Assiut Journal of Agriculture Science 53 (5) 2022 (65-77)
ISSN: 1110-0486 / EISSN: 2356-9840
Website: http://ajas.journals.ekb.eg/
E-mail: ajas@aun.edu.eg

(Original Article)

Impact of Silicon and Endophytic Fungi Application for Plant Survival, Biomass and Chemical Compositions of *Moringa oleifera* Lam. Under Salinity Stress Conditions

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DOI: 10.21608/ajas.2022.164423.1180

Abstract

The newly reclaimed soils suffer from some stresses, especially salinity, whether in the soil or irrigation water that severely hinder the growth and production of some crops. A pot experiment was conducted during 2021 and 2022 seasons at Al-Marashda Research Station, Qena, ARC, Egypt to study the influence of silicon and endophytic fungi on germination percentage, survival seedlings rate, biomass and chemical traits of *Moringa oleifera* under salinity conditions. Pots were filled with sandy soil as substrate and arranged in completely randomized design, having nine treatments, including silicon and endophytic fungi together or alone with two levels of salinity. The two salinity levels were significantly affected the germination and survival percentages, biomass as well as chemical compositions of *M. oleifera*. However, addition of silicon at 100 mg/ l or endophytic fungus (*Chaetomium globosum*) at 100 ml, together or alone led to increase germination percentage and survival rate, biomass characters, N, P, K, and total chlorophyll in moringa leaves under saline conditions. The improvement was better with lower salinity level (3 dS m^{-1}) compared to the higher one for the 1st and 2nd seasons. Application of both silicon and endophytic fungi led to better growth results compared to the individual treatments. Leaves of salt- treated plants without any additives contained high levels of Na^+ and Cl^- compared to those treated with saline reducers.

Keywords: *Salinity stress, Moringa oleifera, Silicon, Endophytic fungi, Germination.*

Introduction

Moringa oleifera Lam. (Moringa) is belongs to family Moringaceae and well known for its valuable importance in industrial, medicinal, food and other uses. Its leaves and fruits are widely consumed in the tropical regions of the world (Lockett *et al*., 2000). Moringa contains a high concentration of the crude protein, nutrient

Received: 21 September 2022/ Accepted: 28 November 2022/ Published online: 1 December 2022

elements and vitamins as A, B, and C in its foliage (Makkar and Becker, 1996). It has medicinal properties and nutritional value in various parts of the tree i.e., leaves, seeds, pods, roots and bark. Moreover, the therapeutic effects of moringa are due to the combined actions of the various bioactive components such as alkaloids, flavonoids, saponins, sterols and tannins (Fahal *et al*., 2018). Concerning the nutritional value of moringa leaves for livestock feeding, Ramachandran *et al*. (1980) revealed that feeding could achieve a weight gain and improve the nutritional status. Also, based on the result of Egbewole *et al*. (2017) on moringa wood, it can be concluded that its fiber length falls within short fiber cellulosic materials and axial sampling showed no significant differences in the fiber qualities at any stem height indicating that *M. oleifera* possesses good pulping qualities that suitable for production of pulp and paper.

Salinity is the major limitations that induces desertification and restricts plant sustainability (Siddiqui *et al.*, 2020). Salinity effects on plant development are attributable to a hyperosmotic and ionic imbalance with excessive-production of reactive oxygen species that severely hamper several physiological, biochemical and molecular changes including depletion of chlorophyll, lipid peroxidation, nucleic acid mutilation, and reduction in cell membrane fluidity and selectivity (Ren *et al*., 2020). In Egypt, the notable problems facing the plants growing in the reclaimed soils are drought, salt and heat stresses which adversely affect the growth and productivity of plants (Soliman *et al.,* 2015). The major effects of salt stress in the plants through osmotic stress, Na^+ and Cl^- toxicity and uptake ions of imbalance leading to deficiency in the nutritional elements as N, P, K^+, Ca^{++} and micronutrients (Munns, 2005). All salinity levels significantly affected the emergence parameters of moringa landraces. Higher salinity levels significantly reduced root length, seedling length as well as seedling fresh and dry weights (Farooq *et al*., 2022).

