استجابة نبات الفاصوليا (*Phaseolus vulgaris* cv) للإجهاد الملحي في مراحل امتصاص الماء والإنبات ونمو البادرات

د . أمال فرج احتيوش ، أ . عواطف علي أبوفليحة ـ كلية العلوم الزاوية ـ جامعة الزاوية

الملخص Abstract :

يعتبر الإجهاد الملحي من أهم المشاكل التي تواجه زراعة الكثير من المحاصيل في كثير من مناطق العالم خصوصا في المناطق الجافة وشبه الجافة. وتتأثر عملية امتصاص الماء والتي تعتبر من العمليات المهمة لإنبات البذور وعمليات التمثيل الغذائي في مرحلة الإنبات بملوحة الماء. وقد تم في هذه الدراسة تقييم تأثير الإجهاد الملحي على عملية امتصاص الماء وعلى عملية الإنبات ونمو البادرات لنبات الفاصوليا (*Phaseoulus vulgaris* cv). حيث زرعت بذور الفاصوليا في أطباق بتري (في مرحلة الإنبات) وفي أصص (في مرحلة نمو البادرات). عولجت البذوروالنباتات بثلاث تراكيز مختلفة من الملوحة (0 ، 100 ، 120 ملي مول كلوريد الصوديوم). صممت هذه الدراسة حسب النظام العشوائي الكامل بأربعة مكررات. أظهرت الدراسة الحالية أن الملوحة أثرت سلباً على نسبة امتصاص الماء وإنبات البذور ونمو البادرات. وأوضحت النتائج أن الإجهاد الملحي أدى الى انخفاض النسبة المئوية لامتصاص الماء ، كما أدى إلى انخفاض في صفات الإنبات ونمو البادرات خاصة عند مستويات الملوحة العالية. وأشارت النتائج إلى أن أعلى نسبة مئوية لامتصاص الماء وأعلى نسبة مئوية للإنبات وأعلى متوسط انبات يومي وأعلى سرعة إنبات عند المعاملة بتركيز 0 ملي مولار كلوريد الصوديوم. بينما سجلت أقل نسبة مئوية لامتصاص الماء وأقل نسبة للإنبات وأقل متوسط يومي للإنبات و أقل سرعة إنبات عند المعاملة بتركيز 120 ملي مول كلوريد الصوديوم. كما أوضحت النتائج أن ارتفاع النبات قد تغير بين 33-16 سم ، وتغير عدد الأفرع للنبات الواحد بين 10-4 فروع لكل نبات ، وتغير عدد الأوراق بين 26-9 ورقة لكل نبات، وتغير وزن النبات الطازج بين 23-8 جم ، كما تغير الوزن الجاف للنبات بين 7-2 جم في الفاصوليا بين 0-120 ملي مولار من ملح كلوريد الصوديوم. وأشارت النتائج إلى أن إنبات البذور ونمو وتطور البادرات قد تم تثبيطهم بسبب انخفاض الجهد المائي، مما أدى إلى

انخفاض امتصاص البذور للماء، كما تم تثبيط إنبات البذور بسبب ارتفاع مستوى االملوحة في الماء.

الكلمات الدالة: الفاصوليا (*Phaseoulus vulgaris* cv) ؛ الإجهاد الملحي؛ الإنبات؛ نسبة امتصاص الماء؛ ؛ نمو البادات.

The response of bean (*Phaseolus vulgaris* cv) Plants to Salinity Stress at Water Uptake, Germination and seedling stages

Amal F Ehtaiwesh*, and Awatef A Abuiflayjah[1]

[1]Department of plant science, University of Zawia, Libya

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1. Abstract

Salinity stress plays the main environmental limitation to crop productivity in the arid and semi-arid regions of the world. Water uptake which is an important issue of seeds germination and for metabolic processes is effected by water salinity. In this study the effect of water salinity on: water uptake, germination, and seedling growth of bean plant (*Phaseoulus vulgaris* cv) was evaluated. Seeds of bean were sown in petri dishes (for germination stage) and in pots (for seedling stage). Seeds were treated with three different concentrations of salinity (0, 100, and 120mM NaCl) with completely randomized design in four replications. The present study demonstrated that salinity adversely affected water uptake percentage, seeds germination and seedling growth. Results revealed that salinity decreased water uptake percentage, decreased germination and seedling growth parameters, particularly at high salinity level. The results

*Corresponding author. Amal Ehtaiwesh Tel: 0927869017/ /0914891754
E- mail address: a.ehtaiweshl@zu.edu.ly

indicated that the highest water uptake percentage, highest germination percentage, mean daily germination, and germination speed were found in 0 mM NaCl and the lowest water uptake percentage, germination percentage, mean daily germination, and germination speed were found in 120 mM NaCl. Also the results illustrated that plant height changed between 33-16 cm, number of branches changed between 10-4 branch Plant^{-1}, number of leave changed between 26-9 leaf plant^{-1}, plant fresh weigh changed between 23-8 g, and plant dry weigh changed between 7-2 g in beans between 0-120 mM NaCl salt concentrations. 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 high level of salinity stress.

