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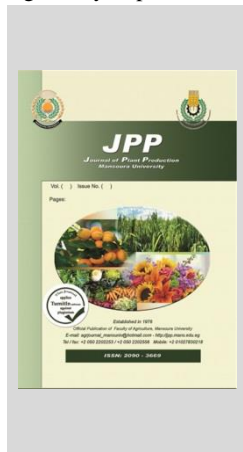
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## Using some Natural Substances to Improve Seedlings Parameters of Sugar Beet under Salinity Conditions

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### ABSTRACT

To investigate the effects of different natural substances (without soaking, soaking in distilled water, 100, 200 and 300 ml/L of moringa leaf extract, alga's extract at 75, 150 and 225 ml/L and 100, 150 and 200 ml/L of yeast extract) to improve seedlings parameters of sugar beet Hossam cultivar under salinity stress conditions (0, 20, 40, 60, 80, 100 and 120 mM of NaCl), at the Faculty of Agriculture, Agronomy Department, Mansoura University, Egypt, an experiment was conducted in an experimental setting in the month of October 2022. The experiment was carried out as a factorial experiment with a completely randomized design. (CRD). The highest values of root & shoot lengths; seedling length and seedlings vigor index of sugar beets were obtained from the control treatment (no salinity stress), followed by treatments with salinity stress at levels of 20, 40, 60, 80, and 100 mM of NaCl. Soaking sugar beet seeds in moringa leaf extract at 300 ml/L resulted in the highest values of root & shoot and seedling lengths and seedling vigor index. It may be advised to soak seeds for 18 hours in moringa leaf extract (MLE) at a concentration of 300 ml/L, yeast extract (YE) at a concentration of 200 ml/L, or algal extract (AE) at a concentration of 225 ml/L to improve sugar beet Hossam cultivar seedling parameters under salinity stress conditions.

**Keywords:** Sugar beet, salinity stress, salinity levels, natural substances, seedlings parameters.

### INTRODUCTION

One of the major sugar crops in Egypt and many other nations around the globe is sugar beet (*Beta vulgaris* var. *saccharifera* L.). The value of sugar beet in cultivation extends beyond just producing sugar; it also produces a wide range of other products. The sugar beet crop has recently taken on a significant role in the Egyptian agricultural rotations as a winter crop, both in rich soils and in low, saline, alkaline, and calcareous soils. One of the commercial products that can withstand salt but is susceptible to it at the germination stage is the sugar beet, thus an intensive research work is needed to improve seedlings parameters of sugar beet under salinity stress conditions by using some natural substances.

Salinity is one important restriction to crop production in the world. It is well known that soil salt inhibits growth and development by causing physiological and biochemical abnormalities in metabolic processes as well as osmotic stress, ion toxicity, and nutrient deficits. Evidently, the capacity of seeds for the best germination under unfavorable circumstances is linked to satisfactory development of plants in dry and semiarid areas, so it is essential to assess salinity resilience at the primary growth stage. According to El-Arqan *et al.* (2002), an excessive abundance of Na and Cl ions in the growth media inhibits sugar beet's ability to absorb important nutrients and may even cause them to move around the plant. High salt amounts in the soil have been linked to serious negative effects, including poor seed germination, poor seedling establishment, and poor agricultural production, according to research by Carvalho *et al.* (2011). Mahdi *et al.* (2012) revealed that various concentrations of NaCl (4, 8, 12

and 16 dSm<sup>-1</sup>) had a significant effect on shoot & root lengths; seedling length and root to shoot length ratio as compared with distilled water as control. Abd El-Hady *et al.* (2014) showed that increasing the salinity level *i.e.* salt concentrations of seawater (2000, 4000, 8000 and 16000 ppm) decreased the seedling fresh weight; seedling length and seedling vigor of sugar beet. According to Moreno *et al.* (2018), salinity is a growing issue that has an impact on agricultural yield in many irrigated deserts and semi-arid regions of the globe. Reduced water availability caused by high concentrations of soluble salts in the root medium, ion toxicity brought on by the buildup of Na<sup>+</sup> and Cl<sup>-</sup>, oxidative stress brought on by an excess of reactive oxygen species (ROS), and acute K<sup>+</sup> deficiency brought on by massive K<sup>+</sup> leak from depolarized cells is just a few of the negative effects that affect plant growth. According to Yang and Guo (2018), there are three different types of adaptive reactions that plants can have to salt stress: osmotic stress tolerance, ion exclusion, and tissue endurance to salinity. The reaction to salinity stress at each stage of development differs not only between plant species but also between genotypes or cultivars, at early development stages, including seedling foundation phases, most agricultural species are vulnerable to salinity stress, according to Feghhenabi *et al.* (2020). Radical, plumule, and seedling length, as well as the seedling vitality index, were significantly increased at 12 dSm<sup>-1</sup> NaCl compared to the control treatment, according to Shokouhian and Omidi (2021).

