ISSN 2229-5518

# GROWTH RESPONSES OF *BRASSICA CAMPESTRIS* L. AT DIFFERENT GRADES OF SODIUM CHLORIDE TOXICITY

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## ABSTRACT

Salinity can cause hyper-ionic and hyper osmotic effect on plant, leading to membrane disorganization and metabolic toxicity. The occurrence of salt affected soil is worldwide problem particularly in arid and semi-arid region including Pakistan. The *Brassica campestris* commonly known as rapeseed mustard is important group of edible oils and vegetables crops belonging to Brassicaceae family. It is the second most important oil crops in world next to soybean. The present investigation was the trail of salt affects on the vegetative growth of Brassica species. Grown Seedlings were transplanted in earthen pots at the depth of 2 cm (each pots were 8cm diameter and 8cm in height) containing 5kg loamy soil. In order to prevent salt accumulation 1cm diameter hole was made at the bottom of pots. After germination seedling were thinned and homogenized per pots. Plants grew in pots and observed it on daily basis at 9 am in the wire house of Botany Department Ghazi University Dera Ghazi Khan .After two week of plants germination; different grades of salinity treatment were started. Plants were subjected to five levels of sodium chloride solution i.e.0 (Control), 0.25, 0.50, 75 and 1 M. Data were subjected to statistical analyzed with one way analysis of variance using SPSS (version 20).The mean values were recorded at 0.05% probability level. It was concluded that the salinity was the problem for the growth and development of all the type of vegetation except halophytes.

**Key Words: Salt effect; Oil crops; Rape seeds; Sodium Toxicity, Different level of NaCl and Hyper ionic effect.**

# 1. INTRODUCTION

The salt affected soil is worldwide problem particularly in arid and semi -arid region including Pakistan. According to an estimate there are up to maximum $950\times10^6$ hectares of salt-affected soil in the world [1]. Another estimate that soil salinity is increases regularly in extent recent data [2] about 800 million hectares of the world land are salt affected; of which 397 million hectares are saline 434 million hectares are sodic throughout the world [3]. A current loss of 12 billion US$ annually due to salinity in the USA is expected to rise in future, because soil are further affected by salinity [4].

Salinity refers to the occurrence of high concentration of dissolved major inorganic ions. It includes solution of $Na^+$, $Mg^{++}$, $Ca^{++}$, $K^+$, $HCO_3^-$, $SO_4^{--}$ and $Cl^-$[5]. The salinity has two main source primary or natural, resulting weathering of mineral and soil derived from saline parent rocks and secondary salinization that is caused by human interference such as irrigation, high evaporation, deforestation, over grazing, or intensive cropping [6], [7]. Primary salinity is more wide spread as compared to secondary salinity [8]. Salinity drastically alters both physical and biologically environments [9].

Salinity affect plant growth and its deleterious effect are attributed to reduced osmotic potential of the growing medium, specific ion toxicity and nutrient deficiency [10], low osmotic potential of saline solution prevent water up take by plant, resulting in Physiological drought,, .Alteration in physiological processes due to osmotic stress cause reduction in growth. Plant dry weight may reduce drastically [11]. Salinity can cause hyper-ionic and hyper osmotic effect on plant, leading to membrane disorganization and metabolic toxicity, including excessive generation of Reactive Oxygen Species (ROS) such as the super oxide anion and hydroxyl radical, particularly in chloroplasts and mitochondria [12].

The most important process that is affected by salinity is photosynthesis [13]. Reduced photosynthesis under salinity is not only attributed to stomatal closure leading to a reduction of intercellular CO2 assimilation, but also to non-stomatal factors like reduction in green pigments and leaf area. There is a potential for many in nutrient interactions in salt stressed plants which may have important consequences for growth [14]. Many researchers across the globe [15], [16], [17], [18], reported that salinity had a major effect on the uptake and internal concentrations of mineral elements and plant growth in many plants. Both stresses would reduce shoot more than root growth mainly due to the osmotic component and actually many studies on water and salt stresses in Brassica species reported a decrease of the shoot/root ratio [19], [20], although there are also opposite evidences for salt stress [21]. Oil seed crops are one of the major crops of Pakistan agriculture. We are exporting a large amount to complete our annual requirements

Keeping in view all this, the present work was done to assess the growth responses of *Brassica campestris* to different grades of sodium chloride toxicity application in the earthen pots experiments. So that a larger area of salt affected lands can be economically utilized by cultivation of oil seed crops.

