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Improving the Tolerance to Salinity Stress in Alfalfa Cultivar Grown in Under Different Levels of Sea Water Using Exogenous Application of Ash

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ABSTRACT

Salinity is a major non-living stressor that impacts agricultural output globally, especially in dry and semi-dry areas. This research analyzes how various salinity levels in irrigation water affect the growth and physiological reactions of alfalfa cultivar and examines the potential of palm leaf ash to alleviate these effects. Research was carried out in a controlled greenhouse setting, testing different levels of salinity (0, 6000, and 10000 ppm) both with and without ash foliar treatment. Various growth factors such as shoot and root length, dry matter content, relative water content, soluble sugar, protein, proline, and chlorophyll levels were evaluated. The findings show that salt levels decrease the growth of alfalfa, but applying ash helps counteract this by increasing nutrient availability, enhancing water retention, and stabilizing physiological functions. The research indicates that using ash may be an affordable way to enhance the ability of crops to withstand high levels of salt in their environment.

تحسين تحمل إجهاد الملوحة في صنف البرسيم المزروع تحت مستويات مختلفة من مياه البحر باستخدام تطبيق خارجي للرماد

خديجة محمد المصراتية مفتاح الطيب كريم

الملوحة هي أحد مسببات الإجهاد الرئيسية غير الحية التي تؤثر على الإنتاج الزراعي على مستوى العالم، وخاصة في المناطق الجافة وشبه الجافة. يحلل هذا البحث كيف تؤثر مستويات الملوحة المختلفة في مياه الري على نمو وردود الفعل الفسيولوجية لصنف البرسيم ويفحص إمكانات رماد أوراق النخيل للتخفيف من هذه التأثيرات. تم إجراء البحث في بيئة دفيئة خاضعة للرقابة، واختبار مستويات مختلفة من الملوحة (0, 6000, 10000 جزء في المليون) مع وبدون معالجة الأوراق بالرماد. تم تقييم عوامل نمو مختلفة مثل طول البراعم والجذور ومحتوى المادة الجافة ومحتوى الماء النسبي والسكر القابل للذوبان والبروتين والبرولين ومستويات الكلوروفيل. تظهر النتائج أن مستويات الملح تقلل من نمو البرسيم، لكن استخدام الرماد يساعد في مواجهة ذلك من خلال زيادة توفر العناصر الغذائية وتعزيز احتباس الماء واستقرار الوظائف الفسيولوجية. يشير البحث إلى أن استخدام الرماد قد يكون وسيلة ميسورة التكلفة لتعزيز قدرة المحاصيل على تحمل مستويات عالية من الملح في بيئتها

INTRODUCTION

Throughout their lives, plants are frequently impacted by a wide range of biotic and abiotic pressures in various parts of the world. A major global threat to agricultural productivity and crop yields is soil salinization. Abiotic stressors such as salt limit the quantity, quality, and growth of numerous crops (Dai *et al.*, 2022). It is commonly known that the main obstacles preventing agricultural expansion in many arid and semiarid locations are the availability and quality of irrigation water (Munns, 2002).The determination of varieties as sensitive or tolerant be an influential strategy to accomplish the salinity stress. 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 (Muhammad *et al*., 2015). Developing varieties capable of maintaining productivity at low or moderate levels of salt stress through breeding investigations may offer a relatively cost-effective short-term solution to this issue (Bahardwaj *et al.,* 2010). Where salinity stress is a brutal environmental stress which decreases the yield production of plants. Salt stress significantly impacts plant growth worldwide. Although alfalfa exhibits some tolerance to salt-alkali soils, the specific salt tolerance levels across different alfalfa varieties remain inadequately understood. Alfalfa is commonly described as "sensitive to salt." When alfalfa is exposed to salt stress, genes associated with other plants' ability to tolerate salt stress are activated. This can lead to various physical effects, such as a decrease in shoot height, foliar area, and mineral content, as well as an increase in the ratio of leaves to stems (Sandhu *et al*., 2017). Salinity can impact forage quality in several ways. For example, protein levels may be affected by reduced nitrogen levels caused by higher salt concentrations in root nodules (Latrach *et al*., 2014). Although alfalfa is comparatively more tolerant of salt stress than most other crops (Song *et al.,* 2019), alfalfa plants' response to salt stress is physiologically and genetically complex due to the fact that salt tolerance is regulated by multiple genes and involves a variety of biochemical and physiological processes. Additionally, because soil salt encourages soil degradation and inhibits plant growth, soil salinization has had a significant impact on alfalfa output worldwide (Bhattarai *et al.,* 2021).

