Effect of salinity on the physiological and biochemical responses of neem

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***Abstract*—** *The present study was conducted to evaluate the plant growth, physiological and biochemical changes of neem under different salinity levels (4, 8, 10 and 12 dS/m) which have multipurpose use in agroforestry. Growth parameters, photosynthetic pigments, carbohydrate, proline and total phenol were investigated 30, 60, 90 and 120 days after treatment imposition. The results revealed that salinity caused significant differences in all the growth parameters and the maximum reduction were observed when plants were exposed to high salinity (12 dS/m) level and minimum were in control treatments. It was found that total dry matter and relative water content were reduced 72% and 40% in 12 dS/m compared to control plants at 120 DATI, respectively. By increasing saliniy 0 to 12 dS/m, the chlorophyll (the photosynthetic pigment) and carbohydrate (the photosynthetic product) content decreased, but increased the level of proline (an amino acid) and total phenol content (an antioxidant) in different days. The highest accumulation of free proline and total phenol content was recorded in 10 dS/m treatment and it was 77% and 59% greater than control plant, respectively. These findings suggest that though growth and biochemical parameters of neem were affected by salt stress, but all the plants survived in different salinity levels. Among all different salinity levels, neem can performed better up to 10 dS/m salinity level could be due to better antioxidant system of neem to cope up with oxidative damage to stressed plants.*

Keywords*— *Chlorophyll, growth, medicinal plant, neem, salinity.

I. INTRODUCTION

Salinity is a wide spread problem across the world and it has been interpreting considerate impacts on crop growth and productivity. The saline land is unfit for crop cultivation and the EC (Electrical Conductivity) value of that saline soil is more than 4 dS/m would adversely affect crop growth and productivity [1]. High content of soluble salt, usually sodium chloride causes high osmotic pressure which results reduction of absorption of water and nutrients that suppress the seedling growth and plant development [2]. These changes are also associated with decrease in chlorophyll and carbohydrate contents in leaves [3]. There is strong evidence that one of the adaptation mechanisms of plants to salinity and water deficit by accumulation of compatible solutes and proline in cytoplasm [4]. Additionally, salinity also induces osmotic and ionic imbalance and toxicity in plants that induces oxidative stress [5], which initiates antioxidant system of the plants to cope up with oxidative damage to stressed plants [6]. Many researchers explored the salt resistant plants and their tolerance mechanism. There have been prompt attempts to generate agricultural crop varieties tolerant to salinity stress. However, very few reports are available on the identification and utilization of perennial tree species, tolerant to salinity stress. Screening salt stress tolerance has been investigated in woody plant species such as olive [7], mango [8], acacia [9] and pine [10].

In Bangladesh, about 650 species have been identified as medicinal plants because of their therapeutic properties [11]. Many government and non-government organizations have had focused attention on improving the medicinal plants sector. But still the medicinal plant cultivation is in a very rudimentary stage. In order to meet the ever increasing demand for medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially. In addition, coastal area of Bangladesh is the potential area for crop cultivation. Coastal area in Bangladesh constitutes 20% of the country of which about 53% are affected by different degrees of salinity. So it seems valuable, to test the important medicinal plants for their salt tolerance capacity. Effect of salt stress has been studied in some medicinal plants such as aloe vera, golden shower, madagaskar periwinkle [12], drumstick [6]. This study is related to the effect of salt stress on our selected medicinal plants, neem.

Neem is an important multipurpose agroforestry species under the family Meliaceae, which is well-known for its medicinal value. The plant is a source of several potent botanical insecticides, soap, lamp oil, lubricants and lumber. It is a good shade tree and reduces soil erosion. Different parts like bark, leaf, seeds, root of neem have very strong medicinal value and the

seeds of this species can also be used as biological control of fungicide and pesticide of agricultural crops. Besides its use in medicine, the neem tree has great importance for its anti-desertification properties and possibly as a good carbon dioxide sinks [13]. Plantations of neem can improve the environmental condition of Bangladesh, if it can be introduced all over the country. Soil of the southern part of the country is saline affected and it is noteworthy that till date there are no reports of salt stress tolerance ability of the important medicinal plants, neem. Therefore, the objectives of the study were to understand the effect of different salinity levels on growth as well as the physiological and biochemical responses of neem.