In contrast to fertilizers, pesticides, and soil additives, natural substances, also known as bio-stimulants, can promote plant development when applied to plants and seedlings. Bio-stimulants that are frequently used include

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advantageous bacteria and fungus, nitrogen-containing substances, biopolymers, and plant preparations. To reduce dependence on traditional herbicides and fertilizers that are less sustainable and frequently abused in agricultural cropping systems, research into and use of bio-stimulants in agriculture has grown recently. Because of the smaller surface area that must be treated, seed treatments use even less active substance than non-treated seed while still promoting more germination and plant development (Rouphael and Colla, 2018).

Using moringa leaf extract (*Moringa oleifera* L.), a miracle species, can increase plant development. Many studies reported that moringa leaf extract is rich in antioxidants; ascorbate; macro and micronutrients, amino acids, zeatin and growth hormones (Latif and Mohamed, 2016). Nambiar (2006) indicated that moringa leaf extract is very good source of zeatin, cytokinin, potassium, calcium, protein, ascorbate; vitamin A and C. Vitamin A and other micronutrient deficiencies can be overcome by Moringa application. Basra et al. (2009) demonstrated that moringa leaf extract, which is high in K, Ca, Fe, amino acids, ascorbic acid, and growth-stimulating hormones like zeatin, proved to be an effective plant growth booster. According to Nouman et al. (2012), a recently found plant growth booster known as moringa (*Moringa oleifera* Lam.) leaf extract enhances rangeland grass seedling emergence as well as seedlings vigor and development when compared to other seed priming methods. Khan et al. (2017) found that used the extract of *Moringa oleifera* L. as a priming agent was reported to enhance seedling vigor.

Algae are an abundant source of minerals, proteins, carbs, and chemical substances, particularly polysaccharides, polyphenols, phlorotannin, plant pigments, unsaturated fatty acids, sterols, and phytohormones. Due to their activity at modest concentrations, algae extract effectively stimulate plant development. Additionally, algae extract enhances the sprouting of seeds, the growth of saplings, the production of photosynthetic pigment, and the ability of plants to withstand external stressors. (Manickavelu et al., 2006). Ghalab and Salem (2001) revealed that a significant class of microbes capable of fixing atmospheric nitrogen were thought to be present in algae extract. Additionally, it significantly increases photosynthetic compounds, growth-stimulating hormones, fresh & dry weights of roots, total biomass, and root extension and development. Raupp and Oltmanns (2006) noted that the significant impact of the extract of algae may be ascribed to its influences in boosting plant efficacy in the uptake of nutrients, which has a direct relationship with chlorophyll concentration, and raising cell membrane permeability. Additionally, the presence of cytokinin in algae extract may help postpone the ageing of plants by slowing down the breakdown of chlorophyll. Additionally, algal extract functions as a bio-regulator that influences the harmony of a plant's photosynthesis and breathing processes. Begum et al. (2018) showed that due to the existence of numerous growth regulators, seaweed and products made from it are frequently used as bio stimulants in agricultural production. For example, the existence of macronutrients like Ca, K, and P as well as minerals like Cu, Fe, Zn, Mn, B, Mo, and Co, which are essential for plant growth and development. Seaweed extract's positive impacts

on plants during germination stage, resilience to biotic & abiotic stress, and early seedling emergence.

