Original Research Article

Morpho-Physiological Alteration of *Mangifera Indica L.* in Response to Sea Water Induced Salt Stress

UNDER REVIEW

ABSTRACT

Salinity is one of the biggest challenges in the southern part of Bangladesh, which is affecting the coastal ecosystem adversely. A pot experiment was conducted to find out the morphological and physiological changes in mango (*Mangifera indica L.*) seedlings in response to sea water induced salt stress at the Agroforestry and Environment research field of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU). The experiment was laid out in a Randomized Complete Block Design (RCBD) with five replications, where four treatments, viz 4, 8, 12 dS m^{-1} salinity level (prepared from sea water), and the tap water (control) were imposed. The results indicated that plant height of mango shortened with the higher level of salinity and the declining magnitude was 28.44% over control at maximum salinity level. Number of leaves per plant of mango were reduced by 27.51% at 12 dS m^{-1} salinity level compared to that tap water, and the size of mango leaf was also reduced for salinity. Both shoot and root biomass of mango seedlings were significantly reduced due to salinity. The Salt tolerance index in mango was 56.76 only at 12 dS m^{-1}. In case of physiological parameters, the lowest relative water content (RWC) was found in 12 dS m^{-1}, while water saturation deficit (WSD) and water uptake capacity (WUC) were found to be the lowest in control for this plant. Water saturation deficit and water uptake capacity were found to be increased with the increment of salinity level. Total chlorophyll content of mango was decreased by 66.27%, respectively, while carotenoid contents were decreased by 61.07%, respectively, at the highest salinity level. Proline content of mango increased by 73.07%, respectively, at 12 dS m^{-1} salt level in comparison to that of seawater devoid control plants. Considering the overall results, it can be concluded that although mango seedlings were significantly affected by high salinity (12 dS m^{-1}), but can survive up to moderate salinity (8 dS m^{-1}) at sapling stage.

Keywords: Salinity;morpho-physiological changes;chlorophyll content; proline content;salt tolerance index.

1. INTRODUCTION

Salinization in both soil and groundwater are some of the ancient and most onerous environmental problems in the world, posing commiserative impacts on natural resources and agricultural productivity [1, 2]. Globally, more than 45 million hectares of irrigated land are affected by salt, which accounts for 20% of total land and 1.5 million hectares of land are taken out of production each year owing to high salinity levels [3]. Due to juxtaposing geographical position, the coastal area of Bangladesh is highly vulnerable to salt stress.

Rising of sea levels, seawater droplet drift, tidal changes causing intermixing of fresh and salt water. Every year the country has experienced catastrophic incursion of sea water due to cyclone. Approximately, one million hectares of land in southwest, south central and southeast zone of coastal belt of Bangladesh are under threat due to different magnitudes of salinities [4] Salinity problem in Bangladesh was started with the construction of coastal embankment in 1960s, since then intrusion of saline water for brackish water shrimp farming angered the salinity level tremendously in that region. Salinization is a key issue and single most significant problem of those areas, affecting the productivity and availability of agricultural lands [5]. Approximately, 50% of coastal lands are somewhat untenable for agriculture in a year due to majority of the crop plants are salt sensitive as well as relatively low salt tolerance [6].

The severity of soil salinity elevates with the dryness of the soil body, salinization also causes a great reduction in growth parameters such as fresh and dry weights of shoots and roots and these changes are associated with decrease in chlorophyll contents in leaves [7]. High content of soluble salt causes high osmotic pressure which results reduction of absorption of water and nutrients by plant [8]. Salinity causes physiological changes of plant that suppress the seedling growth and plant development [9]. In addition to osmotic and ionic imbalance and toxicity, salinity also induces oxidative stress in plants [10], which initiates antioxidant system of the plants to cope up with oxidative damage to stressed plants [11]. Plants grown under saline conditions are stressed and are characterized by increased levels of free proline in different tissues [12] as a response to osmotic adjustment [13]. Proline accumulation is one of the adaptation mechanisms of plants to salinity and water deficit [14]. Beside agricultural productivity, vegetation in the coastal region has also affected tremendously and fruit trees are more sensitive to salinity than timber tree species [15]. Mango (*Mangifera indica L.*) a tropical fruit, belongs to the family Anacardiaceae, is one of the most popular and commercially important fruits in Bangladesh. In Bangladesh, mangoes are grown everywhere, but 'Guti Amm' is a popular and early variety which is grown in Satkhira district, a saline prone area of Bangladesh. The specialty of this variety is, it comes early to the market and farmers get high income. However, the information of saline tolerance limit of the mango is not well documented. The findings will be helpful for the mango growers of coastal districts. Since mango is an important tree species in coastal area of Bangladesh, it is needed to assess the effects of salinity on the growth of M. indica and physiological response to salt stress.

2. MATERIAL AND METHODS

2.1 Experimental Location and Climatic Conditions

The experiment was conducted at the research farm of the Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, which is located at 24°29´ N latitude and 90°26´ E longitude from January to May 2018. The study area has subtropical climate with hot summers and mild winter, and characterized by three distinct seasons; the pre-monsoon (March to April), the monsoon (May to October), and the dry season (November to February).

2.2Plant Collection and Establishment

One-year seedling of Guti Amm were collected from Satkhira district, Bangladesh. Twenty plastic pots (diameter 33 cm and height 34 cm) were prepared by 23 kg of fine river sandy soil and dried cow dung with a ratio of 2:1 and treated with formaldehyde to curtail soil born disease for raising seedling. After well establishment of seedlings in pots, irrigation water was supplied as per treatments at three days interval up to the end of the experiments. The

UNDER PEER REVIEW

treatments were imposed from 16 February 2018. Fertilizer was applied in solution form. Each pot was fertilized with 0.4, 0.3 and 0.3g N, P and K, respectively, with water at 30 days interval.