In view of this, many chemicals, reagents, hormones and growth regulators have been developed. protective effect of ash to resist salt stress Ash derived from plant sources is a cheap and locally available source of plant nutrients. Ash contains varying amounts of organic matter as well as major and minor elements. The addition of ash to plants results in increased nitrogen uptake and soil organic matter values (Abdul Rahim, 2020).

This study aimed to determine the impact of different levels of salinity on crop yield during irrigation and the nutritional value of alfalfa cultivars grown in field conditions. The study also aimed to provide the infrastructure for breeders to develop new alfalfacultivars under high salinity conditions by using ash as a treatment for alfalfa. Additionally, the effects of applying ash through foliar spraying on the growth, physiological, biochemical, and genetic aspects of alfalfa were investigated. The study intended to better understand the potential mechanisms for ash-mediated seawater stress signaling and provide a practical approach to managing alfalfa plants challenged with salt stress. The present study was undertaken to investigate the ameliorative effect of ash on alfalfa.

MATERIALS AND METHODS

Experiment Site:

An agricultural experiment was conducted to evaluate the impact of different seawater irrigation salinity treatments (0, 6000, and 10000 ppm) in a small greenhouse at the Faculty of Education, Misurata University, Libya.

Germination Test:

The seeds used in this experiment were procured from the local market in Misurata in September 2023. All external contaminants, including dust, dirt, straw, and any immature or broken seeds, were removed from the seeds during the cleaning process. The Agricultural Research Station in Misurata examined the soil to ascertain its texture and nutrient availability before the experiment. The results of the soil analysis are shown below.

Table 1: Soil Composition Analysis

Salinity	0.85			
pH	8.47			
Soluble Cations	Na+	K+	Ca++	Mg++
	2.5	0.09	4.5	2.67
Essential Macronutrients	Total Nitrogen (%) *	Available Phosphorus p(mg/k) **	Available Potassium K(ppm) ***	
	0.001	1.8	34.1	
Mechanical Analysis	Sand(%)	Silt(%)	Clay(%)	Soil texture
	88.41	7.812	3.78	Loamy Sand

Palm Tree Leaf Ash Production:
Palm ash, often considered waste, possesses properties that can be advantageous in various industrial and agricultural applications. The palm leaf ash analyzed in the research was sourced from a farm located in Misurata, Libya, where it was assumed that the palm trees were between 15 and 20 years old. The palm leaves were burned outside in a steel drum at a temperature averaging about 300 ± 5 °C. After reaching the desired temperature, the ashes were left to cool outside. The ashes were cooled, then ground for a duration of 30 minutes (Victor and Alabi ., 2020).

Experimental Design:
The experiment was designed with five salinity levels in a completely randomized design. The seeds were surface-sterilized by soaking them for three minutes in 5% sodium hypochlorite solution. The salinity levels were assigned as follows
:

- **T1** = 0 ppm (Control)
- **T2** = 6000 ppm
- **T3** = 6000 ppm with ash foliar spray
- **T4** = 10000 ppm
- **T5** = 10000 ppm with ash foliar spray

Following sterilization, the seeds were planted in a greenhouse at a temperature of 23°C on December 7, 2023. The seeds were directly sown at a rate of 50 seeds per container, using a small drill to plant the seeds in sand-filled pots up to a height of 10 cm. The treatments were irrigated with tap water for the first two weeks. When the seedlings reached a height of approximately 15 cm, they were divided into a control group and several experimental groups. The control group was irrigated with tap water, while the experimental groups were treated with seawater solutions of varying concentrations (6000 and 10000 ppm) and irrigated every two days for an additional two weeks. Foliar spraying with ash was applied to specific groups twice a week for 15 days. Afterward, morphological and chemical measurements were taken to evaluate the effects of seawater and ash treatment on the growth of alfalfa plants.

Root and Shoot Height:
The length of the root was measured from the seed to the tip of the primary root, while the shoot length was measured from the seed to the tip of the leaf blade.

Dry Matter:
Dry matter content was calculated according to the methods of Field and Mooney (1988).

Relative Water Content (RWC):
Relative water content was calculated according to the methods of Lai and Liu (1988).

