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Characterization of NaCl Stress in Young Bismarckia Palm (Bismarckia Nobilis)

CHARACTERIZATION OF NACL STRESS IN YOUNG BISMARCKIA PALM (*BISMARCKIA NOBILIS*)

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ABSTRACT

Salinity is a major problem caused by the accretion of excess salts composites in the form of ions like sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and chloride (Cl^-). The possible out-turns of the salinity on the morphology; physiology and biochemical variables of bismarckia palm (*Bismarckia nobilis*) were investigated in this study. The seedlings of bismarckia palm were grown in pots and periodically irrigated with various concentrations of NaCl solution to assess the various biological variables at the regular intervals. The experiment was performed by following the completely randomized design (CRD) and the results showed that the increased level of salt stress significantly decreased the shoot, length, fresh and dry weight. Conversely, the root length and fresh, dry weight were enhanced under higher salts concentration (150 mM of NaCl). However, there was no effect of stress on leaves parameters particularly for total number of leaves. Moreover, the chlorophyll contents were reduced with the increase of the NaCl concentration. Similarly, the antioxidants activities including SOD, POD and CAT were enhanced at maximum test dosage. Conclusively, it can be concluded that high saline condition of soil may hinder the morphological, physiological and biochemical statutes of bismarckia palm (*Bismarckia nobilis*).

Keywords: Salt stress, Bismarckia palm, morphology, chlorophyll contents, antioxidants.

INTRODUCTION

Plant experiences various abiotic stresses during its course of life. Salt stress is one of the major stresses, among all of them which induces premature flower senescence and reduction of photosynthetic area of leaf which lowers the photosynthetic activity of plant. Generally, charged ionic species of sodium and chloride elements plays a vital role in the physiological processes of plants including the monitoring the cellular trafficking by fluctuating the ion channels. With the elevated level of sodium and chloride ions beyond the threshold level, the cellular trafficking got ceased, resulting in the reduction of crop foliage and biomass production. Salt stress also leads to deterioration of cell by lowering the turgor pressure. Moreover, it has been reported that metabolic and enzymatic activities of many palm trees also interrupted due to the stress caused by salinity (Munns, 2002).

Bismarckia palm (*Bismarckia nobilis*), one of the ornamental plants, a member of family Arecaceae (palmae) bears simple, lobed, star-shaped, palmate and evergreen leaves of silver-gray shade, is being frequently used for landscaping at highways and residential areas. It can attain height of 25-50 ft with 4 ft of flower stalk and 1.5 inches of fruit length. A single plant of the Bismarckia palm is attractive enough and well suited for the indoor landscaping (Gilman & Watson, 2011; Gilman, 1997). Despite its beautification, it has not so much showy appearance to attract the wild life. High drought tolerant and moderate aerosol salt tolerant is the major characters of bismarckia palm. The spread of bismarckia palm along the road-side become the reason of a pleasant dramatic impact (Linz et al., 2004). Bismarckia palm is also considered as the highly profitable palm but requires a long time for its germination due to latent period of dormancy. Furthermore, the sufficient ambient light also supports excessive growth of *B. nobilis* in well-drained soil (Klinger & Rejmánek, 2010).

Salinization is one of the greatest challenges for deteriorating the crop productivity and ecosystems around the world, highly aggravated in regions facing acute and chronic fresh water shortage. Enormous quantity of salts tends to accumulate in the soils due to high evaporation rate and irrigation with brackish water which constrains the agricultural and ornamental crops. In arid and semi-arid regions, salinity along with other abiotic stresses, deteriorates crop yields and food security (Hazzouri et al., 2020; Malash et al., 2008). Nowadays, water shortages, contamination of groundwater with various leachates and soil salinity have threatened the productivity of the various agricultural crops. Palm trees are socio-economically important crops in the arid regions of Pakistan and other part of the world. A number of studies have been conducted to evaluate the effects of salinity in landscape plants. Only a few have investigated the effects of salinity on palms despite their immense value in landscaping (Bañón et al., 2005; Cassaniti et al., 2009; Picchioni & Graham, 2001; Valdez-Aguilar et al., 2011). Salinity might reduce the growth and expansion of leaves of ornamental shrubs (Bañón et al., 2005; Cassaniti et al., 2009; Picchioni & Graham, 2001; Valdez-Aguilar et al., 2011). Presence of the high concentration of Na^+ and Cl^- ions in saline waters poses adverse effects in the form of cellular necrosis and toxicity (Manual, 1992; Vieira et al., 2020).