Yeast extract consists primarily of oxidized amino acids and contains nutrients such as vitamins (especially rich in B vitamins) and minerals. The use of yeast extract as a plant fertilizer has been found to improve plant resilience, particularly resistance to saline-alkali, and to encourage the development of crop side roots and adventitious roots. Improve soil enzyme activity, boost plant defense (especially against disease, bugs, and arid, hot weather), boost microbial activity in the soil, encourage the fast spread of helpful microorganisms, and trigger seed enzyme activity to speed up seed germination. In this regard, Nagodawithana (1991) found that yeast extract is including cytokinin, many nutrient elements, and organic compounds like carbohydrates; protein; lipids and nucleic acid. These elements enhanced vegetative development in wheat and neutralized the inhibiting impact of moisture stress. Nemeat Alla et al. (2015) asserted that the beneficial effects of yeast extract are primarily attributable to its role in facilitating the availability of nutritional elements for plants, as well as the fact that its content of macro- and micronutrients, growth factors, and vitamins encourages the plant to accumulate dry matter. Kasim et al. (2017) revealed that seed priming with 50% yeast extract for 10 h showed a remarkable increase in the measured seedlings growth criteria. Abo-Hamad (2021) reported that soaking in two different concentrations of yeast extract (1% and 2%) significantly increased seedlings parameters of both *Zea mays* and *Helianthus annuus*. Taha et al. (2021) showed that active yeast extract enhanced anatomical structure, promoted vegetative development, and increased levels of chlorophyll, carotenoids, and total soluble sugars while lowering levels of proline and alkaloids.

To better understand how some natural compounds can enhance sugar beet (Hossam cultivar) seedling characteristics under salinity stress in a lab setting, this research was recorded.

## MATERIALS AND METHODS

In the month of October 2022, a laboratory trial was conducted at the Faculty of Agriculture, Agronomy Department Laboratory of Seed Testing, Mansoura University, Egypt. This laboratory experiment aimed to investigate the impact of some natural compounds at different concentrations on sugar beet Hossam cultivar seedling characteristics under salinity stress.

A factorial experiment with a completely randomized design (CRD) was used to perform the laboratory experiment. The first factor included sodium chloride (NaCl) at seven salinity levels: 0, 20, 40, 60, 80, 100, and 120 mM for the baseline therapy. According to Table 1, the salt amounts are made from NaCl.

**Table 1. Weight of NaCl (g) and the salinity stress concentration (mM).**

No.	Concentration	Weight of NaCl (g)
1	0 mM (control treatment)	Without salt (distilled water only)
2	20 mM	1.17 g NaCl/L
3	40 mM	2.34 g NaCl/L
4	60 mM	3.51 g NaCl/L
5	80 mM	4.68 g NaCl/L
6	100 mM	5.85 g NaCl/L
7	120 mM	7.02 g NaCl/L

In the second factor, eleven seed treatments were soaked for 18 hours in a variety of natural substances, including distilled water, moringa leaf extract (MLE) at concentrations of 100, 200, and 300 ml/L, algal extract (AE) at concentrations of 75, 150, and 225 ml/L, and yeast extract (YE) at concentrations of 100, 150, and 200 ml/L.

Moringa leaf extract (MLE) was made by combining 675 ml of 80% ethanol with 20 g of immature moringa leaves (shoots were collected 35 days after emergence). To ensure the most extract possible, the mixture was homogenized and agitated. After that, the fluid was purified by wringing it out with a sheep towel. Using No. 2 Whatman filter paper, the fluid was once again filtered. As per the research rates, the extract was diluted with purified water before the sugar beet seeds were immerse (Culver *et al.*, 2012).

**Table 2. Chemical composition of algas extract (AE).**

Elements	N	P	K	Mg	Ca	Fe	Zn	Mn	Cu
	%		mg/L						
Concentration	8.00	2.45	0.68	0.20	0.93	1986	31	58	88

A method that enabled yeast cells (dry yeast) to grow and multiply effectively under favorable aerobic and nutritional circumstances was used to make yeast extract (YE), a natural bio-stimulant. This process made it possible to create new, advantageous bio-constituents like carbs, sugars, proteins, amino acids, fatty acids, hormones, etc., which could then easily discharge from yeast cells. For activation and replication, active dry yeast was dissolved in water at a rate of 1 g/L, followed by the addition of sugar at a ratio of 1:1. The mixture was then left overnight, and two rounds of chilling and reheating were used to break open the yeast cells and release the contents. This method of making yeast was changed by Spencer *et al.* (1983).

