Research Article

Effect of sodium chloride on the growth parameters of canola plant (*Brassica napus*)

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Abstract
Rapeseed (*Brassica napus*) is one of the most important plant producing essential oil. These oil are edible around the world. An experiment was carried out in triplicate in greenhouse condition at the botanical garden in Islamia College Peshawar. The experimental design consisted of four major groups which were arranged in sequences line and to study the effect of salinity stress on growth parameters, four level of salinity solution (0mM, 125mM, 250mM, 370mM) containing NaCl was applied in irrigation planting in a greenhouse. The results showed that salinity significantly affected the rate of germination, shoot length, leaves number, fresh weight of whole plant, fresh weight of root and number of flowers at P< 0.05. High concentration of NaCl significantly reduced the rate of germination, shoot length, leaves number, fresh weight of root and whole plant, as well as the number of flowers. It is necessary to indicate that canola plant cannot tolerate high concentrations of NaCl and damage under high salt stress condition. The highest value of growth parameters were observed under non-salinity condition (Control) with respect to salinity.

Keywords: Growth Parameters; NaCl; Rapeseed (*Brassica napus*); Salt; Stress

Introduction
Salinity is one of the most significant environmental factor which effect plant productivity extremely in arid and semi-arid climates [1]. Plants are uninterruptedly facing harsh environment, modulated by different environmental component, one is biotic factor such as parasites, pathogens, herbivores, etc. and second one is abiotic factor like nonliving matters such as salinity, drought, temperature, humidity, light, water, mineral nutrients, heavy metal and radiation etc [2]. Correspondingly to the USAD salinity laboratory, saline soil have high concentration of soluble salt. Ions donate soil salinity are Na+, Cl-, Ca++ and Mg++ [3]. Salts also obstruct with seed germination and crop establishment. Germination and seedling characteristics are the most achievable criteria used for selecting salt tolerance in plants. Salinity stress can influence seed germination through osmotic effects [4]. Canola (*Brassica napus* L.) plant can grow under different environmental conditions especially under the drought, salinity and temperature stresses [5]. Salt-affected lands transpired in virtually all climatic regions, from the humid tropics to the Polar Regions.
All soils consist salts and all irrigation waters, from canal pumping, carry some dissolved salt which are necessary elements of soil (nitrates and potassium). Precisely, about 2% of the lands farmed by dry-land agriculture, Mediterranean regions are directly undergo enlarged salt stress issues developed from seawater entrance into aquifers and irrigation with brackish water [6]. Primary salinity results from the agglomeration of salts over long periods of time through natural processes in the groundwater. It is created by two natural actions. The first action is the weathering action which break down rocks and release soluble salts of different types mainly chlorides of Na, Ca, and Mg, and to a lesser extent, sulfates and carbonates. The second action is the deposition of oceanic salt carried by wind and rain [7]. Two basic stresses influence plants under salinity are osmotic and ionic stresses. Osmotic stress occurring immediately in the root medium on exposure to salts can result in inhibition of water uptake, cell expansion and lateral bud development. Ionic stress develops when toxic ions like Na⁺ accumulate in cells causing increase in leaf mortality, chlorosis, necrosis and decrease in the activity of cellular metabolism including photosynthesis. In fact excess of Na⁺ and Cl⁻ have the potential to destroy plant enzymes, refuse energy construction and other physiological processes. Salt stress impact growth and development of plant; yet, the feedback of plants is different with the state of stress and crop growth stage. Seed germination is the most introductory time in seedling establishment. Germination and early seedling growth are additionally sensitive to salinity than later developmental stage. Hyper-osmotic stress and toxic outcomes of sodium and chloride ions on germinating seeds in a saline soil may inhibit germination. It has been reported that salinity adversely affect the process of germination in different plants like *Posidonia*, *Oryza sativa*, *Triticum aestivum*, *Zea mays* and *Brassica*. In *Brassica napus* the rate of germination is reduced with increase in the salt concentration. Photosynthesis is one of the most fundamental biochemical pathway by which solar energy is convert into chemical energy in the form of sugar. Photosynthesis rate decreases due to salt stress, more salt are responsible to reduce the osmotic potential, accumulation of Na and Cl ions in the chlorophyll, so in this way the photosynthesis rate is reduced. Moreover literature exhibited that the *O. sativa* leaves decline the chlorophyll a and b content of the leaves after the treatment of NaCl. It is observed that the chlorophyll b was effected 41% and chlorophyll a was effected 33% [8, 9]. According to the theory Gouy-Chapman, it was concluded that Na: Ca interaction construct at the plane of the membrane. NaCl salinity decreases the surface potential of the plasma membrane, surface charge density and ions activity, reduction in the surface charge density of the membrane, reduce the cation activity at the outside surface of the membrane as result the ions transport across the plasma membrane are effected [10]. Salinity cause the nutrient imbalances, due to the competition of the Na⁺ and Cl⁻ with other plant nutrient such as K⁺, Ca²⁺, and NO₃⁻. The saline soil decreased the plant growth because of ion toxicities (Na⁺ and Cl⁻). NaCl interaction increase with other element and decreased the amount of K, P, Ca, N and Mg [11]. Canola (*Brassica napus* L.) belong to the genus Brassica and family Brassicaceae. It is also called rapeseed and is widely cultivated plant around the world for the production of edible oil and biodiesel fuel. It is third most important crop plant after soybean and palm. The canola yield is reduced due to the salt stress. Several articles have been written about the canola plant morphological, physiological and biochemical response to salt stress [12].
Current research work was conducted on the effects of the salt stress on the growth parameters of the canola plant.