Despite these facts, the bismarkia palm is one of the less explored plants regarding the salinization studies to address the saline stress. The objective of this research work was to assess the morphological, physiological and biochemical characteristics on growth of young bismarkia palm (*B. nobilis*) under different levels of salt stress, mainly posed due to NaCl to explore the bearable threshold level of salt stress that bismarckia palm.

MATERIAL AND METHODS

The experiment was organized at Botanic Garden, University of Agriculture, Faisalabad, to evaluate the impact of sodium chloride (NaCl) stress on bismarckia palm (*B. nobilis*) which was obtained from a horticultural nursery at Pattoki district Qasoor, Pakistan. A total 60 seedlings of palms were planted in pots (diameter: 10 inches, capacity: 5 kg by weight of Approx. 2 mm sized granules) having garden soil, leaf compost and manure from the farm-yard using the ratio of 1:1:1 as a medium in each pots. The palm seedlings were kept under normal conditions for one month for growth stabilization. After one-month, various dosage of NaCl was applied with the concentration of 50 mM, 100 mM and 150 mM periodically. Five replicates with four treatments were used in the whole experiment. Experiment was laid down in complete randomized design (CRD). Data regarding morphological, physiological and biochemical parameters was recorded after every 15 days for a period of three months.

Morphological parameters including length of shoot and root, fresh and dry weight of shoot and root and number of leaves were analyzed. The physiological parameters like chlorophyll contents including chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b and carotenoids were recorded using the green foliage aqueous lysate in a spectrometer at wavelengths of 663 nm, 645 nm and 480 nm. For biochemical analysis, the antioxidants including superoxide dismutase (SOD) with the protocol of (Giannopolitis & Ries, 1977), peroxidases (POD) and catalases (CAT) were analyzed with the protocol of (Chance & Maehly, 1955; Samrana et al., 2020). The data was analyzed by using analysis of variance technique (Steel et al., 1980). The mean values of different parameters were analyzed and compared using least significant difference (LSD) test at 5 % confidence level.

RESULTS

a) Morphological Parameters

To investigate the changes in foliage of *B. nobilis*, shoot length along with fresh and dry weight was recorded (Table 1). The maximum shoot length observed was 86.96 ± 3.19 cm (Mean ± S.E.) in control plants without any salt stress level compared to the shoot length observed at 150 mM of NaCl slat stress i.e. 74.34 ± 1.57 cm (Figure 1a). While, the shoot length of *B. nobilis* treated with 100 mM and 50 mM of NaCl concentration was 80.14 ± 3.15 cm and 85.62 ± 2.92 cm respectively. A significant decrease in shoot length (14.5 %) was noted at the highest salt test dosage i.e. 150 mM of NaCl, when compared to the control group. Similarly, shoot fresh weight decreased with increasing the level of salt concentration. The maximum weight gain of shoot fresh weight was 74.00 ± 6.19 g for control group, while the least weight gain was observed at 150 mM of NaCl i.e. 59.32 ± 2.03 g (Figure 1b). In general, stress level of 150 mM of NaCl cause the most adverse effect of 19.83 % and 10.08 % decrease in the shoot fresh and dry weight, respectively. In case of shoot dry weight, the maximum observed weight was 50.1 ± 4.39 g for control group, while the dry leaves weight was observed for plant treated with 150 mM of salt stress i.e. 40.02 ± 1.39 (Figure 1c).

The numbers of leaves were also counted in the experiment. No significant difference was observed in all cases where salt stress was applied (50 & 100 mM: 7 leaves per plant, 150 mM: 6 7 leaves per plant) along with control group (7 leaves per plant). It infers that NaCl does not adversely affect the leaves of *B. nobilis* (Table 1).