Keywords: Beans (*Phaseoulus vulgaris* cv); Salinity stress; Water uptake percentage Germination; Seedling growth.

2. Introduction

Plant productivity depends on the availability of soil water, in respect of both quantity and quality. However; in arid and sem-iarid regions, water salinity imposes severe threats to plant growth, crop production and soil fertility (1). Salinity reducing crop production up to 35% and making shortage of food for human and animal consumption (2, 3, 4). Due to an increase in demand for irrigation in many regions worldwide with the decrease of fresh water resources, the use of saline water may be needed to maintain maximum agricultural productivity in such regions. However; soils become saline when the soil salt concentration reaches about 40mM NaCl (5). Different plant species show different levels of salinity tolerance that vary from sensitive to tolerant with their growth and development affected to different extents (6). Salinity usually causes an individual and synergistic effects of osmotic, ionic toxicity and nutritional imbalances, which may affect plant productivity (7). Seed germination is directly linked to the amount of water absorbed. Salinity can affect germination of seeds by making osmotic potential that may reduce or prevent water uptake. Salinity decreases seed ability to absorb water and causes a decrease in germination and seedling growth as well as changing plant metabolic processes (8, 9). Many studies indicated that plant sensitivity or tolerance to salinity may differ according to the plant growth stage (10, 11, 12, 13). The most important stages of plant life cycle are germination and seedling stage. However, both stages are negatively influenced by salinity stress (14, 15, 16). In addition, studies have indicated that salinity drastically affects most of physiological and biochemical processes such as photosynthesis process (5, 4), decreases in chlorophyll content (17), changes in transpiration rate, and stomata conductance (18, 19). Also salinity stress may disturb plant mineral nutrition causing an increase in sodium (Na^+) and decrease in potassium (K^+) contents in plant tissues (20). In addition, salinity stress has previously seen to cause a reduction in number of leaves, plant height and plant fresh and dry weight in different plants such as castor beans plant (21).

Beans are an annual legumes and one of the major crop worldwide that providing proteins, vitamins and minerals which are needed by humans and livestock (22). It is also preferred as a sustainable crop due to its ability to fix atmospheric nitrogen

(23). Worldwide, more than 30.4 million tons of dry grain and 24.8 million tons of green bean are produced annually (24). However, abiotic and biotic stress factors such as heat draught, salinity, cold, and pathogens are major constraints to bean production (25, 26, 27). Approximately 60% of beans produced world-wide are grown in areas exposed to water stress (28, 26). As of the limited water resources, it's important to use the available water resources which may have low or medium salinity level for irrigation. However, legumes are classified as a salt-sensitive crop (29). Yet; little attention has been given to improve its production under saline condition. In Libya the water resources for agriculture is limited, therefore, using the available water resources with low or medium salinity level is very important. Therefore, the purposes of this study were to evaluate the effects of water salinity on bean plant productivity and to estimate the salinity level that bean plant can tolerate.

3. Materials and Methods

The experiment was conducted at Alzahra farm under semi controlled environment. Bean seeds obtained from the local market. Salinity concentrations of (0, 100, and 120mM NaCl) prepared using NaCl and fresh water. The electrical conductivities of NaCl solutions were (8.3, and 12.3 dS m^{-1}, and fresh water served as a control. For this study two experiments were conducted, laboratory experiment to evaluate the effect of salinity stress on seed germination traits of bean, and pot experiment to evaluate the influence of salinity stress on seedling growth of bean plants.

