



## Impact of Salinity Stress on Germination and Growth of Pea (*Pisum sativum* L) Plants

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**Abstract:** The aim of the present study was to evaluate the effects of salinity stress on germination and growth of pea (*Pisum sativum* L) plants. A laboratory experiment was conducted to evaluate the effect of salinity stress on germination and growth of pea *Pisum sativum* L plant. Seeds of pea were sown in Petri dishes and pots and treated with four different levels of salinity (0, 50, 100, and 150mM NaCl) with completely randomized designs in four replications. Results revealed that seeds of pea were able to germinate at low salinity levels (NaCl 50mM NaCl) without a significant decrease in germination and growth traits, at the same time as a severe decrease in those traits were recorded at higher levels of salinity (100 and 150mM NaCl). The results indicated that seed germination and seedling establishment were inhibited due to the decrease of water potential, which results in the decline in water uptake by seeds, and seed germination was prevented by a high level of salinity stress (150mM NaCl). The results pointed out that germination percentage (GP), mean daily germination (MDG), germination speed (GS), and vigor index (SVI) varied under moderate and high salinity levels. All the studied parameters were reduced with increasing the NaCl level. The max and min GP, MDG, GS, and SVI were observed under control conditions (0mM NaCl) and highest salinity level (150mM NaCl) respectively. The same trend was seen in plant growth traits including: plant height, branch number, leaf number, leaf area, and shoot fresh and dry weight. The results provided important reference information for research on the impact of salinity on germination and growth of pea.

**Keywords:** Pea (*Pisum sativum* L.); Salinity Stress; Germination; Growth; Seedling Vigor Index.

### INTRODUCTION

Salt injure is one of the most important abiotic stress that affects plant productivity worldwide. About 20% of the global land area and over 50% of agricultural irrigated land is salt-affected soils (Cheng et al., 2016). It is also estimated that about 50% of agricultural land will be affected by salt in 2050 (Mahajan and Tuteja, 2005; Yan et al., 2005). Soils become saline when the soil salt concentration reaches about 40mM NaCl (Munns and Tester, 2008). The majority of crops are highly susceptible to saline soil. Crops are typically sown within the top 10-15cm layer of top soils. These layers are more saline than lower layers (Esechie, 1995).

Therefore, seeds show irregular germination and poor seedling development. In most crop plants, the yields start decreasing even at fairly low salinity in soil with electrical conductivity of (EC<sub>se</sub>> 1 dS/m) (Chinnusamy et al. 2005). Saline soils affect the growth of crop plants in two different ways (I) raising the osmotic pressure of soil solution, which results in an additional decrease in the physiological availability of water to the plant and, (II) the accumulation of toxic quantities of various ions within the plant (Hayward and Wadleigh, 1949). Salinity decreases seeds' ability to absorb water and causes a decrease in germination and plant growth, which leads to

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changes in plant metabolic processes (Munns, 1993; Munns, 2002). Seed germination and seedling growth are the most sensitive stages to salt stress (Bhattacharjee, 2008; Hubbard et al., 2012). Salt stress affects seed germination through osmotic stress, ion-specific effects, and oxidative stress (Hayward and Wadleigh, 1949). Salt stress influences the seed germination and plant growth of many plants. Many studies found that seed germination and early establishment of seedlings were inhibited by increasing salinity stress in different crops such as - wheat (Sadak, 2016; Mujeeb-Kazi et al., 2019), cabbage (Sarker et al., 2014; Yan, 2015), maize (Konuskan et al., 2017), and cowpeas (Abdel-Haleem and El-Shaieny, 2015),

Pea (*Pisum sativum* L.) plant is an important winter season vegetable legumes grown in Libya and the Mediterranean region and used as a source of protein vitamins, minerals, salts, and antioxidants (Nuttonson, 1961; Noreen and Ashraf, 2009). It's economically grown and used for dry grain or fresh fruit (Kaya et al., 2002). Also, the pea plant is grown as a forage crop for farm animals, as a green compost crop for improving soil, and as a cover crop for reducing soil erosion (Wolde and Adamu, 2018).