## 2. MATERIALS AND METHODS

The experiment was conducted at wire house of Botany Department of Ghazi University Dera Ghazi Khan

### 2.1. Seed Collection

Seeds of *Brassica campestris* were collected from local seed corporation of Dera Ghazi Khan. These were surface sterilized with one percent sodium hypochlorite for one minute and then rinsed three times with distilled water.

### 2.2. Growth Experiment

The seeds were sown in petri plates and then 10 selected seedlings of equal length were transplanted in earthen pots (each pots were 8 cm diameter and 8 cm in height) containing 5kg loamy soil. In order to prevent salt accumulation 1cm diameter hole was made at the bottom of pots. After one week of transplantation seedling were thinned per pots. Plants grew in pots and observed it on daily basis at 9 am in wire house of Botany Department Ghazi University Dera Ghazi khan. After two week of transplanting, treatment was started. Plants were subjected to five level of sodium chloride solution: 0(control); 0.25; 0.50; 0.75 and 1.0 M.

The pots containing plants were arranged in a (CRD) design inside a wire house .One liter of saline water with relative concentration was added to each pots on weekly basis up to end of experiment. The effect of sodium chloride was observed on growth of *Brassica campestris* on daily basis.

### 2.3. Harvest-I

After one month of treatment plants were harvested from each pot carefully up rooted and washed thoroughly with water to remove soil particles on the surface of the plants parts and blotted to surface dry.

## 2.4. Measurements of growth parameters

Following growth parameters were studied at different stage of the experiment: Shoot length and root length (cm); Fresh weight of shoot and dry weight of shoot (g); Fresh weight of root and dry weight of root (g); Number of leaf and Leaf area ($cm^2$).

## 2.5. Harvest-II

The treatments were continued on remaining plants for one month. After two months second harvest was taken and following studies was performed and the same procedures as like in first harvest were adopted for the measurements of growth parameters.

## 2.6. Measurements procedures

All the plant samples were dried overnight at 80 $^{O}C$ temperature in oven. At the end of experiment, shoot height was measured from the base of the stem, just above the soil surface, to its apex using a centimeter rule. The total number of leaves on both the stem and branches were also counted and recorded at the end of the study. Leaf area was obtained by measuring the length of the leaf and width of the leaf and calculated following the formula of [22] as shown below: LA=0.5(L x W), where L is the leaf length and Wl is the maximum width measured for each leaf on each plant at the end of the experiment, plants were harvested carefully from the pots. Roots were separated from the shoots and then weighed immediately using an electronic weighing balance. The roots and shoots were packed separately in envelopes and dried in an oven at 80 $^{o}C$ to constant weight for 48 hours. The weights were then determined using an electronic weighing balance. Stem diameter was measured 5cm above soil level of each. Shoot height was significantly at ($P \geq 0.05$) reduced by NaCl.

## 2.7. Statistical Analysis

Data were subjected to statistical analysis with one way analysis of variance using SPSS (version 20).The mean values were recorded at 0.05% probability level.

# 3. RESULTS

## 3.1. Effects of salt treatments on shoot and root length

The shoot growth of the Brassica species was noteworthy response in application of the various concentrations of salts treatments. With the increase of Sodium Chloride salt concentration (M) in the pots experiments, the shoot growth of the particular plant individual in the pots were significant decrease and showed the stunted growth than the growth of control plants. From the one-way analysis of variance and the result of correlation (Tables 1 and2) showed that the $P \geq 0.000$ means the strong significant differences were found in the growth of shoot of Brassica species in connection to different levels of salt concentrations.

The roots of Brassica species were also showed obviously response in variable treatments of sodium salt. The same trends of growth response were found in the roots as like in the shoots of the same species of Brassica. From the one-way analysis of variance and the correlation (Tables 1 and 2, $P \geq 0.000$, Figure- 1) observed the same trends that was seen in the shoot length.

## 3.2. Effects of salt treatments on the number of leaves of Brassica sp.

The effects of sodium chloride on number of leaves were significant. The number and size of the leaves of Brassica sp. were significantly decreased in the salt treatments. But control plants produced more number of leaves and their vigorous growth was observed during the harvest. The one-way analysis of variance and the correlative results (Tables 1 and 2, $P \geq 0.000$; Figure-1) showed that the similar gist was found in the number of leaves of Brassica species which were grown in the different levels of salt concentrations in the earthen pots.