Silicon is one of the most available elements in the nature. It i is found as silicon dioxide or silicates that chemically bond with various elements due to its strong affinity with oxygen. Silicon is beneficial for most plants under biotic and abiotic stress, since it causes a significant increase in the plant growth. Hence, it is suggested to be used in agriculture (Imtiaz *et al*., 2016; Yan *et al*., 2018). In this respect, Carballo-Méndez *et al*. (2022) revealed that salinity reduced emergence, growth, biomass and increased concentration of Na^+ in moringa plants. Meanwhile, Si addition resulted in increased growth of aerial part, biomass and reduced Na^+ concentration in leaves.

Endophytic fungi are microbes that reside inside plants without causing disease (Khan *et al*., 2015), and there is increasing evidence of their high impact on the plant development, physiology, evolution, adaptation and the ability to supply nitrogen to host plant through the biological nitrogen fixation (Compant *et al*., 2010). On the other hand, production of phytohormones have been demonstrated by the plant growth promoting endophytic fungi, which modulate the endogenous levels of auxins in plants and enhancing plant resistance to various stresses (Asad Ullah *et al*., 2021). Therefore, our objectives were to determine the

effect of Si and endophytic fungi addition on germination, survival rate, biomass as well as chemical compositions of *M. oleifera* under salinity stress conditions.

Materials and Methods

A pot trial was undertaken during the 2021 and 2022 seasons at Al-Marashda Research Station, Qena governorate, ARC, Egypt (26° 9' N, 32° 42' E). The main objective was to investigate the effect of silicon and endophytic fungi on germination, growth performance and chemical compositions of moringa under salinity stress.

Fungal isolation, identification, and cultivation

Moringa oleifera leaves were collected and washed carefully by tap water, then by sterilized distilled water. Leaf samples were surface sterilized by sequential immersion in 75% ethanol for 1 min, 4 % sodium hypochlorite for 3 min finally, 75% ethanol for 30 s. The samples were then rinsed by using sterilized distilled water, and shade dried (Filip *et al*., 2003). Leaves were cut for small pieces and placed on prior poured glucose-Czapek's agar, then incubated at 28± 2 °C for 2 - 3 weeks in a microbiological incubator until the desired *Chaetomium globosum* fungi growth (Smith and Dawson, 1944). It contained mycelia and spores; the filtration was done with the Watman filter paper No. 2 for a spore suspension. Concentration of 5×10^7 spores/ml was adjusted to be used in the treatments.

Table 1. Some physical and chemical analysis of the used soil at the beginning of the experiment (average of 2021and 2022 seasons)

Soil type	E.C. (m.mohs/cm^3)	pH	P_2O_5%	Anion (meq/L.)			Cation (meq/L.)		
				HCO_3^-	Cl^-	SO_4^-	Ca^{++}	Mg^{++}	K+
Sand	1.95	7.6	0.14	4.22	7.20	1.17	4.21	3.34	5.11

Seeds of *Moringa oleifera* Lam. were collected from healthy tree from Faculty of Agriculture, Beni-Suef University, and soaking for 24 h. in tap water and planted on poly pots (20 x 20 cm), filled with sandy, some physical chemical properties of the soil used were done according to the methods described by Jackson (1973) as shown in Table (1). Pots were arranged in a completely randomized design, having nine treatments with three replicates, each pot filled with 2 kg of soil and 5 seeds sown. The nine treatments including: 1) control soil without salinity or Si addition; 2) saline-treated soil at 3 dS/m-1 NaCl (3g NaCl/ kg soil); 3) saline-treated soil at 6 dS/m-1 NaCl (6 g NaCl/ kg soil); 4) 3 dS m-1 NaCl + 100 mg/ l Si as foliar spraying; 5) 6 dS m-1 NaCl + 100 mg/l Si; 6) 3 dS m-1 NaCl + 100 ml endophytic fungi; 7) 6 dS m-1 NaCl + 100 ml endophytic fungi; 8) 3 dS m-1 NaCl + 100 mg/ l Si + 100 ml endophytic fungi; 9) 6 dS m-1 NaCl +100 mg/ l Si + 100 ml endophytic fungi. Salinity treatments were added weekly for the two levels, while silicon as potassium silicate and EF as *Chaetomium globosum* treatments were added together or individually one time every two weeks (3 times). However, after germination, seedlings were supplied three doses with nitrogen, phosphorus and potassium as: 1.5 g NH4NO3 and 1.5 g

KH2PO4 per pot, respectively. The first dose was added 15 days after germination, while the next two doses were applied every 15 days. All treatments were performed on the top surface of the soil, the pots were placed under open field, and normally irrigated for 50 days from sowing seeds, which were on 1st March for both seasons.