Pea plants can be grown on a wide range of soil types, from light sandy to heavy clay (McKay et al., 2003). Pea is a legume and has the inherent ability to obtain its nitrogen requirement from the atmosphere by forming a symbiotic relationship with Rhizobium bacteria in the soil (Schatz and Endres, 1999). The pea plant has quite a short growing season and uses less water than many other broadleaf crops (Johnson et al., 2002). However, the plant has been accounted as a salt-sensitive plant as compared with other legumes, such as broad bean, common bean, and soybean (Zahran, 1999; Khan et al., 2015). Nevertheless, the effect of salinity on growth and yield of peas has been investigated in many studies (Hernhdez et al., 1995; Martí, 2011; Pandolfi,

2012; Shahid, 2012; Husen et al., 2016; Wang et al., 2016; Desoky et al., 2017), however the impact of salinity on germination and seedling growth has not been well investigated and the relative importance of the effects of salt stress on seed germination of peas is not clear. Therefore, the aim of the present study was to evaluate the effect of salinity on germination and growth of pea plants.

## MATERIAL AND METHODS

This study was carried out at the Department of Plant Science, University of Zawia. Pea seeds were obtained from the local market. Salinity concentrations were (0, 50, 100, and 150mM NaCl) prepared using NaCl and fresh water. The electrical conductivities of NaCl solutions were 4.4, 8.3, and 15.3 dS m<sup>-1</sup>, and fresh water served as a control. For this research, a pair of experiments were conducted. A laboratory experiment to evaluate the effect of salinity stress on seed germination of peas, and a pot experiment to evaluate the influence of salinity stress on seedling growth of pea plants.

**Experiment I:** Pea seeds were first sterilized for 5 min with 5 % commercial bleach (Pandolfi et al., 2012), then thoroughly washed with distilled water. Germination tests were conducted in Petri dishes (containing one Whatman filter papers with 20 ml of respective test solutions) with four treatments by four replications (10 seeds per replication). The Whatman filter papers were replaced every 2 days to prevent the accumulation of salts. Seeds were allowed to germinate at room temperature and in darkness for 10 days. During this period, the Petri dishes were monitored daily, and 5 ml of the appropriate solution was added to the Petri dishes. A seed was considered to have germinated when the emerging radicle was 10 mm long (Cokkizgin, 2013).

**Experiment II:** A pot experiment was conducted to investigate the effect of salinity on the growth of pea plants. Pea seeds were

grown in pots filled with loamy soil, which was collected from the soil surface (0-10 cm). The soil was air-dried and passed through a 5-mm mesh screen, and filled in small plastic pots without a leaching possibility. Four pea seeds were sown in each pot and irrigated with fresh water for 10 days. After seedling establishment, only two seedlings of each pot were kept. Pots were then divided into four groups with four replications. Each group represented one saline treatment, which includes 0, 50, 100, and 150 mM NaCl. Pots were kept under semi-controlled conditions and irrigated with an appropriate saline solution for 30 days. At the end of the experiment, one plant from each pot was collected and used for data collection.

**Data Collection:**

**Germination traits:** Germinated seeds were counted daily for 10 days, and the number of germinated seeds was recorded every 24 h for each replicate of the treatment. After 10 days the germination percentage (GP) was calculated using the formula below (Nasri et al., 2011).

$$GP \% = (NSG \div TNSS) \times 100$$

Where *NSG* is the number of seeds germinated. *TNSS* is the total number of seeds sown.

The germination speed (GS) was calculated according to the equation given by Rubio-Casal *et al.* (2003). The number of germinated seeds was recorded every day from sowing, and lasted for 10 days, and was used to calculate GS. The following formula was used to calculate GS:

$$GS = n_1/d_1 + n_2/d_2 + n_3/d_3 + \dots$$

Where  $n_i$  is the number of seeds germinated in day one of sowing,  $d_i$  is the number of days taken for germination from the day of sowing.

Mean daily germination (MDG) was calculated as per Gairola *et al.* (2011). The following formula was used to calculate MDG:

$$MDG = TNGS \div TNDG$$

Where *TNGS* is the total number of germinated seeds and *TNDG* is the total number of days taken for final germination.

**Growth traits:** Morphological traits include plant height, number of branches per plant, number of leaves per plant, leaf area, and aboveground fresh biomass weight per plant, and their dry weight. Aboveground fresh and dry biomass weight were subsequently measured from 4 uniform seedlings per each treatment at the seedling stage. The plant height was measured using a measuring ruler from the surface of the soil to the top of the last leaf blade. The leaf area was calculated by measuring each leaf. Each leaf has two leaflets (left, and right), and the traits length (L) and maximum width (W) of leaflets were measured using a measuring ruler. The product of the length times width (LW) of the leaflet was calculated. The fresh weight of aboveground biomass was recorded using a weighing balance and then dried in an oven at 50 °C till a stable weight had been attained. Subsequently, the aboveground dry weights were recorded using a weighing balance. Using the morphological traits, the salinity tolerance index (STI) and seedling vigor index (SVI) were calculated as follows.