2.3Experimental Design and Treatments

The experiment was conducted in a Factorial Randomized Complete Block Design (RCBD) with five replications and four treatments. The different salinity intensity of irrigated water were treatments i.e., 4, 8, 12 dS m^{-1} along with tap water as control treatment.

2.4Samplingand Data Collection

Relevant data were recorded at different days after treatments (DAT) imposition during the period of five months of field research and lab work for determining the plant performance under salinity stress.

2.4.1Phenological Data

Plant height was measured at 30 days interval after treatment imposition from the base of the plant (top of the soil) to the leaf primordia. At 30 days interval, total number of leaves per plant was counted. Leaf area was measured at 30 days interval after treatment imposition till end of the study by Area Meter (AM 200). For determining shoot and root masses, plants were removed from the soil and washed to eliminate loose soils and then placed on dry polythene sheets to allow any free surface moisture to dry out. After that, plants were divided into root and shoot and measure weighed on an electric balance. Then plant materials were placed in paper bags and oven dried at 80˚C for 72 hours and allowed to cool in a dry environment (in a paper bag to keep moisture out) and again weighed on an electronic balance. In order to determine total dry weight of a plant, month wise dried leaves were collected. After that, total dry weight per plant was calculated by summing up the dry weight of collecting leaves, shoots and roots of plants. Shoot and root distribution, shoot and root density were measured at the end of the experiment according to the procedure of Arduini et al., [16] Salt Tolerance Index (STI) was calculated by using the formula of Seyedi, [17].

2.4.2Physiological Data

Water Saturation Deficit (WSD) is the deviation of the water content from the saturation level and Water Uptake Capacity (WUC) quantifies the capacity of plants to absorb a greater quantity of water per unit of dry weight in relation to turgid weight.Relative Water Content (RWC), WSD and WUC were determined according to Weatherly [18] and calculated as follows:

$RWC = \frac{FW - DW}{TW - DW} \times 100$; WSD % = 100 – RWC; WUC = (TW-FW)/DW

Where,FW = Fresh weight, DW = Dry weight, and TW = Turgid weight of the leaf

Stomatal conductance was measured at 90 days after treatments imposition by Steady State Diffusion Prometer (Model No.SC-1).Chlorophyll and Carotenoids content were estimatedfrom the fully expanded uppermost leaves at 30 days interval after treatments imposition till the end, by following the procedure developed by Witham et al. [19]. Proline content was determined by using the method described by Bates [20].

2.5DataProcessingandStatistical Analyses

UNDER PEER REVIEW

Data recorded for different parameters of plant and soil were processed by Microsoft Excel and statistical analysis were carried out by using "Statistix10". Two-way analysis of variance (ANOVA) was used to determine mean comparison and interaction between treatments and measurement periods. Least significance difference (LSD) test was used at 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Growth Parameters

Growth parameters of mango were found to be significant in salinity induced stress to *Mangifera indica* seedling after 90 days of treatment imposed (Table 1). Plant height was significantly influenced by the salinity level, the tallest plant was recorded in control (121.40 cm) and it shortened with the increase of salinity level. The results that found in this study have been confirmed by the results of Kapoor and Srivastava [21] on *Vigna mungo L.* and Jamil et al., [22] on radish plant. Number of leaves per plant decreased with the higher concentration of saline treatments. The lowest number of leaves was observed in 12 dS m^{-1} (11.80), which was significantly lower than that of other sampling treatment. In salinity stress plants accumulate sodium chloride in the cell walls and cytoplasm of the older leaves of plants, which triggers leaf senescence. Inhibition of the formation of leaf primordia under salinity stress could be probable reason for low leaf number. Munns and Tester [3]also stated that the ion-specific phase of plant response to salinity starts when salt accumulates to toxic concentrations in the old leaves, which are no longer expanding and so no longer diluting the salt arriving in them as younger growing leaves do, and they die and causes a decrease in leaf number.

Table 1.Response of plant height (cm), leaves per plant and leaf area (mm^2) of *Mangifera indica L.* to different salinity levels at 90 days after treatment (DAT) imposition

Salinity level	Plant height (cm)	Leaves per plant	Leaf area (mm^2)
Control	121.40a (±4.43)	23.60ab (±3.90)	17904a (±285)
4 dS m^{-1}	103.18b (±2.05)	26.20a (±4.00)	11657b(±250)
8 dS m^{-1}	96.10c (±1.31)	16.40b (±3.50)	9475c(±213)
12 dS m^{-1}	86.32d (±1.40)	11.80c (±4.30)	7213d(±248)

Values are mean five replicates for each treatment (±SE). Values in a column with different small letters are significantly different by LSD (P≤0.05)

Size of leaf of mango seedlings greatly affected by saline water treatments, leaves became smaller with the higher concentration of salinity. Similar reports were found in two different study such as *Moringa oleifera* [23] and milk thistle [24]. Seawater stress cause a reduction in meristem activity as well as cell elongation, thereby inhibiting leaf expansion after the loss of cell turgor pressure [25]. Generally, reduction of cell turgor pressure leads to stomatal closure and limits CO_2 assimilation and reduced photosynthetic rate resulting lessening of chlorophyll content which is responsible for reduction in leaf area [26].