Germination and growth parameters

Germination percentage (%) = Number of emerged plants/ Number of sown seeds x 100. The survival seedlings rate was measured by recording dead and alive seedlings on 19th April i.e., after 50 days from sowing seeds and using the formula:

Survival seedling rate (%) = Number of survived seedlings⁄Number of seeds sown x 100. Biomass: herb fresh and dry weight as well as root fresh and dry weight (g).

Chemical Analysis

The fresh and dry weights of moringa seedlings were recorded. Three plants from every replicate were picked, cleaned and oven dried at 70°C till constant weight. Leaf samples were ground in a stainless-steel mill and a portion of the dried leaves was digested with di-acid mixtures (sulfuric and perchloric acids) in laboratory of Medicinal and Aromatic plants Department, Faculty of Agriculture, Beni-Suef Univ. Then the digested aliquot was analyzed for N, P, K, Na and Cl. Percentages of N, P and K were determined as described by Anderson and Ingram (1993), while Na^+% and Cl^- contents were determined using a flame spectrophotometer according to Jameel and Kahayri (2002). Total chlorophyll content was extracted using the method described by Nornai (1982).

Statistical analysis

The obtained data were subjected to the statistical analysis of variance and means were compared using LSD test at the 5% level, according to Little and Hills (1978).

Results

Germination and survival (%)

There were significant differences between the used treatments for germination and survival percentages for both seasons (Table 2). Seed germination percentage and survival seedlings rate were higher in the control treatment, followed by that receiving salinity at 3 dS m^{-1} + 100 mg Si + 100 ml/ l EF in the 1st and 2nd seasons. The lowest values of these traits were due to the addition of salinity only, whether at 6 or 3 dS m^{-1} NaCl. The addition of silicon or endophytic fungi, together or alone led to an improvement in the germination percentage and survival rate under saline conditions. Also, the improvement was better with lower salinity level (3 dS m^{-1} NaCl) compared to the higher one for both seasons.

Table 2. Effect of silicon and endophytic fungi addition on germination percentage (%) and survival rate of *Moringa oleifera* under salinity stress

Treatments	Germination (%)			Survival rate		
	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	85.00	87.00	86.00	81.00	84.00	82.50
Salinity 3 dS/m^{-1} NaCl	52.00	55.67	53.84	28.67	34.67	31.67
Salinity 6 dS/m^{-1} NaCl	43.33	50.00	46.67	23.33	22.67	23.00
Salinity 3 dS/m^{-1} + 100 mg/ l Si	66.00	63.00	64.50	58.67	53.00	55.84
Salinity 6 dS/m^{-1} + 100 mg/ l Si	61.33	58.00	59.67	49.67	49.00	49.00
Salinity 3 dS/m^{-1} NaCl + 100 ml EF	66.33	62.67	64.50	58.67	57.67	58.17
Salinity 6 dS/m^{-1} NaCl + 100 ml EF	61.33	58.00	59.67	55.00	53.67	54.34
Salinity 3 dS/m^{-1} + 100 mg Si/l + 100 ml EF	72.33	74.33	73.33	69.33	71.33	70.33
Salinity 6 dS/m^{-1} + 100 mg Si/ l +100 ml EF	68.67	67.33	68.00	66.00	66.33	66.17
Mean	64.04	64.00		54.48	54.70	
LSD5%	3.85	3.61		4.43	4.32	

Table 3. Effect of silicon and endophytic fungi addition on herb fresh and dry weight (g) of *Moringa oleifera* under salinity stress