**Materials and methods**

**Selection of plant**
The selected plant for the research was canola (*Brassica napus*), belong to the genus of *Brassica* and the family of *Brassicaceae*. Canola is commonly called rapeseed and oilseed. Rape is a word derived from Latin *rapum* meaning turnip. Generally it is used as edible oil. From the germination to seed production, the life cycle of canola plant is completed in 3 ½ months, based on temperature, moisture, sunlight and soil fertility.

**Collection of seed**
The canola seeds (Husnain variety) were obtained from the Turnab, Agriculture University Peshawar. The seeds were same size and color. 84 seeds were used in the research.

**Experimental design**
A total of 12 pots were used in the experiment. The experiment was conducted in triplicate in the greenhouse condition at the botanical garden in Islamia College Peshawar. The experimental design consisted of four major groups which were arranged in sequences line. The main groups were Control group, Experiment 1, Experiment 2 and Experiment 3. Seven seeds were sown in each pot of each one group.

**Concentration of salt (NaCl) used in experiment**
Different concentration of sodium chloride (NaCl) were used in the experimental groups. No salt was added to the control group while 125mM, 250mM and 370mM NaCl was added in solution form to the each one pot of rest of the group’s i.e. Experiment 1, Experiment 2 and Experiment 3 respectively. The three concentration of the salt was prepared using the following formula,

\[
1\text{Molar solution of NaCl} = 1000\text{mM solution of NaCl} = 58.5\text{g NaCl}/1000\text{ ml}
\]

**Procedure**
For 125mM solution, 3.65g of sodium chloride was dissolved in 1000ml of distilled water while for 250mM solution, 7.31g of sodium chloride was dissolved in 1000ml of distilled water. For 370mM solution, 10.96g of sodium chloride was dissolved in 1000ml of distilled water.

**Effects of NaCl on the germinations**
The seeds of canola plant were grown on 2 Nov, 2019 and give solutions of NaCl to the experimental groups. First I visited after 9 days to the field and observed that the seed has started germination. The effects of the used salts on the germination of the canola seeds were recorded by counting the number of germinated seeds in each pot. By this way I visited to the field after every three days and after nine days I recorded the data by the same method.

**Effects of NaCl on the shoot length**
The shoot of both control and those which were treated with different concentrations of sodium chloride was measured with the help of ruler in centimeter. The shoot length was considered from the soil of the pot to the apex of the plant.

**Effects of NaCl on Fresh and dry weight**
When the plants were fully matured, they were harvested and the fresh weight of the plants was determine in gram (g) using digital balance.