II. MATERIAL AND METHOD

2.1 Experimental site

A pot experiment was designed at the research field of the Department of Agroforestry and Environment (24^0 09` N; $90^0$ 26` E) for a period of 5 months (April 2017 to October 2017). The minimum and maximum temperatures of the research area were fluctuated between 22 to 33^0C and 15 to 28^0C, respectively, during the experimental period. One-year-old plant was transplanted into each pot (26.5 cm in height and 27.5 cm in diameter). The pots were previously filled with soil mixture that was prepared by mixing oven-dried soil and cow dung (4:1). Each pot contained 12.04 kg of soil mixture and the soil moisture content was approximately 17% at field capacity. One-year-old neem seedlings of uniform size were collected from BRAC nursery, Gazipur, Bangladesh.

2.2 Treatments and design

After transplanting the seedlings into pots, the plants were allowed to grow for 15 days for adaptation prior to being treated with salt stress. The treatments of salinity levels were 4, 8, 10 and 12 dS/m and the control (tap water). The EC of about 4, 8, 10 and 12 dS/m were maintained by adding 4.41g, 8.81g, 11.02g and 13.22g NaCl respectively with mixing 1.7 liter of tap water. The irrigation was supplied on every 3 days interval up to 120 days. The treatments were imposed in 24 April, 2017. At first week 4 dS/m saline treatment was given to all the plants except control. At 2nd week, 8 dS/m saline treatment was given to all the plants except control and 4 dS/m. At 3rd week, 10 dS/m saline was given to the plants of 10 and 12 dS/m treatment. At 4th week, 12 dS/m saline was given only to the plants of 12 dS/m treatment. The experiment was laid out in Randomized Complete Block design (RCBD) with six replications in each treatment, and each replication comprised one plant.

2.3 Data collection

Data of growth contributing characters such as plant height, number of leaves, branches per plant and collar diameter were collected at 15 days interval up to 120 days. Growth parameters namely total fresh and dry matters (stem dry weight + leaf dry weight + root dry weight) of plants were measured after 120 days. All plants were measured for diameter over bark at collar region of above ground level by slide calipers and height was measured by meter scale. Above ground biomass or shoot dry weight was calculated by summing up the dry weights of stem and leaf of plants. Roots were washed thoroughly in tap water and dry before weighted. Soil salinity was measured by the conductivity meter (Model CD-4301). For biochemical analysis leaf samples were collected from plants of different treatment at 30 days interval up to 120 days after treatment imposition (DATI).

2.4 The relative water content (RWC)

Relative water content (RWC) was measured according to [14] and calculated as follows: RWC (%) = $[(FW-DW)/(TW-DW)]\times100$. For the determination of turgid weight (TW), leaf samples were submerged for 24 h in distilled water, then, they were blotted dry on a paper towel and weighed.

2.5 Determination of chlorophyll, proline, carbohydrate and total phenolic content

The levels of chlorophyll (Chl), proline (Pro), carbohydrate and total phenolic content (TPC) were determined following the methods described by [15], [16], [12] and [17], respectively.

2.6 Data analysis

The experiment had a Randomized Complete Block design (RCBD), and the values obtained for each plant and each variable were considered as independent replicates. The means were compared by one way analysis of variance and by using the Least Significance Difference (LSD) test at $P < 0.05$, using the Statistics 10 Software package.

