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Lower Doses of NaCl Promotes Growth in Alfalfa (*Medicago Sativa* L. cv. Ek Sali) Cultivated on the Soil Amended with Farmyard Manure

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Abstract Salinity is the key problem for crop cultivation in several regions of India. To study the plant growth and photosynthetic pigments of alfalfa under salinity stress, an experiment was conducted in a factorial design wherein alfalfa seeds were grown in polythene bags in triplicate, containing 3.5 kg sandy-loam soil, poor in NPK amended with 5%, 10%, 15%, 20% and 25% (w/w) Farmyard manure (FYM). NaCl doses of 0, 10, 20, 50, 100, 200 and 500mM were given for one month after the plants attained enough strength. Lower salinity doses of 10, 20 and 50mM with FYM proved to be the growth promoter for the plant. Peaks of shoot height (103.00cm), root depth (24.00cm), shoot dry biomass (8.97g/plant), root dry biomass (2.33g/plant) and leaf area (2467.17cm^2/plant) were recorded in plants grown on soil amended with 15% FYM and treated with 50mM NaCl. Plant growth started to decrease at 100mM NaCl and afterwards. Comparatively, better performance of the plant and overall growth recorded in plants raised on soil amended with 15% FYM. Similarly, the highest chlorophyll *a* (0.71mg/g), chlorophyll *b* (0.86mg/g), carotenoids (0.48mg/g), chlorophyll *a*+*b* (1.57mg/g) were found in plants raised with 20% FYM at 50mM salt stress. Plants failed to survive at 500mM NaCl. The outcomes suggest that the interaction of fertilizer and lower salinity doses prove to be instrumental for the growth of alfalfa.

Keywords Salt Stress, Photosynthetic Pigments, Growth, Farmyard Manure (FYM), Dry Biomass

1. Introduction

Alfalfa or lucerne (*Medicago sativa* L.) is a perennial herb of Fabaceae, cultivated as primary fodder in many regions across the world [1]. Because of its extensive adaptability, high yield, superior fodder quality, and resilience to repeated cuts, alfalfa forms an essential forage source for dairy industries all over the world. It is frequently used to make pasture, hay, silage and dehydrated products [2], [3]. Globally, alfalfa is cultivated approximately on 30 million hectares of land [4]. Soil salinity is one of the most significant agricultural challenges for plant cultivation in India. Salinity negatively impacts 77 million out of 1.5 billion hectares of the world's arable land, rendering it unfit for crop production [5]. Plants are affected by salinity in a variety of ways, including osmotic damage, specific ion toxicity, and/or nutritional problems [6]. Na^+, Ca^{2+} and Mg^{2+} are the most frequent cations linked with salinity, whereas Cl^-, SO_4^{2-} and HCO_3^- are the most prevalent anions. However, Na^+ and Cl^- are the most significant because Na^+ in particular, degrades the physical structure of the soil and both Na^+ and Cl^- are harmful to plants [7]. When plants are exposed to excessive salt concentrations, all major physiological processes relating to plant growth and development *viz.* germination, root growth, and crop yields are altered [8]. According to

Carvajal *et al*. [9], the salt imposes the stress on plant through following ways: (i) reducing the osmotic potential of the soil solution, thereby limiting the accessible water to plants (ii) deteriorating the soil structure, thereby lowering the water permeability and soil aeration (iii) increasing the concentration of certain ions that inhibit plant metabolism. Salt stress reduces plant growth, number of leaves, leaf area and plant dry biomass, hence decreasing the overall yield [10]. Salt stress generates a nutritional imbalance in plants and the better growth can be achieved by using fertilizers. Organic fertilizers are very useful for improving plant performance [11]. They are increasingly being employed in arable and natural lands due to their advantages both for plant growth and improved soil structure. Organic fertilizers endowed with organic acids not only increase the crop production but also improve the physical, chemical and biological aspects of the soil. Because of quasi-hormonal components, these acids are also effective in increasing plant output and improving plant quality. Application of organic fertilizers has been proved to minimize the environmental stress for the better plant performance [12].

We have made a contrast by employing varying levels of farmyard manure (FYM) and salt concentrations of NaCl to address the following questions:

(I) How does FYM impact the growth performance of alfalfa (*M. sativa*) under salt stress?
(II) What are the effective FYM doses mitigating/minimizing the impact of salt stress on the growth performance of alfalfa?

To seek out the answers of our questions, the plant of *M. sativa* was tested for the growth parameters of shoot height, root depth, shoot/root dry biomass, leaf area, and photosynthetic pigments i.e. chlorophyll *a*, chlorophyll *b* and carotenoids.

Our key hypothesis was that deleterious impact of salinity on the overall growth of alfalfa can be mitigated and growth can be improved by organic manure.

2. Materials and Methods

2.1. Preparation of Soil and the Study of Soil Properties

Plants were raised on the sandy-loam soil under the pot culture experiment. Soil used in the experiment was dug out in November 2020 after removing the litter and upper 10cm thick layer from the conserved forest land of Government Science College, Gwalior, India. It was thoroughly mixed, crumbs were broken and pebbles were removed by sieving a 5mM sieve. Soil mechanical composition and chemical properties were studied prior to the setting of the experiment and five samples were randomly taken for determining the soil properties. Soil mechanical composition was determined by hydrometer method as described by Bouyoycos [13]. Soil pH was determined in a soil water suspension of 1:5 ratios by a pH meter duly calibrated with standard buffer solution. Electro-conductivity meter was used to measure the EC after calibration with standard KCl solution and the suspension of soil water in the ratio 1:2 was used for this purpose. Available N was estimated by using alkaline $KMnO_4$ [14], whereas the soil phosphorus was estimated as per the method of Olsen [15] and soil potassium was estimated through Ammonium acetate method [16]. Well decomposed farmyard manure was obtained from a local agriculture cum dairy farm and the same was tested for N, P and K by similar methods.

2.2. Experimental Design, Preparation of Pots and Seed Sowing

Pot culture experiment was based on a factorial design. 5 sets of soils, amended and thoroughly mixed with 5, 10, 15, 20 and 25% farmyard manure, were prepared. One set of control was also kept without any fertilizer amendment. Black polythene bags of 16 × 12 inches' size were taken, each filled with 3.5 kg soil amended with respective doses of FYM. Three replicates were taken for each dose of salinity. The seeds of *Medicago sativa* marketed as "Ek Sali" cultivar by a Gujarat-based firm (Narayani seeds of Shah Bhupendra Kumar Ramniklal, Ahmedabad, India) were procured from the company outlet of old Delhi.

Pots were prepared and seeds were sown in a week time between 21^{st} – 27^{th} December 2020 and the same were kept in bright sunshine in the open lawn of the botanical garden of Government Model Science College, Gwalior, India (26°11′38″N and 78°10′23″E). Five seeds were sown in each pot and three healthy plants were finally retained in each pot after the seedling establishment. Each set of plants with a specific percentage of organic fertilizer were put under seven NaCl treatments *viz*. 0, 10, 20, 50, 100, 200 and 500mM. The Plants were treated with salt dose 30 DAS (Days after sowing) when they had gained enough strength to withstand salt stress. To minimise osmotic shock, the initial salt amount of 5mM was applied and then progressively raised over a week time for the respective doses. Plants were irrigated at the interval of three days with respective salt doses for a period of thirty days and distilled water was used for the remaining days between the consecutive salt doses to remove the additive effect of the accumulated salt. 100ml of salt solution of each treatment was used for irrigation, whereas the control was watered with distilled water only.