Treatments	Stem fresh weight (g)			Stem dry weight (g)		
	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	3.34	3.47	3.41	1.09	1.10	1.10
Salinity 3 dS/m^{-1} NaCl	1.57	1.37	1.47	0.52	0.53	0.53
Salinity 6 dS/m^{-1} NaCl	1.17	1.19	1.18	0.44	0.46	0.45
Salinity 3 dS/m^{-1} + 100 mg/ l Si	1.99	1.98	1.19	0.64	0.67	0.66
Salinity 6 dS/m^{-1} + 100 mg/ l Si	1.74	1.67	1.71	0.55	0.57	0.56
Salinity 3 dS/m^{-1} NaCl + 100 ml EF	1.96	1.84	1.90	0.68	0.64	0.66
Salinity 6 dS/m^{-1} NaCl + 100 ml EF	1.77	1.75	1.76	0.56	0.53	0.55
Salinity 3 dS/m^{-1} + 100 mg Si/l + 100 ml EF	2.87	2.82	2.85	0.93	0.95	0.94
Salinity 6 dS/m^{-1} + 100 mg Si/ l +100 ml EF	2.33	2.30	2.32	0.78	0.74	0.76
Mean	2.08	2.04		0.69	0.68	
LSD5%	0.14	0.12		0.09	0.06	

Herb fresh and dry weight

The herb fresh and dry biomass of the moringa obtained at 50 days after sowing seeds presented in (Table 3) indicated that there was a significant difference between treatments ($p < 0.05$). The greatest herb biomass was obtained from the treatment without any additives (control), followed by the addition of silicon with EF under 3 dS m^{-1} NaCl treatment. Treatments with 6 or 3 dS m^{-1} NaCl recorded low fresh and dry aerial plant weight compared to the other treatments for both seasons. Addition of both silicon and EF together or individually led to an increase of herb fresh and dry weights under salinity stress compared to the control.

Table 4. Effect of silicon and endophytic fungi addition on root fresh and dry weight (g) of *Moringa oleifera* under salinity stress

Treatments	Root fresh weight (g)			Root dry weight (g)		
	1st season	2nd season	Mean	1st season	2nd season	Mean
Control	1.55	1.46	1.51	0.49	0.50	0.50
Salinity 3 dS/m^{-1} NaCl	0.64	0.63	0.64	0.23	0.24	0.24
Salinity 6 dS/m^{-1} NaCl	0.52	0.56	0.54	0.18	0.18	0.18
Salinity 3 dS/m^{-1} + 100 mg/ l Si	0.94	0.94	0.94	0.33	0.31	0.32
Salinity 6 dS/m^{-1} + 100 mg/ l Si	0.80	0.79	0.80	0.26	0.24	0.25
Salinity 3 dS/m^{-1} NaCl + 100 ml EF	0.90	0.87	0.89	0.34	0.34	0.34
Salinity 6 dS/m^{-1} NaCl + 100 ml EF	0.83	0.86	0.85	0.27	0.25	0.26
Salinity 3 dS/m^{-1} + 100 mg Si/l + 100 ml EF	1.19	1.17	1.18	0.47	0.46	0.47
Salinity 6 dS/m^{-1} + 100 mg Si/ l +100 ml EF	1.06	1.04	1.05	0.34	0.32	0.33
Mean	0.94	0.92		0.32	0.31	
LSD5%	0.103	0.058		0.050	0.042	

Root fresh and dry biomass

Data in Table (4) shows the influence of adding silicon and EF on the biomass of moringa roots affected by salinity. There were significant differences between the used treatments for germination and survival percentages for both seasons. Salinity at rate of 3 or 6 dS m^{-1} NaCl considerably decreased root fresh and dry weight (58, 64%) and (52, 64%) in the mean of seasons relative to the control, respectively. Nevertheless, this inhibition of root biomass was alleviated by adding both Si and EF together or alone in the 1st and 2nd seasons. With Si and EF addition, root fresh and dry weights were significantly higher than that in salt-affected plants in both seasons.

Nitrogen, phosphorus and potassium content

Nitrogen, phosphorus and potassium percentages in salinity-stressed moringa leaves were decreased relative to those of the control plants. Conversely, silicon and EF supplementation to salinity-stressed plants increased these element concentrations more than that of the stressed plants (Figures 1, 2 and 3). Addition of silicon and EF was more effective with a salinity level of 3 or 6 dS m^{-1} NaCl than in the rate of 6 dS m^{-1} in increasing the percentages of N, P and K in moringa leaves.