3.2Biomass Status

Effects of salinity on shoot-root growth shown in table 2. It was found that salinity inhibited the length, fresh and dry weight of shoot-root. Shoot and root length, fresh and dry weight of shoot and root were significantly highest in control treatments whereas lowest values of

UNDER PEER REVIEW

these parameters were observed in 12 dS m^{-1} treatments. Growth of *M. indica*, gradually retarding with the increase of salinity level. The presence of high salt concentrations in plant tissues increases the osmotic potential of tissues, leading to low plant water potential. Such osmotic stress leads to reduced cell expansion and cell division rates. Ion toxicity may also have a role in decreasing the rates of cell division and cell expansion; hence retarded shoot and root length and reduced dry weight. This reduction in shoot biomass of seawater-stressed plants could be attributed to inadequate availability of nutrients present in growth medium and the decreased water entry rate into the plants and the decreased in photosynthetic output with suppressed supply of CO_2. In this study, the deleterious effect of salinity on root biomass may be attributed to the inhibitory effect of abscisic acid (ABA), induced by salinity, on cell division and cell expansion as stated by Hassanein [27] or reduced water absorption due to osmotic effect, specific ion toxicity and nutritional imbalances as mentioned by Tahir et al. [28] and Joseph et al., [29]. Two authors have been reported similar effect of salinity on root and shoot length of *Triticum aestivum L.* Rahman et al., [30] and of *Solanum melongena L.* Basalah [31]. Chaparzadeh et al., [32] stated that the reduction in shoot biomass may be a consequence of turgor limitation or cell wall hardening which may be due to altered wall structure induced by salinity. Similar decreases in the root biomass due to salt stress have been reported in pepper and guava [33]. The present findings of the study also corroborate to the findings of Memon et al., [34] on *Brassica campestris L*.

Table 2. Effects of different salinity levels on shoot and root length (cm), fresh and dry weight (g) of Mangifera indica L. at 90 days after treatment (DAT) imposition

Salinity level	Shoot length (cm)	Rootlength (cm)	Shoot fresh weight(g)	Shoot dry weight(g)	Root fresh weight (g)	Root dry weight(g)
Control	121.40a (±4.43)	37.80a (±2.78)	132.92a (±4.00)	65.50a (±1.94)	60.96a (±4.34)	30.40a (±1.81)
4 dS m^{-1}	103.18b (±2.05)	29.60b (±0.75)	110.75b (±2.61)	53.96b (±1.60)	44.31b (±1.28)	22.46b (±0.64)
8 dS m^{-1}	96.20bc (±1.25)	28.00b (±0.45)	101.21c (±1.63)	48.65c (±0.85)	38.13b (±2.04)	18.63b (±2.04)
12 dS m^{-1}	86.32c (±1.40)	22.60c (±1.29)	88.93d (±1.68)	42.18d (±1.23)	23.28c (±2.32)	12.27c (±2.32)

Values are mean five replicates for each treatment (±SE). Values in a column with different small letters are significantly different by LSD (P≤0.05)

3.3Shoot and Root Distribution and Density

Shoot and root distribution was significantly decreased with increasing salinity level (Table 3). Significantly, the lowest shoot distribution was observed in 12 dS m^{-1} (1.030 g cm^{-1}) and the highest shoot distribution was observed in control (1.096 g cm^{-1}) plants. The lowest root distribution was found in 12 dS m^{-1} (1.020 g cm^{-1}), and the highest root distribution was found in control plants (1.619 g cm^{-1}). Shoot and root density were significantly higher in control plants (0.540 g cm^{-1} and 0.809 g cm^{-1}), and lower in 12 dS m^{-1} (0.488 g cm^{-1} and 0.540 g cm^{-1}). Shoot/root density relates dry mass production to the unit shoot/root length and shoot/root distribution represents the fresh mass accumulated per unit of shoot/root length, the reduction in both density and distribution of plants shoot/root may reflect the effect of salinity on decreasing shoot/root biomass (fresh and dry masses). In this respect, Chopart et al., [35] stated that evaluation of shoot/root density and distribution could be considered as a key factor for water and nutrient uptake by a plant in soil. These results were in harmony with those obtained by Seckin et al., [36] on barley cultivars and Ali [37] on

UNDER PEER REVIEW

wheat cultivars; they reported that elevated level of salinity caused considerable decrease in shoot/root biomass which ultimately triggers lessening of shoot/root distribution and density.

Table 3. Effects of different salinity levels on shoot distribution, root distribution, shoot density and root density (gcm^{-1}) of *Mangifera indica L.* at 90 days after treatment (DAT) imposition

Salinity level	Shootdistribution (g cm^{-1})	Root distribution (g cm^{-1})	Shoot density(g cm^{-1})	Root density(g cm^{-1})
Control	1.096a (±0.02)	1.619a (±0.06)	0.540a (±0.00)	0.809a (±0.03)
4 dS m^{-1}	1.073b (±0.01)	1.497ab (±0.01)	0.520b (±0.01)	0.759a (±0.01)
8 dS m^{-1}	1.052c (±0.01)	1.359b (±0.05)	0.506c (±0.00)	0.663b (±0.04)
12 dS m^{-1}	1.030d (±0.00)	1.020c (±0.04)	0.488d (±0.01)	0.540c (±0.01)

Values are mean five replicates for each treatment (±SE). Values in a column with different small letters are significantly different by LSD (P≤0.05)

3.4 Salt Tolerance Index (STI)

The STI was decreased significantly as the level of salinity increased. Significantly the highest (79.85) and the lowest (56.76) STI values were found in 4 dS m^{-1} and 12 dS m^{-1} treatments, respectively (Fig. 1). This result agrees with the result of Carpici et al., [38]. Salt tolerance index, which is a function of total dry weight, is considered to be a reliable criterion for salt tolerance [39]. Al-Thabet et al., [40] stated that plant growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance.