2.3. Growth Parameters

The plants were harvested after sixty days of seed sowing and before bud bursting. Before harvesting the plants, the leaf area (LA) was measured by paper weight method. The plants were randomly uprooted from the pots for each dose of fertilizer and salt. The plants were thoroughly washed under the running tap to remove the soil adhered to the root system. They were wrapped in a newspaper to remove the excess amount of water. The root and shoot portion of plants were fractionated and the root depth (RD), shoot height (SH) were carefully measured with a scale. Fractionated portions were air dried for ten hours and then kept in hot-air oven overnight at 60℃ for the measurement of root dry biomass (RDB) and shoot dry biomass (SDB).

2.4. Photosynthetic Pigments

Chlorophyll *a*, chlorophyll *b*, chlorophyll *a*+*b* and carotenoids were estimated as per the method given by Arnon [17]. 100mg of leaf tissues of fully expanded fresh leaves of the upper canopy were taken and crushed in triplicate using a pestle and mortar with 5ml of 80% acetone. The extract was removed to centrifuge tubes and the mortar residue was rinsed twice with 5ml of 80% acetone. Following that, the tubes were centrifuged for 10 minutes at 5000rpm and the solution was transferred to a volumetric flask. The solution volume was adjusted to 25ml using 80% acetone. The absorbances for chlorophyll *a* and *b* were recorded at 663 and 645nm by using a spectrophotometer (Systronics model 118). The absorbance of carotenoids was recorded at 470nm. The pigment contents (mg/g) were calculated by using the following formulae.

$$\text{Chlorophyll } a\ (C_a) = (12.25 \times A_{663} - 2.79 \times A_{645})\ V/W \times 1000$$

$$\text{Chlorophyll } b\ (C_b) = (21.5 \times A_{645} - 5.10 \times A_{663})\ V/W \times 1000$$

$$\text{Carotenoids (C)} = (1000 \times A_{470} - 1.82 \times C_a - 85.02 \times C_b/198)\ V/W \times 1000$$

A = Absorption of light at wavelengths of 663, 645 and 470nm

W = Fresh weight of sample (g)

2.5. Data Analysis

The experiments were statistically evaluated and the test of analysis of variance (ANOVA) was employed. Multivariate general linear model and LSD test for homogeneity of variance were used to determine the equality of variation among the treatments, followed by LSD test post hoc analysis to determine if specific mean pairings differed significantly. The statistical analysis was carried out with the help of the SPSS software of IBM version 23.

3. Results

3.1. Soil Mechanical Composition and Texture

The data of soil mechanical composition is depicted in Table 1. Data reveals that the soil used in the experiment contains 42.42±2.85% of sand, 18.00±2.46% silt and 39.58±5.23% clay components. Thus, the soil taken in experiment can be considered as sandy loam. Sandy loams are traditionally considered the best soils for crop cultivation in India.

3.2. Chemical Properties of Soil and farmyard Manure (FYM)

Table 2 shows chemical properties of the soil. It reveals that the soil exhibits a good amount (0.48±0.047%) of organic carbon (OC), poor available N (0.00972±0.00%) and P content (0.000626±0.00%). Although soil was found to be moderate (0.013±0.00%) in K. Soil pH was registered as 7.2 and the electro-conductivity (Ec) was found to be 0.32dS/m.

FYM revealed 25.00±4.35% dry weight and 75.00±4.35% moisture (Table 3). It was very rich in N (0.57±0.05%), P (0.27±0.04%) and K (0.58±0.05%).

Table 1. Soil mechanical composition and texture

Sand (%)	Silt (%)	Clay (%)	Texture
42.42±2.85	18.00±2.46	39.58±5.23	Sandy-Loam

Mean±SD

Table 2. Chemical properties of the soil used in the experiment.

OC (%)	N (%)	P (%)	K (%)	pH	EC(dS/m)
0.48±0.047	0.00972±0.00	0.0006262±0.00	0.013±0.00	7.2	0.32

Mean±SD

Table 3. Chemical properties of farmyard manure used in the experiment

N (%)	P (%)	K (%)	Dry Matter (%)	Moisture (%)
0.57±0.05	0.27±0.04	0.58±0.06	25±4.35	75±4.35

Mean±SD

Table 4. Growth parameters of alfalfa (*Medicago sativa* L. cv. Ek Sali) grown in soil amended with farmyard manure

NaCl treatments (mM)	Farmyard Manure (FYM)														
	5%					10%					15%				
	SH (cm)	RD (cm)	SDB (g)	RDB (g)	LA (cm^2)	SH (cm)	RD (cm)	SDB (g)	RDB (g)	LA (cm^2)	SH (cm)	RD (cm)	SDB (g)	RDB (g)	LA (cm^2)
Control	70.67	11.00	4.32	0.33	668.33	70.67	11.00	4.32	0.33	668.33	70.67	11.00	4.32	0.33	668.33
0	76.67	15.33	5.41*	0.40	1116	87.67*	17.67*	5.64*	0.70*	1270.83*	90.67*	19.67	6.53*	1.71*	1682.33
10	79.33	17.33*	5.61*	0.68*	1502*	90.00*	19.33*	6.47*	1.02*	1651.67*	95.00*	22.67*	6.95*	1.93*	1945.5*
20	82.67*	18.67*	5.80*	0.95*	1785.83*	95.00*	20.67*	8.00*	1.39*	2036.33*	101.67*	23.33*	8.6*	2.31*	2046.33*
50	81.67*	17.67*	5.73*	0.79*	2000.17*	97.00*	22.00*	8.21*	1.15*	1883.17*	103.00*	24.00*	8.97*	2.33*	2467.17*
100	72.00	17.33*	4.58	0.68*	1115	77.33	16.00*	5.28	0.97*	1111.67	94.67*	17.67	7.39*	1.28*	1186
200	63.67	15.33	4.31	0.52*	574.5	71.67	13.67	4.77	0.80*	835.33	72.33	15.00	4.77	1.13*	784.5
500	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

NaCl treatments (mM)	Farmyard Manure (FYM)									
	20%					25%				
	SH (cm)	RD (cm)	SDB (g)	RDB (g)	LA (cm^2)	SH (cm)	RD (cm)	SDB (g)	RDB (g)	LA (cm^2)
Control	70.67	11.00	4.32	0.33	668.33	70.67	11.00	4.32	0.33	668.33
0	90.00*	17.67	6.43*	1.62*	1289*	86.33*	16.33*	5.50	1.45*	1289
10	93.67*	20.00*	7.17*	1.75*	2022.5*	88.67*	19.33*	6.30	1.67*	2022.5*
20	96.67*	21.00*	7.97*	1.90*	2230*	94.33*	19.67*	7.05*	1.71*	2230.83*
50	101.67*	22.00*	8.10*	1.99*	1915.5*	93.67*	15.67	7.01*	1.02*	1915.5*
100	94.00*	16.00	6.77*	1.02*	1256.5*	86.33*	15.33	6.37*	0.90*	1256.5
200	70.67	14.00	4.95	0.92*	761.17	67.33	13.67	4.57	0.78*	761.17
500	L	L	L	L	L	L	L	L	L	L