Plant Fresh and Dry Weight (g/pot):
The roots were washed with tap water, followed by distilled water, to remove any soil particles. Both the shoot and the root were weighed for fresh weight using a digital electronic balance. To determine the plant dry weight, the same plants were oven-dried at 70°C for 96 hours, and then weighed on the same balance. The average weight per pot was then calculated.

Biochemical Parameters

. Determination of Total Sugars:
Total soluble sugars were measured according to (Dubois ,1956). Fresh material was mixed with 3 ml of 80% ethanol for sugar extraction. The absorbance at 640 nm was measured by a spectrophotometer according to a calibration curve. Results are expressed in mg/g of fresh material.

. Determination of Soluble Protein Content:
Soluble protein was determined using Folin-Ciocalteau according to(Lawry *et al.* 1951) assay, and protein was expressed as mg protein/g dry weight.

Determination of Proline Content:
for estimating free proline:

1. Take 0.5 g of small sliced shoot and homogenize it in 10 ml of 3% sulfosalicylic acid.
2. Filter the homogenate using Whatman No.2 filter paper.
3. Take a 2.0 ml aliquot of the filtrate and react it with 2 ml of acid ninhydrin solution (1.25 g ninhydrin in 30 ml of glacial acetic acid), 20 ml of 6M phosphoric acid, and 2 ml of glacial acetic acid for 1 hour at 100°C.
4. Terminate the reaction in an ice bath followed by extraction with 4 ml of toluene, mixed vigorously by passing a continuous stream of air for 1-2 minutes.
5. Aspirate the chromophore-containing toluene from the aqueous phase, warm it to room temperature, and measure the absorbance at 520 nm using a scanning spectrophotometer (Model UV1700 UV-VIS)(Bates et al. 1973).

Determination of Photosynthetic Pigment:
The leaf samples from both the treatment and control seedlings were combined with 80% (v/v) acetone using the technique described by (Lichtenthaler and Wellburn in 1983). To measure the chlorophyll (a and b) content, the mixture was then spun in a centrifuge at 5,000 rpm for 15 minutes. The absorbance of the resulting solution was measured at 646 and 663 nm. The pigment content was then determined using the following formulas:

Chlorophyll a = 12.7xO.D 663 2.69XO.D645
Chlorophyll b (mg/ml) = 22.9xO.D.645 4.68XO.D663
Total chlorophyll (mg/ml) =20.2xO.D.645+18.2 O.D633

Analytical statistics:

The test of least significant difference (L.S.D.) was used to compare treatment means and interactions at the 0.05% significance level. SPSS software was utilized for statistical analysis.

RESULTS AND DISCUSSION

Shoot and Root Length:

The effectiveness of different sea water concentrations significantly reduced the shoot and root length of alfalfa cultivar. Compared to control (tap water)indicating optimal growth conditions without salinity stress(Figure 1). In contrast, unfilled grains T2 (6000 ppm)there is a noticeable reduction in shoot and root length compared to T1, showing the adverse effects of moderate salinity on plant morphology. On the other hand, the treatment showed salt concentration.T3 (6000 ppm with ash foliar spray). This treatment shows a significant improvement in both shoot and root lengths compared to T2. The ash spray appears to mitigate the negative effects of salinity, resulting in lengths closer to T1.The same results apply to the treatment T4and T5. This is consistent with the results (Zhang *et al*. 2017) found that organic amendments like ash can enhance plant growth under saline conditions by improving soil structure and nutrient availability, leading to better shoot and leaf development. Also(Basho *et al*. 2019) reported that applying ash could improve root growth under saline conditions by enhancing soil properties and reducing salt stress on plant roots. Another hand(Yang *et al*. 21) showed that the shoot and roots of alfalfa were significantly reduced when salty water with a salinity of >5.0 SS m−1 was used for irrigation, with an average reduction of 20–46% compared with the control treatment. Salinity, particularly from seawater, can significantly affect the growth and development of alfalfa. Mechanism Action-Salinity typically reduces shoot and root length in alfalfa by creating osmotic stress, which limits water uptake, and by causing ionic toxicity that disrupts cellular functions. The application of ash can mitigate these effects through several mechanisms. For example, Nutrient Enrichment where ash is rich in essential nutrients such as potassium (K), calcium (Ca), and magnesium (Mg). These nutrients play crucial roles in cell elongation and division, processes that are directly related to shoot and root growth. also pH Regulation where ash has an alkaline nature, which can neutralize the acidic conditions created by saline water and This pH adjustment improves nutrient availability, promoting healthier and more robust shoot and leaflet development(Cloned Al. 2001).

