

INFLUENCE OF SALINITY WITH DIFFERENT Cl:SO₄²⁻ RATIOS ON WHEAT (*Triticum aestivum* L.) GROWTH

Abstract

A significant threat to sustainable agriculture is the continuous increase in salt-affected areas. Salt stress causes land degradation, ecological imbalance, environmental pollution and reduces crop production. It changes different physical and chemical processes in the plant body, such as seed germination and different nutrients and water uptake. Wheat is the main cereal crop and primary diet for one-third of humans. It can tolerate the salinity effect to some extent, but its production reduces significantly at a higher level of salinity. Salt-affected soils have more salts that are easily solubilized in water and exchangeable sodium than the average soil. These salts are ionized and produce different cations (Mg²⁺, Ca²⁺, and Na⁺) and anions (Cl⁻, CO₃²⁻, SO₄²⁻, and HCO₃⁻). Sulfate salinity is also toxic for plant growth as chloride salinity. This research aims to assess the effect of salinity with various Cl:SO₄²⁻ ratios on wheat growth. A pot trial was conducted on wheat by creating salinity with different levels of chloride and sulphate (4:1, 3:1, 2:1, 1:1, 1:2, 1:3, and 1:4). The experimental design was a complete randomized design (CRD). Different physicochemical parameters of the soil before and after crop harvesting was determined. Different growth parameters will also be determined. Data were analyzed using statistical software. Based on these results, it was determined that both types of salinity reduced the wheat growth significantly, but chloride type salinity has a more injurious effect than sulphate salinity. It was concluded that chloride ion has more toxic effects than sulphate ion for wheat growth and development.

Keywords: Wheat, salinity, chloride, sulfate, abiotic stress

1. Introduction

These days, water shortages and environmental degradation are significant issues. Salinization and urbanization pose a danger to the long-term viability of agriculture (Gould *et al.*, 2021). Salinity may lead to a decrease in agricultural productivity, an imbalance in the ecosystem, and changes in physiochemical soil characteristics (Machado and Serralheiro,

2017). The annual increase in salinized area is estimated at 10% each year by factors like high surface evaporation, low precipitation, breakdown of the rocks, poor cultural activities, and irrigation with water having more salts (Kumar and Sharma, 2020). A saline-sodic soil has electrical conductivity (EC_e) of saturated extract more than 4 dS m^{-1} and 15 exchangeable sodium percentage (ESP) at 25°C . The production of most of the crops is reduced at this EC_e and ESP (Munns, 2005). Consequently, the Plant undergoes morphological, biochemical, and physiological modifications (Wang *et al.*, 2021). Seed germination is inhibited by soluble salts, which restrict seeds from taking water from the soil due to osmotic action (Tavares *et al.*, 2021). Salinity and sodicity result in a decrease in crop production, ecological imbalance, and change in soil physiochemical properties, which ultimately causes low income, soil erosion, and environmental pollution (Gould *et al.*, 2021).

More than half of the world's irrigated land and over a quarter of the world's cultivated land are suffering from salt stress. About 14 % of Pakistan's irrigated land has been salinized, and salinity is responsible for 64 % of the country's output losses. There are now only 23 million hectares (23 million acres) of land used for farming (Sanower and Sultan, 2019; Syed *et al.*, 2021). Almost 3 percent of irrigated land in Punjab, 18 percent in Sindh, and 2 percent in the KPK suffer from severe surface salinity (Syed *et al.*, 2021). Pakistan's geographic size is estimated to be 79.61 million square miles, however only roughly 4.5 million square miles is salt affected (Tavares *et al.*, 2021).

Chlorine and sulphur both are essential nutrients for plants but due to their higher concentration and mobility in irrigated water caused toxicity as a micronutrient it is crucial to regulate the osmotic pressure, photosynthesis, and enzyme action (Reich *et al.*, 2015; Geilfus, 2018). Sulphur is essential to produce several biomolecules, including methionine, cysteine, chlorophyll, proteins, and the oil content of seeds, among others. Understanding how sulphur interacts with other macro components is critical to maximise crop yields (Aziz *et al.*, 2019; Geilfus, 2018). There is an adverse association between salinity and germination rate of seeds. When there is an increase in salinity and sodicity, the germination rate can be reduced and delayed (Yan *et al.*, 2019). Salinity causes to reduce the osmotic potential, protein and nucleic acid metabolism, uses of stored food by seeds (Helal and Mengel, 1979, Machado and Serralheiro, 2017, Yan *et al.*, 2019). It alters the enzymatic activates during germination process (Tavares *et al.*, 2021). It not only delays the germination but also affects the vegetative and propagative growth of the Plant by creating osmotic and oxidative stresses as well as deficiency and toxicity in them (Benedetti *et al.*, 2020).

