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Impact of Saline Irrigation Water Levels on the Early Stages of Alfalfa Growth (*Medicago sative* L.)

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Abstract:

An agricultural experiment was conducted to measure the effects of different salinity treatments of water irrigation (0, 1, 5,10 dS/m) at 25^{0}C on leaf number, root length, plant height, fresh and dry biomass of alfalfa (*Medicago sative* L.) in a small greenhouse at a laboratory of Agriculture College of Sirte University, Libya. Treatments were designated as (S1, S2, S3, S4) respectively. Alfalfa seeds were planted in 24 pots using a random block distribution method with 6 replicates. 12 pots were harvested after one month and 12 after two months of cultivation. Samples of fresh materials were weighed and then dried in an oven under 70^{0}C for 48 hrs to estimate the constant weight of plants per pot and determine whether they were affected by various salt treatments. In general, the results of this study emphasized alleviated effects of growth with salt- -tolerance on growth aspects under salinity in alfalfa (*Medicago sative* L.). Salinity reduced the total length and total biomass of alfalfa with the increase in water stress. Leaf number decreased by about 46.02% after 10 dS/m compared to the control treatment.

Keywords: Water Salinity; Salt Tolerance; Plant height; Biomass; Alfalfa.

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تأثير ماء الري الملحي على مراحل النمو الاولى لنبات البرسيم الحجازي (*Medicago sative* L.)

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الملخص:

أجريت تجربة لتحديد تأثير مستويات الملوحة المختلفة (0, 1, 5, 10 ديسيسمنز/م) عند 25م^0 على عدد الاوراق، طول الجذر، ارتفاع النبات، الكتلة الحيوية الطازجة والجافة للبرسيم الحجازي (*Medicago sative* L.) في صوبة زراعية مصغرة في مختبر بكلية الزراعة بجامعة سرت، ليبيا. اعطيت للمعاملات الرموز الاتية بالترتيب (S1, S2, S3, S4). استخدم 24 وعاء زراعي وفقا لتصميم القطاعات العشوائية في 6 مكررات لكل معاملة. تم حصاد 12 وعاء زراعيا بعد شهر واحد من النمو و12 وعاء زراعيا بعد شهرين من عمر النباتات. تم وزن العينات المحصودة الطازجة، ثم جففت في

فرن تجارب تحت درجة حرارة 70 م^0 لمدة 48 ساعة زمنية، لتقدير الوزن الثابت للنباتات لكل وعاء وتحديد ما إذا كانت قد تأثرت بالمعالجات الملحية. بشكل عام، أكدت نتائج الدراسة تأثر النباتات بانخفاض الارتفاع الكلي واطوال الكتلة الحيوية الكلية للبرسيم مع زيادة الاجهاد المائي. انخفض عدد الاوراق بنسبة 46.02 % بعد تأثرها بالمعاملة 10 ديسيسيمنز مقارنة بمعاملة السيطرة.

الكلمات المفتاحية: ملوحة المياه، تحمل الملح، ارتفاع النباتات، الكتلة الحيوية، البرسيم.

Introduction

Saline irrigation water is considered one the main causes of major problems that are common in dry and semi-arid lands, which are widespread in the Arab world in particular and the world in general [1]. According to [2] determined that between 80 countries live in semi-arid areas where salinization is a major problem. Salt stress is a major obstacle to global food security [1]. Salinity water is the most important and difficult factor that limits the quality and productivity of plants. Salinity affects more than 800 hectares of irrigated areas and is a significant limiting factor in agricultural productivity worldwide [3]. There are many methods of plant cultivation that can facilitate the production of results and comparisons between experiments performed in different laboratories and the relevance of the experiments to real-life situations. land [4]. N and P are essential nutrients for the normal growth and development of plants. [5] found that nitrogen application significantly increases chlorophyll content, thereby encouraging increased photosynthetic rates, while also facilitating the entry and exit of CO_2 and water in photosynthesis, which is also consistent with early research on wheat [5]. Alfalfa is a perennial plant that is cultivated as forage in large areas of irrigated lands in the world, primarily used for livestock feed because of its high protein content [6]. Alfalfa (*Medicago sative* L.) is grown under a wide spectrum of soil and climatic conditions; however, it is moderately sensitive to salinity. Alfalfa has high economic importance due to its high nitrogen fixation ability [6,7]. This plant can consume a large amount of water, and irrigation is necessary to maintain the feed production. Alfalfa can be grown in sandy soil and saline-alkali land (pH > 7) with poor soil fertility, but it is not suited to acidic soils and low-lying land where water stands [8,9,10,11]. In another study, Alfalfa with active nodules showed a higher survival rate [12]. Information on the responses of aboveground biomass, water use efficiency, and osmolytes to water salinity at the branching stage of alfalfa is urgently required. The response of alfalfa to water salinity differs during the growing period, also water salinity has the most severe effects on the yield of alfalfa at the branching stage. However, the soil salt presence can enhance its drought resistance and alleviate the impact of water stress on yield [13].