Table 1: Morphological profiling of *B. nobilis* under NaCl salt stress conditions

NaCl Dosage	Shoot Length	Shoot Fresh Weight	Shoot Dry Weight	Number of Leaves	Root Length	Root Fresh Weight	Root Dry Weight
Control (no NaCl)	86.96 ± 3.19	74.00 ± 6.19	50.10 ± 4.39	6.60 ± 0.51	23.12 ± 1.72	10.86 ± 0.48	5.80 ± 0.31
50 mM	85.62 ± 2.92	70.00 ± 5.10	46.40 ± 3.87	7.20 ± 0.37	21.32 ± 2.78	11.66 ± 1.02	5.84 ± 0.54
100 mM	80.14 ± 3.15	65.80 ± 4.30	45.46 ± 4.01	6.00 ± 0.71	26.84 ± 2.58	13.40 ± 1.68	7.10 ± 0.90
150 mM	74.34* ± 1.57	59.32 ± 2.03	40.02 ± 1.39	5.40 ± 0.40	28.48 ± 2.65	14.56 ± 1.65	7.70 ± 1.16

*Mean ± Standard Error of N = 5; *P < 0.05, Dunnett's test relative to control*

Table 2: Physiological profiling of *B. nobilis* under NaCl salt stress conditions

Treatment	Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll	Chlorophyll *a/b*	Carotenoids
Control	0.94 ± 0.09	0.34 ± 0.02	1.28 ± 0.11	2.74 ± 0.12	0.04 ± 0.01
50 mM	0.70 ± 0.06	0.31 ± 0.01	1.01 ± 0.07	2.27 ± 0.15	0.03 ± 0.00
100 mM	0.69 ± 0.07	0.29 ± 0.02	0.97 ± 0.09	2.32 ± 0.12	0.03 ± 0.00
150 mM	0.54* ± 0.08	0.30 ± 0.01	0.84* ± 0.08	1.69* ±0.23	0.02* ± 0.01

*Mean ± Standard Error of N = 5; *P < 0.05, Dunnett's test relative to control.*

Further, to investigate the changes in rhizopheric parts of *B. nobilis,* root length, root fresh and dry weight was recorded. Surprisingly, the root length showed the positive correlation with the increase of NaCl salt level (Table 1). It was observed that the maximum root length was achieved for 150 mM of NaCl i.e. 28.48 ± 2.65 cm, while the least root growth was observed in control group i.e. 23.12 ± 1.72 cm (Figure 1d). In case of root fresh weight, the least weight gain was observed to be 10.86 ± 0.48 g for control *B. nobilis.* However, the maximum weight gain was observed at highest NaCl dosage i.e. 14.56 ± 1.65 g at 150 mM (Figure 1e). Similar results were observed for the root dry weight (Figure 1f), where the maximum weight was observed for highest tested NaCl concentration (7.70 ± 1.16 g) compared to control group (5.80 ± 0.31).

Table 3: Biochemical profiling of *B. nobilis* under NaCl salt stress conditions

Treatment	Superoxide dismutase (SOD)	Peroxidase (POD)	Catalases (CAT)
Control	2.66 ± 0.22	0.29 ± 0.03	3.98 ± 0.23
50 mM	4.18* ± 0.33	0.49* ± 0.03	5.00 ± 0.28
100 mM	5.15* ± 0.33	0.63* ± 0.04	6.26* ± 0.51
150 mM	5.69* ± 0.23	0.89* ± 0.07	7.69* ± 0.47

*Mean ± Standard Error of N = 5; *P < 0.05, Dunnett's test relative to control.*

In general, it can be inferred that high NaCl concentration does affect the growth of *B. nobilis.* Moreover, a negative correlation was observed between root and shoot length at highest dosage of NaCl tested in this experiment (Figure 2).