In sterile Foam dishes (20\*30 centimeters), 400 seeds for each treatment were planted on top of filter paper. Four foam dishes holding a total of 25 seeds were examined under the circumstances of the Laboratory for Seed Testing in the Agronomy Department, Faculty of Agriculture, Mansoura University, Egypt, during the first week of October 2022. This was done in accordance with the standards of the International Seed Testing Association. (ISTA, 1996). A water solution with varying NaCl amounts, excluding the control, was used to moisten foam plates as needed during daily inspections. Every two days, the papers in each container were changed to avoid sodium buildup. The quantity of seeds that germinated on the fourth day was the first tally of the germinated seeds. Once the germination test was complete, the quantity of seeds that had germinated every 24 hours was tallied. (14 days). According to ISTA (1996), seeds were classified as either germinated (radical 2 mm long) or nonviable (abnormal, deceased, or diseased seeds). At the end of germination test five seedlings randomly chosen from each replicate to estimate: 1) Root length (cm); 2) Shoot length (cm); 3) Seedling length (cm); 4) Seedling vigor index (SVI). It was computed using the AbdulBaki and Anderson (1973) recommended method.:

$$SVI = \frac{(\text{radical} + \text{plumel length}) \times \text{Germination percentage}}{100}$$

Using the Statistix-9 computer software program, the acquired data were statistically analyzed in accordance with the analysis of variance (ANOVA) method for the factorial

Prepared algal extract (AE): At the Algal Biotechnology Unit of the National Research Center, three open pools were used to massively build up the original isolated blue-green alga *Spirulina platensis* from Wadi El-Natron, El-Buhira Governorate. (75 m<sup>3</sup> of a final capacity). Algal mass that includes 75–80% moisture was harvested using a continuous centrifuge (Westifalia Separator), and it was then frozen for 48 hours at 25 °C. Algal slurry was severely stressed by high nutritional dosages prior to centrifugation to satisfy the requirements for nutrient accumulation within algal cells. The frozen mass was then homogenized, re-melted at ambient temperature, and aerobically fermented for 72 hours. Following fermentation, the material was homogenized and purified until it was used. Table 2 lists the major components of the utilized algae extract.

completely randomized design as published by Gomez and Gomez (1984). 5% degree of probability, Duncan (1955) multiple range tests, as outlined by Duncan, were used to compare the treatment means.

## RESULTS AND DISCUSSION

### 1- Effect of salinity levels:

From the study's findings, shown in Table 3, there were substantial variations among the salinity levels examined in terms of root length, shoot length, seedling length, and seedling vigor index (SVI). (0, 20, 40, 60, 80 and 120 mM of NaCl). Overall, increasing salinity levels from 0 to 20, 40, 60, 80 and 120 mM of NaCl significantly reduced root length, shoot length, seedling length and seedling vigor index (SVI) of sugar beet (Table 1). The control treatment (without salinity stress) yielded the highest values for root length, shoot length, seedling length, and seedling vigor index (SVI) of sugar beet as compared with other salinity treatments. The lowest readings of sugar beet's roots, stalks, seedlings, and seedlings vigor index (SVI) were obtained from the plant at the maximum salinity level, 120 mM of NaCl. Salt and osmotic stressors are responsible for both inhibiting or delaying seed germination and seeding settlement, as evidenced by the decrease in sugar beet seedling parameters caused by rising NaCl levels. The amount of water absorbed during imbibition decreases under these conditions, and salt stress may result in an increased ion absorption (Yang and Guo, 2018). These findings are in brilliant consistency with those recognized by Abd El-Hady *et al.* (2014), Yang and Guo (2018), Feghhenabi *et al.* (2020) and Shokouhian and Omid (2021).

### 2. Effect of soaking seed treatments in some natural substances:

Averages of sugar beet seedling parameters, such as root length, shoot length, seedling length, and seedling vigor index (SVI), were significantly influenced by soaking seed treatments in various natural substances for 18 hours, including distilled water, moringa leaf extract (MLE) at various concentrations of 100, 200, and 300 ml/L, alga's extract (AE), yeast extract (YE), and moringa leaf extract at concentrations of 75, 150, and 225 m (Table 3). The results