Salinity tolerance index (STI) was calculated according to the equation given by Tregay *et al.* (2014).  $STI = \text{Seedling dry weight of NaCl treated} / \text{seedling dry weight in control} \times 100$ .

Seedling vigor index (SVI) was calculated according to the equation given by Abdoli *et al.* (2013)  $SVI = \text{Seedling length (cm)} \times \text{germination percentage} / 100$ .

**Statistical analysis:**

The experimental design was completely randomized (CRD) with four replications. Analysis of variance was performed using the generalized linear model (GLM) procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for seed germination and growth related traits. Separation of means was carried out using the least significant differences (LSD;  $P < 0.05$ ). The means were compared using Duncan's multiple range test.

## RESULTS

The Probability values for germination and plant growth traits obtained with SAS PROC GLM are presented in Table 1. The obtained results clearly illustrated that salinity stress induced a significant reduction in germination and growth parameters in pea plants. Significant reduction ( $P < 0.05$ ) was observed in almost all the studied germination and plant growth traits when pea plants were grown under salinity stress. Germination percent, mean daily germination, germination speed, plant height, number of branches, number of leaves, leaf area, fresh and dry branches weight, salinity tolerance index and vigor index were the traits which showed significant differences as shown in Table 2.

**Table (1):** Probability values of the effects of Salinity (S) on germination and plant growth traits of pea plants.

Traits	Salinity (S)
Germination percent (%)	0.0458
Mean daily germination (MDG)	0.0453
Germination speed	0.0425
Plant height (cm)	0.0495
number of branches	0.0357
number of leaves	0.0416
Leaf area (cm <sup>2</sup> )	0.0477
Shoot fresh weight (g)	0.0504
Shoot dry weight (g)	0.0370
Salinity tolerance index	0.0355
Vigor index	0.0348

**Germination traits:** Although, the germination percentage and speed of germination were not significantly affected at a low salinity level (50mM NaCl), however, germination traits including germination percentage, speed of germination, and mean daily germination, decreased gradually with increasing salinity stress levels. Under a salinity level of 150mM NaCl, germination percentage, speed of germination, and mean daily germination

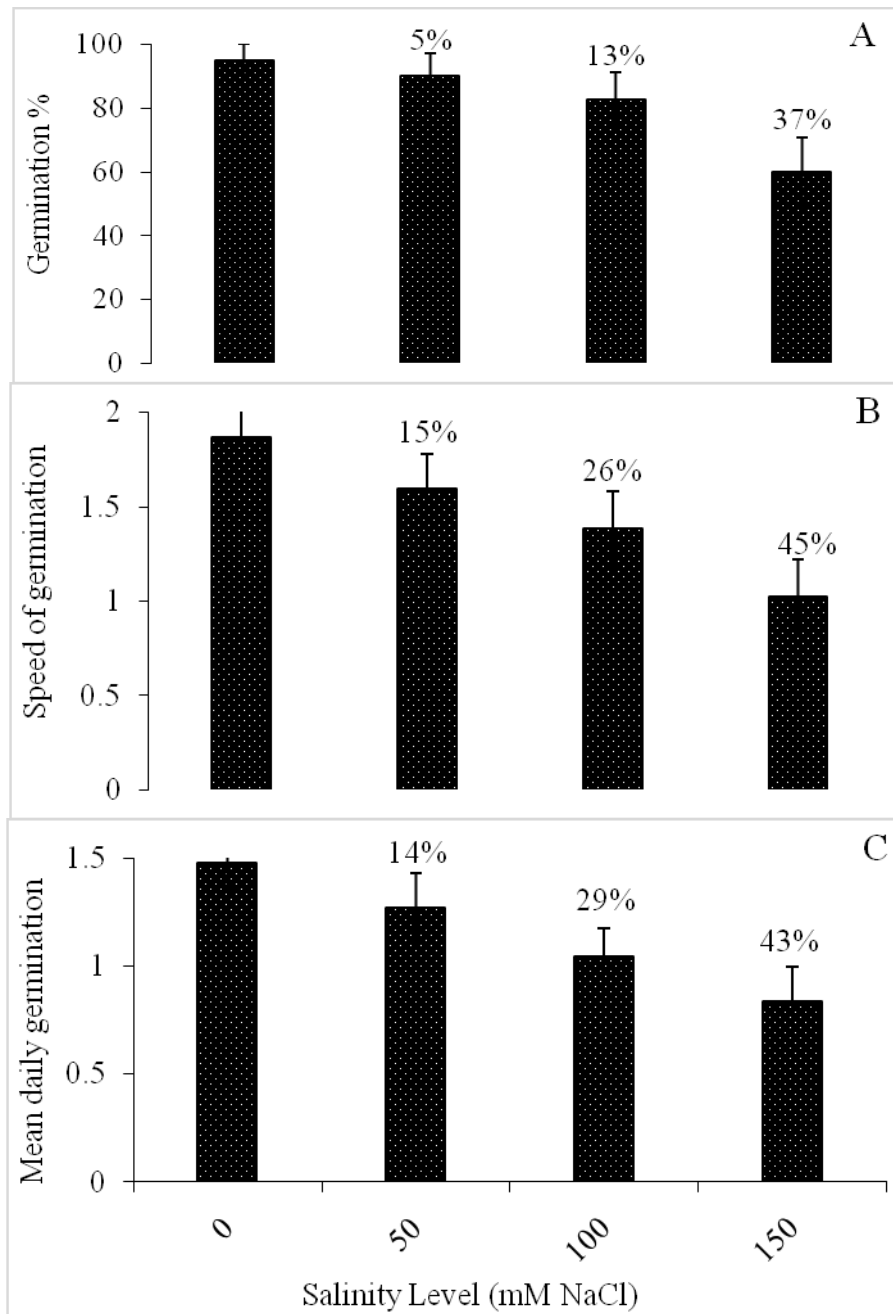
decreased by 37 %, 45 %, and 43 % respectively (Fig 1a, b, and c).