III. RESULTS AND DISCUSSIONS

3.1 Effect of salt stress on plant growth parameters and RWC

3.1.1 Plant height (cm)

The results revealed that salinity had significant negative effects on the growth of neem, and the overall growth performance gradually declined upon increasing the level of salt stress for a period of 120 days (Fig. 1). The plant height of the neem was significantly influenced by different saline treatments at different DATI (Fig. 1, A). At 120 DATI, the maximum plant height (180.5 cm) was noticed in control treatment, which was statistically significant compared to different saline treatments. The tallest plant (180.5 cm) was observed in control treatment, which was statistically differed to all other saline treatments at 120 DATI. Conversely, the smallest plant (119.5cm) was noticed in 12 dS/m saline level, which was significantly differing from other treatments (Fig. 1, A). Though the smallest plant (99.5 cm) was recorded in 12 dS/m, but it was statistically identical to 10 dS/m saline treatment. At 90 DATI, the tallest (152.67 cm) plant was recorded in control treatment, while the lowest plant height (111.83) was found in 12 dS/m, which was statistically dissimilar to other three saline treatments. Relative plant height of neem seedlings gradually decreased over time and it was greater in 60, 90 and 120 DATI than 30 DATI. By increasing salinity level 0 (control) to 12dS/m the value of relative plant height were 100, 91, 78, 74 and 66 at 120 DATI, respectively. The decreasing plant height with increasing salinity was an indication of osmotic stress that was created by the pressure of saline ions. In case of some medicinal plants it has been reported that the affected growth of these plants at seedling stage as a result of salinity stress and this is considered the most essential developmental stage for plants until they establish as fully grown individuals [18]. In the present study salinity affects the plant height due to the occurring of deficit metabolism in plant cells. The negative effect of salinity on plant growth could be explained by two ways. Firstly, the plant water uptake ability reduces by the presence of high salt in the soil solution; this leads to slower growth and other one excessive amount of specific salts entering the transpiration stream which ultimately injure cells in the transpiring leaves, and this may further reduce photosynthesis and growth [19].

3.1.2 Number of leaves

In the present study, number of leaves per plant was statistically similar before treatment imposition (Fig. 1, B). Number of leaves was statistically similar between 4 dS/m and 12 dS/m; it was also similar between 8 dS/m and 10 dS/m treatments at 30 DATI. After 60 DATI, leaves of the salt stressed neem plants showed damaging symptoms such as chlorosis, necrosis, leaf burn etc. Similar observation was reported in Thai neem [20] that its leaves of salt stressed plants expressed damage symptoms such as chlorosis with patches of necrosis, leaf burn, and senescence. At 120 DATI, number of leaves of control, 4 dS/m, 8 dS/m, 10 dS/m and 12 dS/m were 171.17, 113.83, 89.83, 82.17 and 49.50, respectively. It shows that in 12 dS/m number of leaves per plant highly decreased than other saline treatments. The relative number of leaves was 67%, 52%, 48% and 29% in the plants under 4 dS/m, 8 dS/m, 10 dS/m and 12 dS/m treatments at 120 DATI, respectively compared to control plant. Similar reports were found in some other plants such as *Moringa oleifera* [6], *Mentha piperita* var. *officinalis* [21] and milk thistle [22]. The higher accumulation of sodium chloride in the cell walls and cytoplasm of the leaves could be the reason of decrease number of leaves. Concurrently, that leaves vacuole sap may not be accumulating more salt which ultimately decreases the concentration of salt inside the cells that leads to quick death of leaves [1].

3.1.3 Number of branch

Effect of salt stress on number of branches was most prominent among the growth parameters of neem plants. Number of branches of control plant was much higher than stressed plants in all dates of measurements (Fig. 1, C). At 30 and 60 DATI, number of branches varied significantly but variation was little among the plants under different salt stress. Instead of 90 and 120 DATI, number of branches was statistically different in all stressed seedlings. Similar effect on number of branches of seedlings under salt stress was reported in *Chamomilla recutita* [23], *Nigella sativa* [24] and *Withania somnifera* [25]. It was evident that formation of new buds was highly susceptible to increasing salinity and in case of neem this parameter could be a good trait to study the salinity response using other tree species.