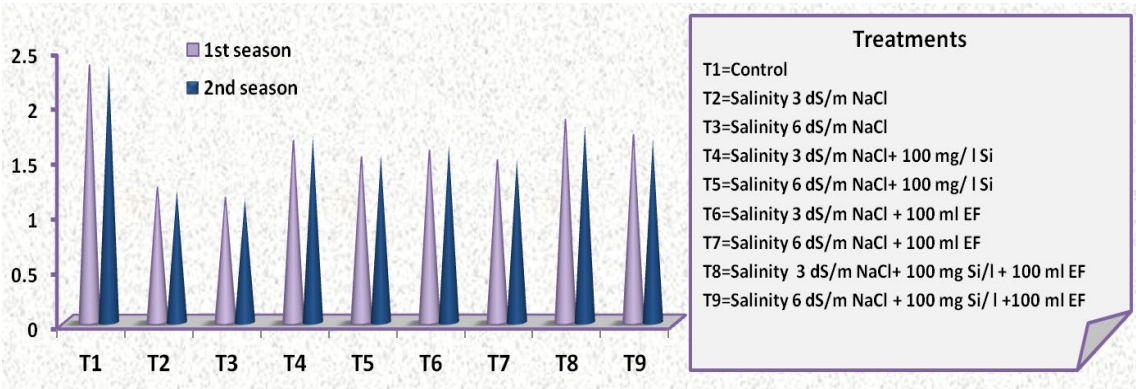

Figure 1. Effect of silicon and endophytic fungi addition on nitrogen content (%) of *Moringa oleifera* leaves under salinity stress.

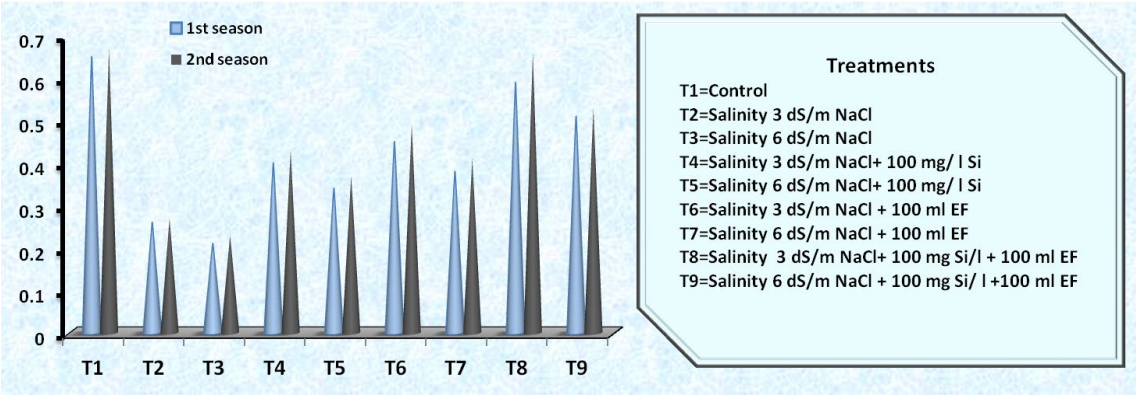

Figure 2. Effect of silicon and endophytic fungi addition on phosphorus content (%) of *Moringa oleifera* leaves under salinity stress.

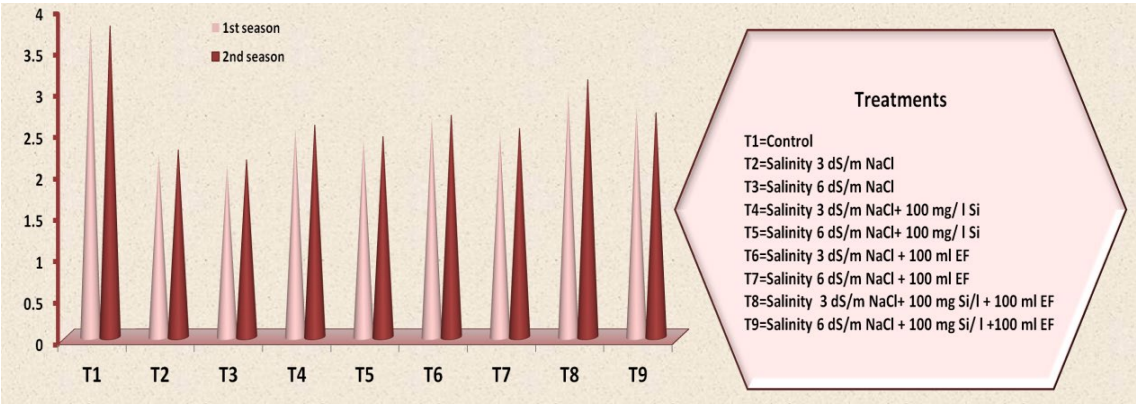

Figure 3. Effect of silicon and endophytic fungi addition on potassium content (%) of *Moringa oleifera* leaves under salinity stress.

