version).

#### Data analysis

Number of new leaf production and leaf area were evaluated by two-way ANOVA followed by LSD and Bonferroni (Sig.<sup>b</sup> at 0.05) by using IBM SPSS Statistics 20 with a view to examining the impacts of salinity and parental origins on new leaf production and leaf area of *A. officinalis* seedlings of less, moderate and high salinity zones of Sundarbans at different salinities. Pearson correlation (2-tailed) (at 0.01 level) were performed by using SAS (6.12.0.1) statistical software to find the relationship between the studied parameters and salinities.

## Results

### New leaf production

At 0 ppt (control) salinity treatment level, significantly highest ( $p < 0.05$ ) number of new leaves were produced by the seedlings of *A. officinalis* generated from less ( $385 \pm 17$ ), moderate ( $393 \pm 22$ ) and high ( $402 \pm 16$ ) salinity zones (Figure 3).

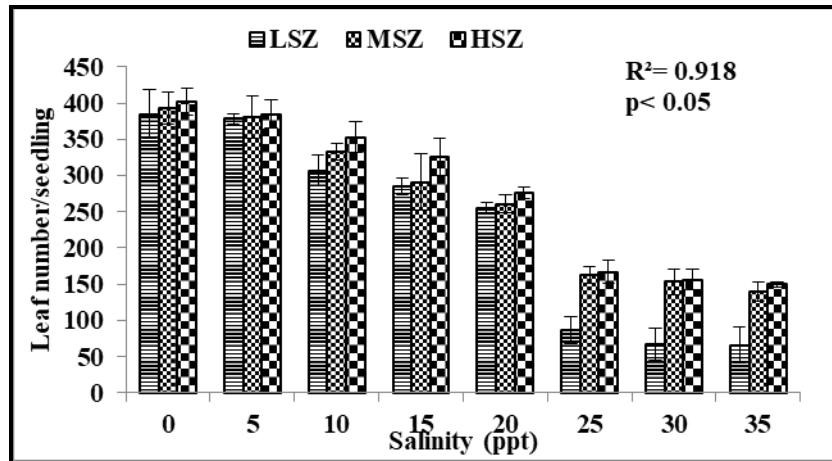


Figure 3. New leaf production of *A. officinalis* seedlings

The number of new leaves produced by *A. officinalis* decreased significantly ( $F_{9, 62} = 77.02$ ;  $p < 0.05$ ) and was negatively correlated ( $-0.86$ ) with increasing salinities. This decreasing trend of new leaf production significantly ( $p < 0.05$ ) differed among and between the three salinity zones (Figure 3). Least significant difference (LSD) as Post ANOVA test with Bonferroni adjustment for determining the interaction between salinity and parental origins (Table-1) exhibited no significant differences in new leaf production among the LSZ, MSZ and HSZ from 0-20 ppt salinities ( $\text{sig.}^b > 0.05$ ). However, from 25 ppt and upwards, the new leaf production (leaf demography) (LD) of *A. officinalis* seedlings from the moderate and high salinity zones was significantly more than those from the less salinity zone (Table-1).

### Leaf area

At 0 ppt (control) salinity treatment level, significantly largest ( $p < 0.05$ ) leaf area (LA) was produced by the seedlings of *A. officinalis* of LSZ ( $7.02 \pm 0.27 \text{ cm}^2$ ), MSZ ( $7.64 \pm 0.12 \text{ cm}^2$ ) and HSZ ( $8.07 \pm 0.52 \text{ cm}^2$ ) (Figure 4).

Area of individual leaf (LA) of the seedlings of *A. officinalis* decreased significantly ( $F_{9, 62} = 71.94$ ;  $p < 0.05$ ) and was negatively correlated ( $-0.81$ ) with increasing salinities. The decrease in leaf area significantly ( $p < 0.05$ ) differed among and between the salinity zones (Figure 4). Least significant difference (LSD) as Post ANOVA test with Bonferroni adjustment for determining the interaction between salinity and parental origins (Table 1) exhibited no significant variation ( $\text{sig.}^b > 0.05$ ) among the less, moderate and high salinity zones from 0-20 ppt salinities in respect of individual leaf area. However, from 25 ppt and onwards, LA of *A. officinalis* seedlings grow up from the moderate and high salinity zones were significantly larger than that grow up from the less salinity zone (Table 1).