3.1.4 Collar diameter (mm)

Collar diameter of neem seedlings was affected by different saline levels over time (Fig. 1, D). In control treatment, collar diameter was increased with time at all the measurement dates. At 30 DATI, collar diameter of the stressed seedlings was similar to each other. However, at 90 and 120 DATI, collar diameter was significantly different in control and all saline treatments. Reduction of collar diameter of neem was 48% in the highest saline treatment (12 dS/m) compared to control at

120 DATI. Affected collar diameter due to salinity is also reported in *Achillea fragratissima* [26] and *Withania somnifera* [25].

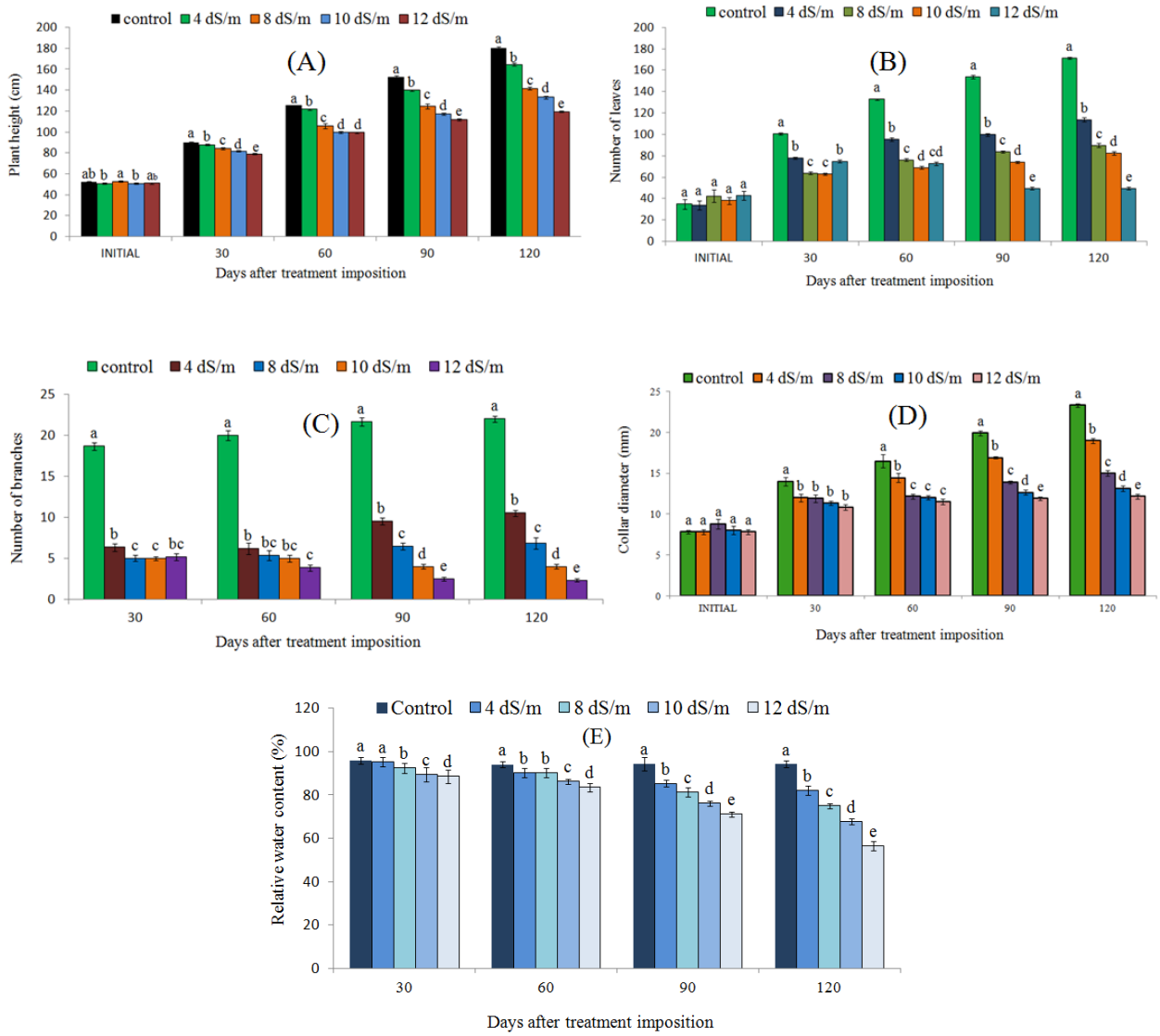

FIGURE 1. EFFECT OF DIFFERENT SALINITY LEVELS ON PLANT HEIGHT (A), NUMBER OF LEAVES (B), NUMBER OF BRANCHES (C), COLLAR DIAMETER (D) AND RELATIVE WATER CONTENT (E) OF NEEM PLANTS AT DIFFERENT DAYS AFTER TREATMENT IMPOSITION (DATI). (DATA REPRESENTS MEANS ± SE OF 6 INDEPENDENT REPLICATES AND MEANS FOLLOWED BY UNCOMMON LETTER(S) DIFFER SIGNIFICANTLY BY LSD AT 5% LEVEL.)

3.1.5 Fresh and dry weight (g)

Leaf, stem, root and total fresh weights (TFW) of neem plants were highly affected by saline treatments (Table 1). Total fresh weight was the highest (577.42 g) in control plant and the lowest (171.30 g) was noted in 12 dS/m saline level at 120 DATI. Total fresh weight of neem was reduced 70 % under 12 dS/m saline treatment as compared to control plants. Dry weights of leaf, stem and root of neem plants were