b) Physiological Parameters

Photosynthetic pigments have the great importance in plant physiological processes. In this experiment, chlorophyll content along with the carotenoids was also profiled to estimate any significant effect of NaCl salt stress. The chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b along with carotenoid approximates were estimated as 0.94 ± 0.09 (Mean ± S.E.), 0.34 ± 0.02, 1.28 ± 0.11, 2.74 ± 0.12 and 0.04 ± 0.01g^{-1} f. wt. for control group (having no saline treatment) respectively (Table 2). In case of NaCl treatment, all the variables were significantly decreased gradually with the increase of NaCl dosage i.e. 50 mM to 150 mM (Figure 3). Alike the morphological parameters, it was observed that with the increase of NaCl dosage, all the physiological parameters (i.e. chlorophyll a, total chlorophyll and chlorophyll a/b and carotenoids) except chlorophyll b showed significant decrease particularly at 150 mM dosage. This indicates that the high saline conditions of NaCl, reduces the amount of total chlorophyll content.

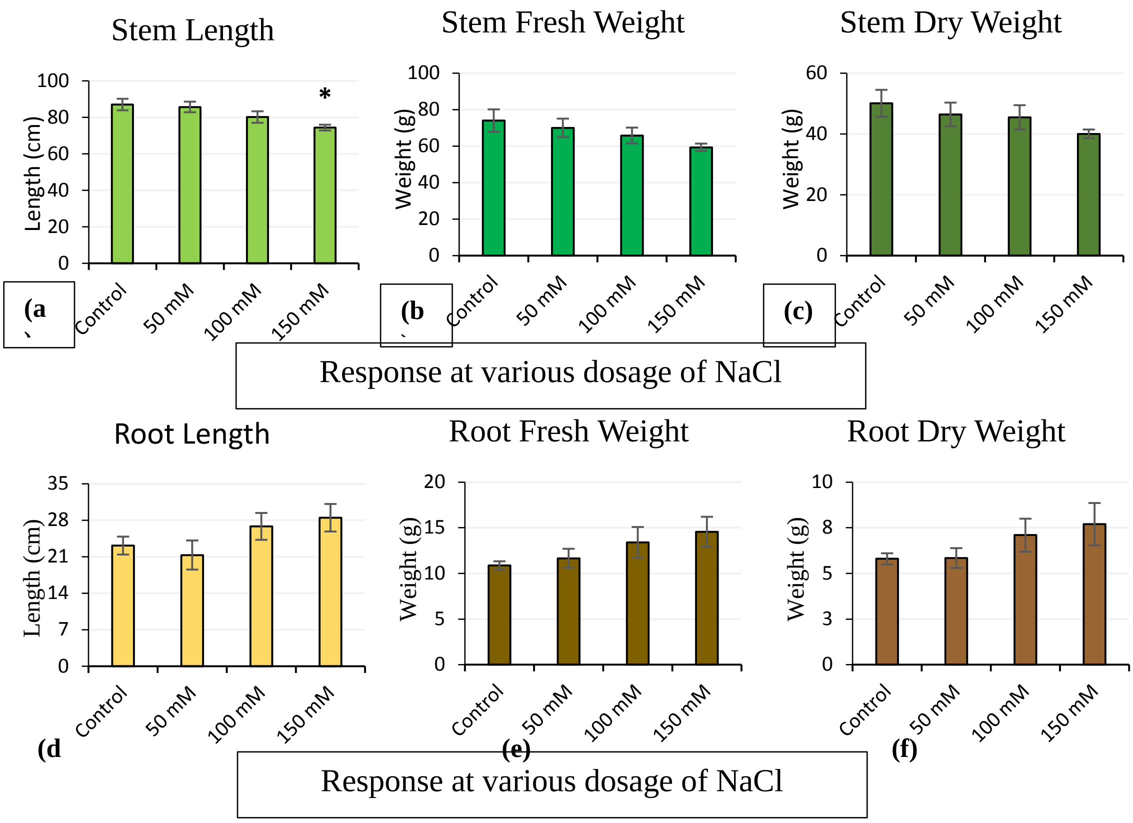

Figure 1: The effect of NaCl salt stress on the morphology of *B. nobilis*

*Stem length, (b) Fresh stem weight, (c) Dry stem weight, (d) Root length, (e) Fresh root weight, (f) Dry stem weight; Bars indicates the Mean ± Standard Error of N = 5; *P < 0.05, Dunnett's test relative to control.*