showed that sugar beet seeds soaked in moringa leaf extract (MLE) at a concentration of 300 ml/L outperformed other seed treatments in some natural substances in seedling parameters and produced the highest values of sugar beet root length, shoot length, seedling length, and seedling vigor index (SVI). After that, sugar beet seeds were soaked in yeast extract (YE) at a concentration of 200 ml/L (Table 1). The arrangement of other studied soaking seed treatments in some natural substances was as follows; soaking in algal extract (AE) at the level of 225 ml/L, moringa leaf extract (MLE) at the level of 200 ml/L, yeast extract (YE) at the level of 150 ml/L, moringa leaf extract (MLE) at the level of 100 ml/L, yeast extract (YE) at the level of 100 ml/L, algal extract (AE) at the level of 150 ml/L, algal extract (AE) at the level of 75 ml/L and distilled water. Whereas, the lowest values of root length, shoot length, seedling length and seedling vigor index (SVI) of sugar beet were produced from without soaking (untreated "control treatment"). The positive benefits of moringa leaf extract, a very excellent source of zeatin, cytokinin, potassium, calcium, protein, ascorbate, vitamin A, and C, may be responsible for these outcomes. Application of moringa leaf juice can treat deficits in vitamin A and other micronutrients (Nambiar, 2006). Yeast extract includes nutrients such as vitamins especially rich in B vitamins, and minerals, thus yeast extract is found to be enhance plant resistance, control in enzyme activity. Because yeast extract can induce endogenous hormones like GA3 and IAA as well as vitamins, enzymes, amino acids, and minerals, it has been shown to have positive impacts on the speed germination index. (Wanas, 2002). Algal extract is also an abundant source of minerals, proteins, carbs, and chemical substances, particularly polysaccharides,

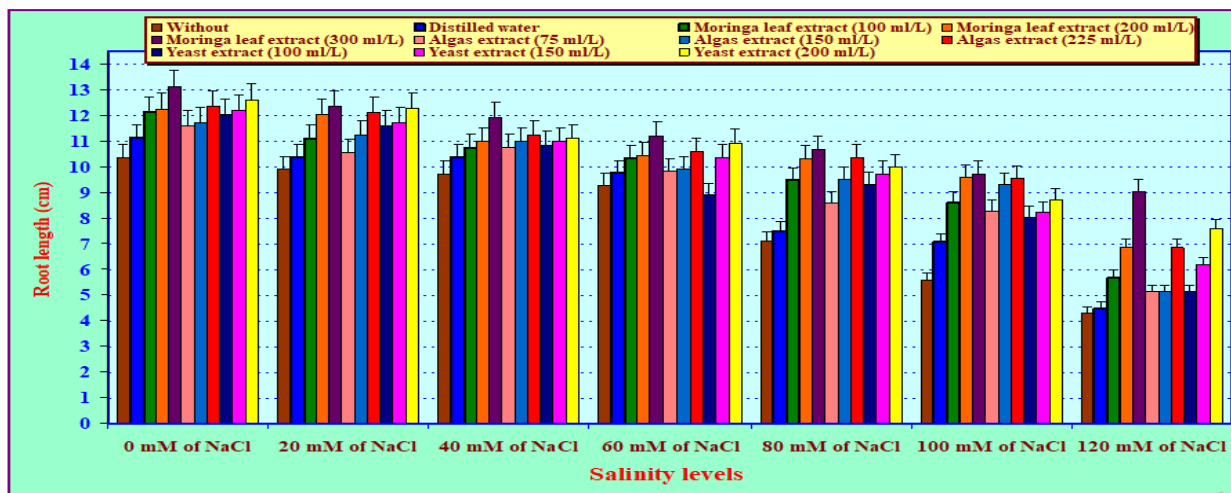
polyphenols, phlorotannin, plant pigments, unsaturated fatty acids, sterols, and phytohormones. Algal extract therefore enhances seed viability, seedling growth, and plant resistance to external stressors (Manickavelu *et al.*, 2006). These outcomes are quite consistent with those recorded by Nouman *et al.* (2012), Nemeat Alla *et al.* (2015), Khan *et al.* (2017), Begum *et al.* (2018), Kasim *et al.* (2017), Abo-Hamad (2021) and Taha *et al.* (2021).