SH: Shoot height; RD: Root Depth; SDB: Shoot dry biomass; LA: Leaf area, L: Lethal, *Significant at $p \leq 0.05$

3.3. Growth Parameters

3.3.1. Shoot Height

Growth parameters of alfalfa raised on soil amended with FYM and treated with different levels of salinity are depicted in Table 4. The data reveals that shoot height (76.67, 79.33 and 82.67cm) was found to be consecutively increased at 0, 10 and 20mM salinity and then started to decrease for 5% of soil amendment. For 10% soil amendment with FYM, the shoot height (87.67, 90.00, 95.00 and 97.00cm) significantly ($p \le 0.05$) increased at 0, 10, 20 and 50mM salinity when compared with the control (70.67cm). The shoot height (90.67, 95.00, 101.67, 103.00 and 94.67cm) further significantly ($p \le 0.05$) increased at 0, 10, 20, 50 and 100mM salinity for 15% FYM amendment. Similar trends of significantly ($p \le 0.05$) increasing shoot heights (90.00, 93.67, 96.67, 101.67 and 94.00cm) were recorded for 20% FYM at 0, 10, 20, 50 and 100mM and for 25% FYM amendment where it was recorded as 86.33, 88.67, 94.33, 93.67 and 86.33cm at 0, 10, 20, 50 and 100mM salinity when compared with control (70.67cm). 500mM salinity level was found to be lethal for the survival of the plant with all FYM additions. Overall peak of the shoot height (103.00cm) was observed at 50mM salinity for 15% FYM amendment.

3.3.2. Root Depth

Root depth was also significantly ($p \le 0.05$) and successively increased with 0, 10, 20 and 50mM salinity for 10, 15, 20% FYM applications, whereas it successively increased with 0, 10 and 20mM with 5 and 25% FYM applications (Table 4). Highest root depths (22.00, 24.00 and 22cm) were observed at 50mM salinity for 10, 15 and 20% FYM amendments, whereas for 5 and 25% soil amendment the maximum root depth (18.67 and 19.67cm) was recorded at 20mM NaCl. The peak of root depth (24.00cm) was recorded at 50mM salinity for 15% FYM amendment.

3.3.3. Shoot Dry Biomass

Shoot dry biomass (SDB) is depicted in Table 4 and Fig 1. It was observed to be significantly ($p \le 0.05$) increased at 0, 10, 20 and 50mM salinity for 5, 10, 15 and 20% FYM amendments. Highest shoot dry weights 8.21, 8.97, 8.10 and 7.01g/plant were recorded at 50mM salinity, respectively, for 10, 15, 20 and 25% soil manure. Lower values of shoot dry biomass (4.31g at 5%, 4.77g at 10%, 4.95g at 20% and 4.57g at 25% FYM) were recorded at 200mM stress. Overall peak (8.97g/plant) of SDB was observed at 50mM NaCl for 15% manure amongst all salinity levels and soil amendments.

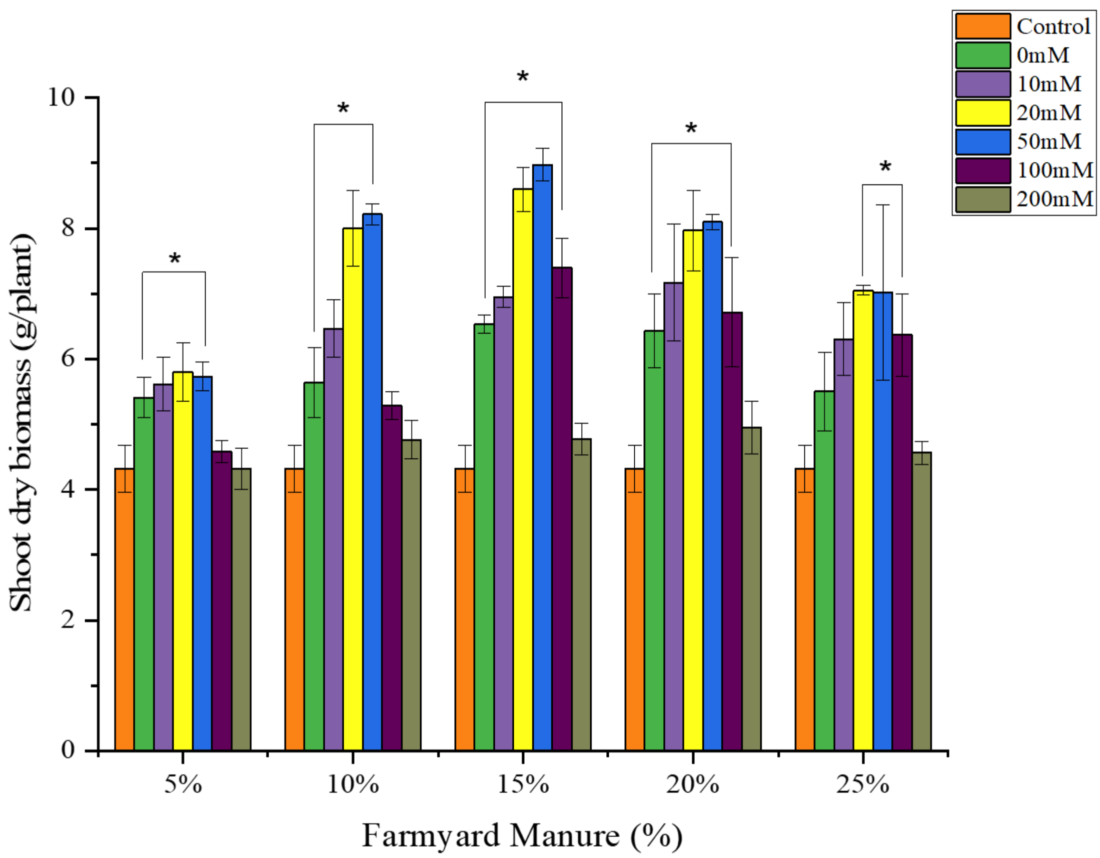

Figure 1. Shoot dry biomass (g/plant) of the plants grown on the soil amended with different levels of FYM and under Salinity treatments. Vertical bars indicate ±SE; *Significant at $p \le 0.05$.

3.3.4. Root Dry Biomass

Significant ($p \leq 0.05$) changes were observed in root dry biomass (0.68, 0.95, 0.79, 0.68, 0.52g/plant) in the plants grown at 10, 20, 50, 100 and 200mM salinity with 5% FYM amendment when compared with the 0.33g/plant of the control. Similar trends were observed for 10% manure, here, the root dry biomass (RDB) was recorded as 0.7, 1.02, 1.39, 1.15, 0.97 and 0.80g/plant respectively at 0, 10, 20, 50, 100 and 200mM salinity. The best results of root dry weight (1.71, 1.93, 2.31, 2.33, 1.28 and 1.13g/plant) were found at 0, 10, 20, 50, 100 and 200mM NaCl in the plants raised on 15% FYM amended soil amongst all FYM amendments. RDB was also significantly ($p \leq 0.05$) increased and recorded as 1.62, 1.75, 1.90, 1.99, 1.02 and 0.92g/plant at 0, 10, 20, 50, 100 and 200mm salinity for 20% FYM amended soil when compared with 0.33g of the control. Similar trends of RDB were observed in the plants grown with 25% FYM (Table 4). Overall study reveals that the best growth results of RDB were obtained in the plants grown with 15% FYM with a peak (2.33g/plant) at 50mM salinity (Table 4 and Fig 2).