**Effects of NaCl on the root weight and number of leaves**
When experimental plants were harvested from the pots after maturity. Then they were separated into parts (shoots and roots). The root weight was taken in gram (g) using digital balance. The number of leaves were counted in the plants grown in control group and those which were treated with different concentration.

**Results**
The experiment was carried out in the Botanical Garden Department of Botany Islamia College, Peshawar to find out the
**Effects of NaCl on germination**

The effects of the used three different concentration of NaCl (125mM, 250mM, and 370mM) on the rate of germination was determined. After seed sowing and addition of different concentration of NaCl to the respective pots for a few days, the germination and seedling growth appeared differently in control and in those which were treated with different concentration of sodium chlorides (NaCl) as shown in the (Fig. 1). Error bars shows standard deviation which were found statistically significant. The maximum reduction in germination was observed at a high concentration of sodium chloride in soil (Exp3) followed by (Exp2) while low concentration (Exp1) of NaCl in soil increased the rate of germination slightly with respect to without treated soil (Control group). Overall statistically significant difference was found in the rate of germination in the seeds grown in control and experimental groups using one way anova (at P<0.05 and P value summary of ****).

**Effects of NaCl on the shoot length**

At the time of harvesting, selected seven plants from each pot. The shoot elongation was measured in centimeter at top of shoot from the base with the help of digital ruler. The shoot length of both control group and which were treated with different concentration of sodium chlorides was measured as shown in (Fig. 2). Error bar shows standard deviation which were found statistically significant. The maximum declination in shoot elongation was observed at high concentration of sodium chloride (Exp3) followed by (Exp1) while to a small extent (Exp2) of sodium chloride promoted the shoot elongation with respect to untreated group (control). It means that low concentration of sodium chloride is beneficial for shoot elongation. Overall statistically significant difference was found in shoot elongation in control and experimental groups using one way anova (at P<0.05 and P value summary of ****).

**Effects of NaCl on number of leaves**

The matured plants after completing their life cycle were harvested from groups of each pot and counted the number of leave of *Brassica napus* of every plant. Observed different data regarding the number of leaves in treated and untreated groups as shown in (Fig. 3). Standard deviations were found statistically significant. Maximum reduction in the leaves number in plants was observed at high concentration of sodium chloride in the soil (Exp3) followed by (Exp2) and (Exp1) while a maximum number of leave was found in untreated soil (control group) with respect to experimental groups. It indicates that salinity is responsible for reduction in the leaves number. Overall statistically significant difference was found in the number of leaves in the seed grown in control and experimental groups using one way anova (at P<0.05 and P value summary of ****).

**Effect of NaCl on the fresh weight of the plant**

After plant harvesting, directly determined the fresh weight with the help of digital balance. Measured the fresh weight of all groups (control and treated) of all parts of the plant canola (shoot and root system) in gram as shown in (Fig. 4). Standard deviations were found statistically significant. Maximum reduction in fresh weight was observed at high concentration of sodium chloride in soil (Exp3) followed by (Exp1) and then (Exp2). While plant grown in non-saline soil (control group) exhibit high fresh weight.
weight with respect to experimental groups. Statistically significant difference was found in the fresh weight in seed grown in control and experimental groups (at P<0.05 and P value summary of ****).

**Effect of NaCl on the initiation and maturation of flowering**

At the start of the flowering period up to maturity flowers were counted in plants in treated and untreated groups. Data of initiation of flowers were collected in five days interval. After ten days the collected data is shown in (Fig. 5a). Error bars shows standard deviation which were found statistically significant. The maximum reduction in the initial of flowering was observed at a high concentration of sodium chloride in soil (Exp3) followed by (Exp2) and (Exp1). While maximum number of flowers were found in non-salinity (control group) with respect to salinity in soil (Experimental groups). Statistically significant difference was found in control and experimental groups using one way anova (at P< 0.05 and P value summary of **). After the maturation period of the plant, counted the number of flower as shown in (Fig. 5b). Error bars shows standard deviation which were found statistically significant. The maximum reduction in number of maturation of flowers was observed at high concentration of sodium chloride in soil (Exp3) followed by (Exp2) while to a small extent of sodium chloride concentration increased the number of maturation slightly with respect to non-salinity (control) in soil. Overall statistically significant difference was found in non-salinity (control group) and salinity (Experimental groups) (P< 0.05 and P value summary of **).