**Table (2)** The effect of Salinity (S) on germination and plant growth traits of pea plants..

Traits	Salinity Level mM NaCl			
	0	50	100	150
Germination percent (%)	95 <sup>a</sup>	90 <sup>a</sup>	83 <sup>ab</sup>	60 <sup>b</sup>
Mean daily germination	1.5 <sup>a</sup>	1.3 <sup>ab</sup>	1 <sup>ab</sup>	0.8 <sup>b</sup>
Germination speed	1.9 <sup>a</sup>	1.6 <sup>a</sup>	1.4 <sup>ab</sup>	1 <sup>b</sup>
Plant height (cm)	14.9 <sup>a</sup>	13 <sup>a</sup>	8.6 <sup>ab</sup>	5.6 <sup>b</sup>
number of branches	4.5 <sup>a</sup>	3.5 <sup>ab</sup>	2 <sup>b</sup>	1.5 <sup>b</sup>
number of leaves	10.5 <sup>a</sup>	8.5 <sup>ab</sup>	5.5 <sup>bc</sup>	3.5 <sup>c</sup>
Leaf area (cm <sup>2</sup> )	23 <sup>a</sup>	19 <sup>ab</sup>	12 <sup>ab</sup>	8 <sup>b</sup>
Shoot fresh weight (g)	4.8 <sup>a</sup>	4 <sup>ab</sup>	2.8 <sup>ab</sup>	2 <sup>b</sup>
Shoot dry weight (g)	2.6 <sup>a</sup>	1.9 <sup>ab</sup>	1.3 <sup>ab</sup>	0.7 <sup>b</sup>
Salt tolerance index	100 <sup>a</sup>	84 <sup>a</sup>	54 <sup>ab</sup>	27 <sup>b</sup>
Vigor index	14 <sup>a</sup>	12 <sup>a</sup>	7 <sup>ab</sup>	4 <sup>b</sup>

Individual value is the mean of 4 plants under different salinity levels. Values followed by different letters are significantly different according to Duncan's multiple range test ( $P < 0.05$ ).