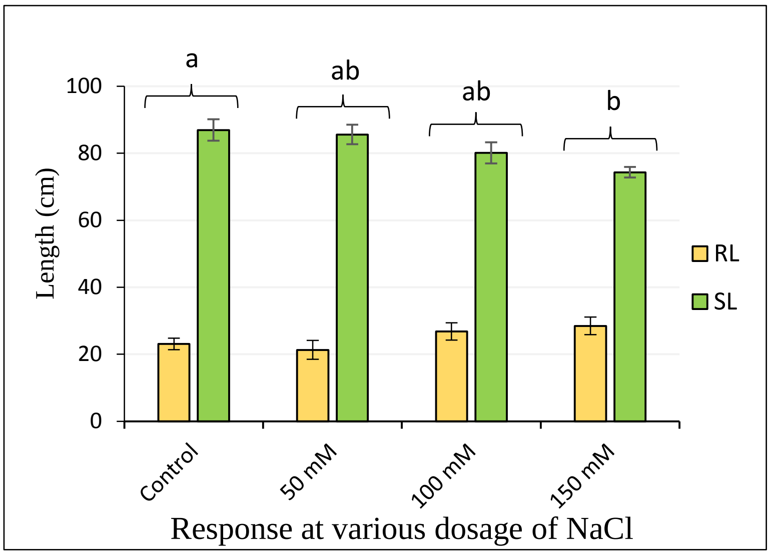

Figure 2: The antagonist effect of NaCl salt stress on stem and root length of *B. nobilis.*
(Bars indicates the Mean ± Standard Error of N = 5; Different letters indicate P < 0.05, ANOVA followed by Tukey's HSD test.).