also significantly diminished compared to control plant at 120 DATI (Table 2). In all cases like leaf, root, stem and total dry matter (TDM), the maximum dry weight was observed in control plant and the lowest was recorded in 12 dS/m salinity level at 120 DATI. Relative TDM of 4 dS/m, 8 dS/m, 10 dS/m and 12 dS/m was 45%, 36%, 31% and 27%, respectively. These results agreed with the findings of [27] that leaf, stem and root dry weight of *Satureja hortensis* were decreased compared to control plants in different levels of salinity. It can be explained that significant decline of total dry matter due to reducing photosynthesis, slow or less mobilization of reserve foods and suspending the cell division in plants [28].

TABLE 1
EFFECT OF DIFFERENT SALINITY LEVELS ON FRESH WEIGHT (G) OF NEEM SEEDLINGS AT 120 DAYS AFTER TREATMENT IMPOSITION

Treatment	Leaf	Stem	Root	TFW
Control	166.90 a	290.29 a	120.23 a	577.42 a
4 dS/m	110.02 b	131.71 b	64.48 b	306.21 b
8 dS/m	64.40 c	102.51 c	57.32 c	224.23 c
10 dS/m	63.98 c	87.73 d	45.33 d	197.05 d
12 dS/m	50.95 d	76.13 e	44.22 d	171.30 e
LSD(0.05)	3.9685	3.2405	2.4977	8.9432

TABLE 2
EFFECT OF DIFFERENT SALINITY LEVELS ON DRY WEIGHT (G) OF NEEM SEEDLINGS AT 120 DAYS AFTER TREATMENT IMPOSITION

Treatment	Leaf	Stem	Root	TDM
Control	45.80 a	138.53 a	45.37 a	229.70a
4 dS/m	24.60 b	57.40 b	21.67 b	103.67 b
8 dS/m	18.15 c	43.36 c	20.30 c	81.81 c
10 dS/m	17.10 c	38.32 d	15.95 d	71.37 d
12 dS/m	14.07 d	33.93 e	15.15 d	63.15e
LSD(0.05)	1.8864	1.3591	1.0691	2.4520