### 3. Effect of interaction:

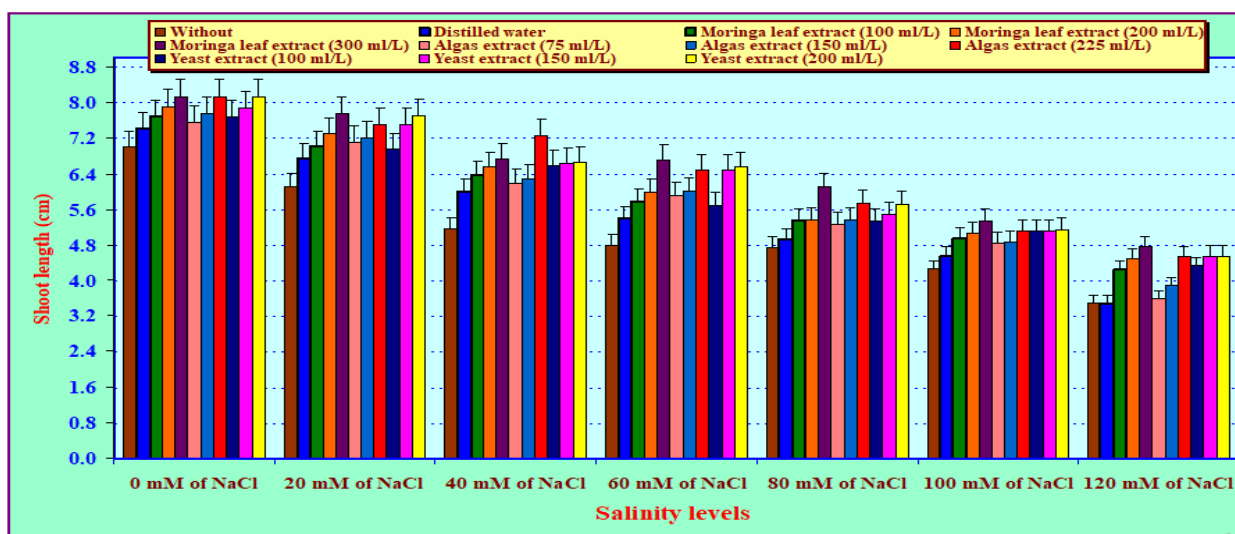
The obtained results clearly demonstrated that the interaction between salinity levels and soaking seed treatments in some natural substances at various levels had a significant impact on sugar beet seedling parameters such as root length, shoot length, seedling length, and seedling vigor index (SVI), (Table 3). From obtained results, the maximum values of root length (Fig. 1), shoot length (Fig. 2), seedling length (Fig. 3) and seedling vigor index (Fig. 4) of sugar beet were recorded when soaking sugar beet seeds in moringa leaf extract (MLE) at the level of 300 ml/L under without salinity stress (control treatment). However, soaking sugar beet seeds in yeast extract (YE) at the level of 200 ml/L under without salinity stress followed by soaking sugar beet seeds in algal extract (AE) at a concentration of 225 ml/L, moringa leaf extract (MLE) at a concentration of 200 ml/L, yeast extract (YE) at a concentration of 150, and algal extract (AE) at a concentration of 150 ml/L under salinity stress of 20 mM of NaCl. On the other hand, the lowest values of root length, shoot length, seedling length and seedling vigor index (SVI) of sugar beet were recorded from control treatment (without soaking seeds in natural substances) under salinity stress of 120 mM of NaCl.

**Table 3. Averages of root and shoot lengths, seedling length and seedling vigor index (SVI) of sugar beet as affected by salinity levels and soaking seed treatments in some natural substances at various levels as well as their interaction.**