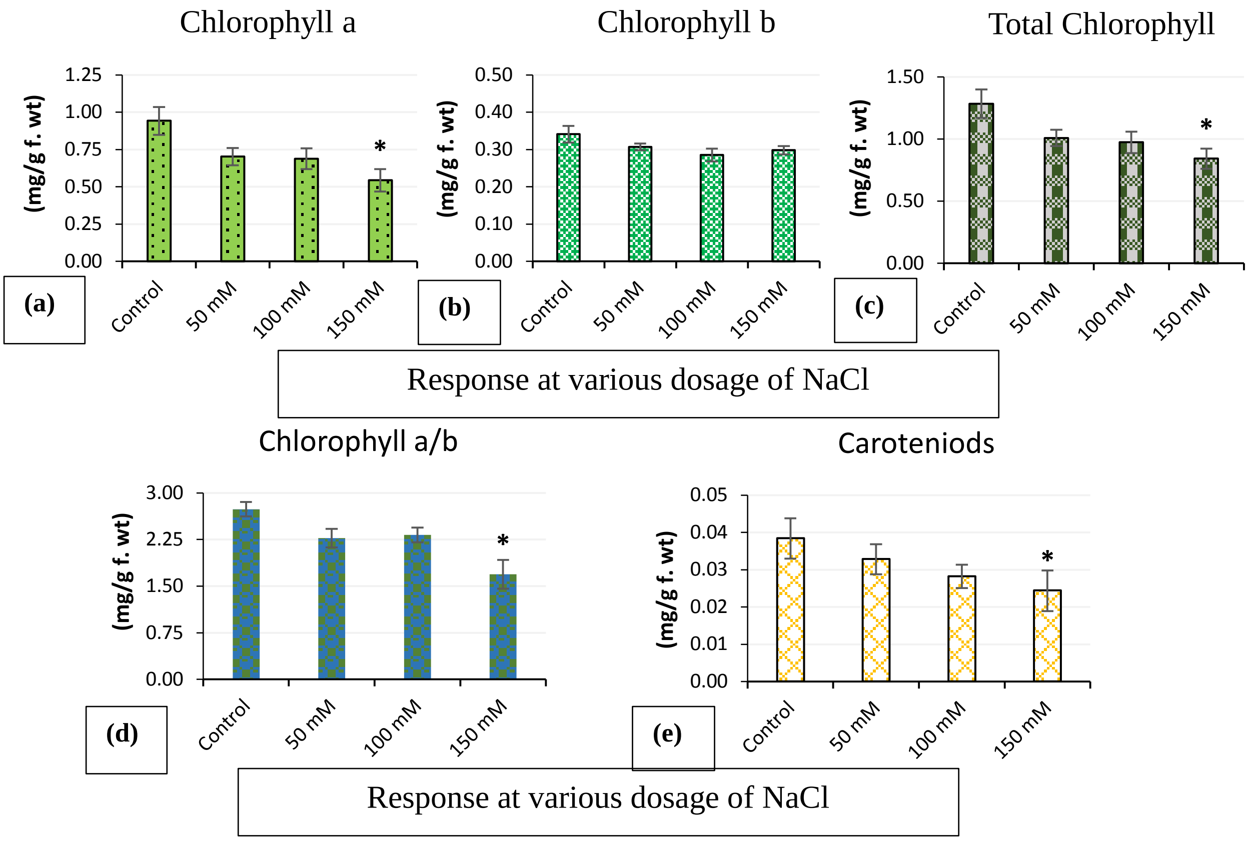

Figure 3: The effect of NaCl salt stress on the Physiology of *B. nobilis*.

*(a) Chlorophyll a, (b) Chlorophyll b, (c) Total chlorophyll, (d) Chlorophyll a/b, (e) Carotenoids; Bars indicates the Mean ± Standard Error of N = 5; *P < 0.05, Dunnett's test relative to control.*

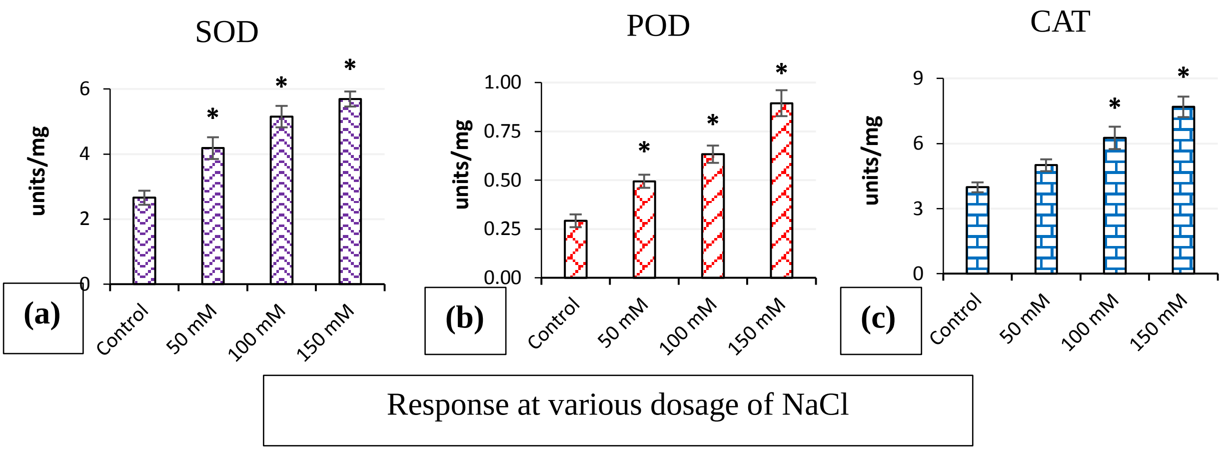

Figure 4: The effect of NaCl salt stress on the Biochemical aspects of *B. nobilis*.

*(a) Superoxide dismutase (SOD), (b) Peroxidase (POD), (c) Catalases (CAT); Bars indicates the Mean ± Standard Error of N = 5; *P < 0.05, Dunnett's test relative to control.*

a) ***Biochemical Parameters***

Besides the morphological and physiological fluctuation due to salinization, various enzyme activities are also got affected. To estimate the effect of NaCl salt stress in biochemical statutes of *B. nobilis*, superoxide dismutase (SOD), peroxidase, (POD) and catalases (CAT) were also profiles. It was observed that a slightest application of artificial saline conditions, all the biochemical parameters got affected severely. Even at least applied dosage of NaCl i.e. 50 mM, all the parameters showed enhance level of biochemical parameters compared to control group (Table 3, Figure 4). It infers that SOD, POD and CAT significantly increase with the increase of NaCl concentration.