3.1.**Experiment I**

Bean seeds were surface sterilized with for 5 min with 5 % commercial bleach, rinsed with fresh water several times, and briefly blotted onto paper and then let to dry (30). Then seeds were weighted (5g) and subjected to corresponding treatment as following: (0mM NaCl) value of < 0.7 dSm^{-1} control), 100 mM NaCl [EC] value of 8.3 dSm^{-1}) and 120 mM NaCl [EC] value of 12.5 dSm^{-1}. Seeds allowed for water imbibitions for 24h. During the imbibitions period (24h) water uptake was calculated after 6, 9, 12 and 24h (31). Each time the seeds were removed from the water solutions, followed by draining and blotting dry with a paper towel for 1 min and reweighing and water uptake percentage (WUP) was calculated. Four replicates of 10 seeds of beans were germinated in Petri dishes contain one Whatman filter papers with 10 ml of respective test solutions. The papers were replaced every 2 days to prevent accumulation of salts (30). The experimental design with the three treatments as following: Control – seeds germinated in fresh water, seeds germinated in a solution of 100mM NaCl, and seeds germinated in a solution of 120mM NaCl. Seed 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 appropriate solution was added to the Petri dishes. A seed was considered to have germinated when the emerging radicle elongated to 2 cm (32).

3.2.Experiment II

Pots experiment was conducted to investigate the effect of salinity on growth of bean plants. Seeds of bean were grown in pots filed with loamy soil and Peat moss (1-3). Loamy soil was collected from the soil surface (0-10 cm). The soil was air-dried and passed through a 5-mm mesh screen, mixed with Peat moss and filled in10 L plastic pots without a leaching possibility. 6 seeds of beans were sown in each pot and irrigated with fresh water for 7 days. After seedling establishment only three seedlings of each pot were kept. Then pots were divided into three groups with four replications, each group represents one saline treatment which include 0, 100, and 120mM NaCl. Pots were kept under semi- controlled condition and irrigated as needed with appropriate saline solution. Plants were harvested 40 days after sowing. At the end of the experiment one plant from each pot was collected and used for data collection.

3.3.**Data Collection**

3.3.1. Water uptake percentage

During the first 24h of seed socking water uptake was calculated after 6, 9, 12 and 24h water uptake percentage (WUP) was determined as following the equation (31). The increase in weight of the soaked beans was considered as result of water absorption.

WUP = W2-W1/W1 x100

Where W1= initial weight of seed, and W2= weight of seed after absorbing water in a particular time.

3.3.2. 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 was calculated using the formula below (33)

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

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 (34). The number of germinated seeds was recorded every day from the start of sowing and last for 10 days and used to calculate GS. The following formula was used to calculate GS:

$$GS = n1/d1 + n2/d2 + n3/d3 + \cdots$$

Where n_1 is the number of seeds germinated in day one of sowing, t_1 is the number of days taken for germination from day of sowing.

Mean daily germination (MDG) was calculated as per (35). 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.

3.3.3. **Growth traits**

Morphological traits include: plant height, number of branches per plant, number of leaves per plant, and plant fresh and dry biomass per plant were subsequently measured from 4 uniform seedlings from each treatment. The plant height measured by using measuring ruler from the surface of the soil to the top of last leaf blade. The fresh weight of plant was recorded using a weighing balance, and then dried in an oven maintained at 50 °C till it attains stable weight. Subsequently plant 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 following.

Salinity tolerance index (STI) calculated according to the equation given by (36).

STI= Seedling dry weight of NaCl treated / seedling dry weight in control x 100.

Seedling vigor index (SVI) calculated according to the equation given by (37) *SVI= Seedling length (cm) x germination percentage / 100.*

3.4.Statistical analysis

The experimental design was a randomized complete design (RCD) with four replications. Analysis of variance performed using generalized linear model (GLM) procedure in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) for water uptake percentage, 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.

4. Results

The P-values for water uptake percentage, germination and seedling traits obtained with SAS PROC ANOVA are presented in table 1. The analysis of variance for water uptake percentage, germination percent, mean daily germination, germination speed of bean showed that increasing NaCl concentrations had significantly ($p<0.05$) adverse effect on water uptake percentage, final germination, mean daily germination and speed of germination. Also, the varietal difference in plant growth parameters included in this study were significant ($P<0.05$).

Results of salinity stress on water uptake percentage, germination percent, mean daily germination, germination speed, plant height, plant fresh and dry weight, salinity tolerance index and seedling vigor index are presented in (Tables 2). Water uptake percentage, germination and seedling growth parameters decreased under both saline condition (100 and 120 mM NaCl) as compared with control (0mM NaCl).

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

Traits	Salinity (S)
Water uptake percentage (%)	0.0005
Germination percentage (%)	0.0320
Mean daily germination (MDG)	0.0312
Germination speed	0.0116
Plant height (cm)	0.0006
Number of branches	0.0023
Number of leave	0.0008
Shoot fresh weight (g)	0.0351
Shoot dry weight (g)	0.0205
Salinity tolerance index	0.0292
Vigor index	0.0002

Table:(2). The effect of Salinity (S) on germination and seedling growth traits of bean plants.