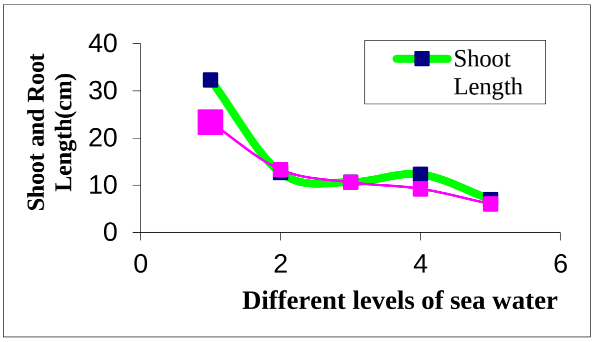

Figure 1. Shoot and root growth of alfalfa cultivar as affected by sea water concentrations

Dry Matter:

The experiment assessed the effect of salinity and ash application on the dry matter content of alfalfa plants through various treatments. The highest dry matter content was observed in the control group (T1, 0 ppm), indicative of ideal growth conditions without the presence of salinity-induced stress. On the flip side, T2 (6000 ppm) exhibited a decline in dry matter content relative to T1, suggesting that moderate salt stress hinders the accumulation of biomass. Dry matter content was higher in the presence of ash foliar spray at the same salinity level (T3, 6000 ppm) compared to T2. This indicates that ash helps alleviate certain detrimental impacts of salinity by potentially enhancing soil structure, increasing nutrient access, and decreasing osmotic stress. This allows plants to sustain improved growth and dry matter accumulation even in the presence of salinity stress. At the elevated salinity level of T4 (10000 ppm), there was a notable reduction in dry matter content, suggesting the presence of intense stress. The impact of salt concentration is more noticeable in this area, potentially causing significant decreases in photosynthesis and overall plant strength, ultimately resulting in less dry matter being accumulated. In T5, despite dry matter content being less than T3, it was greater than T4. This shows that ash foliar spray offers some relief from extreme salinity stress, but its effectiveness decreases with higher salinity levels. Additional research by(Rady *et al*. 2016) found that incorporating organic amendments like ash can boost plant dry matter content in saline conditions through improved nutrient absorption and decreased osmotic stress. Showed improved growth compared to those without any amendments.

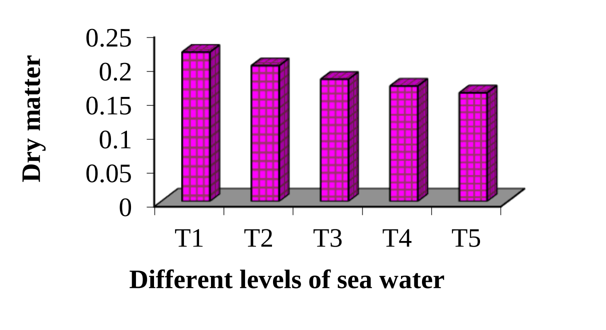

Figure 2. Dry matter of alfalfa cultivar as affected by sea water concentrations

Fresh and Dry Weight:

The results showed that T1 (Control, 0 ppm) had the highest fresh and dry weight, indicating healthy biomass accumulation in the absence of salinity stress. In T2 (6000 ppm), both fresh and dry weight decreased significantly, reflecting the impact of salinity on overall plant health and biomass production. However, in T3 (6000 ppm with ash foliar spray), there was a significant improvement in fresh and dry weight compared to T2, suggesting that ash application mitigates some of the salinity-induced biomass reduction. Similar results were observed in T4 and T5 treatments. Supporting Studies (Li *et al*. 2015) demonstrated that the use of ash improved fresh weight in plants under saline conditions by enhancing nutrient uptake and reducing osmotic stress. Additionally, (El-Mageed *et al*. 2016) reported that ash amendments could increase dry weight in crops exposed to salinity stress by improving soil properties and reducing salt toxicity.