Salt-affected soils have high concentration of cations (Ca^{2+} , Mg^{2+} and Na^+) and anions (Cl^- , CO_3^{2-} , SO_4^{2-} , and HCO_3^-) (Geilfus, 2018). Chlorine and sulphur toxicity is widespread in salt-affected soil because it is the most common anion in salt-affected soil (Reich *et al.*, 2015; Tavares *et al.*, 2021). High concentration of Cl^- ion in chloroplast or cytosol tissue limits the growth of many glycophytic plants (Geilfus, 2018). It also retards the growth of root and tuber in pea, potato and avocado (Duan *et al.*, 2013). Chlorine (Cl^-) has an antagonistic impact on numerous nutrients, such as nitrate absorption by *Brassica rapa*, which is reduced due to increased soil Cl^- concentrations (Reich *et al.*, 2017). When combined with phosphate and sulphate, it has an antagonistic impact on plant cells, decreasing their absorption and transport (Massa *et al.*, 2009). NaCl is a dominated salt in most of the salt-affected soils (Soriano *et al.*, 2014) but many areas of the world have sodium sulphate (Na_2SO_4) as dominated salt. Sulphate salinity may occur in marine soil, in volcanic soils, in agriculture soils which are irrigated with saline water or cause by human inputs such as industrial waste or due to atmospheric sulphur gases deposition (Chang *et al.*, 1983). It is more toxic than chloride salinity in many species of the plant (Renault *et al.*, 2001). The effects of salts on the growth and production of the plant are investigated using single salt (NaCl) (Song *et al.*, 2005; Hanif *et al.*, 2017) but in saline-sodic soil, different types of cations and anions are present (Qadir *et al.*, 2000). There are some studies, those investigate the response of different plants using mixture of all possible salts that may occur in salt affected soils (Sosa *et al.*, 2005; Yang *et al.*, 2007; Zhang and Mu, 2009; Li *et al.*, 2010; Guo *et al.*, 2011; Lin *et al.*, 2014; Zhao *et al.*, 2014; Zhang *et al.*, 2015). Reported data shows that Cl^- inhibits germination more strongly than SO_4^{2-} in three species from Mediterranean salt marshes (Vicente *et al.*, 2009). On the other hand, the germination of an inborn species of salt-affected area of Argentina are more strongly suppressed by SO_4^{2-} than by Cl^- (Sosa *et al.*, 2005). There is lot of contradiction that which ion (Cl^- or SO_4^{2-}) is deadlier for plant development under saline conditions. Therefore, this study was conducted to examine the wheat tolerance against Cl^- and SO_4^{2-} type salinity.

2. Methodology

2.1. Growth condition and Experimental Layout

A pot trial was conducted in the wire house of Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad (UAF) was conducted during the year 2018-19. The test crop was wheat (Galaxy 2013).

Soil for the experiment taken from farm area of ISES. For the soil preparation, it was first air-dried in a shade to remove the water contents. Then it was grounded and to remove the material other than soil (stones, plant debris) it was sieved through 2 mm sieve. This prepared soil was brought in the lab for different analyses like saturation percentage, texture, carbonate, bicarbonate, chloride, sulphate, calcium, magnesium, pH, EC and SAR before the start of the experiment (Richards, 1954). According to the treatments, salinity ($EC = 10 \text{ dS m}^{-1}$) was created artificially using Na_2SO_4 and NaCl salts.

2.2. Development of Salinity and Crop Growth

Soil was weighed, and a quadratic equation was used to calculate the amount of salts per the above treatments. The amount of water for dissolving the salts was equal to SP of the soil. Then soil was saturated by applying different salt solutions according to their treatments. After one month, incubated soil was dried and filled in pots (4.5 kg capacity).

At field capacity, 5 seeds of wheat were sown in each pot (24 pots). Recommended amounts of N, P and K for wheat were applied using urea, DAP and SOP as their sources. After germination, 3 plants were kept in each pot. At maturity, crop growth parameters were recorded. From each pot, soil samples were obtained with stainless steel tube and were analysed for chemical properties. The plant material was analyzed for N, P, K, Cl^- and SO_4^{2-} .

2.3. Soil Analysis

2.3.1. Saturation Percentage

For saturation percentage determination, soil saturated paste was prepared first. Saturated paste was prepared by taking 250 to 300 g prepared soil in a plastic beaker. Add DI water slowly in it and mixed continuous with spatula. When the paste was prepared, it was taken in pre-weight petri plate. Again, the weight of the petri plate plus soil paste was measured with electronic balance. Then this plate was placed in an oven at a temperature of 105°C for an hour. After an hour, the plate was taken out from an oven and weighed again. The saturation percentage of the soil was determined with the help of following equation after subtracting the weight of petri plate from both weights.

$$\text{Saturation percentag} = \frac{\text{Weight of the soil paste} - \text{weight of the oven dry soil}}{\text{weight of the oven dry soil}} \times 100$$

2.3.2. Soil Texture

For Texture determination, Hydrometer method (Bouyoucos, 1962) was used. Following this method, a solution was prepared by adding 40 g sodium hexametaphosphate [(NaPO₃)₁₃] with 10 g of sodium carbonate (Na₂CO₃) in a Volumatic flask of one liter (L) and made the volume of one L by adding DI water. Then, prepared soil (40 g) was taken in a beaker and add 60 ml of above solution. The beaker was covered by a lid and keep for an overnight. Then, the material of the beaker was transferred in to a soil-stirring cup and fill this cup up to 1/3 by water. Suspension was mixed with the help of stir. After stirring, the suspension was not disturbed for 2 min. Then transferred the suspension into the hydrometer jar (1L) and made the volume with water. Mixed the suspension with paddle and insert the hydrometer after mixing. Reading was noticed after 40 sec. was CHR1. Another reading was noticed after 4 hours abbreviated as CHR2. The following formula was used to determine the relative percentage of sand, silt and clay (Cowley and Moodie, 1959).

$$\text{Silt + Clay (\%)} = [(\text{CHR1} * 100) / \text{soil weight (g)}]$$

$$\text{Clay (\%)} = [(\text{CHR2} * 100) / \text{soil weight (g)}]$$

$$\text{Silt (\%)} = \% (\text{clay + silt}) - \% (\text{clay})$$

$$\text{Sand (\%)} = 100 - [\% (\text{clay} + \text{silt})]$$

2.3.3. Carbonate and Bicarbonate

For carbonate (CO₃) and bicarbonate (HCO₃) determination, 10 ml of soil saturated paste extract was used. This extract was taken in a beaker and 2 to 3 drops of an indicator (1% phenolphthalein) was added for CO₃ determination. If the colour of this extract changed from colourless to pink then it was titrated with 0.01 N sulfuric acid (H₂SO₄) till the colourless end point. After that, 2 to 3 drops of another indicator (0.1% methyl orange) was added in a same extract sample to determine HCO₃. Again, titrate it with 0.01 N H₂SO₄ till the colour of the sample turned orange or pinkish yellow. The following equations were used to determine their concentration.