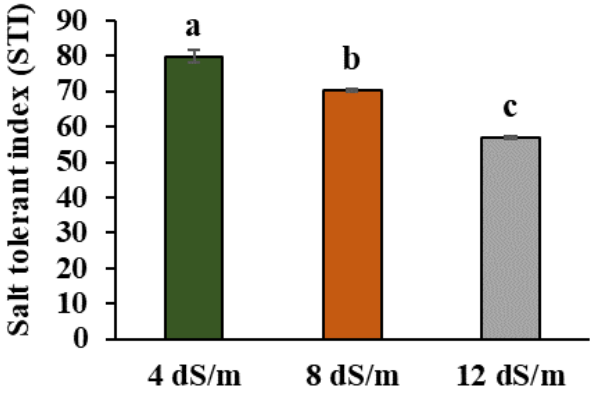

Fig. 1.Effects of different salinity levels on Salt tolerant index of *Mangifera indica L.* at 90 days after treatments (DAT) imposition

3.5Relative Water Content (RWC)

Water stress is one of the first and most obvious effects of salinity and thus the determination of water relations is crucial for understanding salinity tolerance mechanisms of a plant. RWC in mango was significantly influenced by the salinity level and it was decreased with increasing the salinity level and salinity exposure duration (Table 4). Among the treatment means, the highest mean RWC was recorded in control (90.53%) which were

UNDER PEER REVIEW

significantly different from each salinity levels. At 90 DAT imposition the lowest RWC was observed in 12 dS m^{-1} (51.68%) which was significantly different from other treatments and sampling dates. Salt induced-reduction of tissue water content may be caused by low leaf water potential [41]. The relative water content of leaves might be decreased due to decreasing of leaf water potential due to salt stress. The Chemlali olive tree tends to cope with salt stress conditions by decreasing enormously its leaf water potential [42]. Plants with high relative water content in leaf has a more stable osmotic balance [43]. The decreasing relative water content of leaves indicate the less capacity to uptake water. However, Islam [44] observed similar results for Mahagony and Eucalyptus.

Table 4. Effects of different salinity levels on relative water content (RWC%) of*Mangifera indica L.* at different days after treatments (DAT) imposition

Salinity level	30 DAT	60 DAT	90 DAT	Mean
Control	90.58a (±0.33)	90.59a (±1.46)	90.41a (±0.12)	90.53A (±0.64)
4 dS m^{-1}	84.76ab (±2.52)	81.82bc (±0.74)	75.11c-e (±3.70)	80.56B (±2.32)
8 dS m^{-1}	78.46b-d (±1.55)	74.25de (±0.49)	64.86f (±2.28)	72.52C (±1.44)
12 dS m^{-1}	71.81de (±3.03)	71.42ef (±3.09)	51.68g (±4.55)	64.97D (±3.56)

Values are mean three replicates for each treatment (±SE). Values in a columns and rows with different letters are significantly different by LSD (P≤0.05). Different capital letters beside the mean values are significantly different by LSD (P≤0.05)

3.6Water Saturation Deficit (WSD)

Water saturation deficit (WSD) indicates the degree of water deficit of plants. The WSD was remarkably influenced by the salinity level and it increased with increasing the salinity level and progressing of days after treatment imposition (Table 5). Significantly the maximum WSD was found in 12 dS m^{-1} (48.33%) at 90 DAT. In average, the highest (35.03%) WSD was recorded in 12 dS m^{-1} which was 3.7 times higher than control treatment. It was observed that WSD remain constant in control treatment throughout the growing period. Seawater stress responsible for changing the situation because of restricted transpiration. Katerji et al., [45] and Kaya et al., [46] observed similar result that sea water stress accountable for altering the water status of plants viz. diminution of Relative Water Content (RWC), while promoting Water Saturation Deficit (WSD).

3.7Water Uptake Capacity (WUC)

Water uptake is essential for cell expansion and plant growth. The WUC quantifies the ability of a plant to absorb water per unit dry weight in relation to turgid weight. The WUC followed the similar trend of WSD (Table 6). In the interaction effect, the maximum WUC rate was observed in 12 dS m^{-1} (1.04) at 90 DAT, which was significantly different from other treatments at all measurement dates. The minimum WUC rate was found in control (0.08) at 30 DAT, which was almost similar with other dates in control treatment. The decrease in water uptake indicates a loss of turgor that results in limited water availability for expansive growth of cells. Letting down transpiration hinders water uptake from the soils because of injury in the root systems. A higher WUC under saline condition means a plant is subjected to water stress at a greater degree, because the plant would absorb more water to reach turgidity than a plant under control condition [47]. The presents findings agreed with those obtained by Stoyanov [48] on young bean, and Kabir et al., [49] in mungbean. Poor cell

UNDER PEER REVIEW

growth due to disparity water status causing disruptions of overall morpho-physiological growth of a plant.