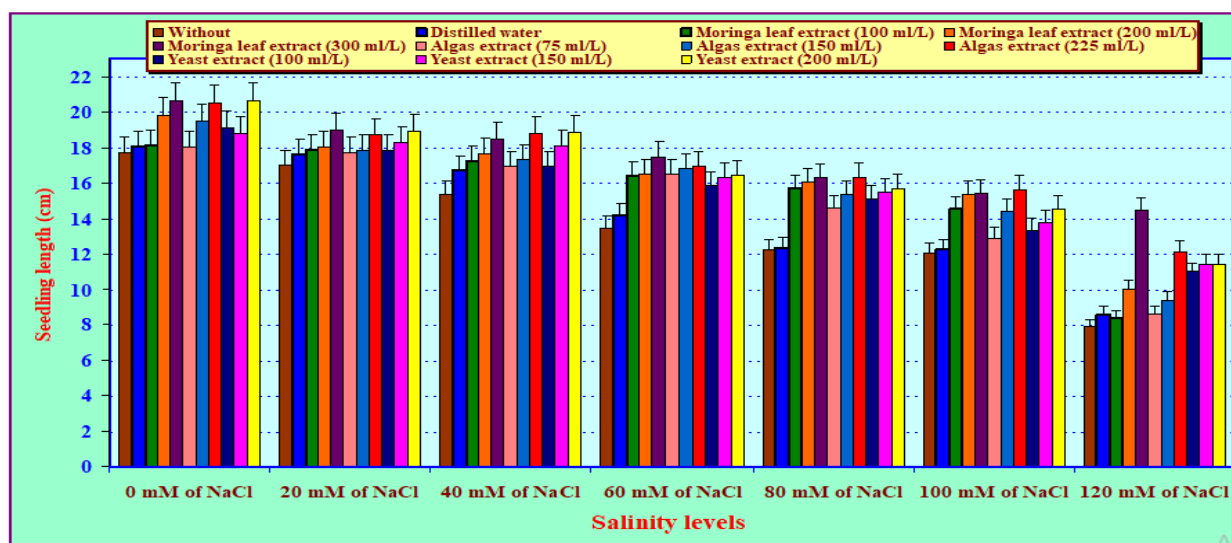
Characters Treatments	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling vigor index (SVI)
A. Salinity levels:				
0 mM of NaCl	11.57 a	7.45 a	18.51 a	17.02 a
20 mM of NaCl	11.06 ab	6.93 b	18.12 a	16.82 a
40 mM of NaCl	10.75 b	6.54 c	17.00 b	15.34 b
60 mM of NaCl	9.83 c	6.25 c	16.34 b	15.16 b
80 mM of NaCl	9.67 c	5.45 d	15.12 c	12.31 c
100 mM of NaCl	9.40 c	4.96 e	14.80 c	7.47 d
120 mM of NaCl	6.03 d	4.26 f	10.29 d	4.65 e
F. test	*	*	*	*
B. Soaking seeds in some natural substances at various levels:				
Without	8.27 d	5.27 d	14.43 d	11.00 d
Distilled water	9.20 c	5.78 c	14.47 d	11.32 d
Moringa leaf extract (100 ml/L)	9.68 bc	6.10 abc	15.69 bc	12.91 bc
Moringa leaf extract (200 ml/L)	9.88 bc	6.16 abc	16.05 abc	13.19 ab
Moringa leaf extract (300 ml/L)	10.77 a	6.37 a	17.05 a	14.24 a
Algal extract (75 ml/L)	9.30 c	5.82 bc	15.08 cd	11.62 d
Algal extract (150 ml/L)	9.82 bc	5.84 bc	15.47 bcd	11.97 cd
Algal extract (225 ml/L)	10.17 ab	6.16 abc	16.34 ab	13.40 ab
Yeast extract (100 ml/L)	9.65 bc	5.86 bc	15.67 bc	12.86 bc
Yeast extract (150 ml/L)	9.82 bc	6.11 abc	16.00 abc	12.92 bc
Yeast extract (200 ml/L)	10.77 a	6.28 ab	16.87 a	14.08 a
F. test	*	*	*	*
C. Interaction (F. test):				
A × B	*	*	*	*



**Fig. 1.** Averages of root length (cm) of sugar beet as affected by the interaction between salinity levels and soaking seed treatments in some natural substances at various levels.



**Fig. 2.** Averages of shoot length (cm) of sugar beet as affected by the interaction between salinity levels and soaking seed treatments in some natural substances at various levels.



**Fig. 3.** Averages of seedling length (cm) of sugar beet as affected by the interaction between salinity levels and soaking seed treatments in some natural substances at various levels.