CO_3^{2-} (meq/ l) = 2 (volume of H₂SO₄ used for CO₃ titration) *(normality of H₂SO₄) * 1000/ volume of extract used.

$\text{HCO}_3^- (\text{meq/l}) = [(\text{volume of H}_2\text{SO}_4 \text{ used for HCO}_3 \text{ titration}) - 2 (\text{volume of H}_2\text{SO}_4 \text{ used for CO}_3 \text{ titration})] * (\text{normality of H}_2\text{SO}_4) * 1000 / \text{volume of extract used.}$

2.3.4. Chloride analysis

For chloride (Cl) determination, the same extract sample which was used. 2 to 3 drop of an Indicator (5% potassium chromate solution) was added in the sample and titrated against the 0.01 N silver nitrate (AgNO_3). The end point was the reddish-brown or brick-red colour.

The volume of AgNO_3 used for titration was measured. Following equation was used to determine the Cl concentration.

$\text{Cl}^- (\text{meq/l}) = (\text{Volume of AgNO}_3 \text{ used for titration}) * (\text{normality of AgNO}_3) * 1000 / \text{volume of extract used.}$

2.3.5. Sulphate

For sulphate (SO_4) determination, turbidimetric method was used in which 10 ml of soil saturated paste extract was taken into 250 ml Pyrex breaker and diluted it by adding 40 ml of DI water. Then added 1 to 2 ml of 1: 1 HCl solution in it. After that, 2 to 3 drops of 0.1% methyl orange indicator was added. Then this Pyrex break was put on the hotplate to boil the sample. After boiling, 10 ml of 1 N Barium Chloride Solution ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) was added. After boiling, covered and cooled the solution. Ash less filter papers were used for the filtration of this solution. The precipitate of barium sulphate was collected on the filter paper by giving washing with hot DI water many times. Then porcelain crucibles were weighed (wt_1) and filter paper with precipitate were placed on these crucibles. Then these crucibles were put into an oven at 105°C for an hour. After that, transferred them into a muffle furnace at 550°C for 2 to 3 hours to make dry ash. Then after 2 to 3 hours, these crucibles were put out from the muffle furnace and cooled down. After cooling, again weighed (wt_2) them. The following equation was used to determine the SO_4 concentration.

$\text{SO}_4^{2-} (\text{meq/l}) = [(\text{wt}_2 - \text{wt}_1 / \text{volume of extract used}) * (1000 / 0.1165)]$

2.4. Plant and Grain Samples Preparation

The plant shoots and grains samples were also taken to analyse different parameters (N, P, K, Cl⁻ and SO₄²⁻).

2.4.1. Grinding

For the preparation of plant and grain samples, grinding of these samples were done with the help of electric grinder (small in case of grains grinding) to get the material in powder form. After grinding, the digestion of plant material was done.

2.4.2. Di-acid Digestion

Di-acid digestion was used to prepare the Plant or grain sample for all other analysis except N. For di-acid digestion process, the mixture of two acids (hydrochloric acid (HNO₃) and perchloric acid (HClO₄) at the ratio of 2:1 respectively) were used. 0.5 g plant sample or grain sample was taken in flask. 5 ml of di-acid was used for each sample and keep overnight. After 24 hours, these flasks were put on the hot plates at 300°C until the color of inside material became white or white fumes started to come out. After that, put out these flasks from the hot plate and cooled them. After cooling, the material inside these flasks was filtrated with Whatman filter paper number 40. 50 ml volume was made in Volumatic flask with DI water of each sample. Now the sample was prepared for further analysis.

2.5. Kjeldahl Method for N Determination in Plant

Kjeldahl method for N determination has following two steps;

2.5.1. Digestion

To prepare the sample for N analysis, digestion of that sample was done. 0.5 g grinded plant or grain samples were kept in digestion flasks or tubes. 5 g digestion mixture (K₂SO₄ + Cu SO₄ · 5H₂O + Se at the ratio of 100: 10: 1 respectively) and 15 to 20 ml concentrated H₂SO₄ (0.1 N) were added in these flasks. Put these flasks in digestion block and heat them at 400°C for 3 to 4 hours until the transparent or light yellowish colored material was observed in these flasks. After that, put out these flasks from the digestion block and cooled them. After cooling, the material inside these flasks was filtrated with Whatman filter paper number 40. 50 ml volume was made in Volumatic flask with DI water of each sample. Now the sample was prepared for further analysis.

2.5.2. Distillation

After the digestion of the sample, the next step for the N determination was the distillation. For distillation process, 10 ml of digested filtrate was kept in beaker. Added 10 ml of 4% boric acid (H_3BO_3) in this beaker. 2 to 3 drops of indicator (methyl red) was added. The color of the sample became purple. After that, placed the beaker on the distillation unit for 3 min during which the color was changed from purple to light yellow. After 3 min, take the breaker out from the distillation unit and titrate it against the 0.1 N H_2SO_4 until the sample's color again becomes purple. The volume of 0.1 N H_2SO_4 used for titration was noticed, which was used to calculate N concentration in this sample.