3.3.5. Leaf Area

Leaf area of alfalfa is shown in Table 4 and Figure 3. The leaf area (cm^2/plant) significantly ($p \leq 0.01$) increased with most of the applications of FYM in the plants grown under 10, 20 and 50mM NaCl. Leaf areas of 1502.00, 1785.83, 2000.17cm^2/plant were recorded for 5% FYM fertilizer at 10, 20 and 50mM NaCl. It again significantly ($p \leq 0.01$) increased and recorded as 1651.67, 2036.33 and 1883.17cm^2/plant in the plants raised with 10% FYM at 10, 20, 50mM salinity. Leaf area of 1945.50, 2046.33 and 246.17cm^2/plant for 15% and again 1459, 2078.67 and 2016.67cm^2/plant were recorded for 20% FYM application again under 10, 20 and 50mM NaCl with a significant ($p \leq 0.01$) increase over the control (668.33cm^2/plant). The peak 2467.17cm^2/plant) was observed under 50mM NaCl that for 15% FYM among all the FYM amendments.

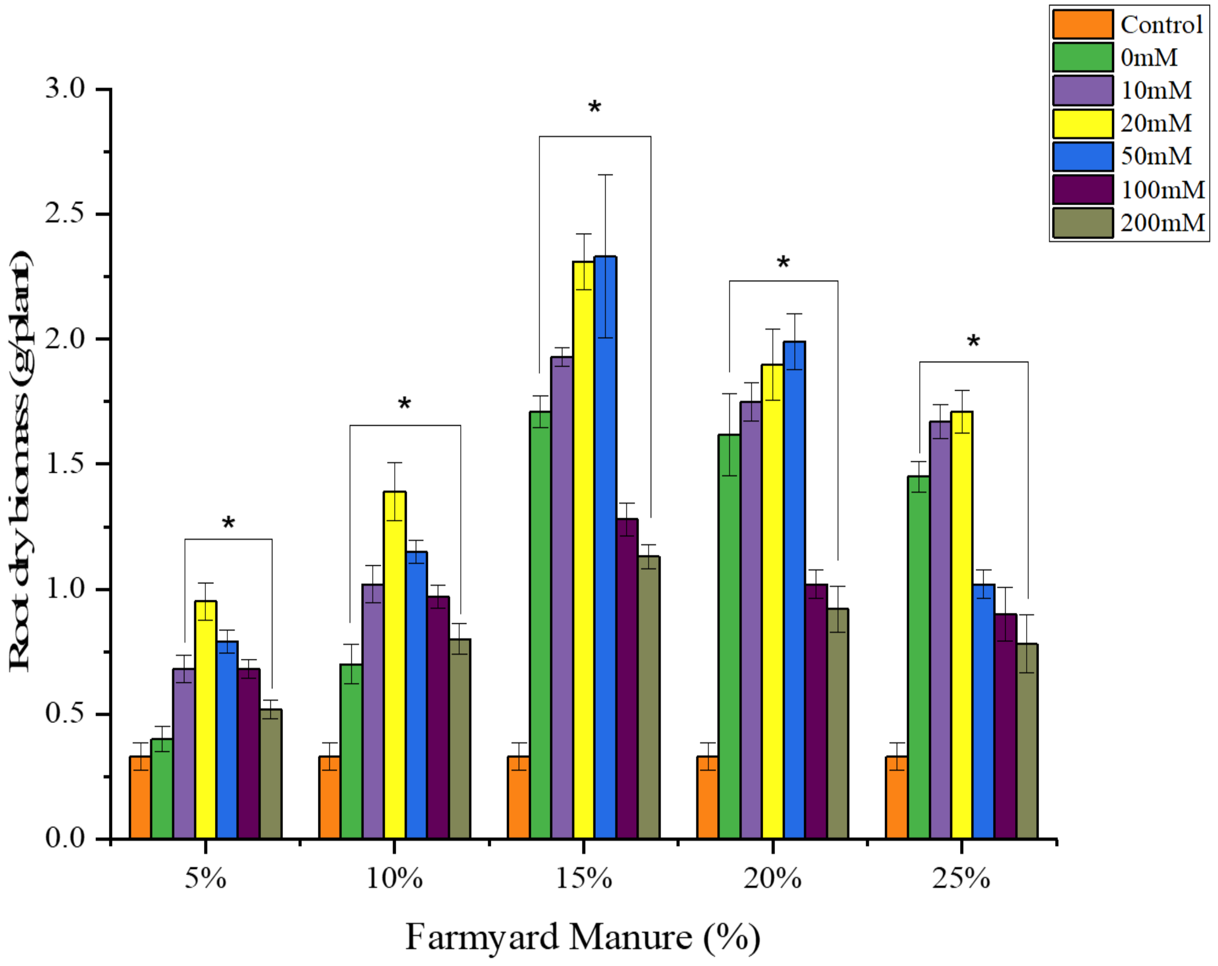

Figure 2. Root dry biomass (g/plant) of the plants grown on the soil amended with different levels of FYM and under Salinity treatments. Vertical bars indicate ±SE; *Significant at $p \leq 0.05$