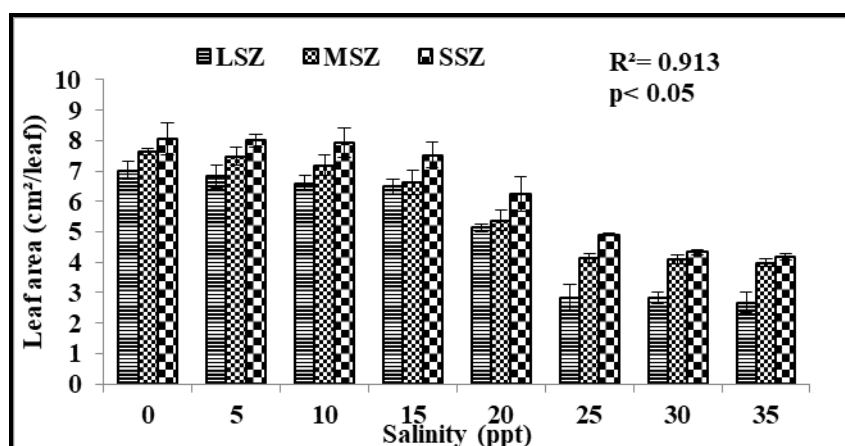


Figure 4. Individual leaf area (LD) of *A. officinalis* seedlings

Table 1. LSD for Pairwise comparisons (Bonferroni) (sig.<sup>b</sup>) between the salinity zones in respect of LD and LA. Mean differences were significant (\*) at 0.05.

Between saline zones at different salinities (ppt)	LD (sig. <sup>b</sup> )	LA(sig. <sup>b</sup> )
0	LSZ and MSZ	1.00
	LSZ and HSZ	1.00
	MSZ and HSZ	1.00
5	LSZ and MSZ	1.00
	LSZ and HSZ	1.00
	MSZ and HSZ	1.00
10	LSZ and MSZ	0.94
	LSZ and HSZ	0.92
	MSZ and HSZ	0.94
15	LSZ and MSZ	0.87
	LSZ and HSZ	0.85
	MSZ and HSZ	0.89
20	LSZ and MSZ	0.82
	LSZ and HSZ	0.86
	MSZ and HSZ	0.79
25	LSZ and MSZ	0.007*
	LSZ and HSZ	0.003*
	MSZ and HSZ	0.868
30	LSZ and MSZ	0.002*
	LSZ and HSZ	0.003*
	MSZ and HSZ	0.713
35	LSZ and MSZ	0.000*
	LSZ and HSZ	0.001*
	MSZ and HSZ	0.62

## Discussion

In this study, we observed that new leaf production and leaf area of *A. officinalis* seedlings generated from the less, moderate and high salinity zones of Sundarbans were significantly ( $p < 0.05$ ) reduced with the increase in the levels of salinities. Surface area of leaf as well as leaf turnover rates are largely regulated by the production of new leaf and leaf shedding, which are intimately associated with the sequestration of carbon as well as nutrient use and re-translocation efficiency that bear significant physiological effects on plants. (Reich et al., 1992; Casper et al., 2001). Leaf demography implicates the salt balance of mangroves in the saline environments (Clough et al., 1982).

Several mangrove species, having produced new leaves satisfactorily, can grow optimally from 5-25% salinities (Ball, 1988; Ball & Pidsley, 1995). Suarez and Medina (2006) in their study opined that *A. officinalis* exhibits

significant decrease in leaf area and leaf number with increasing salinity levels. The leaf morphology of *Heritiera fomes* (Buch.-Ham.) changes in response to salinity stress in the Sundarbans (Khan et al., 2020). *Heritiera fomes*, *Excoecaria agallocha* and *Ceriops decandra* exhibit plasticity in leaf morphology with fluctuating salinities in the Sundarbans (Mollick et al., 2021). Hence, it is confirmed that salinity impacts on the leaf neogenesis and leaf area of *A. officinalis*. Waisel (1972) reported that salinity affects physiology, morphology and growth of mangroves. Greenway & Munns (1980) and Rawson & Munns (1984) proved that high salt concentrations cause a reduction of leaf area of mangroves, which in turn, result in the decrease in growth rate of mangrove species. Presumably, for this reason, Alam et al. (2018b) observed reduced growth characteristics of *A. officinalis* seedlings with increasing salinities.