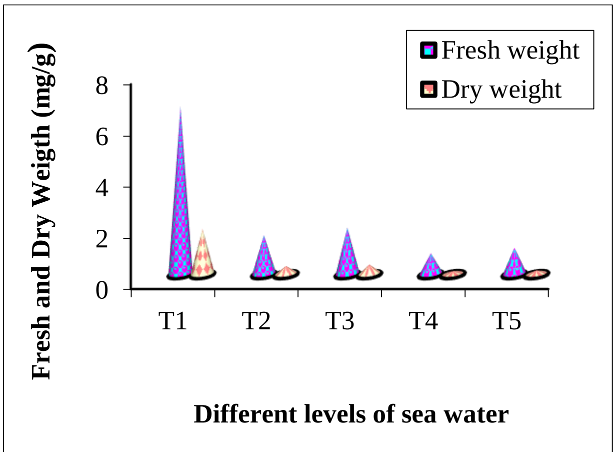

Figure 3. Fresh and dry weight of alfalfa cultivar as affected by sea water concentrations.

Relative Water Content (RWC):

The sea water concentration treatments significantly affected the water content of the alfalfa cultivar. Maximum water content was recorded in seedlings grown in non-saline water (T1). Under saline conditions, the interaction between the alfalfa cultivar and seawater concentrations showed a sharp decline in water content (T2 and T4). However, both the alfalfa cultivar and seawater concentrations with ash spray (T3 and T5) demonstrated a salinity-alleviating effect, increasing relative water content compared to non-ash-treated.

Lower RWC indicates water deficiency in plant tissues, leading to impaired cell turgor and physiological functions. Research by (Ashraf ,2004) demonstrated that RWC in alfalfa significantly decreased with increasing salinity levels, corresponding with reduced growth and biomass accumulation. High salt concentration reduces plant water availability, leading to dehydration at the cellular level and causing osmotic stress. Supporting Studies (Kumar *et al.* 2014) found that organic amendments, like ash, can improve RWC in plants under saline conditions by enhancing soil moisture retention and reducing osmotic stress.

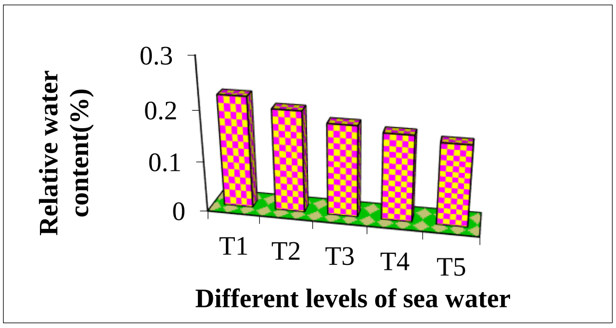

Figure 4. Relative water content of alfalfa cultivar as affected by seawater concentrations

Determination of Total Sugars:

Salt treatments significantly impacted the levels of soluble sugar in the subject. A greater rise in sugar levels was observed with the seawater concentration (T2) compared to T4 without ash, while the control group showed the least amount of soluble sugar, suggesting that the initial sugar levels were naturally low without salinity or ash. This finding aligns with the study conducted by (Parida and Das, 2005) which demonstrated that sugar content is decreased as a result of salinity stress impacting photosynthesis. Using organic amendments like ash can help improve soil conditions and increase plant stress resistance, reducing harmful effects. Impact of Ash Application: T3 and T5 treatments, which included ash foliar spray, greatly increased the amount of soluble sugars in the alfalfa variety when compared to T2 and T4 treatments. A study conducted by (Rady *et al*., 2019) backs up this discovery, indicating that organic materials such as ash can enhance the soluble sugar levels in plants subjected to salinity stress by improving soil characteristics and nutrient accessibility

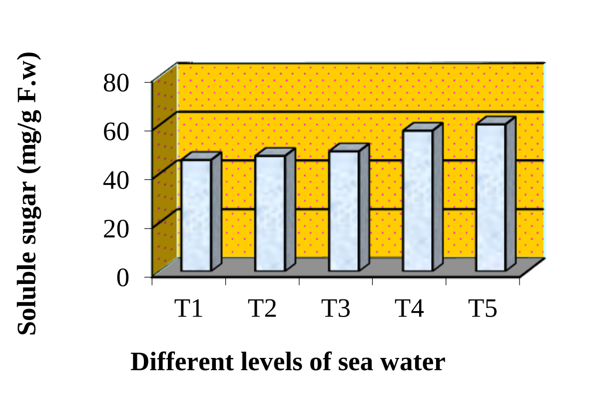

Figure 5. Soluble sugar content of alfalfa cultivar as affected by sea water concentrations.