The N concentration (%) was determined with following formula;

$$N (\%) = (V_0 - B) * 0.1 * V_1 * 14.01 * 100 / \text{Wt.} * V_2 \times 1000$$

Where;

V_0 = vol. of H_2SO_4 used for titration of sample

B= vol. of H_2SO_4 used for titration of blank

V_1 = total vol. of digest

W_t = weight of sample

14.01= N atomic weight

2.6. Determination of P in Plants

For P determination, 10 ml of the filtrate sample was put into a flask (100 ml) after di-acid digestion. 10 ml of reagent ammonium-vanadomolybdate (ammonium heptamolybdate $[(\text{NH}_4)_6 \text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ + ammonium vanadate (NH_4VO_3) + nitric acid (HNO_3) was added in this flask and made the volume with DI water. The standards were formed by potassium dihydrogen phosphate salt, having the concentration of P (0.5, 1, 1.5, 2 and 2.5 ppm). 10 ml of each standard was also taken in flask (100 ml) and used reagent (10 ml). Samples, standards, and blank were kept for an hour to develop their color. After an hour, the absorbance of all the samples, standards and blank was read on spectrophotometer set at the wavelength of 410 nm (Chapman and Pratt, 1961). A standardized curve was designed against the spectrophotometer reading and respective P concentration in standards. This curve was used to find out the unknown concentration of P in the samples. The following formula was used to determine the P concentration in the sample.

$$P (\%) = (\text{Conc. of P (ppm) from curve}) * (V_0 / V_1 * W_t * 100)$$

Where;

V_0 = Total ml of digested sample

V_1 = Volume of the sample (ml) used for P determination

W_t = Plant material weight used for digestion (g)

2.7. Determination of K in Plants

For K determination in plants, Filtrate sample after di-acid digestion was used. Standards of K (10, 20, 40, 60, 80 and 100 ppm) were prepared by using KCl salt. The samples and the standards were run on flame photometer and emissions of these samples were noted. A standardized curve was designed against the flame photometer reading and respective K concentration in standards. This curve was used to find out the unknown concentration of K in the samples. The following formula was used to determine the K concentration in the sample.

$$K (\text{ppm}) = (\text{Conc. of K (ppm) from curve}) * (V/W_t)$$

Where;

V = Total ml of digested sample.

W_t = Plant material weight used for digestion (g).

2.8. Determination of SO_4 in Plants and Grains

For the determination of SO_4 in plants and grains material, the extraction process was done first before using the turbidimetric method (discussed on the above portion). For extraction, 1g of sample (plant material and grain) was taken in evaporating basin. 10 ml of magnesium nitrate [$Mg(NO_3)_2 \cdot 6H_2O$] was applied on this sample. Then, placed this sample on hot plate at $180^\circ C$. When the sample was completely dried, the hot plate temperature was raised at $280^\circ C$. At this temperature, the color of the sample changed from brown into yellow. Then, sample was kept in a muffle furnace at $450^\circ C$ overnight. Sample had put out from the furnace and cooled. Then, 10 ml of HCl was used in this sample and boiled the sample for 3 min. After boiling, 10 ml of DI water was used in it. Then sample was shifted in 100 ml Volumetric flask. It was up to the marked with DI water and then filtered through whatman

filter paper number 541. The Extraction process was completed here, further the turbidimetric method was used for SO_4 determination in plant and grains.

2.9. Statistical analysis

Complete Randomize Design (CRD) with three replications was used. Data from the experiment was analyzed using ANOVA, while the significance of treatment means was separated by applying the LSD test at a 5 % level of significance (Steel and Torrie, 1996).

3. Results

3. Growth Parameters

3.1. Plant Height

Data showed that the treatments significantly ($P < 0.05$). Maximum plant height (98 cm) was noted with T1 and minimum with T2 (95.667 cm). When the ratio between Cl^- and SO_4^{2-} was decreased from T2 to T8, the wheat plant height was increased but remained less than T1 (Control). The percentage decrease in plant height as compared to T1 was 23.8%, 23.8, 23.8 %, 20.4 %, 13.6 %, 13.6 % and 6.8 % in treatments, from T2 to T8 respectively. The comparison between the treatments showed that T2, T3, and T4 treatments were statistically similar. T5, T6, T7 and T8 treatments were in between the response of treatment T1 and treatments T5, T6, T7 and T8. The effect of treatment (T1) was different than the other treatments (Figure 1(a)).

3.2. Yield

The data disclosed that the impact of treatments on wheat yield was significant at $P = 0.000$. Wheat yield was maximum with treatment T1 (27.25 g) while minimum with T2 (17.42 g). When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the yield of wheat was increased but remained less than T1 (Control). The percentage decrease in yield as compared to T1 was 36 %, 34.12 %, 34.75 %, 34.61 %, 29.17 %, 26 % and 25.81 % in treatments, from T2 to T8, respectively. The comparison between the treatments showed that the response of treatments (T3, T4, and T5) and (T7 and T8) on yield was relatively similar (Figure 1(b)).