There is likely to be a close relationship between osmotic tolerance and tissue Na^+ tolerance, as genotypes that tolerate high Na^+ concentrations within leaves by partitioning them in vacuoles would also be able to ability to withstand higher osmotic pressure due to their high osmotic fine-tuning ability. However, this speculation remains to be tested [4]. Salt stress negatively affects alfalfa (Medicago sativa L.) production and biological nitrogen fixation was studied by [12] to investigate whether rhizobium symbiosis affects the ability of the host plant's ability to tolerate salt stress or not. The effect of salinity alters general metabolism and enzyme activity, leading to oxidative stress in alfalfa. [14] noted that salt-tolerant genotypes tend to maintain stomatal conductance at a certain level to continue photosynthesis and support growth; Alfalfa has moderate salt tolerance (3 g kg-1).

Alfalfa with higher proline, SOD, POD, and Na^+ activity have better fertility under salt stress. Meanwhile, combined with moderate irrigation (70–85% FC), alfalfa yield improved. The results can provide a theoretical basis for the use of alfalfa in saline soils. On the other hand, many studies have shown that alfalfa is particularly sensitive to salinity at the seedling and early growth stages [15,16,17,18,19]. [20] measured the effects of salt (1:1 molar ratio of NaCl to Na_2SO_4, pH 7.01 to 7.05) and alkali (1:1 molar ratio of $NaHCO_3$ to Na_2CO_3, pH 9.80 to 10, 11) for germination, growth, photosynthesis, and ions. accumulation in alfalfa and the results from this study both highlight a significant reduction in seed germination and elongation; indicating that alfalfa is relatively sensitive to stresses during seed germination and early seedling development. Relative growth rate, water content, chlorophyll content, intracellular CO_2 concentration, stomatal conductance, net photosynthetic rate (NP), and transpiration rate decreased slightly with increasing salinity under stress. salt stress, but significantly reduced under alkaline stress.

Therefore, this study aims to provide understanding and knowledge to relate new molecular techniques to whole plant physiology. So, providing more information about alfalfa is relatively sensitive to stress during seed germination or the early stages of seedling development.

Material and Methods

Experimental Site:

The experiment was carried out in a small greenhouse at a laboratory of Sirte University in Sirte City, Libya. The experiment started on the 17th of March. Until the 20th of May. The climate was mild and warm. The temperatures were between 22-33^{0}C on middays.

Treatments:
Four salinity water levels were used for irrigation as shown in Table (1):

Table (1): Treatments and frequencies.

Treatments	Water salinity (dS/m)	Pots number	Number of seeds per pot
S1	0	6	12
S2	1	6	12
S3	5	6	12
S4	10	6	12

Plantation and Harvesting Methods:
Alfalfa (*Medicago sative* L.) seeds were sterilized in an aqueous solution of 0.1% $KMnO_4$ for 10 min and subsequently rinsed with distilled water before plantation on the 17th of March. Seed and modular compost were put in 24 pots plus sand. The compost has a medium base level of fertilizer to meet the individual requirements of crops and their growing regimes. After washing, seeds were planted in the pots and irrigated with fresh water for 7 days (500 ml) for each pot from the 18th of March until the 25th of March. Plants of treatments 2, 3, and 4 were irrigated with saltwater treatments on the 26th of March after 7 days of sowing the seeds. NaCl was used, because it is the salt most responsible for the salinity of seawater and of the extracellular fluid of many multicellular organisms. Salt was increased gradually in the water every irrigation (1 dS/m) time until it arrived at the required 5 dS/m for treatment 3 and 10 dS/m for treatment 4.