Table 5. Effects of different salinity levels on water saturation deficit (WSD%) of*Mangifera indica L.* at different days after treatments (DAT) imposition

Salinity level	30 DAT	60 DAT	90 DAT	Mean
Control	9.42g (±0.33)	9.41g (±1.46)	9.59g (±0.12)	9.47D (±0.64)
4 dS m^{-1}	15.25fg (±2.52)	18.18ef (±0.74)	24.89c-e (±3.70)	19.44C (±2.32)
8 dS m^{-1}	21.54d-f (±1.55)	25.76cd (±0.49)	35.14b (±2.28)	27.48B (±1.44)
12 dS m^{-1}	28.19cd (±3.03)	28.58bc (±3.09)	48.33a (±4.55)	35.03A (±3.56)

Values are mean three replicates for each treatment (±SE). Values in a columns and rows with different letters are significantly different by LSD (P≤0.05). Different capital letters beside the mean values are significantly different by LSD (P≤0.05)

Table 6. Effects of different salinity levels on water uptake capacity (WUC%) of*Mangifera indica L.* at different days after treatments (DAT) imposition

Salinity level	30 DAT	60 DAT	90 DAT	Mean
Control	0.08f (±0.01)	0.08ef (±0.01)	0.09d-f (±0.00)	0.08C (±0.00)
4 dS m^{-1}	0.15c-f (±0.03)	0.17c-f (±0.01)	0.31c-e (±0.08)	0.21C (±0.04)
8 dS m^{-1}	0.23c-f (±0.02)	0.31cd (±0.01)	0.62b (±0.06)	0.39B (±0.03)
12 dS m^{-1}	0.37c (±0.08)	0.35c (±0.05)	1.04a (±0.22)	0.58A (±0.11)

Values are mean three replicates for each treatment (±SE). Values in a columns and rows with differentletters are significantly different by LSD (P≤0.05). Different capital letters beside the mean values are significantly different by LSD (P≤0.05)

3.8Stomatal Conductance

A diminution of stomatal conductance was observed in above and below part of the leaf due to different salinity at 90 DAT imposition (Fig. 2). In the upper part of leaf stomatal conductance reduced drastically, whereas slightly diminished in lower part of leaf. For reduction of stomatal conductance, the probable reason might be due to the 'osmotic effect' of salinity induces abscisic acid (ABA) accumulation. Salt induced reduction of stomatal conductance can be caused by stomatal limitation with stomatal closure [50] and a disturbance of photosynthetic activity at high tissue salt concentration [51]. This is consistent with previous observations on the effect of salinity on stomatal conductance of non-halophytes by Farquhar et al., [52].

UNDER PEER REVIEW

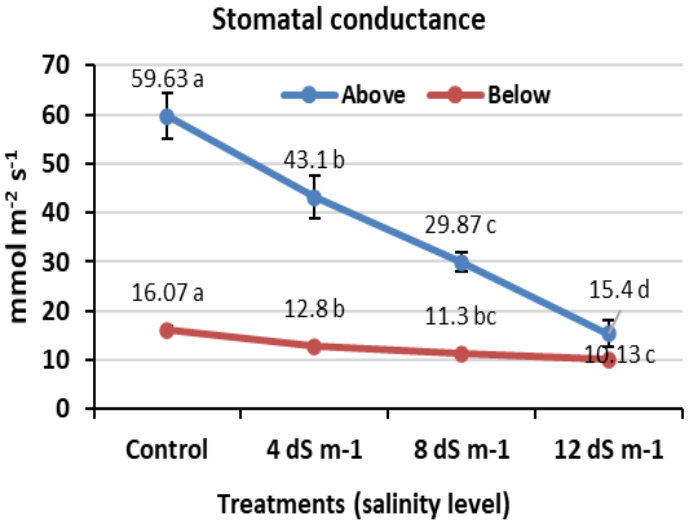

Fig.2.Response of stomatal conductance (mmol m^{-2} s^{-1}) in above and below portion of the leaf of *Mangifera indica L.* under different salinity levels at 90 days after treatments(DAT) imposition

3.9Total Chlorophyll and Carotenoid Content

Total chlorophyll content was greatly affected by the salinity level, and significantly decreased with increasing the salinity level (Fig. 3A). The chlorophyll content decreased with DAT imposition in all saline affected plants, in contrast it increased with times in control treatment. It was observed in interaction effect of total Chlorophyll content that significantly the highest (7.48 mg g^{-1}) and the lowest (1.17 mg g^{-1}) values were recorded in control and in 12 dS m^{-1} at 90 DAT. Carotenoid content was also gradually decreased with increasing salinity level (Fig.3B). Among the treatments mean, the highest carotenoid was recorded in control (1.31 mg g^{-1}) and the lowest (0.49 mg g^{-1}) was found in 12 dS m^{-1}. In the interaction effect it was observed that the highest result was found in control at 90 DAT (1.77 mg g^{-1}) which was significantly higher than other treatments, while the lowest result was found in 12 dS m^{-1} (0.31mg g^{-1}) at 90 DAT imposition.

The decrease in Chlorophyll content under stress is a commonly reported phenomenon and in various studies this is may be due to different reasons, one of them is related to membrane deterioration [53, 54]. Similar results were found in *faba* bean [55] and in *Satureja hortensis* [56]. Another reason for reduction in chlorophyll content in most plants may be due to disorganization of thylakoid membranes with more degradation than synthesis of chlorophyll via the formation of proteolytic enzymes, such as Chlorophyllase, which is responsible for degrading chlorophyll, as well as damaging the photosynthetic apparatus, reducing photosynthetic rate [57] and inhibiting accumulated ions [58].