Traits	Salinity Level mM NaCl		
	0	100	120
Water uptake percentage (%)	155^{a}	140^{b}	130^{c}
Germination percentage (%)	88^{a}	67^{ab}	58^{b}
Mean daily germination	1.6^{a}	1.2^{ab}	0.98^{b}
Germination speed	2.1^{a}	1.6^{b}	1.3^{b}
Plant height (cm)	33.3^{a}	19^{b}	15.5^{b}
Number of branches	9.5^{a}	4.8^{b}	3.8^{b}
Number of leave	26^{a}	12^{b}	9^{b}
plant fresh weight (g)	23^{a}	14^{ab}	8^{b}
plant dry weight (g)	6.7^{a}	3.8^{ab}	2^{b}
Salt tolerance index	100^{a}	64^{ab}	35^{b}
Vigor index	29^{a}	13^{b}	9^{b}

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

Water salinity resulted in significant decrease in water uptake by seeds. The results showed that water uptake percentage was declined under both saline condition as compared with control. After 6, 9, 12 and 24h the percentage of water uptake under 100mM NalCl was decreased by 25 %, 15 %, 13 %, 10 % respectively over the control. At the same time, the percentage of water uptake under 120mM NalCl after 6, 9, 12 and 24h was decreased by 29 %, 22 %, 19 %, 16 % respectively over the control (Fig 1).

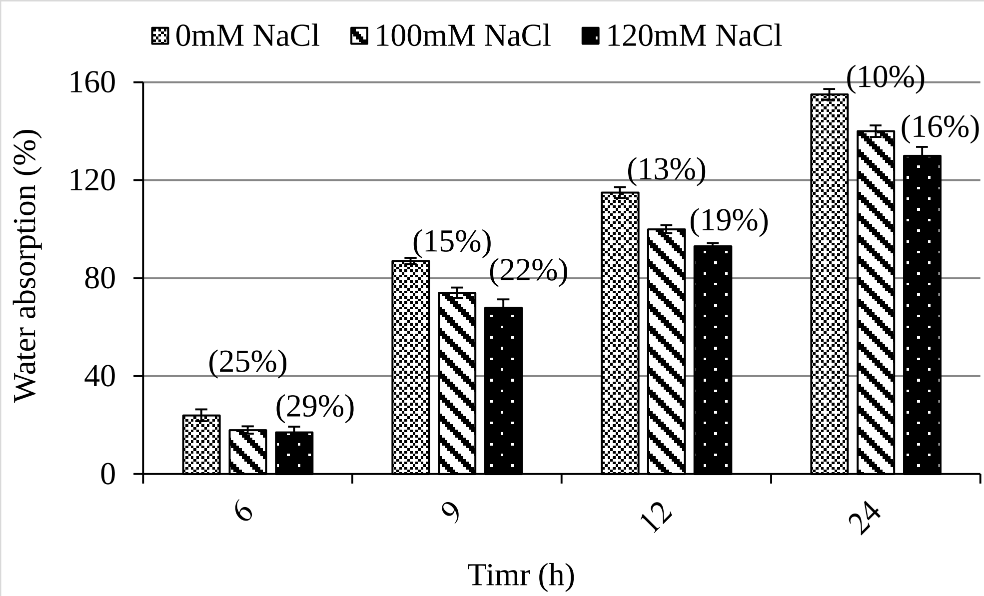
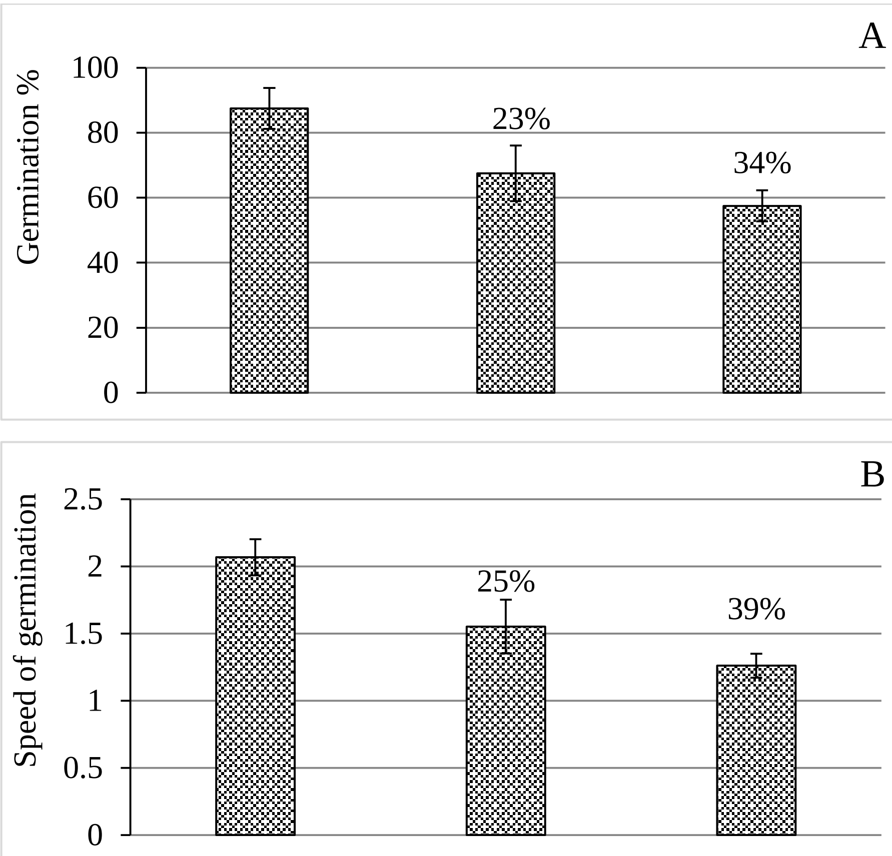