Sodium and chloride content

Figures (4 and 5) illustrated the sodium and chloride contents in moringa leaves at 50 days and were in general, more increased in all treatments than of the control in the 1st and 2nd seasons. The most significant increase in Na and Cl contents were recorded when moringa were treated with salinity, whether at 3 or 6 dS m-1 NaCl. Application of silicon and EF together or separately led to a decrease in Na and Cl contents in moringa leaves compared to plants affected by salinity alone.

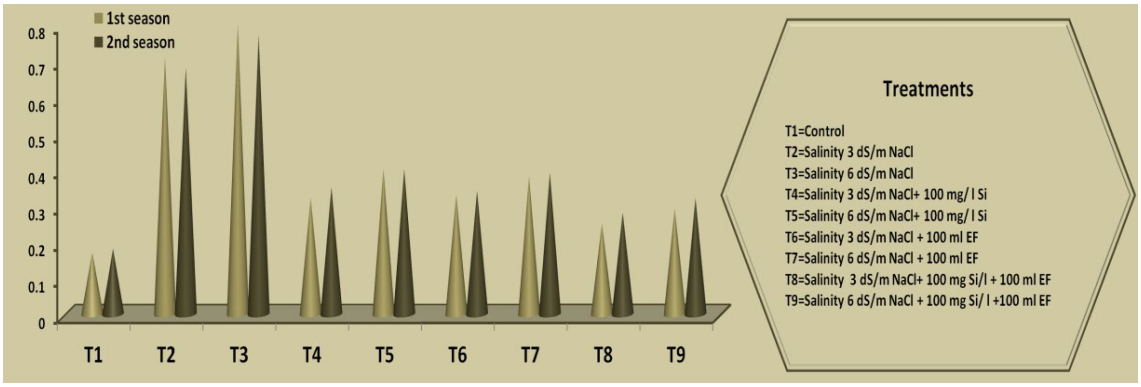

Figure 4. Effect of silicon and endophytic fungi addition on Na^+ content (mg/g DW) of *Moringa oleifera* leaves under salinity stress.

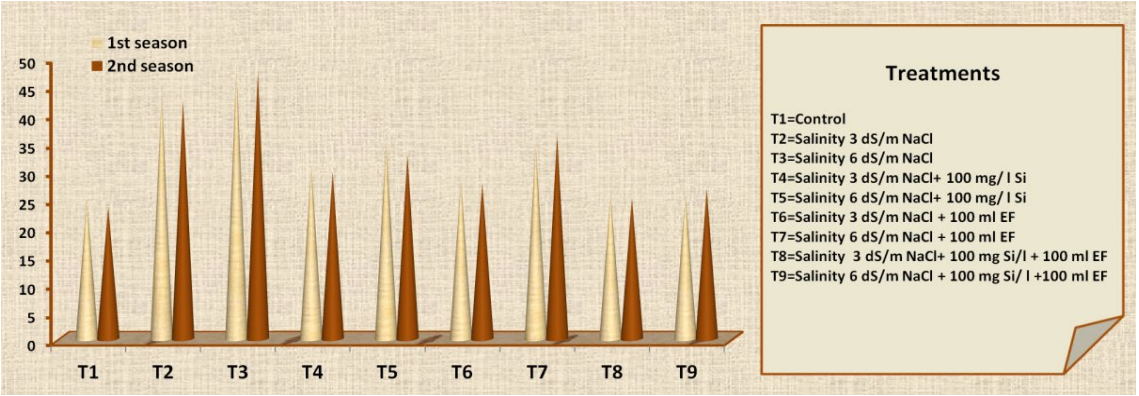

Figure 5. Effect of silicon and endophytic fungi addition on Cl^- content (mg/g DW) of *Moringa oleifera* leaves under salinity stress.