Soil Moisture Measurement:
Theta Meter (type HH1) was used for measuring soil moisture by pushing its stainless-steel measurements into the soil before the irrigation of plants. The amount of water irrigation applied was decreased at the highest treatments (S3, S4) after 25 days of plantation (12th April), because of the high moisture in soils, also soils had a strong smell because of the extra water. At the same time, pots of treatments S1 and S2 had too dry soil and required irrigation constantly every 3 days.

Plant Harvest:
12 pots of plants were harvested on the 19th of April and others on the 20th of May by cutting the above grounds of the plants and saving them in medium-sized bags, as well as, their soils.

Laboratory methods:
Firstly, plants were transferred to the laboratory for testing their aboveground and underground for laboratory tests needed to investigate reflected changes on plants which affected and non-affected by salinity levels.

Aboveground and underground measurements:
The number of leaves was accounted per pot, as well as the length of aboveground and underground were recorded. Total biomass was determined, and after that total plants were immediately weighed to obtain the fresh weight. Plants were kept in falcon tubes, then plants were kept in a refrigerator for 24 hours at 3 ^{0}C after distilled water was added to each tube. Plants were blotted with tissue paper to remove moisture and immediately were weighted to obtain the turgid biomass. The turgid plants were oven-dried at 70 ^{0}C for 48hr in a ventilated oven. Dry plants were weighted and reported in grams per plant.

Statistical analysis:
Statistical analysis was performed using Microsoft Excel ©. IBM SPSS AMOS 24 was used to compare mean values between different levels within a factor, and comparisons with p values > 0.05 were considered significantly different.

Results and Discussion
results and Discussion Comparison of alfalfa between one month and two months after exposure to treatments (0, 1, 5 and 10 dS/m), as well as all data presented as mean standard error. The results showed that both stresses significantly reduced seed germination and elongation, suggesting that alfalfa is relatively sensitive to both stresses during seed germination and early seedling development stages. In soil treated with high salinity, Na^+ content increased and K^+ content decreased as salinity increased under both stresses, indicating competitive inhibition between Na^+ and K^+ uptake. The intracellular imbalance of Na^+ and K^+ due to the high pH of alkaline stress may be one of the reasons for the apparent decrease in PN. Ca^{2+} and Mg^{2+} contents decreased with

increasing salinity under both stress conditions. Our study shows that the deleterious effects of alkaline stress are more severe than those of salt stress [20].

The mean of leaf number:
The effects of different salinity levels on plant leaf numbers are presented in Figure (1). Leaf number decreased 46.02% after 10 dS/m compared to the control treatment. The average number of plant leaves ranged from 6.8 to 4 in the first month of growing, there was no significant difference between them. On the other hand, there was a significant difference after 2 months which is determined between 35.2 and 19. The highest treatment (10 dS/m) had the lowest number of leaves (about 19) after two months of growing. The decreased rate of leaf growth after the increase in water salinity is primarily due to the osmotic effect of salt around the roots because a sudden increase in salinity causes leaf cells to lose water [4].

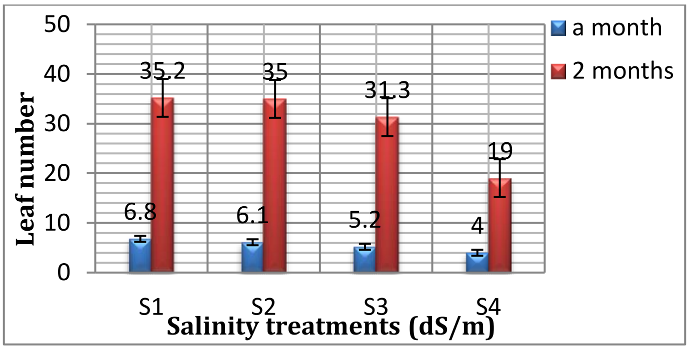

Figure (1): The effects of salinity treatments on the average of leaves number per plant

Total plant height (cm):
One month after treatment, under the highest salinity conditions (10 dS/m), the total plant height was 46.38% lower than that of the control conditions as shown in (Figure 2). Also, total plant height decreased by about 31.8% significantly with increasing salt stress treatment when it was affected by 10 dS/m compared with S1 treatment after two months of growth. Salt stress more strongly suppressed the growth of the first month than that of the second month of plants growing.