Figure (1). Water uptake percentage after 6, 9, 12, and 24h of bean plant as effected by salinity. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control

According to the results obtained from the Petri dishes trials salinity stress effect seeds germination. This decrease started appear at 100mM salinity levels. Yet the decrease was more pronounced at high level of salinity (120mM NaCl). Salinity level of 100 mM NaCl and 120 mM NaCl caused reduction in germination percentage by 23 % and 34 % respectively over the control ((Fig 2a). Also, speed of germination was decreased with respect to the control by 25 % and 39 % under salinity stress of 100 and 120 NaCl respectively (Fig 2b). The same trend was seen with mean daily germination which was decreased by 25 % and 38 % over the control ((Fig 2c).

Salinity affected the early development of the tested bean plants, and this effect was pronounced at plant height, number of leave and number of branches per plant. However, this effect was more pronounced at high level of salinity (120mM NaCl). According to the result obtained from the pots trials salinity stress caused reduction in plant height by 43% at 100 mM and by 53% at 120mM over the control (Fig. 3a). And branches number was reduced by 53% at 100 mM and by 61% at 120mM over the control (Fig. 3b). The same trend was seen with leave number per plant which reduced by 54% at 100mM and by 64% at 120mM over the control (Fig. 3c).



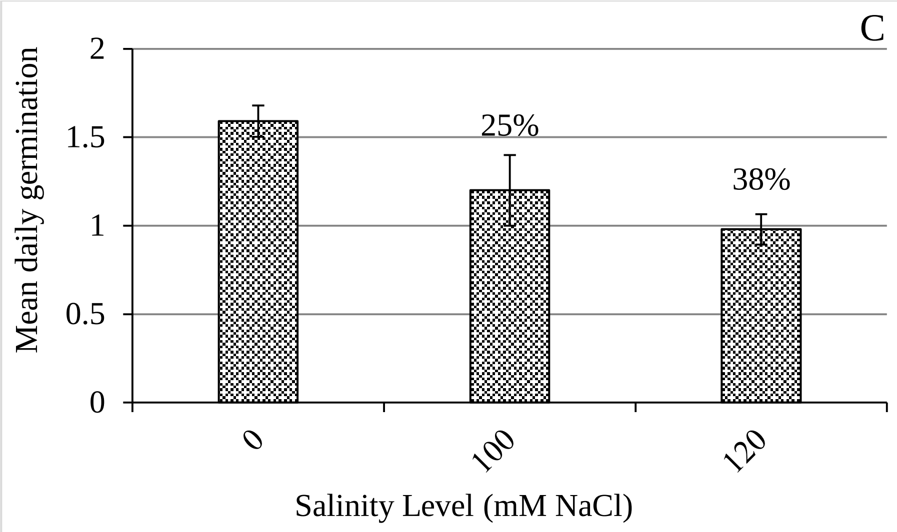
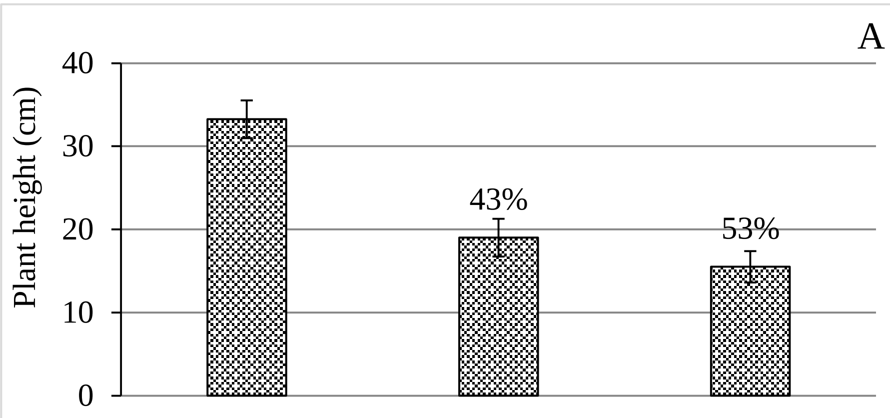