UNDER PEER REVIEW

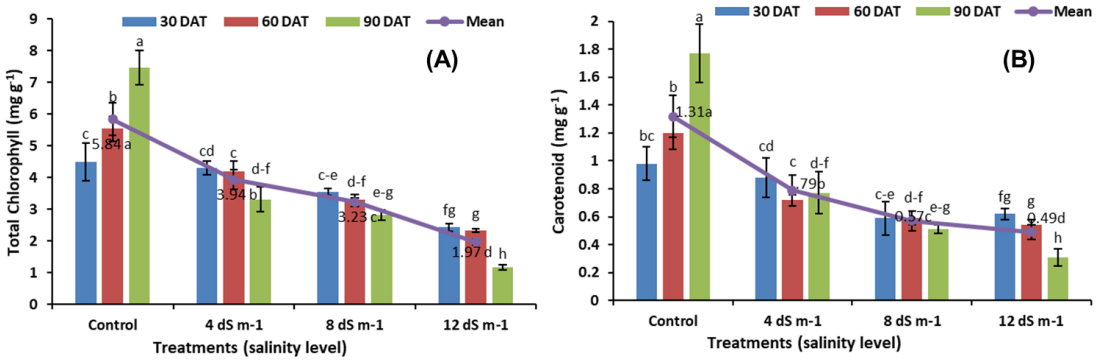

Fig.3.Effects of different salinity levels on total chlorophyll content (A) and carotenoid (B) of *Mangifera indica L.* at different days after treatments(DAT) imposition

3.10Proline Content

Salinity had a great influence on proline accumulation in leaves (Fig.4). Proline accumulation at 90 DAT imposition increased with increasing salinity level. Maximum proline accumulation was observed in 12 dS m^{-1} (13.37 µmol g^{-1}fw); in contrast, minimum was measured in control (3.60 µmol g^{-1}fw) plants. Proline accumulation in leaves as a response to salt stress were observed in several medicinal plants e.g., *Catharanthus roseus* [59] and *Salvia officinali* [60]. The increased proline content might be attributed to a decrease in proline oxidase activity in saline conditions [61]. Several reports indicate that proline content facilitate rapid mechanism for maintaining the turgor and affects the solubility of various proteins [62] and protects them against denaturation under saline condition [63]. Proline serves as a membrane protectant and accumulates in cytoplasm at higher concentration under stress conditions without interrupting cellular structure and metabolism due to its zwitter ions characteristic feature [64].

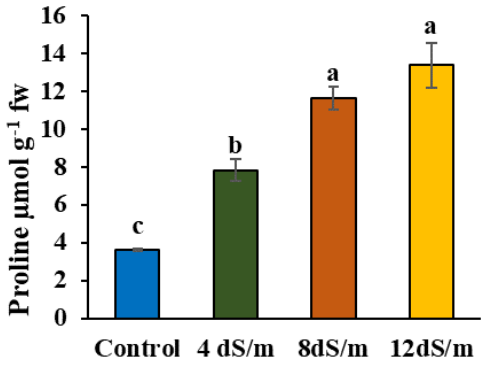

Fig.4.Effects of different salinity levels on proline (µmol g^{-1}fw) accumulation of *Mangifera indica L.* at 90 days after treatments imposition

4. CONCLUSION

The growth performance of mango seedlings was remarkably affected by salinity level. The adverse effect of salinity level was expressed on seedling during whole study period. The growth was stunted gradually over time due to salinity and ultimately reduced total biomass

UNDER PEER REVIEW

of mango sapling. The seawater induced salt stress adversely affected physiological processes of the plants, such as relative water content (RWC) was decreased but water saturation deficit (WSD) and water uptake capacity (WUC) were increased with the elevated concentrations of salinity. The highest total chlorophyll and carotenoid contents were found in control plants and lowest were recorded in 12 dS m^{-1} salinity level. The species produced higher proline with increasing salinity level. The increment of proline content in leaf helped the seedlings to survive under salt stress.

REFERENCES

1. Zakery-Asl MA, Bolandnazar S, Oustan S. Effect of salinity and nitrogen on growth, sodium, potassium accumulation, and osmotic adjustment of halophyte *Suaedaaegyptiaca* (Hasselq.) Zoh. Archives of Agronomy and Soil Science. 2014;60(6):785-792.

2. Shivanna N, Naika M, Khanum F, Kaul VK. Antioxidant, anti-diabetic and renal protective properties of *Stevia rebaudiana*. Journal of Diabetes and its Complications. 2013;27(2):103-113.

3. Munns R, Tester M. Mechanisms of salinity tolerance. Annual Review of Plant Biology. 2008;59:651-681.

4. Bala BK, Hossain MA. Integrated management of coastal zone for food security. Department of Farm Power and Machinery, Bangladesh Agricultural University, Dhaka. 2009;134.

5. SRDI. (2010). Saline Soils of Bangladesh; Soil Resources Development Institute (SRDI), Ministry of Agriculture, Dhaka, Bangladesh.

6. Sheekh-El MM, Omar HH. Effect of high salt stress on growth and fatty acids content of the unicellular green algae Chlorella vulgaris. American Journal of Microbiology. 2002;55:181-191.

7. Balal RM, Ashraf MY, Khan MM, Jaskani MJ, Ashfaq M. Influence of salt stress on growth and biochemical parameters of citrus rootstocks. Pakistan Journal of Botany. 2011;43(4):2135-2141.

8. Ahmed CB, Rouina BB, Akhtar HUR, Boukhriss M. Olive tree (*Olea europaea* L. cv. "Chameli") under salt stress: water relations and ions content. Pakistan Journal of Botany. 2006;38(5):1477-1484.

9. Ramoliya PJ, Pandey AN. Effect of salinization of soil on emergence, growth and survival of seedlings of *Cordia rothii*. Forest Ecology and Management. 2003;176(1-3):185-194.