Chlorophyll content

Figure (6) shows the effect of adding silicon and EF on the chlorophyll content in moringa leaves affected by salinity. The highest values of chlorophyll content were obtained from control plants, followed by salinity- stressed plants, which treated with silicon and EF together in both seasons. On the other hand, the lowest values of chlorophyll content were recorded with salinity- stressed plants, and the decrease was greater with 3 or 6 dS m^{-1} NaCl compared to other treatments.

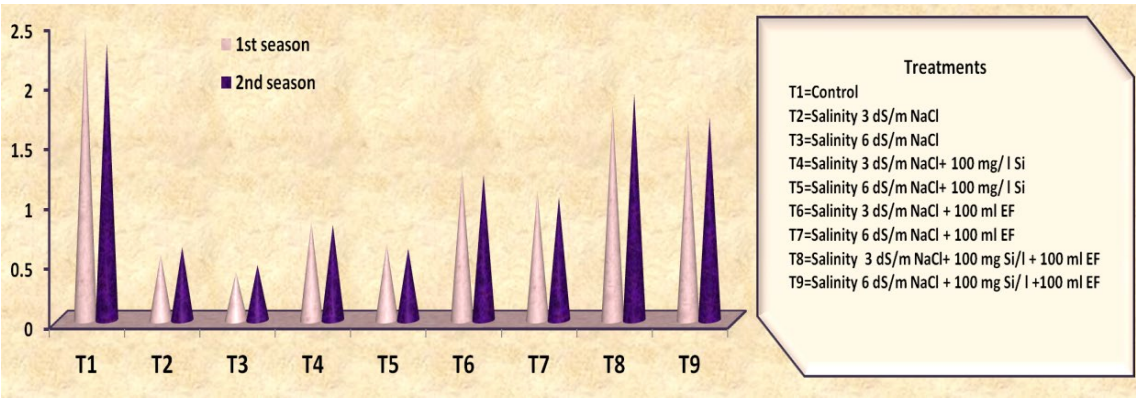

Figure 6. Effect of silicon and endophytic fungi addition on total chlorophyll content (mg/g FW) of *Moringa oleifera* leaves under salinity stress.

Discussion

The seed germination and plant growth are greatly affected under stress conditions such as salinity stress. Our study showed that germination percentage, survival rate, and biomass as well as chemical compositions of moringa plants were drastically suppressed under salinity conditions. Similar results have been observed in previous studies of Elhindi *et al.* (2016); Farouk *et al.* (2020); Sofy *et al*. (2020) and Ren *et al*. (2020). Also, Farooq *et al*. (2022) revealed that all salinity levels significantly affected the germination and growth parameters of *M. oleifera* landraces. Soliman *et al.* (2015) pointed out that salinity levels reduced growth parameters and chlorophyll content in *M. peregrina.* However, the reduction in germination, biomass and chemical compositions under salinity stress may be as a consequence of some physiological processes as photosynthetic activity, water