## 3.3. Effects of salt treatments on the leaf area of Brassica sp.

The leaf area index reduced in high concentration of salt concentration. The control plants had lager leaf area than the plants which were treated in different concentration of sodium chloride. During this trail experiment it was observed that the leaf lamina of the Brassica species was reduced due to application of high salt concentration, and then instinctively the leaf area index was reduced. The unstacked one-way analysis of variance and correlation between the treatment and growth variables (Tables 1 and 2, $P \geq 0.000$, Figure-1) showed that the significant differences and the negative correlative effect were observed the confirmatory evidence about the strong effect of salt on the leaf area index of Brassica species.

## 3.4. Effects of salt treatments on shoot and root fresh weight

The shoot and root fresh weights of Brassica species were showed strikingly sensitive response in various treatments of sodium salt. The obtained mass of plants of Brassica was showed highly sensitivity in the salt stress condition. The one-way analysis of variance (Tables 1 and 2, P≥ 0.000, Figure-1) showed that the strong significant differences were found in the fresh weight of shoot and root of Brassica species which were grown in the different levels of salt concentrations in the earthen pots

## 3.5. Effects of salt treatments on shoot and root dry weight of Brassica sp.

The shoots and roots dry weight of Brassica species was showed noticeably sensitive response in various treatments of sodium salt. The obtained dry mass of plants of Brassica was showed highly sensitivity in the salt stress condition. The one-way analysis of variance and the confirmatory results of correlations (Tables 1 and 2, P ≥0.000, Figure- 1) showed the similar nature of results towards the decline of the growth of Brassica species due to application of salt treatments.

**Table 1. One way analysis of variance (Unstacked) among the all parameters**

| SOV | DF | MS | F-Value | P-Value |
|---|---|---|---|---|
| Factor | 11 | 3377.4 | 217.05 | 0.000*** |
| Error | 456 | 15.6 | | |
| Total | 467 | | | |

**Table .2. Correlations (Pearson) among all measuring growth parameters and treatments**

| | H | SL | RL | NOL | LA | SFW | RFW | SDW | RDW |
|---|---|---|---|---|---|---|---|---|---|
| T | -0.1695 | -0.7919 | -0.5560 | -0.8430 | -0.8690 | -0.9244 | -0.7384 | -0.6562 | -0.5384 |
| P.V. | 0.3022$^{NS}$ | 0.0024$^{***}$ | 0.0002$^{***}$ | 0.000$^{***}$ | 0.0023$^{***}$ | 0.000$^{***}$ | 0.006$^{***}$ | 0.0020$^{***}$ | 0.0004$^{***}$ |