3.3. 100-Grains Weight

Data was analyzed statistically, and it showed that the treatment's impact on the 100-grain weight was significant ($P = 0.000$). The T1 treatment has the maximum 100 grains weight (6.2033 g) while the minimum (3.2033g) was with T2. When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the grain's weight of wheat was

increased but remained less than T1 (Control). The percentage decrease in gains weight as compared to T1 was 44.7 %, 41.59 %, 34.54 %, 33.9 %, 33.9 %, 26.2 %, and 17.9 % in treatments, from T2 to T8, respectively. The comparison between the treatments showed that the response of T2 and T3 treatments on wheat grain weight was relatively similar, and the T7 and T8 response was also relatively similar (Figure1 (b)).

3.4 Plant Nitrogen (%)

Data were analyzed statistically, and it showed that the treatment's impact on Plant N was significant ($P = 0.000$). The maximum concentration of N (0.9523%) in Plant was T1 and minimum N concentration (0.4587%) was T2. When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of N increased but remained less than T1 (Control). The percentage decrease in N concentration as compared to T1 was 51.83%, 50.43%, 49.7%, 48.96%, 46.26%, 42.45% and 40.17% in treatments, from T2 to T8 respectively. Treatments comparison showed that response of treatments (T7 and T8) on N concentration was relatively similar. All the other treatments relative response was different from each other's (Figure 1(d)).

3.5. Plant Phosphorus

Data was analysed statistical, and it showed that the treatments impact on plant P was significant ($P = 0.000$). Maximum concentration of P (0.0887%) was with T1 and minimum (0.0537%) with T2. The ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of P increased but remained less than T1 (Control). The percentage decrease in P concentration as compared to T1 was 39.45%, 37.65%, 37.65%, 34.27%, 32.35, 30.1% and 28.97% in treatments, from T2 to T8 respectively. The treatments comparison showed that response of all the treatments remained different from each other (Figure 1(e)).

3.6. Plant Potassium (%)

The data showed that the treatment's impact on Plant K was significant ($P = 0.000$). The T1 has the maximum concentration of K (0.9057 %), while treatment T2 has a minimum K concentration (0.5643 %). When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of K was not changed significantly, and it remained less than T1 (Control). The percentage decrease in K concentration as compared to T1 was 37.69 %, 36.7 %, 36.36 %, 36.81%, 35.51 %, 35.07 % and 35 % in treatments, From T2 to T8 respectively. The comparison between the treatments showed that the response of treatments (T2, T3, T4, T5, T6, T7, and T8) on K concentration was relatively similar (Figure 1(f)).

3.7. Soil Chloride (ppm)

Data were analyzed statistically, and it showed that the impact of the treatment on soil chloride was significant ($P = 0.000$). The maximum concentration of Cl in soil (45.66 ppm) was with T2, and treatment (T1) had a minimum Cl concentration in soil (7.2 ppm). When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of Cl also decreased but remained more than T1 (Control). The percentage increase in Cl concentration as compared to T1 was 84.23 %, 83.12 %, 77.5 %, 72.65 %, 67.76 %, 62.75 % and 59.24 % in treatments, from T2 to T8 respectively. The comparison between the treatments showed that the response of treatments (T2 and T3) was relatively similar while all treatments' relative response was different from each other's (Figure 1(g)).

3.8. Chloride in Plant (ppm)

Data was analysed statistical and it showed that the treatments impact on chloride concentration in wheat plant was significant ($P = 0.000$). The treatment (T2) have the maximum concentration of Cl in plant (233.67 ppm) while treatment (T1) have minimum Cl concentration in Plant (94.66 ppm). When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of Cl also decreased but remained more than T1 (Control). The percentage increase in Cl concentration as compared to T1 was 59.48 %, 52.66 %, 41.8 %, 37.99 %, 33.48 %, 25.06 % and 23.03 % in treatments, from T2 to T8 respectively. The comparison between the treatments presented that response of all the treatments was relatively different from each other's (Figure 1(h)).

3.9. Chloride in Wheat Grain

Data was analysed statistical and it showed that the treatments impact on soil chloride was significant ($P = 0.000$). The treatment (T2) have the maximum concentration of Cl in wheat grain (195.33 ppm) while treatment (T1) have minimum Cl concentration in wheat grain (73.66 ppm). When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of Cl also decreased but remained more than T1 (Control). The percentage increase in Cl concentration as compared to T1 was 62.28 %, 60.18 %, 58.84 %, 56.91 %, 53.66 %, 45.29 % 42.14 % in treatments, from T2 to T8 respectively. The comparison between the treatments showed that response of the treatments (T2 and T3) on Cl concentration in wheat grain was relatively similar. T7 and T8 also showed the relatively similar response on Cl concentration in wheat grain (Figure 1 (i)).

3.10. Sulphate in Plant (ppm)

Data was analysed statistical and it showed that the treatments impact on sulphate

concentration in wheat plant was significant ($P = 0.000$). The treatment (T8) has the maximum concentration of SO_4 (1256.9 ppm) while treatment (T2) has minimum SO_4 concentration (576.28 ppm). When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of SO_4 increased in wheat plant. The percentage decrease in SO_4 concentration as compared to T1 was 14.52%, 9.7% and 3.06% in Treatments, from T2 to T4 respectively. The percentage increase in SO_4 concentration as compared to T1 was 13.82 %, 30.25 %, 39.16 % and 46.36 % in treatments, from T5 to T8 respectively. The comparison between the treatments showed that response of all the treatments was relatively different from each other (Figure 1(j)).