Figure: (2). The effects of salinity treatments on (A) germination percentage, (B) speed of germination and (C) mean daily germination of bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.



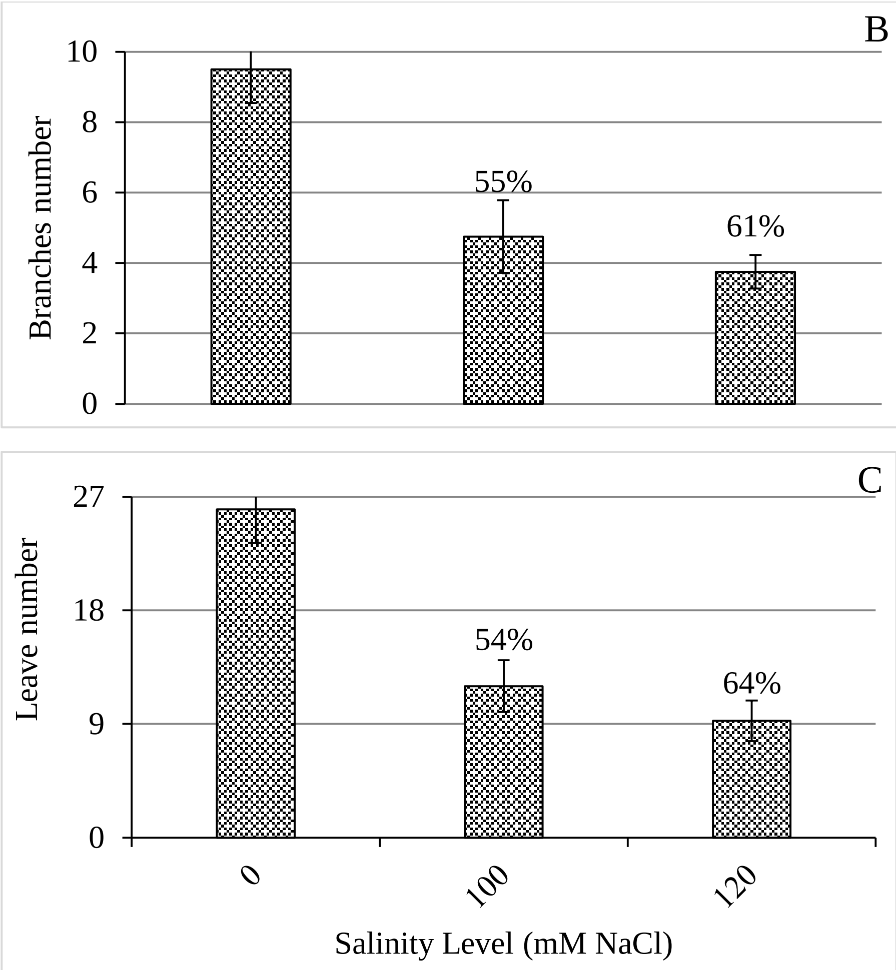


Figure: (3). The effects of salinity treatments on (A) plant height (cm), (B) branches number and (C) leave number of bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

In addition, salinity stress caused decreasing in fresh and dry weight of bean plants grown for 40 days under salinity condition. The fresh weight was less affected than dry matter accumulation. The fresh weight was reduced by 41% at 100mM and by 65% at 120% (Fig4a), whereas he reduction in dry weight was by 43% at 100mM and by 69% at 120% (Fig4b).