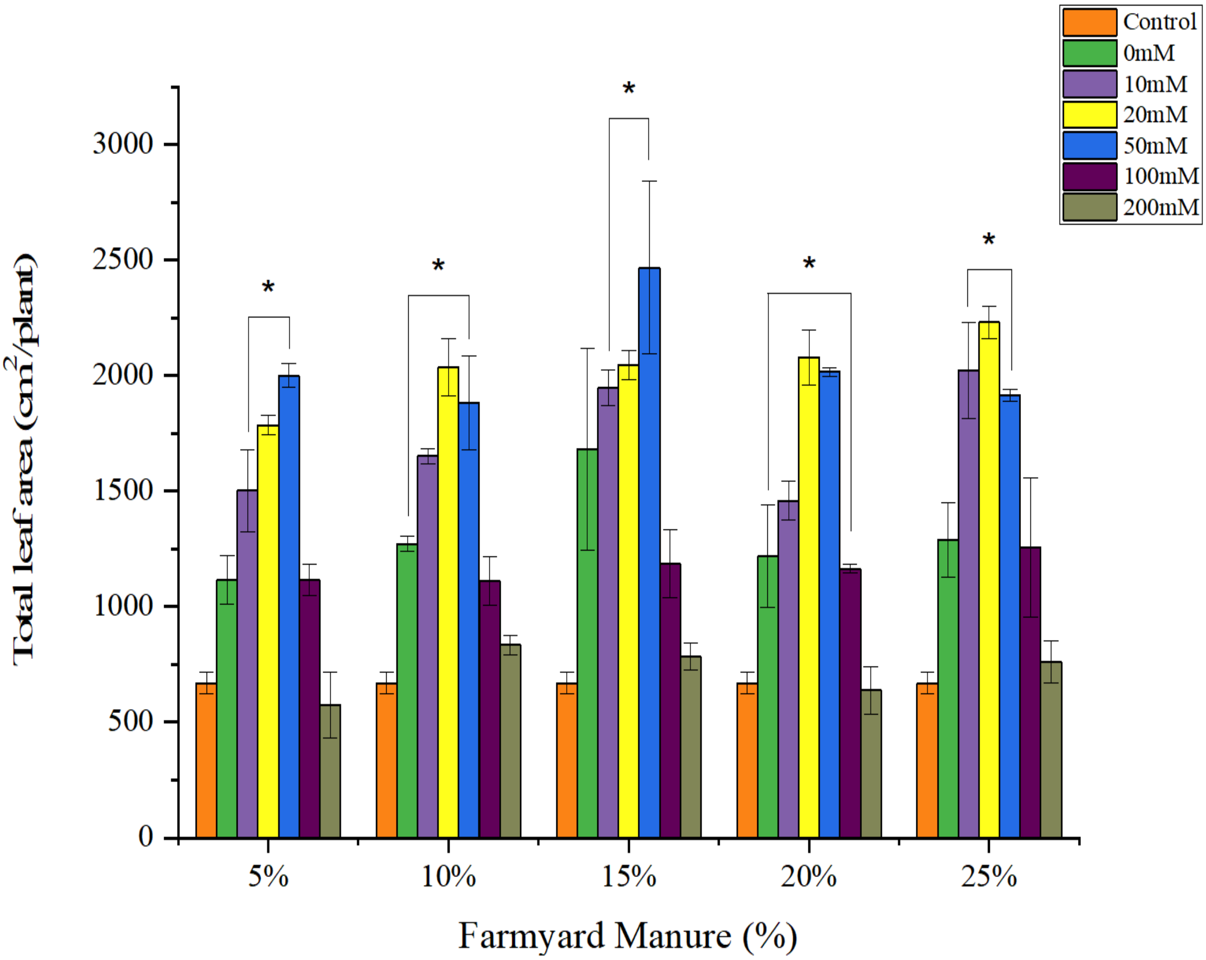

Figure 3. Leaf area (cm^2) of the plants grown on the soil amended with different levels of FYM and under Salinity treatments. Vertical bars indicate ±SE; *Significant at $p \leq 0.05$.

3.4. Photosynthetic Pigments

3.4.1. Chlorophyll *a*

Pigments of alfalfa (*Medicago sativa* L. cv. Ek sali) cultivated on the soil with various formulations of FYM and under different levels of NaCl are given in Table 5. Chlorophyll *a* (0.37mg/g) was found to be significantly ($p \leq 0.05$) increased at 20 and 50mM NaCl treatment in the leaf tissue of the plants grown with 5% FYM when compared with 0.27mg/g of the control. Chlorophyll *a* (0.36, 0.43, 0.49 and 0.39mg/g) at 10, 20, 50 and 100mM NaCl were also significantly ($p \leq 0.05$) increased in the plants raised with 10% compost. Chlorophyll *a* (0.35, 0,44, 0.48, 0.59 and 0.46mg/g) again significantly ($p \leq 0.05$) increased at 0, 10, 20, 50 and 100mM salinity in the leaf of the plant raised after 15% FYM amendments. Chlorophyll *a* (0.38 and 0.46mg/g) was also significantly increased at 20 and 50mM salinity in the leaves of plants grown with 25% FYM when compared with 0.27mg/g of the control. Peak (0.71mg/g) of chlorophyll *a* was observed at 50mM NaCl for 20% FYM. There was a gradual increase in chlorophyll *a* with increasing doses of NaCl up to 50mM after that it started to decline and the 500mM dose was found to be lethal.

3.4.2. Chlorophyll *b*

Chlorophyll *b* (0.52 and 0.54mg/g) was found to be significantly ($p \leq 0.05$) increased in the leaves of plants at 20 and 50mM salinity grown with 5% FYM when compared to 0.38mg/g of the control (Table 5). Higher content (0.55, 0.66, 0,76 and 0.57mg/g) with significant ($p \leq 0.05$) increase was recorded at 10, 20, 50 and 100mM NaCl doses in the leaves of plants raised with 10% manure. Significant ($p \leq 0.05$) increase in chlorophyll *b* (0.56, 0.57, 0.67, 0.77 and 0.64mg/g) at 0, 10, 20, 50 and 100mM salinity was observed in the leaves of plants after 15% FYM amendment against the 0.38mg/g of the control. A very good amount of chlorophyll *b* (0.62, 0.65, 0.74, 0.86, 0.85 and 0.57mg/g) with a significant ($p \leq 0.05$) increase when compared to the 0.38mg/g of the control was recorded at 0, 10, 20, 50, 100 and 200mM salinity in the plants grown with 20% FYM. The overall peak (0.86mg/g) of chlorophyll *b* was recorded at 50mM NaCl after 20% FYM. Chlorophyll *b* was found to be decreased after 50mM salinity in most of the soil amendments.

Table 5. Photosynthetic pigments of alfalfa (*Medicago sativa* L. cv. Ek Sali) grown in soil amended with farmyard manure

NaCl treatments (mM)	Farmyard Manure (FYM)														
	5%					10%					15%				
	A (mg/g)	B (mg/g)	A/B	A+B (mg/g)	C (mg/g)	A (mg/g)	B (mg/g)	A/B	A+B (mg/g)	C (mg/g)	A (mg/g)	B (mg/g)	A/B	A+B (mg/g)	C (mg/g)
Control	0.27	0.38	0.71	0.65	0.22	0.27	0.38	0.71	0.65	0.22	0.27	0.38	0.71	0.65	0.22
0	0.29	0.41	0.70	0.7	0.23	0.32	0.48	0.66	0.80	0.27	0.35*	0.56*	0.62	0.91	0.29*
10	0.30	0.42	0.71	0.72	0.26	0.36*	0.55*	0.65	0.91	0.28*	0.44*	0.57*	0.77	1.01	0.31*
20	0.37*	0.52*	0.71	0.89	0.28*	0.43*	0.66*	0.65	1.09	0.31*	0.48*	0.67*	0.71	1.15	0.34*
50	0.37*	0.54*	0.68	0.91	0.34*	0.49*	0.76*	0.52	1.25	0.34*	0.59*	0.77*	0.76	1.36	0.42*
100	0.36*	0.41	0.87	0.77	0.24	0.39*	0.57*	0.68	0.96	0.25	0.46*	0.64*	0.71	1.10	0.30*
200	0.28	0.35	0.80	0.63	0.19	0.25	0.37	0.67	0.62	0.20	0.28	0.42	0.66	0.70	0.22
500	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L

NaCl treatments (mM)	Farmyard Manure (FYM)									
	20%					25%				
	A (mg/g)	B (mg/g)	A/B	A+B (mg/g)	C (mg/g)	A (mg/g)	B (mg/g)	A/B	A+B (mg/g)	C (mg/g)
Control	0.27	0.38	0.71	0.65	0.22	0.27	0.38	0.71	0.65	0.22
0	0.42*	0.62*	0.67	1.04	0.36*	0.28	0.50	0.56	0.78	0.24
10	0.52*	0.65*	0.80	1.17	0.37*	0.28	0.58*	0.48	0.86	0.28*
20	0.50*	0.74*	0.72	1.28	0.40*	0.38*	0.62*	0.61	1.00	0.31*
50	0.71*	0.86*	0.82	1.57	0.48*	0.46*	0.55	0.83	1.01	0.32*
100	0.58*	0.85*	0.68	1.43	0.38*	0.30	0.39	0.76	0.69	0.26
200	0.30	0.57*	0.52	0.87	0.26	0.28	0.27	1.03	0.55	0.20
500	L	L	L	L	L	L	L	L	L	L