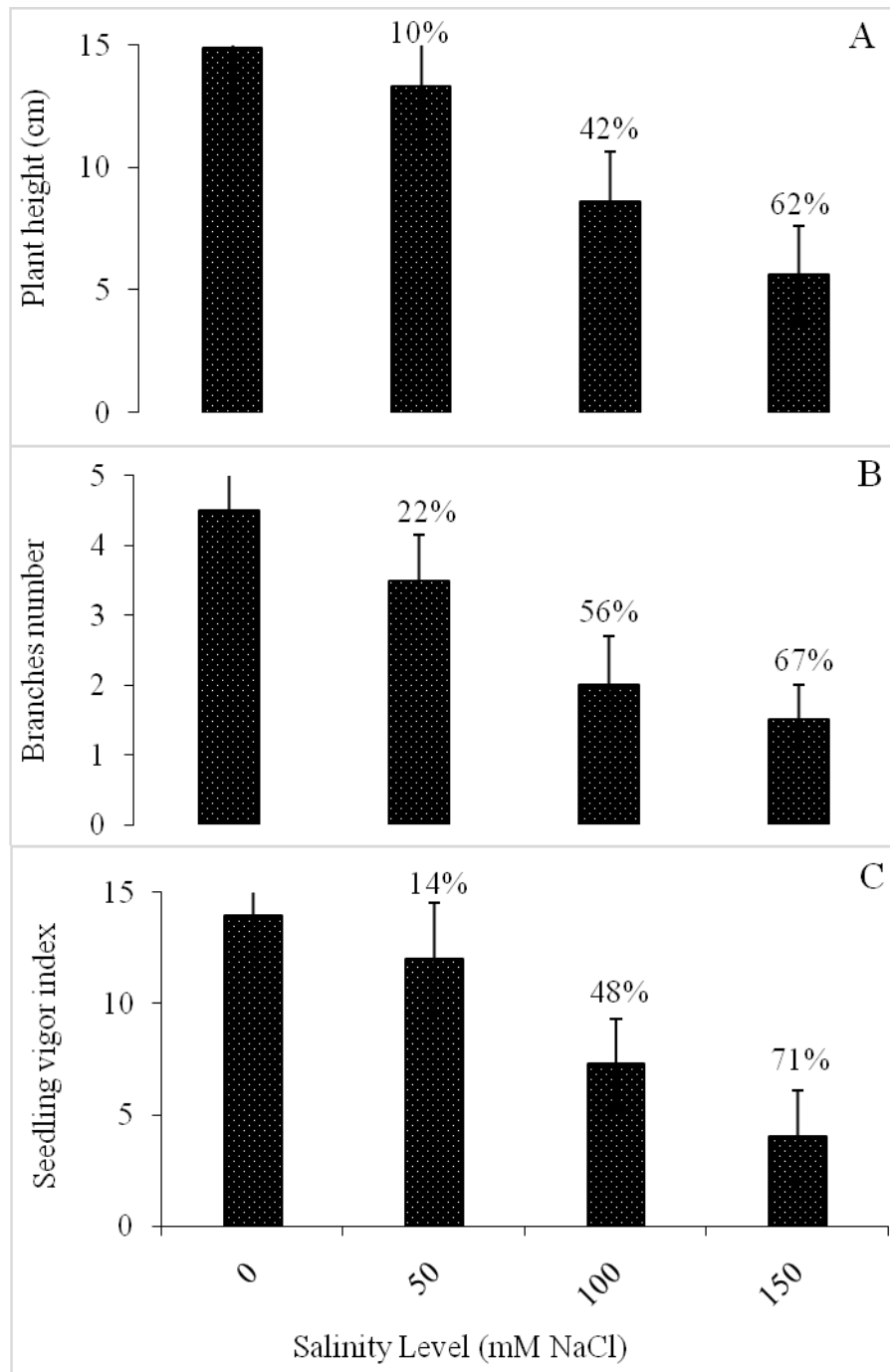
**Growth traits:** Growth data presented in Tables 1 and 2 show that plant growth traits were significantly affected ( $P < 0.05$ ) by salinity stress. The data pointed out that under increased salinity levels, the plant height and number of branches per plant was significantly reduced. At a high level of salinity (150mM NaCl), both traits (plant height and branch number per plant) were reduced by 62% and 67% respectively (Fig 2a and b).



**Figure (1)** The effects of salinity treatments on (A) germination percentage, (B) speed of germination and (C) mean daily germination of pea plant. Each datum indicates mean value, and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

Raising the salinity level from 50 to 150mM NaCl gradually decreased the vigor index. The highest vigor index was observed in control, while salinity at 50, 100, and 150mM NaCl decreased the vigor index. A significant