3.1.6 Relative water content (RWC)

The relative water content (RWC) was measured in order to evaluate the effects of salinity on the water status of neem at different DATI. At 30 and 60 DATI, a little variation was found in relative water content among stressed plants (Fig. 1, E). But at 60, 90 and 120 days after treatment imposition, the relative water content of the plant leaves under salt stress decreased significantly to each other. At 120 DATI, relative water content of control, 4 dS/m, 8 dS/m, 10 dS/m and 12 dS/m were 94.29%, 81.99%, 75.03%, 67.83% and 56.71 %, respectively. Islam, (2013) [29] observed similar results for mahagony and eucalyptus. Plant tends to cope with salt stress conditions by decreasing tissue water content (measured as RWC) which may be caused by low leaf water potential [30]. In this study, may be the decreased relative water content of neem leaf was caused by decreasing of leaf water potential. The decreasing relative water content of leaves indicate the less capacity to uptake water.

3.2 Effect of salt stress on chlorophyll and carbohydrate content

3.2.1 Chlorophyll content

Chl a, b and total Chl in leaves of neem were affected significantly under salt stress (Fig. 2). At 120 DATI, Chl a, b and total Chl in all salt stressed plants were highly decreased as compared to control plants (Fig. 2, A, B, C). However, it is also noticeable that, Chl a was more affected by salinity than Chl b. In accordance with other reports, our results also implied that Chl a is appears to be more sensitive to salinity than Chl. 'b' [6]. But Chl a is mainly responsible for photochemical phase of photosynthesis process and vital part of the light-harvesting compound, whereas Chl b as an accessory pigment acts indirectly in photosynthesis [31]. In general, decrease of these pigments under salt stress is considered to be a result of accelerated degradation and the inhibited synthesis and/or fast plastid breakdown of that pigment [32]. Rapid maturing of leaves is stated to be another reason for the decrease of Chl content under salinity [33]. So, reduction of Chl a and total Chl content in neem leaves may be one of the causes of less photosynthetic product and low biomass production of stressed neem seedlings.

3.2.2 Carbohydrate content

Data depicted in Fig. 2D, significant decrease in carbohydrate content with increasing salinity levels in all measuring dates. At 30 DATI, carbohydrate content of neem leaves were similar in all saline treatments, but it varied significantly in control. At 60 DATI, carbohydrate content was statistically similar in 4 dS/m, 8 dS/m and 10 dS/m saline treatment. But at 90 and 120 DATI, carbohydrate content slightly varied under 4 dS/, 8 dS/m and 10 dS/m (Fig. 2, D) saline treatment. According to [34] explained that increasing salinity decrease the carbohydrate content in *Foeniculum vulgare* , this can be attributed to the

reduced Chl content, nutritional imbalance due to the specific toxic effects of salinity, hyperosmotic stress and reduced photosynthesis. In this study, though reduced Chl content caused decreases in carbohydrate content but ultimately the decreasing carbohydrate content may had possitive effects in tolerance mechanism against salt stress.