Although the leaf demography and area of leaf significantly ( $p < 0.05$ ) decreased with the increase in salinities, it did not significantly ( $p > 0.05$ ) differ among the less, moderate and high salinity zones up to 20 ppt salinities (Table 1). The maternal trees and the seedlings usually experience low saline (0.5-5 ppt) in the LSZ of the Sundarbans. The salinity levels in Sundarbans are increasing gradually with time due to reduction of freshwater flow from the upstream (Basar, 2012; Alam et al., 2017; Alam et al., 2018b). Under such situation, it can be predicted that *A. officinalis* seedlings produced from the LSZ can persist in their natural habitat even if the salinity rises up to 20 ppt in the LSZ of the Sundarbans. And the seedlings from other two saline zones can survive as usual in their natural habitats in the Sundarbans.

Greenway & Munns (1980) and Munns & Termaat (1986) stated that a decrease in leaf production leads to ultimate death of mangroves under saline environments. Indeed, a decrease in the number of leaves per plant is associated with an increase in soil salinity (Ball & Pidsley 1995). In this study, with the increasing of salinity, leaf number and leaf area of *A. officinalis* decreased significantly. Suarez and Medina (2006) reported similar observation for *Avicennia germinans*. It has been stated that increasing salinity hampers leaf production as well as longevity of leaf, which results in the reduction of total area of the leaves (Greenway & Munns, 1980; Clough, 1984; Rawson & Munns, 1984; Ball, 1988; Ball & Pidsley 1995; Suarez & Medina 2006). Medina and Francisco (1997) observed similar result on new leaf production. They stated that the leaves of *A. germinans* become shorter as the salinity in soil increases. Clough (1984) found that as the seawater salinity increases from 25 to 50‰, leaf mortality increases.

Further, though there was no variation among the three salinity zones in terms of leaf production and leaf area up to 20 ppt salinities, new leaf production and leaf area of *A. officinalis* of MSZ and HSZ origins are significantly higher ( $p < 0.05$ ) than that of LSZ origins from 25 ppt salinity and upwards. The effect of salinity on seeds and propagules started while they are attached with their parental trees. The propagules normally take in salt during their developmental stage so that they can cope with the saline environments during their further development (Zheng et al., 1999; Alam et al., 2018a, b). presumably, for this reason, the seedlings generated from the MSZ and HSZ produced more new leaves having more surface area than those of coming up from LSZ of the Sundarbans. And for this reason, the performances of growth of the seedlings of *A. officinalis* coming up from the moderate and high salinity zones was higher than those of less saline zone of Sundarbans (Alam et al., 2018b).

According to Alam et al. (2018b), under increasing saline environments due to sea-level-rise and decrease in fresh water flow, as in the Sundarbans, if the level of salinity exceeds above 25 ppt, the seedlings of *A. officinalis* come up from the moderate and high salinity zones can grow more satisfactorily than those of less salinity zone. This information will also be a better help in selecting suitable propagule sources for coastal plantations in variable salinity areas in the coast of Bangladesh.

## Conclusion

New leaf production and leaf area of *A. officinalis* of less, moderate and high salinity zones decreased significantly with higher salinities. However, no significant differences in leaf production and leaf area were observed among the salinity zones up to 20 ppt salinities. From 25 ppt and above, leaf production and leaf area of *A. officinalis* of moderate and high salinity zones were higher than those of less salinity zone. Therefore, salinity and parental-of-origins apparently influenced new leaf production and leaf area of the seedlings of *A. officinalis* of different salinity zones in the Sundarbans.

**Conflict of Interest:** From the part of the authors, there is no conflict of interest on any issue of the article.

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