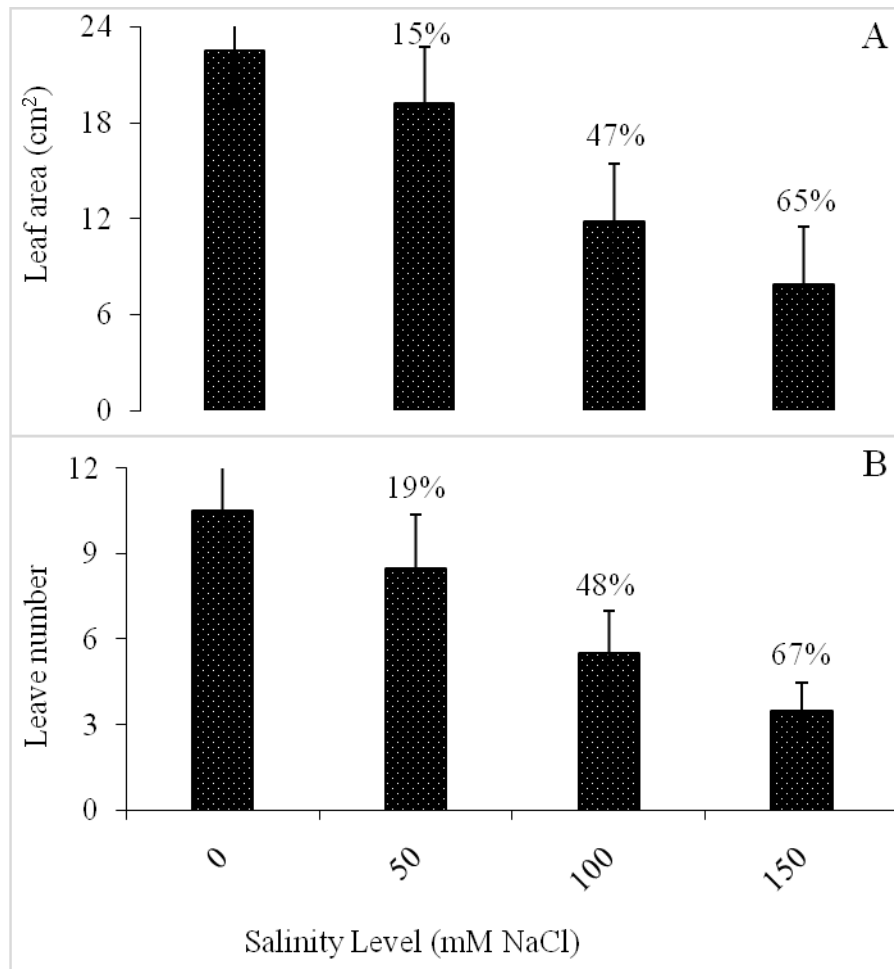
decrease in the vigor index was observed at 150 mM NaCl salinity, which caused a 71 % reduction over the control (Fig 2c).



**Figure (2):** The effects of salinity treatments on (A) plant height (cm), (B) branch number and (C) seedling vigor index of pea plant. Each datum indicates mean value, and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

The same reduction tendency was seen in leaf number per plant and leaf area per plant. As shown in Figure 3a and b, both traits, leaf number per plant and leaf area per plant, were strongly influenced by the high salinity level.

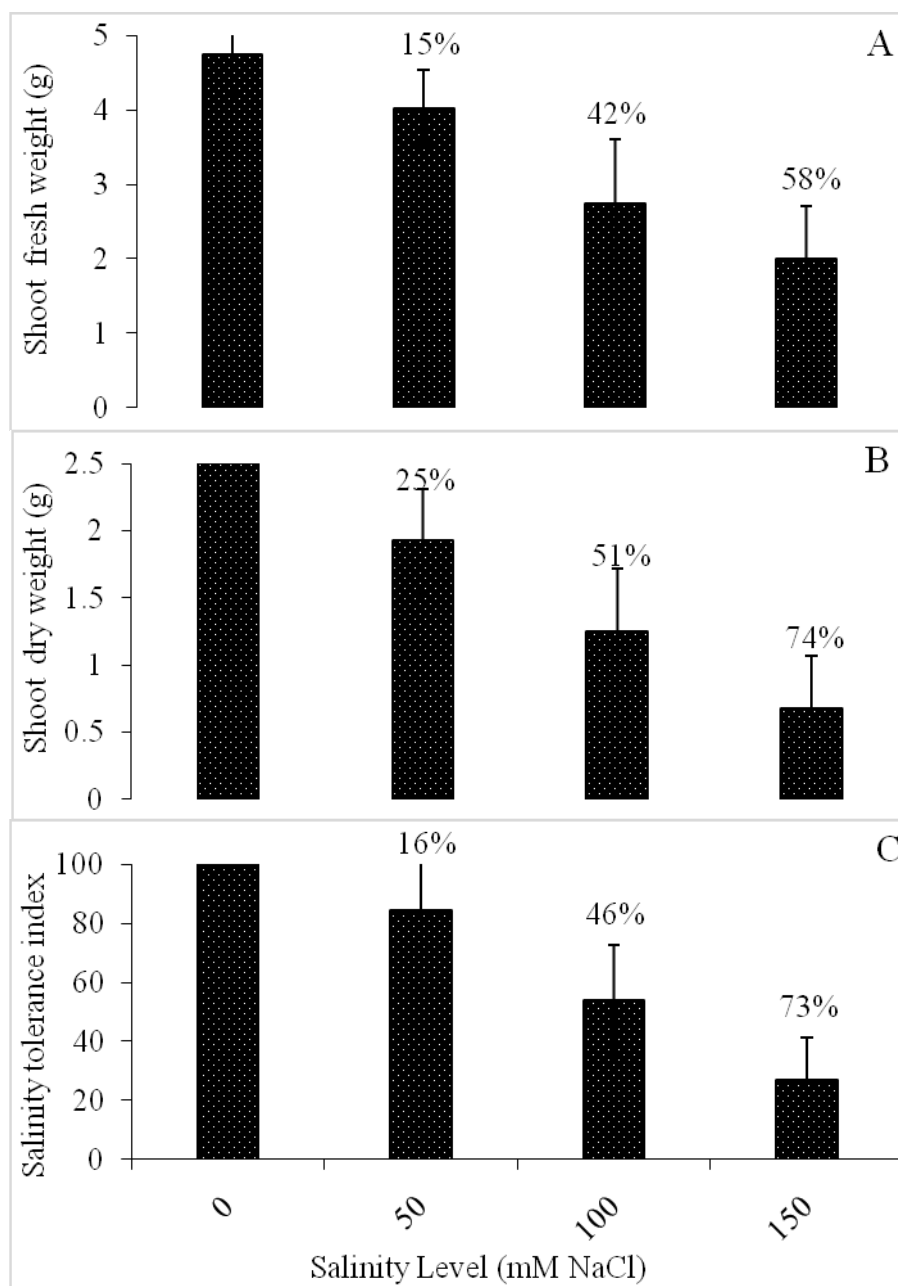
The percent reduction over the control of leaf number per plant and leaf area per plant were 65 % and 67 % respectively (Fig 3a and b).