A: Chlorophyll *a*; B: Chlorophyll *b*; C: Carotenoids, L: Lethal, *Significant at $p \leq 0.05$

3.4.3. Chlorophyll *a*+*b*

Chlorophyll *a*+*b* (0.7, 0.72, 0.89 and 0.91mg/g) successively increased at 0, 10, 20 and 50mM NaCl in the leaf tissue of the plants raised on the soil with 5% FYM (Table 5). Similar trends of chlorophyll *a*+*b* were also observed for other FYM formulations. It was found as 0.80, 0.91, 1.09 and 1.25mg/g at 0, 10, 20 and 50mM salinity in the leaf tissue of the plants with 10% manure. A good amount of chlorophyll *a*+*b* (0.91, 1.01, 1.15 and 1.36mg/g) with a successive increase at 0, 10, 20 and 50mM NaCl was recorded in the leaf tissue of plants raised with 15% FYM. It was found to be further increased as 1.04, 1.17, 1.28 and 1.57mg/g at 0, 10, 20 and 50mM salinity for 20% manure. Chlorophyll *a*+*b* (0.78, 0.86, 1.00 and 1.01mg/g) was also successively increased with 0, 10, 20 and 50mM NaCl treatment in the plants grown on 25% FYM. The peak (1.57mg/g) of chlorophyll *a*+*b* was recorded at 50mM salinity for 20% manure (Table 5 and fig. 4). In most of the cases, chlorophyll *a*+*b* decreased after 50mM salinity for different FYM additions.

3.4.4. Chlorophyll *a*/*b*

Chlorophyll *a*/*b* was found to be very close to the control in most of the plants raised with various FYM amendments. However, higher values 0.87 and 0.80 of Chlorophyll *a*/*b* were found at 100 and 200 NaCl treatment for 5% soil amendment. Higher values 0.82 and 0.83 of chlorophyll *a*/*b* were found at 50mM NaCl, respectively, for 20 and 25% FYM addition. Lower values of chlorophyll *a*/*b* were observed for 10% FYM when compared to the control (Table 5). Study reveals that at lower levels (10 and 20mM) of salinity, chlorophyll *a*/*b* was very close to the control or lesser than the control for most of the soil amendments.

3.4.5. Carotenoids

Carotenoids were found to be 0.22mg/g in the leaf tissue of the control. It increased to the levels of 0.23, 0.26, 0.28 and 0.34mg/g at 0, 10, 20 and 50mM NaCl treatments when grown with 5% FYM. However, lower values 0.24 and 0.19mg/g were recorded at 100 and 200mM NaCl doses of the same formulation of FYM. For 10% FYM soil amendment, carotenoids were found in the range of 0.27-0.34mg/g, the highest being 0.34 mg/g with a significant ($p \leq 0.05$) increase at 50mM salinity whereas, the lowest value (0.28mg/g) was recorded at 200mM NaCl concentration for this soil amendment. Significant ($p \leq 0.05$) increase in the carotenoids was observed at 0, 10, 20, 50 and 100mM salinity when compared to the control (0.22 mg/g) and recorded as 0.29, 0.31, 0.34, 0.42 and 0.30 mg/g in the leaf tissue of plants grown with 15% FYM. Similar trends with 0.36, 0.37, 0.40, 0.48 and 0.38mg/g with a significant increase ($p \leq 0.05$) were recorded at 0, 10, 20, 50 and 100mM salinity in the plant raised with 20% manure. Comparatively low amounts (0.24, 0.28, 0.31 and 0.32mg/g) at 0, 10, 20 and 50mM NaCl were recorded in the plants raised with 25% composting. The peak (0.48mg/g) of carotenoids was observed in the plants raised with 20% FYM mixing.

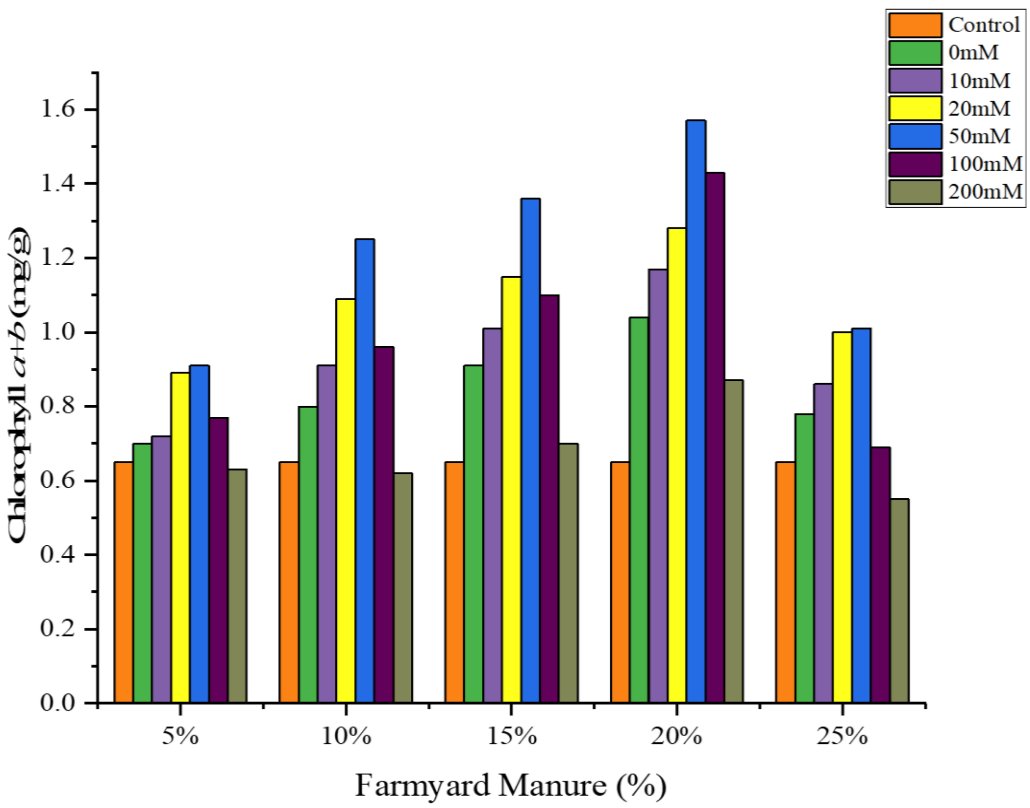

Figure 4. Chlorophyll *a*+*b* (mg/g) in the fully expanded young leaf tissue of upper canopy

4. Discussion

The sandy-loam soil showing pH 7.23 and the Ec $0.462dSm^{-1}$ with 0.568±0.07% organic carbon was suitable for the cultivation of alfalfa. Smith and Doran [18] have described the soil having Ec < $2dSm^{-1}$ as non-saline and good for alfalfa cultivation. They have further prescribed a threshold value of Ec > 0.7 after which microbial respiration, decomposition, nitrification, and denitrification are severely affected, thereby affecting the growth of alfalfa. These scientists have also recommended the range of pH 7 – 7.5 best for crop cultivation. Rogers *et al.* [19] have studied the growth of different cultivars of alfalfa (*Medicago sativa* L.) with increasing levels (2 – $17dSm^{-1}$) of electro-conductivity by the application of NaCl. They have observed 33% of dry weight reduction in the plants grown at $17dSm^{-1}$. As per the requirements of pH (7.3), Ec ($0.462dSm^{-1}$) the sandy-loam soil used in our experiment was fairly suitable for alfalfa cultivation but containing lower amounts of N (0.0107%) and P (0.0011%) the soil fertilization with organic manure having fair amount of N (0.5±0.02%), P (0.27±0.04%) and K (0.55±0.07%) was desirable.