Determination of Soluble Protein Content:

The findings indicated that various levels of saltiness and ash spray on leaves played a significant role in protein accumulation in the alfalfa variety (Figure 6). In reaction to the stress of salinity, plants frequently increase production of specific stress proteins as a defense mechanism, resulting in higher protein levels in certain instances. The findings of (Hasegawa *et al.* 2000) suggest that plants may increase stress proteins in reaction to salinity, leading to higher protein levels in specific circumstances. Research by (Ghasemi *et al.* 2014) showed that organic amendments such as ash can enhance protein synthesis in plants under salinity stress by increasing nutrient absorption and diminishing oxidative harm.(Debouba *et al.* 2006) observed that the impact of organic amendments can differ based on plant species and salt stress levels, which could result in unpredictable outcomes. This indicates that ash could have a protective effect by increasing nitrogen absorption and protein production, or by decreasing the breakdown of current proteins during stressful situations.

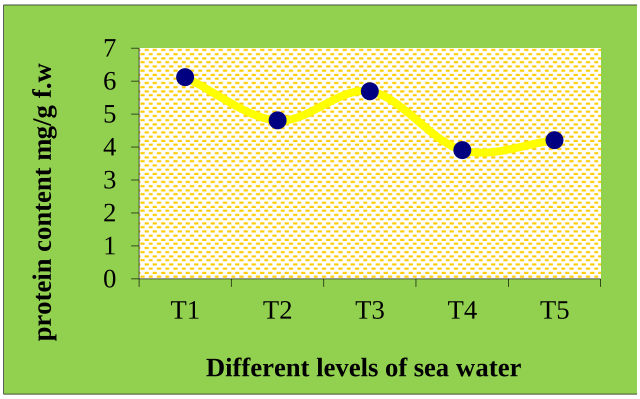

Figure 6. Protein content of alfalfa cultivar as affected by sea water concentrations.

Determination of Proline Content

The amount of proline in plants is frequently used as a marker for their ability to withstand stress. This study found that the control group (T1, 0 ppm) showed initial proline levels. In T2 (6000 ppm), the level of proline rose in reaction to moderate salinity, showing that the plant's stress response mechanisms were activated. Treatment T3 (6000 ppm with ash foliar spray) exhibited increased proline levels, indicating that ash could potentially boost the plant's capacity to generate and store proline, thereby enhancing its resistance to salinity stress. The highest amount of proline was found in T4 (10000 ppm), showing the plant's increased stress response in harsh salinity. In T5 (10000 ppm with ash spray), proline levels dropped slightly from T4 but still stayed high, showing ash's continued support for proline buildup even though its effectiveness may decrease in severe salinity. Additional research (Abdel-Fattah *et al.* 2015) showed that using organic amendments, such as ash, can improve proline buildup in plants when facing salinity stress, helping with osmoprotection and stress resilience. (Verbruggen and Hermans, 2008) verified that the buildup of proline is a typical reaction to salinity in plants under stress, playing various protective functions. On another hand indicate conflicting research found that under specific circumstances, increased proline levels may not necessarily lead to enhanced stress resistance, indicating that additional factors may influence a plant's ability to withstand salt stress (Rai and Rai,2001) Including ash in a foliar spray reduces the negative effects of salt on the levels of soluble sugar, protein, and proline in alfalfa plants. This implies that applying ash can improve the plant's metabolic stability and ability to withstand stress, but its efficacy decreases with increased salinity. The results are consistent with previous research showing that organic amendments can help alleviate salinity stress, but the extent of their effectiveness may differ based on the conditions and type of plants involved.

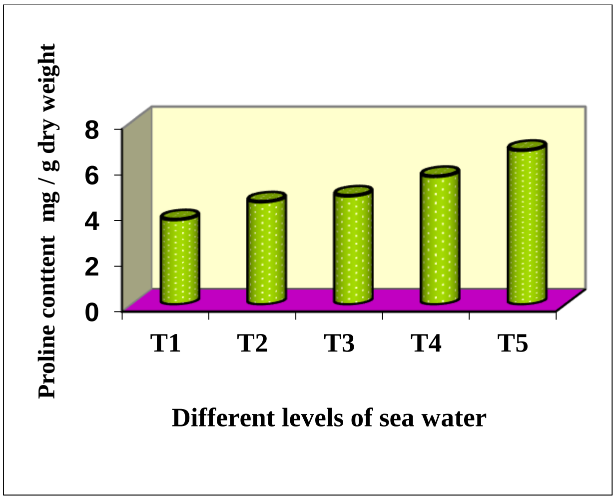

Figure 7. Proline content of alfalfa cultivar as affected by sea water concentrations