10. Rout NP, Shaw BP. Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Science. 2001;160(3):415-423.

11. Nouman W, Siddiqui MT, Basra SMA, Khan RA, Gull T, Olson ME, Hassan M. Response of *Moringa oleifera* to saline conditions. International Journal of Agriculture and Biology. 2012;14(5).

UNDER PEER REVIEW

12. James RA, Rivelli AR, Munns R, von Caemmerer S. Factors affecting CO2 assimilation, leaf injury and growth in salt-stressed durum wheat. Functional Plant Biology. 2002;29(12):1393-1403.

13. Ueda A, Kathiresan A, Inada M, Narita Y, Nakamura T, Shi W, Bennett J. Osmotic stress in barley regulates expression of a different set of genes than salt stress does. Journal of Experimental botany. 2004;55(406):2213-2218.

14. Heuer B, Nadler A. Physiological response of potato plants to soil salinity and water deficit. Plant Science. 1998;137(1):43-51.

15. Miah MD, Foysal MA, Koike M, Kobayashi H. Domestic energy-use pattern by the households: A comparison between rural and semi-urban areas of Noakhali in Bangladesh. Energy Policy. 2011;39(6):3757-3765.

16. Arduini I, Godbold DL, Onnis A. Cadmium and copper change root growth and morphology of *Pinus pinea* and *Pinus pinaster* seedlings. Physiologia Plantarum. 1994;92(4):675-680.

17. Seydi AB. Determination of the salt tolerance of some barley genotypes and the characteristics affecting tolerance. Turkish Journal of Agriculture and Forestry. 2003;27:253-260.

18. Weatherly PE. Studies in the water relations of cotton plant. I. The field measurement of water deficits in leaves. New Phytol. 1950;(49):81-97.

19. Witham H, Blades DF, Devin RH. Exercise in Plant Physiology (2nd Edition). PWS Publishers, Boston, USA. 1986;128-131.

20. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39(1):205-207.

21. Kapoor K, Srivastava A. Assessment of salinity tolerance of *Vinga mungo* var. Pu-19 using ex vitro and in vitro methods. Asian Journal Biotechnology. 2010;2(2):73-85.

22. Jamil M, Lee KJ, Kim JM, Kim HS, Rha ES. Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. *Scientia Agricola*. 2007;64(2):111-118.

23. Nouman W, Siddiqui MT, Basra SMA, Khan RA, Gull T, Olson ME, Hassan M. Response of *Moringa oleifera* to saline conditions. International Journal of Agriculture and Biology. 2012;14(5)557-562.

24. Ghavami N, Ramin AA. Grain yield and active substances of milk thistle as affected by soil salinity. Communications in Soil Science and Plant Analysis. 2008;39(17-18): 2608-2618.

25. Iqbal M, Ashraf M. Changes in growth, photosynthetic capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. Plant Growth Regulation. 2005;46(1):19-30.

UNDER PEER REVIEW

26. Netondo GW, Onyango JC, Beck E. Sorghum and salinity: I. Response of growth, water relations, and ion accumulation to NaCl salinity. Crop Science. 2004;44(3):797-805.

27. Hassanein AA. Physiological responses induced by shock and gradual salinization in rice (*Oryza sativa* L.) seedlings and the possible roles played by glutathione treatment. *Acta botanica hungarica*, 2000;42: 139-159.

28. Tahir MA, Rahmatullah T, Aziz M, Ashraf S, Kanwal S, Maqsood MA. Beneficial effects of silicon in wheat (*Triticum aestivum* L.) under salinity stress. Pakistan Journal of Botany. 2006;38(5):1715-1722.

29. Joseph B, Jini D, Sujatha S. Development of salt stress-tolerant plants by gene manipulation of antioxidant enzymes. Asian Journal of Agricultural Research. 2011;5(1):17-27.

30. Rahman M, Soomro UA, Haq MZU, Gul S. Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. World Journal of Agricultural Sciences. 2008;4(3):398-403.

31. Basalah MO. Action of salinity on seed germination and seedling growth of *Solanum melongena* L. Journal of Agricultural Research Kafer El-Sheikh University. 2010;36:64-73.

32. Chaparzadeh N, D'Amico ML, Khavari-Nejad RA, Izzo R, Navari-Izzo F. Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiology and Biochemistry. 2004;42(9):695-701.

33. Rui L, Wei S, Mu-xiang C, Cheng-jun J, Min W, Bo-ping Y. Leaf anatomical changes of Burguieragymnorrhiza seedlings under salt stress. Journal of Tropical and Subtropical Botany. 2009;17(2):169–175.

34. Memon SA, Hou X, Wang LJ. Morphlogical analysis of salt stress response of pak choi. Electronic Journal of Environmental, Agricultural and Food Chemistry. 2010;9(1):248-254.

35. Chopart JL, Le Mezo L, Mezino M. Software application for processing root data from impact counts on soil profiles. User Guide, Technology Document. 2008;26- 28.

36. Seckin B, Turkan I, Sekmen AH, Ozfidan C. The role of antioxidant defense systems at differential salt tolerance of *Hordeum marinum*Huds. (sea barleygrass) and *Hordeum vulgare* L. (cultivated barley). Environmental and Experimental Botany. 2010;69(1):76-85.

37. Ali A. Influence of silicon on wheat grown under saline environments. A *PhD* Thesis, University of Agriculture, Faisalabad, Pakistan. 2009.

38. Carpici EB, Celik N, Bayram G. The effects of salt stress on the growth, biochemical parameter and mineral element content of some maize (*Zea mays* L.) cultivars. African Journal of Biotechnology. 2010;9(41):6937-6942.