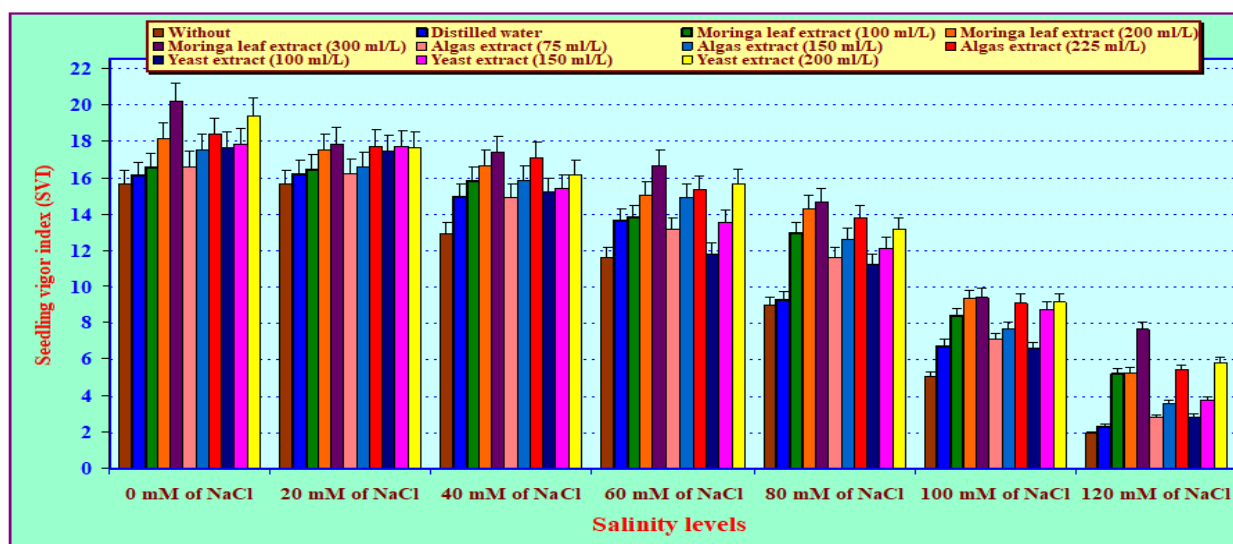


Fig. 4. Averages of seedling vigor index (SVI) of sugar beet as affected by the interaction between salinity levels and soaking seed treatments in some natural substances at various levels.

## CONCLUSION

It may be suggested that seeds be soaked for 18 hours in moringa leaf extract (MLE) at the level of 300 ml/L, yeast extract (YE) at the level of 200 ml/L, or algal extract (AE) at the level of 225 ml/L if it is decided to improve sugar beet Hossam cultivar seedling parameters under salinity stress conditions.

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## استخدام بعض المواد الطبيعية لتحسين صفات بادرات بنجر السكر تحت ظروف الملوحة

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### المخلص

أجريت تجربة معملية بقسم المحاصيل، كلية الزراعة، جامعة المنصورة، مصر، خلال شهر أكتوبر 2022 م، وذلك لدراسة تأثير بعض المواد الطبيعية (بدون نقع، النقع في الماء المقطر، النقع في مستخلص أوراق المورينجا بمعدلات 100، 200 و300 مل/لتر، النقع في مستخلص الطحالب بمعدلات 75، 150 و225 مل/لتر والنقع في مستخلص الخميرة بمعدلات 100، 150 و200 مل/لتر) لتحسين صفات بادرات بنجر السكر صنف Hossam تحت ظروف الملوحة (0، 20، 40، 60، 80 و120 ملليمول كلوريد الصوديوم) في الظروف المعملية. تم إجراء التجربة المعملية في تجربة عاملية في تصميم تام العشوائية (CRD). نتجت أعلى القيم من صفات طول الريشة، طول البادرة ومعدل قوة البادرة من بنجر السكر من معاملة المقارنة (بدون إجهاد ملحي)، يليها الإجهاد الملحي عند مستوى 20 ملليمول كلوريد الصوديوم، ثم الإجهاد الملحي عند مستوى 20، 40، 60، 80 و100 ملليمول كلوريد الصوديوم. أدى نقع بنور بنجر السكر في مستخلص أوراق المورينجا بمعدل 300 مل/لتر للحصول على أعلى القيم من صفات طول الجذير، طول الريشة، طول البادرة ومعدل قوة البادرة. تبع ذلك نقع بنور بنجر السكر في مستخلص الخميرة بمعدل 200 مل / لتر. لتحسين صفات بادرات بنجر السكر صنف Hossam تحت ظروف الإجهاد الملحي، يمكن التوصية بنقع البنور لمدة 18 ساعة في مستخلص أوراق المورينجا بمعدل 300 مل / لتر أو مستخلص الخميرة بمعدل 200 مل / لتر أو مستخلص الطحالب بمعدل 225 مل / لتر.