3.11. Sulphate in Wheat Grains (ppm)

Data was analysed statistical and it showed that the treatments impact on sulphate concentration in wheat plant was significant ($P = 0.000$). The treatment (T8) has the maximum concentration of SO_4 (583.09 ppm) while treatment (T2) has minimum SO_4 concentration (376.43 ppm) in wheat grains. When the ratio between Cl^- and SO_4^{2-} was decreased from treatment (T2) to treatment (T8), the concentration of SO_4 increased in wheat grain. The percentage decrease in SO_4 concentration as compared to T1 was 7.08%, 14.09% and 14.86% in treatments, from T2 to T4 respectively. The percentage increase in SO_4 concentration as compared to T1 was 7.49 %, 15.16 %, 22.62 % and 24.17 % in treatments, from T5 to T8 respectively. The comparison between the treatments showed that response of the treatments (T2 and T3) on SO_4 concentration in wheat grain was relatively similar. T7 and T8 also showed the relatively similar response on SO_4 concentration in wheat grain (Figure1 (k)).

4. Discussion

4.1. Physiological Parameters

The major problem of an irrigated area is soil salinity. The agricultural potential of arid and semiarid regions like in Pakistan is decreasing due to continues increase in salinity. The annual rainfall of these regains is low and net evaporation rate is high. Different types of salts (NaCl , Na_2SO_4 , NaHCO_3 and Na_2CO_3), mostly dominated with NaCl and Na_2SO_4 are accumulated on soil surface and causes the soil salinity (Blaylock *et al.*, 1994). The effects of salts on the growth and production of the Plant are investigated using single salt (NaCl) but in salinized area, different types of cations and anions are present (Song *et al.*, 2005; Hanif *et*

al., 2017, Qadir *et al.*, 2000). There are some studies, those investigate the response of different plants using mixture of all possible salts that may occur in salt-affected soils (Sosa *et al.*, 2005; Yang *et al.*, 2007; Zhang and Mu, 2009; Li *et al.*, 2010; Guo *et al.*, 2011; Lin *et al.*, 2014; Zhao *et al.*, 2014; Zhang *et al.*, 2015). There is lot of contradiction that which ion (Cl^- or SO_4^{2-}) is more toxic for plant growth and development under saline conditions. The present study examined the wheat tolerance against Cl^- and SO_4^{2-} salinity.

The parameters like respiration rate, photosynthetic rate, evaporation and evapotranspiration were affected by both types of salinities (chloride and sulphate salinity). The decrease in these parameters was due to specific ion toxicity, osmotic effect and deficiency of some nutrients. Under salinity stress, the amount of some ions (Na^+ , Ca^{2+} , Cl^- and SO_4^{2-}) were increased. This increase was toxic for plant development because more concentration of these ions inhibits the function of many enzymes during photosynthesis. Moreover, the relatively more numbers of these ions in soil solution were reduced the uptake of many important nutrients (like NH_4^+ , NO_3^- , K^+ , HPO_4^{2-} and $\text{H}_2\text{PO}_4^{2-}$) so, the deficiency of these nutrients were occurred during the salt stress. Salinity also create osmotic and oxidative stress. The osmotic stress severely reduced the roots' uptake of major nutrients and water (Munns and Tester, 2008). These stresses reduced the stomatal conductance, photosynthesis, respiration and evapotranspiration of the wheat plant (Bieet *al.*, 2004; Akbarimoghaddamet *al.*, 2011; Hussain *et al.*, 2013; Chen *et al.*, 2016). When we compared between the two types of salinities (chloride and sulphate salinity), It was found that the effect of chloride salinity (treatments having the dominant ion was chloride) have a more negative affect on wheat physiological parameters than sulphate salinity (treatments have dominant ion was sulphate). These results favour the results of Geilfus (2018) as Cl^- ion was more mobile than SO_4^{2-} ion, the uptake of Cl^- ion was also more than SO_4^{2-} ion. The plasma membrane was more permeable for Cl^- ion than SO_4^{2-} ion. Cl^- also have an antagonistic effect with many plant nutrients (N, P and SO_4). So, it used their pathways as its concentration in soil solution was increased. When the uptake of Cl^- was increased in xylem cell, it caused an injury of leaf cells of the wheat plant. This damage of leaf cells ultimately reduced the process of photosynthesis. Slabu and his colleagues (2009) found that the field bean showed the signs of chlorosis (yellowing of the leaves) or necrosis (leaves burring) due to higher storage of Cl^- ion in chloroplast. Tavakkoli and his co-workers (2010) found that the higher concentration of Cl^- ion in chloroplast was related with the reduction or degradation of chlorophyll contents in Plant. This accumulation of Cl^- ion in chloroplast and reduction in chlorophyll contents causes several problems for photosynthesis. Higher concentration of Cl^- also caused a photo

inhibitory damage of photosystem II reaction centre (Foyer *et al.*, 1994). Bose and his colleagues (2017) found that due to excessive concentration of Cl^- in chloroplast, the electron acceptors were destroyed during the photosynthesis process, which ultimately enhanced ROS production. The process of photosynthesis is an energy producing process used during respiration and other growth functions. Moreover, the chloride salinity affected the respiration and electron transport pathway during respiration (Jacoby *et al.*, 2016). Overall, the physiological parameters were more damaged by chloride salinity than sulphate salinity in my research.