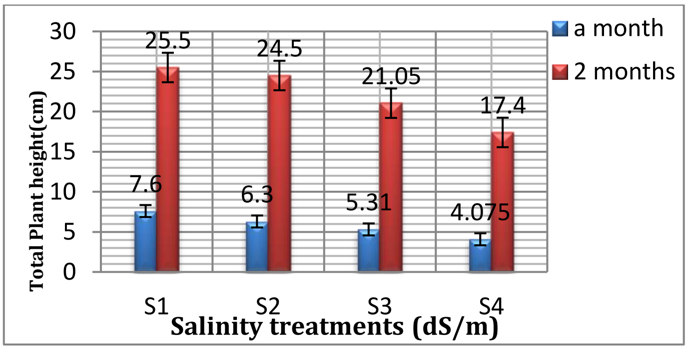

Figure (2): The effects of salinity treatments on total plant height

Total fresh and dry biomass:
Biomass is one of the important growth indices revealing the stress intensity. Decreases in total biomass under drought stress have been observed in many plants. Figure (3) shows that Plant fresh weights decreased with increasing salinity levels. The highest levels of salt treatments (S3, S4) decreased the content of plant weights from the first month of the plant life, was ranged between 0.55 gm in S1 and 0.1 gm in S4 (18.1%). In the second month, Plant weight arrived to 1.2 gm in S1 (control treatment); then decreased to 0.22 gm.

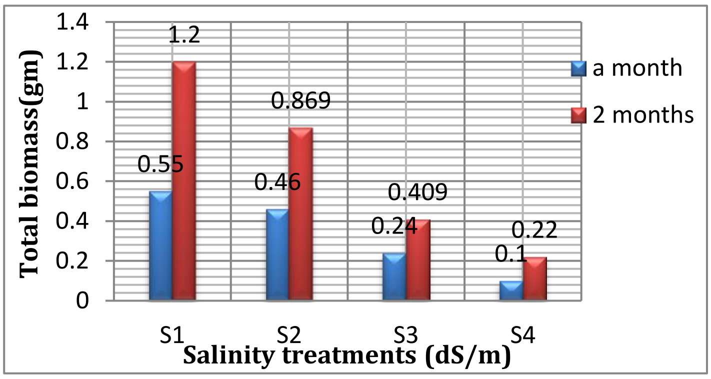

Figure (3): The effect of salinity treatments on total biomass

It was noted from Table (2) that treatments with high salinity have led to two defects in fresh and dry weight after the first and second months compared to the control. The negative effects of salinity on biomass may be due to several reasons: cell growth reduced the value of the water potential – the interference of salinity in the process absorption of essential nutrients – toxic effects caused by the accumulation of sodium chloride ions. etc.

Table (2): The effect of salinity treatments on fresh and dry biomass of plant.

Salinity Treatments	A month		Two-months	
	Fresh biomass (gm)	Dry biomass (gm)	Fresh biomass (gm)	Dry biomass (gm)
S1	0.56	0.24	1.2	0.55
S2	0.46	0.20	0.869	0.26
S3	0.24	0.19	0.409	0.32
S4	0.1	0.67	0.22	0.13

Aboveground heights:

In the second month of growing, aboveground heights were obtained from S2 (17.22), and this was followed by S2, S3, and S4. The lowest aboveground height was obtained from S4 (10.7). but in the first month, the heights performed close to each other. S4 decreased 30.7 % compared with S1.

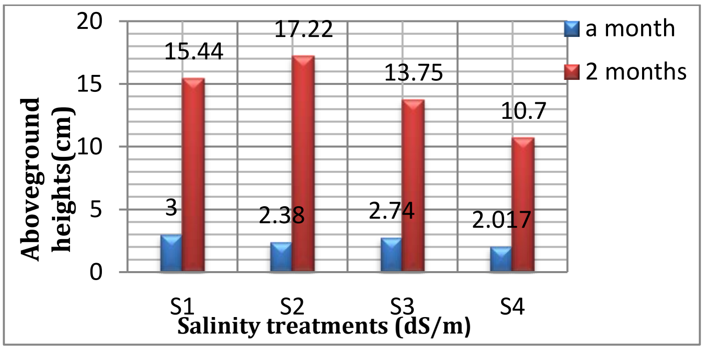

Figure (4): Effect of salinity treatments on aboveground height (cm).