**Figure 3** The effects of salinity treatments on (A) leaf area (cm<sup>2</sup>) and (B) leaf number of pea plant. Each datum indicates mean value, and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

In addition, the result reported that salinity stress significantly decreased aboveground biomass in pea plants in terms of shoot fresh and dry weight per plant. Nevertheless, the shoot dry weight was more strongly affected than shoot fresh weight. At a high salinity level (150mM NaCl), the aboveground fresh weight decreased by 58 % compared with control (0mM NaCl), and likewise, aboveground dry weight decreased by 74 % compared with control (Fig 4a, and b). Results regarding the salt tolerance index (STI) of pea plants showed that pea plants were able to deal with a low salinity level (50mM NaCl), however as the salinity level increased, pea plants became more sensitive to salinity stress. As shown in

the result. At 150mM NaCl, the salt tolerance index decreased by 73 % as compared with control (Fig 4c.).



**Figure: (4).** The effects of salinity treatments on (A) shoot fresh weight (g), (B) shoot dry weight (g) and (C) salinity tolerance index of pea plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control

## DISCUSSION

In the present study, the adverse effect of salinity decreased germination and growth of pea plants as shown in Table 2. Germination and seedling stages are critical life stages for plant survival and appropriate seedling establishment, particularly under stress conditions. The findings of this study indicated

that seeds germination and establishment of pea seedlings were inhibited gradually by increasing salinity stress. At a high salinity level of 150mM NaCl, seed germination was completely inhibited. In this respect, many studies reported that increasing salinity level decreased germination percentage and germination speed in field pea (Wolde and Adamu, 2018)), chick-pea (Ashraf and



Waheed, 1992) wheat (Majid et al., 2013) and other legumes (Esechie, 1995; Morais et al., 2012; Piwowarczyk et al., 2016). Salinity delayed and prevented seed germination through various factors, for instance, a decrease in water uptake, changes in the mobilization of stored food, and disturbing the structural organization of proteins (Ibrahim, 2016). In addition, the present study showed that plant height, branch number, leaf number and area, fresh and dry biomass was severely reduced as salinity level increased and the death of plants was noticed at the high salinity concentration (150mM NaCl) after 4 weeks of plant establishment. These results were consistent with previously published research (Grozeva et al., 2019). From the result of this study, it is evident that the toxicity in the salinity treatments is expressed more clearly in dry weight. This finding supports early findings which indicated that growth inhibition by NaCl treatments was greater for dry biomass production (Cordovilla et al., 1999; Hussain et al., 2002; López-Aguilar et al., 2003). The reduction of growth traits may be attributed to the osmotic effect of salinity stress which causes a decrease of growth promoters (Desoky et al., 2017). Also, the growth inhibition could be due to water deficit, ion toxicity, and nutrient imbalance due to the blockage of other nutrients such as N, P, K, Ca, and NO<sub>3</sub> (Hasegawa et al., 2000). Other studies have shown similar result in pea plants (Grozeva et al., 2019), and other legumes plants such as pea plant (Hernandez et al., 1999), chick-pea (Ashraf and Waheed, 1992), and sesbania (Mahmood et al., 2008) when grown under salinity condition. The result showed a decreasing leaf number per plant, physiologically, salinity stress has a negative impact on many processes, however, the most significant effect is reducing cell division and expansion, which caused a reduction in leaf number. Moreover, the result herein pointed out that a high salinity level caused a reduction in leaf area, which may have resulted due to a reduction in cell division and cell extension. These results agree with another result that