4.2. Growth Parameters

Weight of the spike and stalk, plant height, number of spikes and grains weight were affected by both types of salinities (chloride and sulphate salinity). The decrease in these parameters was due to osmotic effect, specific ion toxicity and deficiency of some nutrients (Afzal *et al.*, 2006; Iqbal and Ashraf, 2007; Khan *et al.*, 2009). Under salinity stress, the concentration of certain ions (Na^+ , Ca^{2+} , Cl^- and SO_4^{2-}) was increased. This increase was toxic for plant growth because higher amount of these ions inhibits the function of many enzymes during photosynthesis. Moreover, the higher concentrations of these ions in soil solution reduced the uptake of many important nutrients (NH_4 , NO_3 , K^+ , HPO_4^{2-} and $\text{H}_2\text{PO}_4^{2-}$). So, the deficiency of these nutrients were occurred during the salt stress. Salinity also create osmotic and oxidative stress. These stresses reduced the photosynthesis process in the wheat plant (Bie *et al.*, 2004; Hussain *et al.*, 2013; Alomet *et al.*, 2016). When we compared between the two types of salinities (chloride and sulphate salinity), it was found that the effect of chloride salinity (treatments having the dominant ion was chloride) have a more negative affect on wheat growth parameters than sulphate salinity (treatments have dominant ion was sulphate) (Minhas *et al.*, 2007). Moreover, they found that with salinity level at the same electrolyte concentration, SO_4^{2-} had less impact than Cl^- on the yield of rice (*Oryza sativa* L.). Munns and Rawson (1999) found that chloride salinity had more damaging effect on wheat growth during reproductive phase. They stressed the wheat plant by applying different concentration of NaCl (100 to 170 mM). These treatments showed significant effect on the number of spikelet on per spike, emergence of the spike was delayed, and the fertility of the seeds reduced. This all resulted in poor grain yield of wheat. The possible reason behind this result was the plasma membrane was more permeable for Cl^- ion than SO_4^{2-} ion. Cl^- also have an antagonistic effect with many plant nutrients (N, P and SO_4) so, it used their pathways as its concentration in soil solution was increased. When the uptake of Cl^- was increased in xylem

cell, it caused an injury of leaf cells of the wheat plant. This damage of leaf cells ultimately reduced the process of photosynthesis. As photosynthesis is an energy producing process, which was used during respiration and other plant growth functions, all the growth processes were affected by chloride salinity.

4.3. Plant Nutrients

Nitrogen (N) concentration was decreased in wheat plant under salinity stress. As the ratio between Cl^- : SO_4^{2-} increased (4:1, 3:1), the concentration of nitrogen in Plant decreased more. An antagonistic effect between Cl^- and NO_3^- was reported in many studied. This effect based on the nitrate transporters inhibition. These transporters unable to differentiate the NO_3^- over Cl^- especially when the Cl^- concentration was enhanced in soil solution (Wang *et al.*, 2012). So, Cl^- ion was taken up due to its higher concentration by these transporters instead of NO_3^- . While SO_4^{2-} did not have such an antagonistic effect with nitrogen, the concentration of N was more affected by chloride salinity than the sulphate salinity. It was concluded that chloride salinity has more negative effect for plant nitrogen than sulphate salinity.

Phosphorus (P) was reduced in wheat plant under salinity. As the ratio between Cl^- : SO_4^{2-} increased (4:1, 3:1), the concentration of nitrogen in Plant decreased more. An antagonistic effect between Cl^- and H_2PO_4^- or HPO_4^{2-} was reported in many studied. This effect was based on either the competition between these anions to occupy the binding site at phosphate transporter protein or due to more concentration of Cl^- in soil solution was displaced the phosphate from binding site. While SO_4^{2-} did not have such kind of antagonistic effect with phosphate so, the concentration of P was more affected by chloride salinity than the sulphate salinity. It was concluded that chloride salinity has more negative effect for plant phosphorus than sulphate salinity.

Potassium (K) concentration decreased in wheat plant under salinity. The concentration of K was decreased in all the treatment except control in almost same trend. It was because Plant take up K in K^+ ion so, various ratio of Cl^- : SO_4^{2-} at same concentration of Na^+ . There was a direct relation between K^+ and Na^+ and because all the treatments, the reduction in K concentration showed a similar trend in all the treatments except control. Munns and his colleagues (2006) find out that, when the Na^+ concentration enhanced, the K^+ concentration was decreased in wheat. This reduction in K^+ concentration in wheat caused to reduce its growth.

Overall, it was concluded that the chloride ion has more toxic effect than sulphate ion for wheat development. These results favored the work done by Minhas and his co-workers (2007), who concluded that the impact of Cl^- on rice yield was more than SO_4^{2-} when the electrolyte concentration was kept constant. SO_4^{2-} salinity has more toxic effect than Cl^- salinity in *Brassica rapa* and also Cl^- salt resulted in less decrease of pepper plant growth than SO_4^{2-} salt (Navarro *et al.*, 2003; Reich *et al.* 2018). These results also contradict the conclusion of work done by Datta and his co-workers (1995) who found that the Cl^- was less damaging than SO_4^{2-} to barley yield.

5. Conclusion

Agriculture lands are at great threat due to increase in salt affected areas. It not only disturb the soil, but also change chemical and physical processes going on in plant bodies. So, this experiment was done to check the effect of salinity with different $\text{Cl}^-:\text{SO}_4^{2-}$ ratios on the growth parameters of wheat. It was concluded that the both types of salinities (chloride and sulphate) significantly reduced the growth of the wheat. Maximum growth reduction was observed in T2 and T3 (treatments with 4:1 and 3:1 of Cl^- and SO_4^{2-} ratios). Chloride salinity was more injurious than sulphate salinity for wheat growth. Overall, it was concluded that the chloride ion has more toxic effect than sulphate ion for wheat growth.

Availability of data and material: The author will share data on demand.

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UNDER PEER REVIEW

Table 1. Characteristics of Experimental Soil.