Underground length:

It was observed that root lengths decreased drastically at S3 and S4 of treatments in the first month of growing, as well as, there was a significant difference between them (P-value = 0.024). on the other hand, there was no significant difference between root lengths after two months of plants growing (P-value = 0.6). The highest root length was obtained from S3 (7.3 cm) and this was followed by S4, S2 and S1 in the second month of growth.

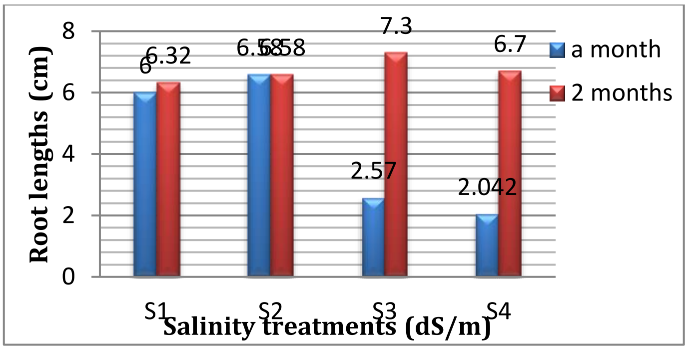

Figure (5): Effect of salinity treatments on underground length (cm).

Ion accumulation:

Alfalfa seeds planted in NaCl conditions accumulate large amounts of Na and Cl ions. Salt treatments cause a decrease in potassium and calcium concentration in alfalfa leaves. In both treatments (S3 and S4), the thermal dissipation showed that this pathway generally played an important role in preventing photodamage in the plant when salt stress was increased. The Na^+ content increased with increasing salinity in the soil of (S3, S4) pots. The K^+, Ca^{2+}, Mg^{2+} content decreased with increasing salinity in the soil of (S3, S4) pots. A high pH of alkali stress might be one of the reasons for the visible decrease in Ca^{2+} and Mg^{2+} [20,21]. Most of the effects of salinity in nutrition balances are mainly related to cationic nutrition, especially because the absorption of cations by plants depends on the absolute amount of them to each other [22]. It was observed that sodium causes a decrease in the calcium level in the plants.

Conclusion

In this study, alfalfa-maintained growth through leaf removal during the first stage of salt stress at the mature growth stage. A careful agricultural experiment was conducted to differentiate between root lengths, green parts lengths, and weights of alfalfa (*Medicago sative* L.) under the influence of different salt treatments. This investigation was based on 4 individuals using the cultivar of alfalfa. (0, 1, 5, 10). The results showed these points:
1- The content of sodium (Na) and magnesium (Mg) increases as salinity increases; but the contents of calcium (Ca), copper (Cu) zinc (Zn), potassium (K), nitrogen (N), and phosphorous (P) were decreased [15].
2- Alfalfa showed a strong salt-tolerant phenotype.
3- Salinity reduced total length, green parts length, leave numbers, and total biomass of alfalfa [12].
4- Salts around roots can affect plant growth not only through their effect on osmotic pressure.
5- The reduced number of leaves is due to salt surrounding the roots, due to the osmotic effect of salt.
6- Alkaline stress resulting from high salinity is more dangerous than the salinity itself.
7- High NaCl concentration in the plant root zone reduces the plant's ability to absorb magnesium, potassium, iron, nitrogen, and calcium.
8- The rising salinity stress leads to a rise in sodium concentration in plant leaves and roots.
9- When salinity treatments increase, plants of alfalfa can improve stress tolerance by increasing root contact, but aboveground biomass and water consumption decrease [13].
10- The decrease in growth is caused by a decrease in the water potential of the rooting solution and not by the presence of a specific salt in it [23].

Recommendations

1- Studies on the tolerance of alfalfa plants to salinity require a lot of study research, and attention because there are still many questions that need solutions.
2- Provide significant future advances for new dimensions aspects in understanding salt tolerance.
3-Soaking seeds with salt is an effective method to improve the germination of alfalfa in saline conditions.
4- Several future studies can be suggested to look into more physiological traits related to salt tolerance that would be beneficial to better understand the physiological adaptation of salt tolerance mechanisms in alfalfa.
5- Instruct farmers periodically about taking care of the soil, maintaining it, and preserving it from salinization that occurs due to irrigation with salt water.

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