Determination of Chlorophyll Contents:

The control treatment (T1, 0 ppm) had the highest chlorophyll content, which showed the best photosynthetic efficiency in stress-free conditions. Nonetheless, in T2 (6000 ppm), a significant decrease in chlorophyll levels was observed at moderate salinity. Salinity stress frequently hinders the production of chlorophyll, resulting in reduced efficiency of photosynthesis. The decrease in chlorophyll a levels indicates that the plant faces difficulty in sustaining photosynthesis in salty environments. On the other hand, T3 treatment (6000 ppm with ash foliar) exhibited rise in chlorophyll levels in comparison to T2. This suggests that ash reduce the harmful impacts of salinity by improving nutrient access and decreasing oxidative stress, which in turn promotes improved photosynthetic efficiency. A noticeable decrease in chlorophyll a content was observed at the higher salinity level (T4, 10000 ppm), possibly resulting from chloroplast damage that hinders chlorophyll a production and maintenance. In T5 (10000 ppm with ash foliar), despite still having lower chlorophyll a content than the control group, there was an improvement compared to T4, suggesting that ash application can partially alleviate the harm from extreme salinity. In a study conducted by (Parida and Das ,2005), it was found that plants under salinity stress experience a decrease in chlorophyll a content as a result of chlorophyll molecule destruction and the inhibition of chlorophyll synthesis. They also observed that specific changes could enhance chlorophyll levels in salty environments elevated salt concentrations hastened the aging of leaves, influencing enzyme function regulating photosynthesis response (Mukami *et al.* 2020). On the other hand, get out conflicting research findings: According to (Kaya et al. ,2013), organic materials such as ash can enhance chlorophyll levels, but their efficacy is restricted when plants are exposed to high salinity levels, which could cause irreversible damage to chlorophyll. Similar patterns were noted in the levels of chlorophyll b and overall chlorophyll content.

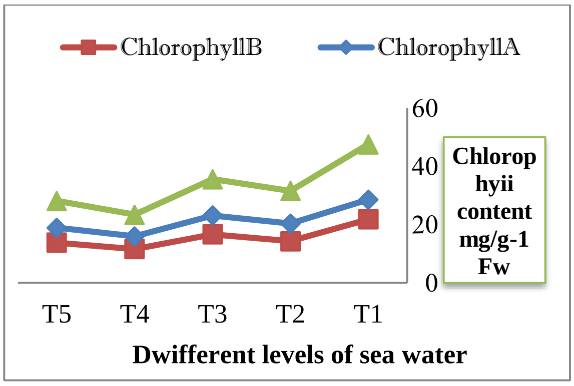

Figure 8. Chlorophyll content of alfalfa cultivar as affected by sea water concentrations

.CONCLUSION

This research highlights the significant effects of salinity stress on the growth and physiological responses of alfalfa, underscoring the challenges posed by saline irrigation in agriculture. The findings demonstrate that salinity, particularly at higher levels, detrimentally affects essential growth characteristics like the length of the shoot and roots, dry matter accumulation, and photosynthetic efficiency. However, the application of palm leaf ash shows promise as an ameliorative agent, mitigating some of the adverse effects of salinity. The ash's role in enhancing nutrient availability, improving soil properties, and stabilizing physiological processes like water retention, sugar accumulation, and protein synthesis was evident across the different treatments. The ash application improved plant resilience under moderate salinity levels (6000 ppm), as evidenced by increased growth metrics and biochemical markers. While the effectiveness of ash diminished under extreme salinity (10000 ppm), it still provided partial relief, suggesting its potential as a supportive treatment in high-salinity environments. These findings align with previous studies that advocate for the use of organic amendments in managing salinity stress in crops. In conclusion, the use of palm leaf ash could serve as a cost-effective, locally available strategy to enhance alfalfa growth in saline soils, providing a practical approach to sustaining agricultural productivity in regions facing salinity challenges. Future research should explore the long-term effects of ash application across different crops and environmental conditions to optimize its use in saline agriculture.

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