UNDER PEER REVIEW

39. Bagci SA, Ekiz H, Yilmaz A. Determination of the salt tolerance of some barley genotypes and the characteristics affecting tolerance. Turkish Journal of Agriculture and Forestry. 2003;27(5):253-260.

40. Al-Thabet SS, Leilah AA, Al-Hawass I. Effect of NaCl and incubation temperature on seed germination of three canola (*Brassica napus* L.) cultivars. Scientific of King Faisal University (Basic and Applied Sciences). 2004;5(1):81-92.

41. Chartzoulakis KS. Salinity and olive: growth, salt tolerance, photosynthesis and yield. Agricultural Water Management. 2005;78(1-2):108-121.

42. Ahmed CB, Rouina BB, Akhtar HUR, Boukhriss M. Olive tree (*Olea europaea* L. cv. "Chameli") under salt stress: water relations and ions content. Pakistan Journal of Botany. 2006;38(5):1477-1484.

43. Morgan JM. Osmoregulation and water stress in higher plants. Annual Review of Plant Physiology. 1984;35(1):299-319.

44. Islam R, Senko C, Campbell WC, Korenblit S, Smith J, Lee A, Monroe C. Emergence and frustration of magnetism with variable-range interactions in a quantum simulator. Science. 2013;340(6132):583-587.

45. Katerji N, Van Hoorn JW, Hamdy A, Mastrorilli M. Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. Agricultural Water Management. 2003;62(1):37-66.

46. Kaya C, Higgs D, Kirnak H, Tas I. Ameliorative effect of calcium nitrate on cucumber and melon plants drip irrigated with saline water. Journal of Plant Nutrition. 2003;26(8):1665-1681.

47. Sangakkara UR, Hartwig UA, Nosberger J. Soil moisture and potassium affect the performance of symbiotic nitrogen fixation in faba bean and common bean. Plant and Soil. 1996;184(1):123-130.

48. Stoyanov Z. Effects of water stress on leaf water relations of young bean plants. Journal of Central European Agriculture. 2005;6(1):5-14.

49. Kabir E, Hamid A, Haque M, Nawata E, Karim A. Effect of nitrogen fertilizer on salinity tolerance of mungbean (*Vigna radiata* L. Wilczek). Japanese Journal of Tropical Agriculture. 2005;49(2):119-125.

50. Goldstein G, Drake DR, Alpha C, Melcher P, Heraux J, Azocar A. Growth and photosynthetic responses of Scaevola sericea, a Hawaiian coastal shrub, to substrate salinity and salt spray. International Journal of Plant Sciences. 1996;157(2):171-179.

51. Yeo AR, Lee AS, Izard P, Boursier PJ, Flowers TJ. Short-and long-term effects of salinity on leaf growth in rice (*Oryza sativa* L.). Journal of Experimental Botany. 1991;42(7):881-889.

52. Farquhar GD. Effects of drought, salinity and soil compaction on photosynthesis, transpiration and carbon isotope composition of plants. Journal of Plant Biochemistry and Physiology. 1987;6:147-155.

UNDER PEER REVIEW

53. Mane AV, Saratale GD, Karadge BA, Samant JS. EmiratusJounral of Food and Agriculture. 2010;23:59-70. 1999;46(3):229-237.

54. Tantawy AS, Abdel-Mawgoud AMR, El-Nemr MA, Chamoun YG. Alleviation of salinity effects on tomato plants by application of amino acids and growth regulators. European Journal of Scientific Research. 2009;30(3):484-494.

55. Dawood MG, Taie HAA, Nassar RMA, Abdelhamid MT, Schmidhalter U. The changes induced in the physiological, biochemical and anatomical characteristics of *Vicia faba* by the exogenous application of proline under seawater stress. South African Journal of Botany. 2014;93:54-63.

56. Najafi F, Khavari-Nejad RA. The effects of salt stress on certain physiological parameters in summer savory (*Satureja hortensis* L.) plants. Journal of Stress Physiology and Biochemistry. 2010;6(1).

57. Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science. 2010;4(8): 580.

58. Jaleel CA, Sankar B, Sridharan R, Panneerselvam R. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. Turkish Journal of Biology. 2008;32(2):79-83.

59. Abd EL, Azim WM, Ahmed STH. Effect of salinity and cutting date on growth and chemical constituents of *Achillea fragratissima*Forssk, under Ras Sudr conditions. Research Journal of Agriculture and Biological Sciences. 2009;5:1121-1129.

60. Ali RM, Abbas HM, Kamal RK. The effects of treatment with polyamines on dry matter, oil and flavonoid contents in salinity stressed chamomile and sweet marjoram. Plant Soil and Environment. 2007;53(12):529.

61. Muthukumarasamy M, Gupta SD, Panneerselvam R. Enhancement of peroxidase, polyphenol oxidase and superoxide dismutase activities by triadimefon in NaCl stressed *Raphanus sativus* L. Biologia Plantarum, 2000;43(2):317-320.
62. Abraham E, Rigo G, Szekely G, Nagy R, Koncz C, Szabados L. Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in Arabidopsis. Plant molecular biology. 2003;51(3):363-372.

63. Tonon G, Kevers C, Faivre-Rampant O, Graziani M, Gaspar T. Effect of NaCl and mannitol iso-osmotic stresses on proline and free polyamine levels in embryogenic Fraxinus angustifolia callus. Journal of Plant Physiology. 2004;161(6):701-708.

64. Goyal M, Asthir B. Polyamine catabolism influences antioxidative defense mechanism in shoots and roots of five wheat genotypes under high temperature stress. Plant Growth Regulation. 2010;60(1):13-25.

UNDER PEER REVIEW