reported that salt stress causes a reduction in leaf surface expansion ratio, leading to the cessation of expansion as salt concentrations keep increasing (Wang and Nii, 2000).

## CONCLUSIONS

Food productivity is decreasing due to the effect of various abiotic stresses. Cold, heat, salinity, and drought are among the major stresses, which adversely affect plants' growth and productivity. For that reason, reducing these losses is a main area of concern for all crop producers to manage increasing crop production. This study was aimed to investigate the impact of salinity stress on pea plants. The study showed that high salt stress inhibited and delayed seed germination and growth of pea plants. The study concluded that pea plants (*Pisum sativum* L.) are resistant to 50 and can withstand salinity at 100 mM NaCl, but this cultivar is strongly sensitive to 150mM NaCl, and damages of salt-stress were significantly observed. However, plant growth was more sensitive to salt stress than germination. Future research must concentrate on molecular, physiological, and metabolic changes induced by salinity stress. Also, comprehensive information is required to understand the physiological responses of this plant under field conditions.

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## تأثير إجهاد الملوحة على إنبات ونمو نباتات البازلاء *Pisum sativum* L

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**المستخلص:** الهدف من الدراسة هو تقييم تأثيرات إجهاد الملوحة على إنبات ونمو نبات البازلاء. التجارب المعملية أجريت على نبات البازلاء *Pisum sativum* L. حيث زرعت بذور البازلاء في أطباق بيترى وفي أصص وعولجت بأربع مستويات مختلفة من الملوحة (0، 50، 100، و 150 ملي مولار من كلوريد الصوديوم). نفذت التجارب وفق التصميم العشوائي الكامل بأربعة مكررات. أظهرت النتائج أن بذور البازلاء كانت قادرة على الإنبات عند مستويات ملوحة منخفضة (50 ملي مولار من كلوريد الصوديوم) دون انخفاض ملحوظ في الإنبات، وبعض صفات النمو، وفي الوقت نفسه أشارت النتائج إلى وجود انخفاض حاد في هذه الصفات عند مستويات ملوحة أعلى (100 و 150 ملي مولار من كلوريد الصوديوم). كما أوضحت النتائج أن ارتفاع مستوى الملوحة قد نتج عنه تثبيط لعملية إنبات البذور وتطور البادرات وذلك بسبب انخفاض الجهد المائي مما أدى إلى انخفاض امتصاص الماء بواسطة البذور. وتم منع إنبات البذور بسبب مستوى عالٍ من إجهاد الملوحة (150 ملي مولار من كلوريد الصوديوم). وتشير النتائج إلى وجود اختلاف في كل من نسبة الإنبات، ومتوسط الإنبات اليومي، وسرعة الإنبات، ومؤشر قوة البادرة تحت مستويات الملوحة المتوسطة والعالية. كما تشير النتائج إلى انخفاض جميع الصفات المدروسة بزيادة تركيز محلول كلوريد الصوديوم. حيث سجلت النتائج أن الحد الأقصى لكل من نسبة الإنبات، ومتوسط الإنبات اليومي، وسرعة الإنبات، ومؤشر قوة البادرة كانت تحت ظروف (0 ملي مولار من كلوريد الصوديوم) والحد الأدنى الصفات نفسها كان عند أعلى مستوى للملوحة (150 ملي مولار من كلوريد الصوديوم). وقد لوحظ التأثير نفسه مع صفات النمو الأخرى والتي تشمل: طول النبات، وعدد التفرعات، وعدد الأوراق، مساحة الورقة، والوزن الطازج والوزن الجاف للمجموع الخضري. توفر نتائج هذه الدراسة معلومات مرجعية مهمة للبحث عن تأثير الملوحة على إنبات ونمو البازلاء.

**الكلمات المفتاحية:** البازلاء (*Pisum sativum* L.)، الإجهاد الملحي، الإنبات، النمو، مؤشر قوة البادرة.