Plant growth is one of the most significant agricultural indicators of salt stress tolerance [20]. Our results revealed that lower doses of NaCl promote the growth of alfalfa raised on the soil amended with different ratios (5, 10, 15, 20 and 25%) of FYM. The growth parameters *viz.* shoot height, shoot dry biomass, root depth, root dry biomass and leaf area increased significantly for most of the FYM amendments with increasing NaCl levels. In a study carried out in *Vicia faba*, Qados [21] found low and medium concentrations of NaCl caused an increase in plant height, fresh and dry weight. Similarly, Chowdhury *et al*. [22] have proposed that soil amendment using farmyard and chicken manure substantially boosted rice cultivar growth, grain and straw yields, K+/Na+ ratio, and nutrient absorption under salt conditions, resulting in improved salt tolerance. In the present study, varied formulations of FYM differentially impacted the growth performance of alfalfa which can be attributed to the improvement of soil aggregation, aeration and its water holding capacity that has provided favorable environmental conditions for its root system. Ng'etich *et al*. [23] found that the response of several growth parameters directly depended upon the application of FYM levels. According to several researchers, the application of farmyard manure improves the physical, chemical, and biological characteristics of soils [24]. FYM additions in soil have been shown to improve soil porosity and aeration [16].

In the present study, the growth parameters revealed a decreasing trend at higher salt doses. The 500mM dose was found to be lethal and the plant succumbed after the very first treatment. This is in agreement with Chowdhury *et al*. [22] who demonstrated in rice cultivars that at 100mM NaCl stress, the negative effect was more apparent. Several workers [25], [26] have explained the suppression of growth under higher salinity. According to them, plant growth is suppressed by a variety of physiological reactions, including changes in ion balance, photosynthetic efficiency, carbon allocation, and consumption. Tester and Davenport [27] reported that plants acquire additional inorganic ions in the leaves under salt stress and store these ions in the vacuoles. These ions can accumulate in the cytoplasm and impair enzymatic functions or they can accumulate in the cell wall causing cell dehydration. In our study, both the root depth and shoot height were inhibited at higher salinity levels, but the shoot height was affected more severely than the root. This is consistent with Esechie *et al*. [28] who obtained similar results in alfalfa. A decline in overall growth at 20 and 25% of FYM in the present study concurs with Mgbeze and Abu [29]; they found that treatment of the soil with 25, 50, and 100% farmyard manure (FYM) significantly reduced the growth and development of African yam bean, whereas 12.5% significantly improved the same.

Leaf area is considered an important aspect of plant growth. Leaf area determines photosynthetic efficiency and surface area, hence, affecting the plant productivity [30]. Leaf area significantly increased with increased (0, 10 and 20mM) salinity for all applications of manure followed by the reduction at higher (100 and 200mM) NaCl levels. However, the findings of our study did not line up with Beinsan *et al*. [31]. They discovered that soil salinity produced a considerable decrease in leaf area. In a separate study in *Vicia faba* by Qados [21], no significant change in the number of leaves and leaf area was registered with low concentrations of NaCl, whereas there was a significant decrease in leaf area when plants were subjected to higher NaCl stress.

Pigments are crucial aspects of plant growth because of their key role in photosynthesis. Both chlorophyll *a* and *b* and chlorophyll *a*+*b* were found increasing with increased levels of salinity for all FYM amendments. Chlorophyll *a*+*b* increased with salt stress as was also observed by in alfalfa [32] and in maize [33]. According to Bojović and Marković [34], increasing chlorophyll with increasing levels of manure might be owing to the plant's excellent absorption and assimilation of nitrogen from manure, which acts as a component of chlorophyll and has been shown to be directly related to the photosynthetic capacity and output of any specific plant.

However, at higher salt concentrations, a declining trend was observed for all pigments in our study. The increasing enzymatic activity responsible for chlorophyll degradation (chlorophyllase), the destruction of the chloroplast structure, and the instability of pigment-protein complexes have been proposed as an explanation for the decreasing chlorophyll at higher salinity [35], [36].

Carotenoids are antioxidants and plant secondary metabolites that protect chlorophyll and plant oxidative stress [37], [38]. Carotenoids are known to act as light energy collectors for photosynthesis as well as quenchers of triplet chlorophyll and O_2. Furthermore, they discharge surplus energy through the xanthophyll cycle and can operate as strong chloroplast membrane stabilizers, lowering membrane fluidity and susceptibility to lipid peroxidation [39]. The rise in carotenoid levels revealed that their protection was one of the most significant mechanisms under salt stress after FYM application.

Chlorophyll *a*/*b* is an important tool to study the performance and behavior of the plant especially under stress conditions. Chlorophyll *a*/*b* showed greater variations for different salinity treatments and FYM amendments without revealing a pattern. However, the ratio was generally less than unity, implying the less molar concentration of chlorophyll *a* than chlorophyll *b*. According to Humphrey [40], chlorophyll *a* is three times more than chlorophyll *b* in plants, but this observation does not match with our study. Kasajima [41], found chlorophyll a is more easily degraded by oxidative stress than chlorophyll *b* in rice and the chlorophyll *a*/*b* ratio severely decreases in such conditions. Thus, it can be concluded that Chlorophyll *b* is more adaptive to stress conditions.

5. Conclusions

Study reveals that the plants grown on the soil amended with most of the FYM amendments performed better at the lower salinity levels (0, 10, 20 and 50mM). The peaks of shoot height (SH), root depth (RD), shoot dry biomass (SDB), root dry biomass (RDB) and the leaf area (LA) were observed either at 20 or 50mM salinity for most of the FYM formulations. The best growth performance was observed at 50mM salinity when the plants were grown with 15% FYM. The peaks of chlorophyll *a*, *b* and carotenoids were also recorded at 50mM salinity for all FYM additions. Thus, it can be concluded that the soil amendment with 15-20% FYM and permissible limits (20 – 50mM) of NaCl be utilized to improve plant nutrition, growth and forage yield in “Ek Sali” cultivar of alfalfa.

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Author Contribution

Both authors have equally contributed to design, acquisition, analysis and interpretation of data and drafting the article and revising it critically.

Conflict of Interest

Conflict of interest is none.

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REFERENCES

[1] Chegni, H., Oshaghi, M., Gharegozlou, B., and Kaviany, M., (2015). The use of alfalfa root instead of streptolysin O in ASO diagnostic test. *International Journal of Herbal Medicine* 2 (6 Part A):10–12.

[2] Goplen, B. P., Baenziger, H., Bailey, L. D., Gross, A. T. H., Hanna, M. R., Michaud, R., Richards, K. W. and Waddington, J., (1982). Growing and managing alfalfa in Canada. Agric. Canada Publ. 1705/E (revised). Agriculture Canada, Ottawa, ON. 50 p

[3] Coburn, F. D., (1907). The book of alfalfa; history, cultivation and merits. Its uses as a forage and fertilizer.