DISCUSSION

This research has investigated that increased level of salt stress caused reduction in the shoot length, mainly at 100 mM of NaCl and 150 mM of NaCl. The current output is according to a previous study (Ashraf & Hafeez, 2004) which showed the significant reduction in the shoot length by applying the salt stress of NaCl in millet and maize. It has been suggested that the different types of stress reduce the total plant biomass especially the shoot fresh weight that caused the crop yield reduction (Ekinci et al., 2012). In most of the plants shoot fresh weight and dry weight reduced remarkably due to the different salt stress levels directly or indirectly (Yildirim et al., 2011). Similar results we found in recent study carried out by Barassi and his colleagues that describe that salinity stress enhanced the root length to decrease the stress pose due to high salt conditions (Barassi et al., 2006).

The current results are according to previous studies (Demiral & Türkan, 2005; Mohammad et al., 1998; Murillo- Amador et al., 2002) that indicates that higher concentrations of sodium chloride have increased the fresh weight of root. Another study illustrated that the active uptake of sodium, potassium and chloride ions enhanced the total biomass of the plant including fresh and dry biomass of the root (Chandrasekaran et al., 2014). There were no adverse effects of salinity stress on leaf count as suggested by Mehta and his colleagues (Mehta et al., 2010).

These conclusions are in accordance with the results of Din and his colleagues (Din et al., 2011) that exposed the suppression of photosystem-I and PS-II because of salt accumulation that reduced the content of chlorophyll a. It was suggested that due to salt stress, any modulation in light observing pigments influenced the photosynthetic patterns (Farooq et al., 2007). Findings of current study are also in accordance with the results of another study which revealed that the stress of NaCl caused deposition of Na^+ and Cl^- that reduced the chlorophyll content and lowered the total photosynthetic products and rate (Turan et al., 2009). High concentrations of sodium and chloride ions produced toxicity in leaves that disturbed all physiological parameters especially the photosynthesis rate by destructing total ratio of chlorophyll a/b (Tiwari et al., 2010). Biochemical analysis included in this study showed the increased trend by the higher salinity level and our findings are according to past researches (Debez et al., 2008; Foyer & Allen, 2003; Perveen et al., 2013) who explained that during salt stressed conditions, plants possess the defense system like antioxidant for scavenging the reactive oxygen species (ROS) and synthesized superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in higher amount by increasing the level of stress.

CONCLUSION

All parameters including in this study respond differently according to different concentrations of salt stress during this short period of evaluation. All morphological parameters decreased by increasing the salt stress including shoot length, shoot fresh weight and shoot dry weight. On the other hand, root length, root fresh and dry weight increased with the salt stress. Chlorophyll contents like chlorophyll a, total chlorophyll, chlorophyll a/b and carotenoids decreased by raising the salt stress of NaCl. Antioxidants including superoxide dismutase (SOD), peroxidase (POD) and catalases (CAT) significantly enhanced by increasing salinity stress more obvious at 150 mM of NaCl to minimize impact of salt stress. In future, a long-term evaluation will be reported to decisively infer the possible adverse effects of NaCl on morphological, physiological and biochemical statutes of *Bismarckia nobilis*.

AUTHORS COMTRIBUTION

Dr. M. Afzal Naeem, designed the experimental study and wrote the drafts of the manuscript, Ayesha Hanif executed the research work and compiled the data, Dr. Hassaan Khan helped in statistical analysis and presentation of research data whereas, Dr. Midrarullah reviewed the earlier drafts of the manuscript**.** Tehmina Nazish provided the technical expertise to conduct the biochemical assays.

CONFLICT OF INTERESTS

All concerned authors declare no conflict of interests either financial or personal relationships regarding this manuscript.

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