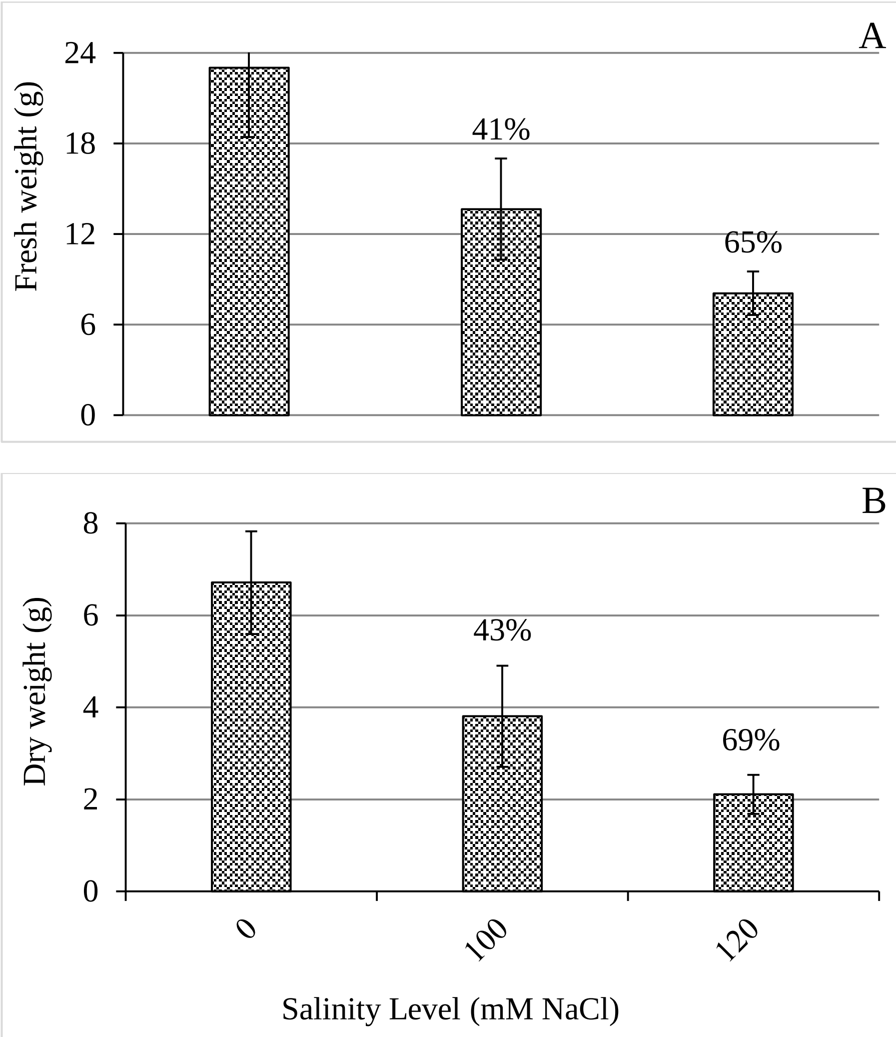


Figure: (4). The effects of salinity treatments on (A) plant fresh weight (g) and (B) plant dry weight (g) of bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

Moreover, the result pointed out that salinity tolerance index was significantly affected by salinity stress, the result showed that salinity tolerance index was significantly decreased as salinity level increases. The results showed that salinity tolerance index was reduced by 36% at 100mM and by 65% at 120mM NaCl (Fig 5a). Likewise, both salinity levels (100 to 120 mM NaCl increasingly decreased seedling vigor index. salinity level (100mM NaCl) showed small reduction on seedling vigor index. Figure 4b showed that the highest seedling vigor index was observed in control, while salinity at 120 mM NaCl decreased significantly seedling vigor index by 70 % (Fig 5b).