[4] Yuegao, H., Cash, D., Kechang, L., Suqin, W., Ping, Z., & Rong, G., (2009). Global status and development trends of alfalfa. *Alfalfa management guide for Ningxia*, 1-14.

[5] Miransari, M., (2017). Arbuscular-mycorrhizal fungi and soil salinity. In *Mycorrhizal mediation of soil* (pp. 263-277). Elsevier.

[6] Läuchli, A., & Epstein, E., (1990). Plant responses to saline and sodic conditions. *Agricultural salinity assessment and management*, *71*, 113-137.

[7] Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J., (2000). Plant cellular and molecular responses to high salinity. *Annual review of plant biology*, *51*(1), 463-499.

[8] Parida, A. K., & Das, A. B., (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and environmental safety*, *60*(3), 324-349.

[9] Carvajal, M., Martínez, V., & Alcaraz, C. F., (1999). Physiological function of water channels as affected by salinity in roots of paprika pepper. *Physiologia Plantarum*, *105*(1), 95-101.

[10] Singh, K. N., Chatrath, R., (2001). Salinity tolerance. In: Reynolds M. P, Ortiz-Monasterio JI, McNab A (eds) Application of physiology in wheat breeding. CIMMYT, Mexico, DF, pp 101–110.

[11] Ebrahimi, M., & Miri, E., (2016). Effect of humic acid on seed germination and seedling growth of *Borago officinalis* and *Cichorium intybus*. *Ecopersia*, *4*(1), 1239-1249.

[12] Rezaei, M. A., Khavari-Nejad, R., & Fahimi, H., (2004). Physiological response of cotton (*Gossypium hirsutum* L.) plants to soil salinity. *In Agronomy and Horticulture*.

[13] Bouyoucos, G. J. (1936). Directions for making mechanical analyses of soils by the hydrometer method. *Soil Science*, *42*(3), 225-230.

[14] Subbiah, B. V., & Asija, G. L., (1956). A rapid method for the estimation of nitrogen in soil. *Current Science*, *26*, 259-260.

[15] Olsen, S. R., (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate* (No. 939). US Department of Agriculture.

[16] Haynes, R. J., Naidu, R., (1998). Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutr Cycl Agroecosys 51:123–137.

[17] Arnon, D. I., (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*, *24*(1), 1.

[18] Smith, J. L., & Doran, J. W., (1996). Methods and use of ph and electrical conductivity for soil quality analysis. *Methods for assessing soil quality.* Soil Science Society of America Special Publication 49:169-185.

[19] Rogers, M. E., Grieve, C. M., & Shannon, M. C., (1998). The response of lucerne (*Medicago sativa* L.) to sodium sulphate and chloride salinity. *Plant and Soil*, *202*(2), 271-280.

[20] Voigt, E. L., Almeida, T. D., Chagas, R. M., Ponte, L. F. A., Viégas, R. A., & Silveira, J. A. G., (2009). Source–sink regulation of cotyledonary reserve mobilization during cashew (*Anacardium occidentale*) seedling establishment under NaCl salinity. *Journal of Plant Physiology*, *166*(1), 80-89.

[21] Qados, A. M. A., (2011). Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*, *10*(1), 7-15.

[22] Chowdhury, S., Bhusan, D., Hashem, M. A., &Hoque, M. A., (2019). Organic amendments for mitigating soil salinity in rice. *Research in Agriculture Livestock and Fisheries*, *6*(1), 11-17.

[23] Ng'etich, O. K., Aguyoh, J. N., & Ogweno, J. O., (2012). Effects of composted farmyard manure on growth and yield of spider plant (*Cleome gynandra*). *Int J Sci Nat*, *3*(3), 514-520.

[24] Narjary, B., Aggarwal P., (2014). Evaluation of soil physical quality under amendments and hydrogel applications in a soybean-wheat cropping system. Commu Soil Sci Plant Anal 45:1167–1180.

[25] Munns, R., & Tester, M., (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, *59*, 651-681.

[26] Abogadallah, G. M., (2010). Insights into the significance of antioxidative defense under salt stress. *Plant signaling & behavior*, *5*(4), 369-374.

[27] Tester, M., & Davenport, R., (2003). Na+ tolerance and Na+ transport in higher plants. *Annals of botany*, *91*(5), 503-527.

[28] Esechie, H. A., Al-Barhi, B., Al-Gheity, S., & Al-Khanjari, S., (2002). Root and shoot growth in salinity-stressed alfalfa in response to nitrogen source. *Journal of plant nutrition*, *25*(11), 2559-2569.

[29] Mgbeze, G. C., & Abu, Y., (2010). The effects of NPK and farm yard manure on the growth and development of the African yam bean (*Sphenostylis stenocarpa* Hochst ex. a rich). *African Journal of Biotechnology*, *9*(37), 6085-6090.

[30] Yoshida, S., (1981). Fundamentals of Rice Crop Science, International Rice Research Institute, Los Baños. *Laguna*.

[31] Beinsan, C., Camen, D., Sumalan, R., & Babau, M., (2009). Study concerning salt stress effect on leaf area dynamics and chlorophyll content in four bean local land-races from Banat area. In *44th Croatian & 4th International Symposium on Agriculture, Opatija, Bosnia and Herzegovina*.

[32] Al-Khanjari, S., Al-Kathiri, A., & Esechie, H. A., (2002). Variation in chlorophyll meter readings, nodulation and dry matter yields of alfalfa (*Medicago sativa* L.) cultivars differing in salt tolerance. *crop research-hisar*, *24*(2), 350-356.

[33] Awad, N., Turky, A., Abdelhamid, M., & Attia, M., (2012). Ameliorate of environmental salt stress on the growth of *Zea mays* L. plants by exopolysaccharides producing bacteria. *J Appl Sci Res*, *8*(4), 2033-2044.

[34] Bojović, B., & Marković, A., (2009). Correlation between nitrogen and chlorophyll content in wheat (*Triticum aestivum* L.). *Kragujevac Journal of Science*, *31*(5827), 69-74.

[35] Jamil, M., Lee, K. J., Kim, J. M., Kim, H. S., & Rha, E. S., (2007). Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. *Scientia Agricola*, *64*(2), 111-118.

[36] Singh, A. K., & Dubey, R. S., (1995). Changes in chlorophyll a and b contents and activities of photosystems 1 and 2 in rice seedlings induced by NaCl. *Photosynthetica (Czech Republic)*.

[37] Delshadi, S., Ebrahimi, M., & Shirmohammadi, E., (2017). Influence of plant-growth-promoting bacteria on germination, growth and nutrients' uptake of *Onobrychis sativa* L. under drought stress. *Journal of Plant Interactions*, *12*(1), 200-208.

[38] Ramadan, M. F., (2007). Nutritional value, functional properties and nutraceutical applications of black cumin (*Nigella sativa* L.): an overview. *International journal of food science & technology*, *42*(10), 1208-1218.

[39] Demmig-Adams, B., & Adams III, W. W., (2002). Antioxidants in photosynthesis and human nutrition. *Science*, *298*(5601), 2149-2153.

[40] Humphrey, A. M., (2004). Chlorophyll as a color and functional ingredient. *Journal of food science*, *69*(5), C422-C425.

[41] Kasajima, I., (2017). Difference in oxidative stress tolerance between rice cultivars estimated with chlorophyll fluorescence analysis. *BMC Research Notes*, *10*(1), 1-12.