status, stomatal aperture, mineral nutrition, ion imbalance and carbon allocation (Bregnoli and Loutari, 1991; Li *et al*., 2015 and Farouk *et al.,* 2020). On the other side, the present study revealed that adding of silicon and EF was more effective with a salt- affected moringa plants in increasing germination, survival rate, and biomass as well as chemical compositions, with decreased of Na^+ and Cl^- content. The positive effects of silicon on the plant growth may be due to the increment in antioxidant capacity (Farouk and Omar, 2020 and Farouk *et al.,* 2020). Our results agree with Carballo-Méndez *et al.* (2022) who suggested that silicon addition should be considered as an alternative to reduce the harmful effects of salinity in *M. oleifera* plants. Li *et al*. (2015) concluded that addition of silicon in plants under salinity conditions, regulates the expression of aquaporins, which increased the root hydraulic conductivity and improved water absorption. They added that silicon application increased the stem hydraulic conductivity, which induces water supply to the leaves and led to increase the growth of leaves and shoots. Ali *et al*. (2021) suggested that the endophytic fungus can serve as a good way to improve growth of maize plants under salinity conditions. Application of *Aspergillus ochraceus* alleviated the negative effects of seawater on the growth and physiological characters of barley plants (Badawy *et al*., 2021). The beneficial effects of Endophytic fungi on growth and plant biomass were found in another species, such as *Solanum lycopersicum* (Azad and Kaminskyj, 2016); *Chrysophyllum oliviforme* (Ebeid and Shebany, 2017); *Zea mays* L. (Rho *et al*., 2018); *Oryza sativa* L. (Saddique *et al*., 2018) and *Triticum aestivum* L. (Zhang *et al*., 2019). The positive effects of endophytic fungi under salinity stress may be due to induce plants to synthesis of hormones that respond to stress (Manasa *et al*., 2020), or enable plants to regulate stress genes and by maintaining a low level of Na^+: K^+ ratio (Abdelaziz *et al*., 2017). Also, endophytic-fungi induced interplay of strigolactones that play regulatory roles in tolerance of salinity by interacting with phytohormones (Gupta *et al*., 2020). Salt tolerance by endophytic fungi coincide with the enhanced gibberellic acid concentration, which stimulated the physiological mechanism and gene in response to salinity stress that improved crop productivity (Siddiqui *et al*., 2022). In conclusion, *M. oleifera* is affected under saline conditions, especially at high levels. Addition of endophytic fungi and silicon together or individually leads to improve the germination, survival rate, biomass and chemical compositions. More studies should be conducted in this field on moringa species and their tolerance to salinity and the use of biological compounds and stimulants that mitigate the harmful effects of salinity.

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تأثير اضـــافة الســـليكون والفطريات الداخلية على بقاء النبات والكتلة الحيوية والمكونات الكيميائية لنبات المورينجا تحت ظروف الاجهاد الملحي

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الملخص

تعاني الأراضـي حديثة الاستصـلاح من بعض الاجهادات، خاصـة الاجهاد الملحي، سـواء بالتربة أو مياه الري الجوفية والتي تؤثر ســـلبا على نمو وانتاج بعض المحاصـــيل. أجريت هذه التجربة خلال موســـمي 2021 و2022 بمحطة بحوث المراشـــدة- قنا- مركز البحوث الزراعية بمصر لدراسة تأثير اضافة السليكون والفطريات الداخلية على النسبة المئوية للإنبات وبقاء النبات والكتلة الحيوية والصـفات الكيميائية لنبات المورينجا النامية بتربة رملية تحت ظروف الملحية. تم تصـميم التجربة بنظام العشـوائية التامة باسـتخدام 9 معاملات في ثلاث مكررات. انخفضـت النسـبة المئوية لكل من الإنبات وبقاء الشـتلة والكتلة الحيوية والخصـائص الكيميائية معنويا عند مسـتويي الملوحة 3 6 ديسـيسـيمنز/ متر ($3, 6\ dS\ m^{-1}$) وكان التأثر أكثر وضـوحا عند المسـتوي الأعلى. اضـــافة الســـليكون بمعدل 100 مجم/ لتر و/او 100 مـل فطريـات داخليـة متمثلا في فطر *Chaetomium globosum* بتركيز $5x10^7$ جرثومة/ مل نتج عنه تحسـن في دلائل النمو لنبات المورينجا مقارنة بالنباتات المتأثرة بالملوحة وبدون تلك الاضـــافات. كان التحســـن في النمو أكثر وضـــوحا عند المســـتوى الأقل من الملوحة في وجود اضـــافات الســـليكون والفطريات الداخلية في موسـمي الزراعة. نتج عن اضـــافة كل من السـليكون والفطريات الداخلية معا تحسـنا ملحوظا في دلائل النمو مقارنة بإضـــافة أي منهما بصـــورة منفردة. احتوت أوراق نبات المورينجا المتأثرة بالملوحة غير المعاملة على مســـتويات عالية من الصـــوديوم والكلور مقارنة بالنباتات المعاملة بالسـليكون والفطريات الداخلية. لذا توصـي الدراسـة بأنه عند زراعة نبات المورينجا بالأراضـي الرملية والمتأثرة بالملوحة فإن اضـافة وسـائل تخفيف أثار الملحية مثل السـليكون و/ أو الفطريات الداخلية يعزز من نموها في مثل هذه الظروف.