**Discussion**

The effects of sodium chloride (NaCl) on the rate of germination of the selected plant is clear from (Fig. 1). Results showed that an increase in the concentration of sodium chloride (NaCl) decrease the rate of germination but to a smaller extent increase in the concentration of the same salt increased the rate of germination. It demonstrated that an increase in soil salinity decreases the rate of germination. Our findings are similar to the results of [1-13]. Similar research work was conducted and found significant maximum reduction in germination at high concentration of sodium chloride. Findings of the current research work regarding the effects of NaCl on germination are in agreement of the findings of [13]. Our finding are similar to the result of [14-16] indicated that the different treatments of salinity stress considerably effected the rate of germination.

The effect of sodium chloride (NaCl) on the shoot elongation is shown in (Fig. 2). Results showed that increase in level of sodium chloride (NaCl) declined the shoot elongation (Exp3) while low concentration of sodium chloride increased the shoot length (Exp2). It is reported that salt stress affect the plant growth, and declined the shoot elongation. Our findings are similar to the results of [17]. Similar research work was conducted and
found significant maximum reduction in shoot elongation of pepper seedling at high level of sodium chloride. Finding of current research regarding the effects of NaCl on shoot elongation are in agreement of the findings of [18]. Similar research work was conducted by [19] and exhibited that reduction in shoot length was found at high concentration of NaCl with respect to non-salinity.

The effect of sodium chloride on the leaves number is shown in (Fig. 3). Results shows that high concentration of NaCl caused significant reduction in leaves number. Similar research work was conducted by [20]. The micro shoot was exposed to salinity. Slight reduction was obtained in leaves and shoot number.

The effect of salt of NaCl on the fresh weight of plant of canola is shown in (Fig. 4). Results showed that increased in concentration of NaCl (Exp3) reduced the fresh weight of rapeseed. It is demonstrated that salt stress caused a significant reduction in length, wet (fresh) and dry weight. Our findings are similar to the results of [21]. Similar research was conducted by [22] and showed that salt stress inhibited plant growth (shoot length, fresh root and shoot weight but root growth was more effected than shoot).

The effect of sodium chloride stress on flowers is shown in (Fig. 5a, b) Results showed that increased in concentration of NaCl caused a significant reduction in flowers of rapeseed. It is indicated that increased in salinity (NaCl) cause reduction in number of branches per plant, flowers per plant, and also significantly reduced the fresh and dry flowers weight. Our finding are similar to the results of [23].

![Growth of canola plant in control and NaCl treated soil](image)

**Figure 1.** Effect of different concentration of NaCl on seed germination of *Brassica napus*
Growth of canola plant in control and NaCl treated soil

Figure 2. Effect of different concentration of NaCl stress on the shoot length of *Brassica napus*

Growth of canola plant in control and NaCl treated soil

Figure 3. Effect of different concentration of NaCl on number of leaf of *Brassica napus*
Growth of canola plant in control and NaCl treated soil

Figure 4. Effect of different concentration of NaCl stress on the fresh weight of *Brassica napus*

Growth of canola plant in control and NaCl treated soil

Figure 5(a). Effect of different concentration of NaCl stress on the initiation of flowering of *Brassica napus*
Growth of canola plant in control and NaCl treated soil

Figure 5(b). Effect of different concentration of NaCl on the maturation of flowering of *Bassica napus*

Growth of canola plant in control and NaCl treated soil

Figure 6. Effect of different concentration of NaCl on the fresh weight root of *Brassica napus*
Conclusion
Based on current research work it is concluded that the rate of germination, shoot length, leaves number, fresh and dry weight of plant and root were negatively effected at a high concentration of sodium chloride stress as compare to the plants grown in non saline soil. However low concentration of sodium choride was found to increse the rate of germination with repect to non-salinity.

Authors’ contributions
Conceived and designed the experiments: MA Sajad, Performed the experiments: Kainat, Analyzed the data: Kainat, Contributed materials/ analysis/ tools: Kainat Wrote the paper: Kainat, MA Sajad.

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