Characteristics	Unit	Results
Texture		Sandy clay loam
EC_e	dS m ⁻¹	2.2
TSS	mmol _C L ⁻¹	22
pH_s		7.48
CO₃⁻	mmol _C L ⁻¹	0
HCO₃⁻	mmol _C L ⁻¹	6
Cl⁻	mmol _C L ⁻¹	11
SO₄²⁻	mmol _C L ⁻¹	5
Ca+Mg²⁺	mmol _C L ⁻¹	14
Na⁺	mmol _C L ⁻¹	8
SAR		2.65
Saturation percentage	%	34

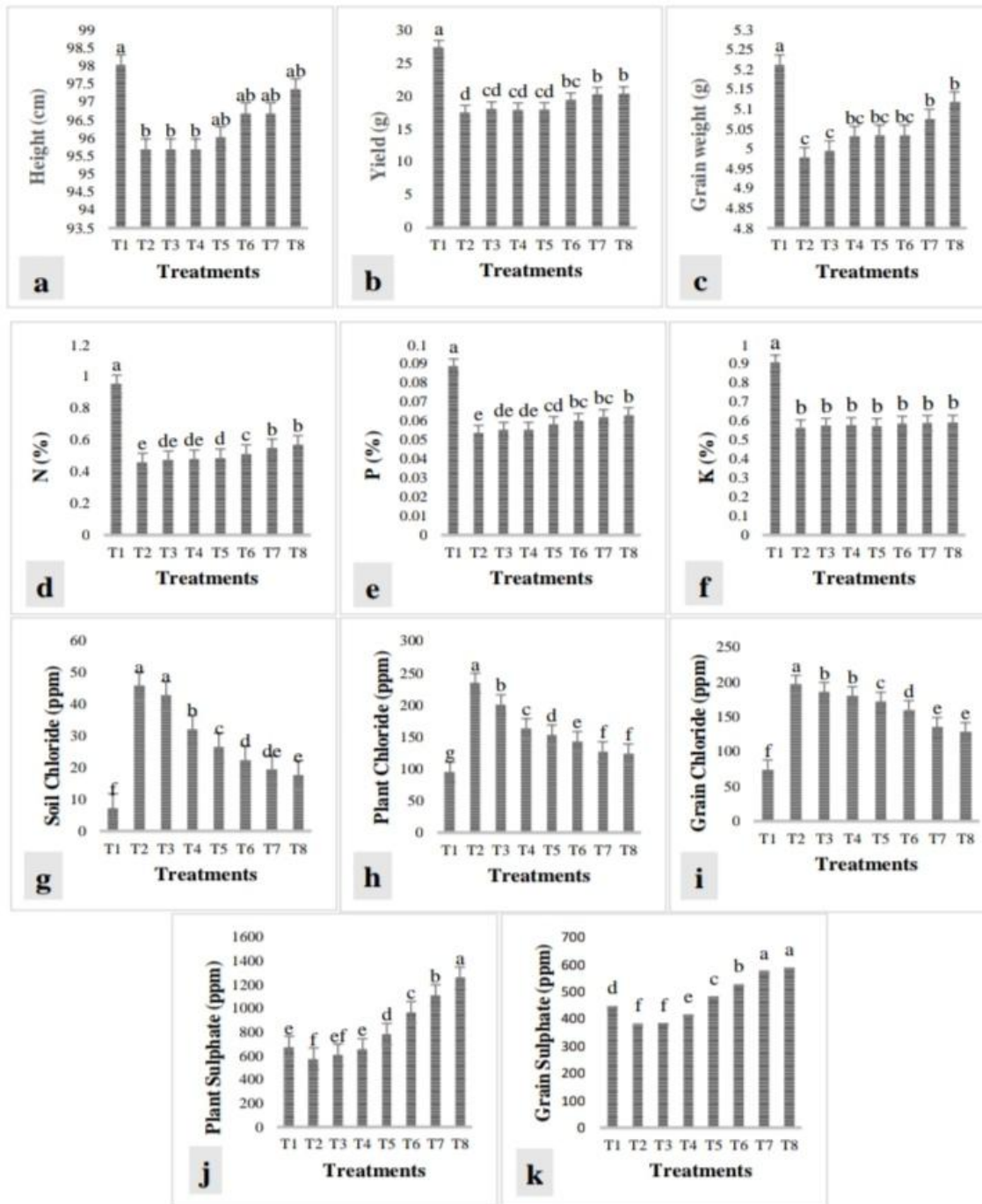


Figure 1. T1= Control, T2=4:1 of Cl⁻: SO₄²⁻, T3=3:1 of Cl⁻: SO₄²⁻, T4=2:1 of Cl⁻: SO₄²⁻, T5=1:1 of Cl⁻: SO₄²⁻, T6=1:2 of Cl⁻: SO₄²⁻, T7=1:3 of Cl⁻: SO₄²⁻ and T8=1:4 of Cl⁻: SO₄²⁻. Mean sharing the same letter(s) do not differ significantly at P ≤ 0.05. (a) Effect of treatments on plant height. (b) Effect of treatments on wheat yield. (c) Effect of treatments on 100 grain weight of wheat crop. (d) Effect of treatments on N concentration in wheat straw. (e) Effect of treatments on P concentration in wheat. (f) Effect of treatments on the concentration of K in wheat. (g) Effect of treatment on the concentration of Cl in soil. (h) Effect of treatments on the concentration of Cl in wheat plant. (i) Effect of treatments on the concentration of Cl in wheat grains. (j) Effect of treatments on the concentration of SO₄ in wheat plant. (k) Effect of treatments on the concentration of SO₄ wheat grain.