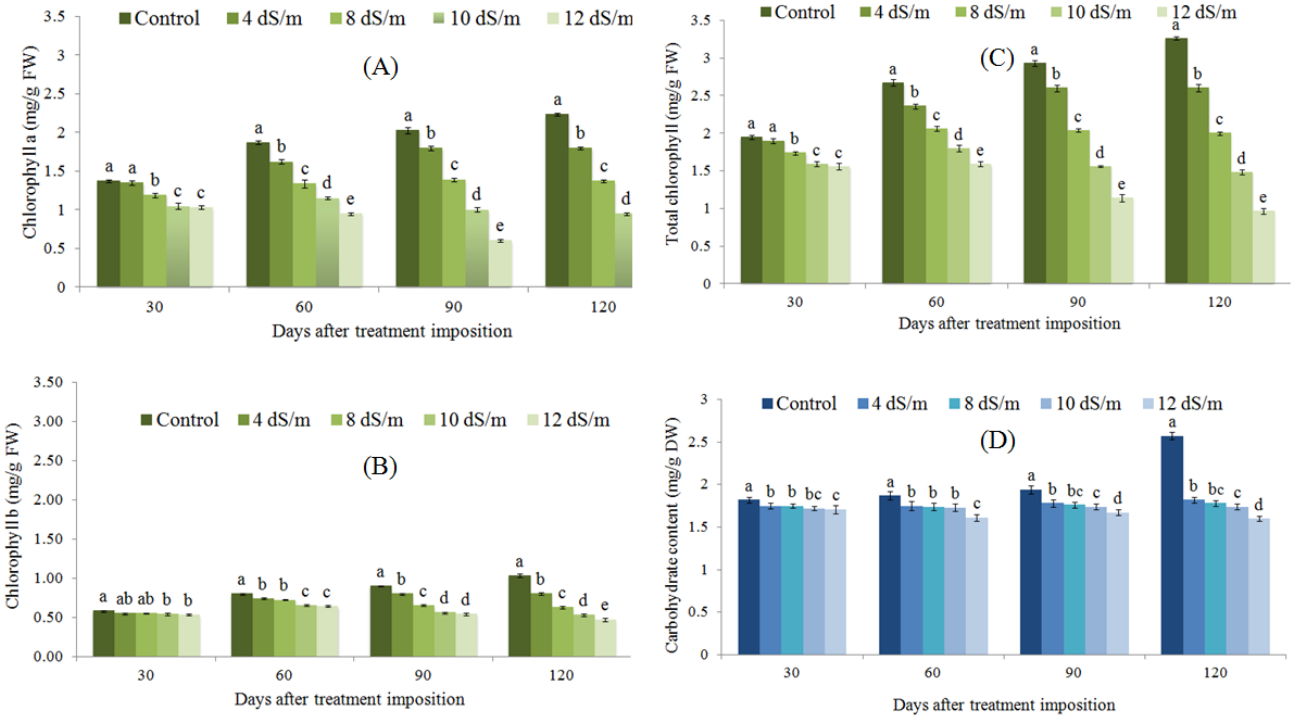

FIGURE 2. EFFECT OF DIFFERENT SALINITY LEVELS ON CHLOROPHYLL A (A), CHLOROPHYLL B (B), TOTAL CHLOROPHYLL (C) AND CARBOHYDRATE CONTENT (D) OF NEEM PLANTS AT DIFFERENT DAYS AFTER TREATMENT IMPOSITION (DATI). (DATA REPRESENTS MEANS ± SE OF 6 INDEPENDENT REPLICATES AND MEANS FOLLOWED BY UNCOMMON LETTER(S) DIFFER SIGNIFICANTLY BY LSD AT 5% LEVEL.)

3.3 Effect of salt stress on proline and total phenol content

3.3.1 Proline content

The Pro content was considerably increased in neem in response to salt stress with time. At 30 and 60 DATI, the highest Pro accumulation (10.545 and 22.29 µmol/g respectively) was found in 12 dS/m saline treatment (Fig. 3, A). But at 90 and 120 DATI, the highest Pro content (23.775 and 25.965 µmol/g respectively) was observed in seedlings under 10 dS/m salinity level. Accumulation of Pro is probably one of the most frequently reported modifications induced by salinity and water deficit in plants. In the present study, Pro content significantly increased with the increase of salinity, which agrees with previous results obtained for several medicinal plants e.g. Chamomile [35], Fennel [26] and sage [36]. The increased Pro may lead to a reduction in stress induced cellular acidification and may also act as a hydroxyl radical and singlet oxygen scavenger. Additionally, the accumulation of high Pro concentrations in the cytoplasm under stress conditions without interrupting cell structure and metabolism may be due to its zwitterion nature [37]; it is thought to be involved in osmotic adjustment of stressed tissues. This may assist plants in their adaptation to salinity stress. It has also been reported that hyperaccumulation of Pro is one of the positive indicators for the salinity resistance of plants; whereas other researchers affirm that it appeared to be a symptom of salt stress [38].