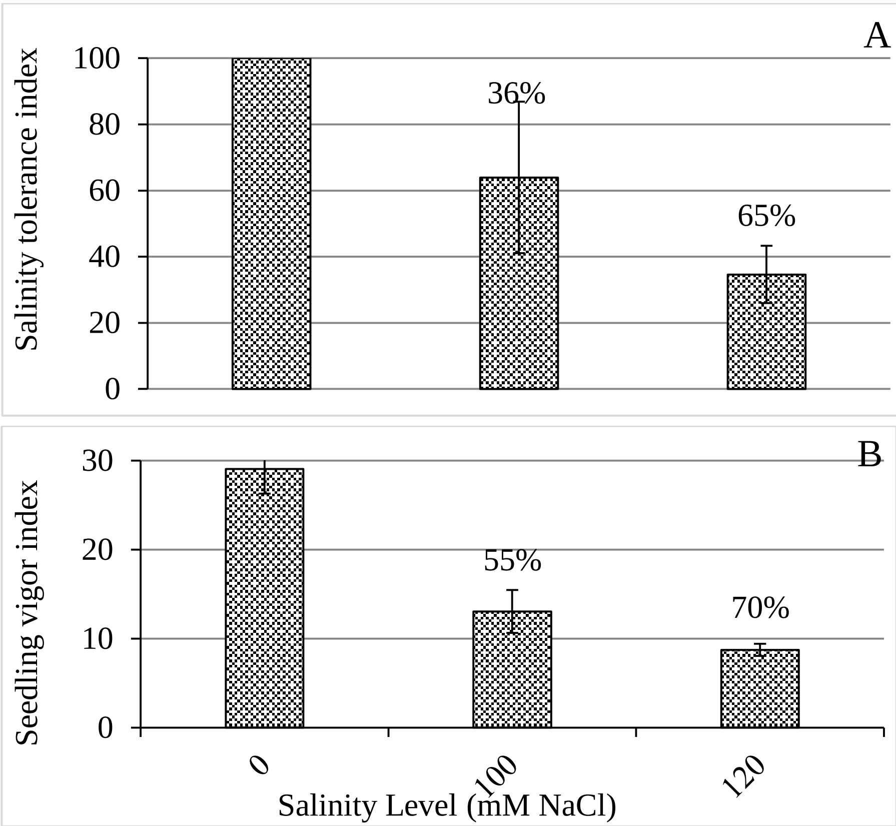


Figure: (5). The effects of salinity treatments on (A) seedling vigor index and (B) salinity tolerance index of bean plant. Each datum indicates mean value and vertical lines on top of bars indicate standard error of means (n = 4). Values in parenthesis indicates the percent reduction from control.

5. Discussion

The response of bean (*Phaseolus vulgaris* L.) plants to salinity stress at germination and seedling growth was investigated. The results herein found that salinity stress negatively affect seeds water uptake, germination and seedling growth parameters. According to our results, the effect of salinity stress on seeds water uptake and germination traits was statistically significant (Tables 1 and 2). The results in figures 1 and 2 were corresponded to these of other studies who also found that salinity stress reduced seed germination and seedling growth (38, 39, 40). Generally, water is taken up by the fine roots of plant by the process of osmosis, which involves the movement of water from states of low salt concentration to states of high salt concentration. However, when salt concentration in the soil is high, the movement of water from the soil to the root is delayed. Figures I and 2 showed that salinity affects water uptake, germination percentage, mean daily germination and germination speed because of the high confederation of salt in the medium which decreased osmotic potential to a point which delayed or prevented the uptake of water essential for nutrient mobilization necessary for germination. Also salinity affects germination because both Na and Cl ions might be toxic to the embryo. Many studies agreed that germination was directly related to the amount of water absorbed by the seed and the delay in germination as due to the salt concentration of the medium (41, 31,42). In addition; figures 3 and 4 reported that all seedling growth traits of bean plants included in the study were reduced due to salinity stress. The effects of salinity stress on seedling growth of bean was reported in early studies (43, 20, 21). The harmful effects of salinity stress on seedling growth of plants could be related to decrease of mobilization of reserve foods, suspending the cell division, and injuring hypocotyls (44). The decrease in growth traits could be a combined effect of the higher ion uptake and osmotic stress as reported in early studies (45, 46). This study conclude that salinity inhibited plant growth, and some metabolic activity and other previous researches have reported similar results in different plants such as beans (47), chickpea (48), sesbania (49), Cowpeas (50), peas (51, 52), wheat (53) and barley (54). Physiologically, salinity stress has a negative impact on many processes however the most significant effect is reducing cell division and cell expansion which caused reduction in plant growth figures 4 and 5 (55).

6. Conclusion.

Salinity is an abiotic stress factor which limits crop production and affects development of plants. Therefore, this study was aimed to investigate the impact of salinity stress on bean (*Phaseoulus vulgaris* cv) plants. According to the results of this study, water uptake (figure 1), germination traits (figure 2), and seedling growth parameters (figure 3 and 4) reduced severely with increasing salinity concentration. The effect of salinity stress on seedling growth traits has been showed in Table 2. Comparison of seedling growth traits mean in different salinity level showed that when salinity level increase, seedling growth traits decrease (figure 3 and 4). The most reduction in seedling growth traits obtained from 120 mM salinity level was seen in plant dry weight with 69% reduction over the control (figure 4). However, seedling growth traits were more sensitive to salt stress than was germination traits.

7. **Recommendations**:

- Future studies need to be focus on molecular, physiological and metabolic changes induced by salinity stress.
- The future focus should be on understanding the physiological and biochemical responses of bean plant under field conditions.

Acknowledgment.

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