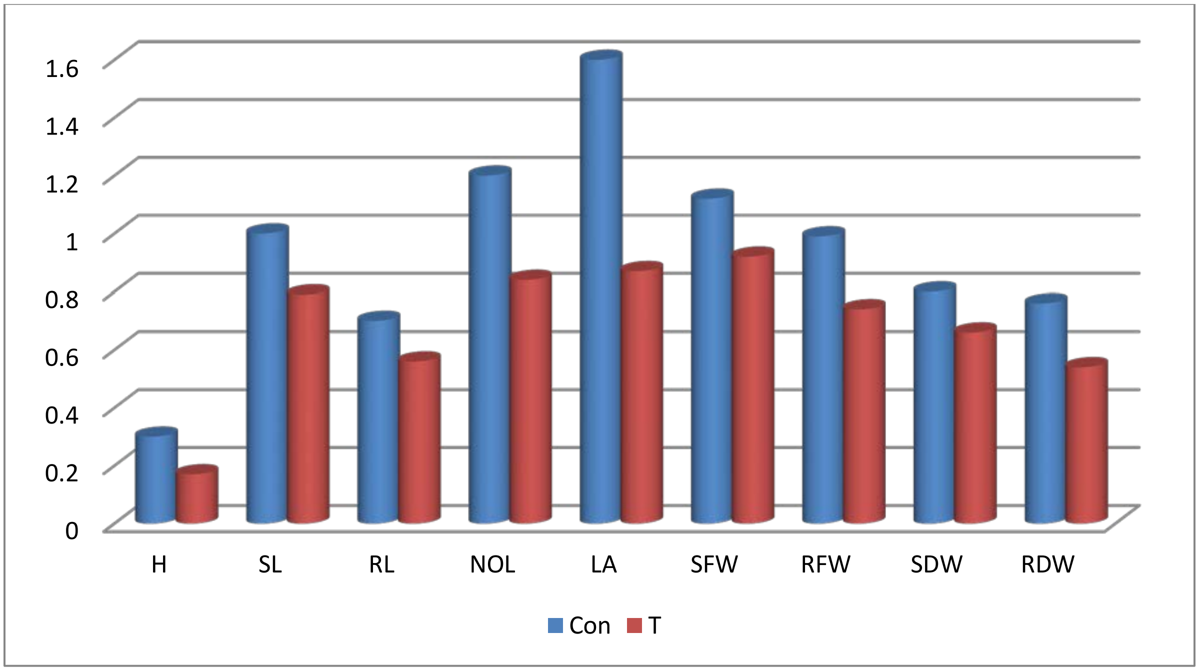


**Figure-1 Graph showed the decline effects of treatments on the growth variables**
***Con= Control; T= Treatments**



## 4. DISCUSSION AND CONCLUSION

The results from this trail experiment was supported the hypothesis that if the sodium chloride concentration in soil increases, then the vegetative growth and development of plant body will be decreased. With high sodium chloride concentrations/grades, the Brassica sp was showed reduction in height, mass, number of leaves and leaf area index. The plants grown in strongly saline environments were significantly shorter, lighter and had significantly fewer numbers of leaves than the non-saline, slightly saline and moderately saline treatment plants. It was also observed that seeds had delayed in germination of strongly saline environments. The study was the continuity and the strong evidence followed by [23].

Besides on these results, the effects of sodium chloride may not be gradual; rather there might be a threshold of sodium chloride tolerance between the moderate salinity and the strong salinity at which the plants could not flourish. The strong saline plants germination was likely delayed due to highly osmotic potential difference among the soil micelles and the germplasm.

Because to high concentration of salt the osmotic potential of the soil is intend to decrease so much, then instead of water absorption into the seeds, the water content was moved from the seed into the soil micelles. So the protoplast of the seed was shrinked due to ex-osmosis and the germination of the seeds was delayed and that was followed by [18].

The height, number of leaves, mass and the leaf area index of the strongly saline plants stems were strongly affected by the chloride. Chloride was accumulating in the stem of the plant and dehydrating the plants. The dehydration caused the plants to stress and stopped the growth and development of height and the number of leaves. The mass was lowered by dehydration; without as much water absorbed in the plants, the mass was less. The osmotic stress caused by chloride results in the plant pushing water out of the plants roots to balance ion concentration in the soil causing dehydration to the plants. Osmotic stress prevents nutrient uptake, causing reduced height, number of leaves, mass and the leaf area index. The root lengths of the strongly saline plants were shorter, likely because high concentrations of salt particles in the soil damage the tissue of the roots and inhibit growth. The salt particles surroundings the roots cause local dehydration and lead to damage of the root system. All these results were confirmed and the supportive documents of [24] and [25].

This study provides important information and the continuity of the previous research about the impacts of sodium chloride on soil and vegetation. The results showed that plants growth was stunted by saline environment. This suggests that when sodium chloride is used in tanneries in urban areas and this wastage was used for the purpose of cultivation, then the surrounding vegetation may be negatively affected. Plants are filters for ground water, absorbing pollutants and keep them from contaminating drinking water. Plants also provide oxygen to many organisms, including man. Furthermore, vegetation helps to prevent soil erosion. If plants are damaged of killed by sodium chloride, it will be easier for erosion to remove the top superfacial layer of soil. Based on the data, sodium chloride is harmful, when used as a deicer, suggesting a need for alternative methods of clearing ice and snow from roads.

From this trial it was concluded that the salinity was the problem for the growth and development of the plants as well as vegetation. So before the cultivation of any crop in the soil, the soil was checked physically and chemically from the Soil and water testing laboratory at any district unit level. If the laboratory confirmed the high salinity level in the soil then subject to add $CaCO_3$ in that soil for obtaining a good yields of the crops.

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