3.3.2 Total Phenol content

Accumulation of total phenol content increased with increasing salinity levels in leaves of neem plants (Fig. 3, B). At 30 and 60 DATI, the highest phenol content (4747.2 and 5678.4 mg/ 100 g dry weight, respectively) was found under 12 dS/m saline treatment, which was statistically different to other stressed plants. But at 90 and 120 DATI, the highest phenol content (23.775 and 25.965 µmol/g respectively) was observed under 10 dS/m salinity level. Similar observation was found in *Moringa oleifera* [6] and *Nigella sativa* [25]. Phenolic compounds are very important plant constituents because of their scavenging ability due to their hydroxyl groups. These compounds are also powerful chain breaking antioxidants and play a vital role in the defense against reactive oxygen species (ROS) [39]. In this study, may be the increased levels of phenols at

elevated levels of salinity induced accumulation of secondary metabolites to tolerate higher levels of salinity stress and aroused adverse conditions.

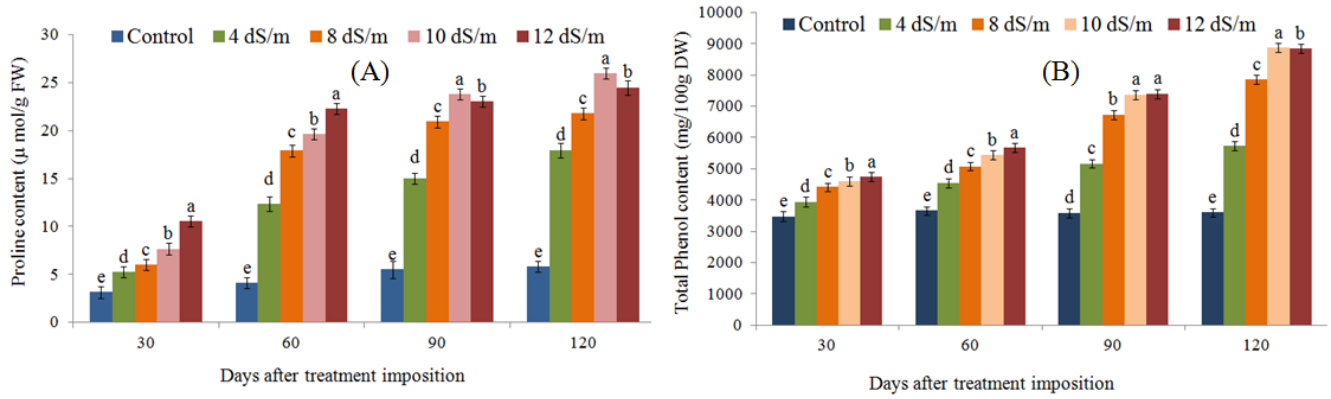

FIGURE 3. EFFECT OF DIFFERENT SALINITY LEVELS ON PROLINE (A) AND TOTAL PHENOL CONTENT (B) OF NEEM PLANTS AT DIFFERENT DAYS AFTER TREATMENT IMPOSITION (DATI). (DATA REPRESENTS MEANS ± SE OF 6 INDEPENDENT REPLICATES AND MEANS FOLLOWED BY UNCOMMON LETTER(S) DIFFER SIGNIFICANTLY BY LSD AT 5% LEVEL.)

IV. CONCLUSION

The results of this study showed that salinity stress had significant effect on growth and photosynthetic pigments of neem plants. However, salinity decreased the amount of Chl a, b, and carbohydrate contents in neem plants. During salinity stress, increasing the accumulation of free Pro and total phenol content in leaf, sustained the plants to better growth and survival under salt stress. The findings of this study show valuable information regarding plant growth and physiological performance of important medicinal tree species in different saline treatments, which may be useful to introduce neem plantation in the saline affected areas. However, based on the findings of the study it can be advocated that on-farm investigation should be conducted in real field conditions of saline prone area to confirm the performance of neem.

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