



## Titanium: An Element of Non-Biological Atmospheric Nitrogen Fixation and a Regulator of Sugar Beet Plant Tolerance to Salinity

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**T**HERE IS UNPRECEDENTED interest in the biological and non-biological atmospheric nitrogen fixation *via* some elements *e.g.*, titanium, nickel, vanadium, *etc.* to reduce the inputs of mineral N-fertilizers in the future, especially under salinity conditions. Also, synthetic nitrogen fertilization could increase the impurities in the sugar beet. So, A field trial ( as an exploratory experiment) was executed in a randomized complete block design (RCBD) to highlight the role of the Ti element in non-biological atmospheric nitrogen fixation and regulating sugar beet plant tolerance to salinity conditions ( soil EC value =6.25 dSm<sup>-1</sup> & irrigation water EC value =4.86 dSm<sup>-1</sup>). Treatments were as follows; **T<sub>1</sub>**: Without Ti (0.0 mg L<sup>-1</sup>); **T<sub>2</sub>**: Adding Ti as foliar application (5.0 mg L<sup>-1</sup>); **T<sub>3</sub>**: Adding Ti as foliar application (10.0 mg L<sup>-1</sup>); **T<sub>4</sub>**: Adding Ti as foliar application (15.0 mg L<sup>-1</sup>); **T<sub>5</sub>**: Adding Ti as soil injection (5.0 mg L<sup>-1</sup>); **T<sub>6</sub>**: Adding Ti as soil injection (10.0 mg L<sup>-1</sup>); **T<sub>7</sub>**: Adding Ti as soil injection (15.0 mg L<sup>-1</sup>); **T<sub>8</sub>**: Combination of both methods (Soil + foliar) [5.0 mg L<sup>-1</sup> , (2.5+2.5 for each method)]; **T<sub>9</sub>**: Combination of both methods (Soil + foliar) [10.0 mg L<sup>-1</sup> , (5.0+5.0 for each method)]; and **T<sub>10</sub>**: Combination of both methods (Soil + foliar) (15.0 mg L<sup>-1</sup> , (7.5+7.5 for each method)]. At the harvest stage, top & roots yield and juice quality were evaluated. The findings illustrate that the difference due to the studied treatments was significant, where the sequence order of the evaluated Ti treatments from the most effective to the less was as follows; **T<sub>8</sub>**>**T<sub>2</sub>**>**T<sub>3</sub>**>**T<sub>9</sub>**>**T<sub>5</sub>**> **T<sub>6</sub>**>**T<sub>1</sub>**> **T<sub>7</sub>**>**T<sub>4</sub>**>**T<sub>10</sub>**. Through the statistical comparison among the studied treatments, it can be noticed that the combined addition method of Ti (foliar plus soil) was the most effective one then the foliar application method solely followed by the soil injection method alone. Also, the best Ti rate was 5.0 mg L<sup>-1</sup> under all studied application methods, while plant yield parameters decreased thereafter as the Ti rate increased . Also, it can be noticed that the plant performance under the control treatment was better than that treated with 15.0 Ti mg L<sup>-1</sup> under all studied application methods. Generally, a better understanding of titanium toxicity in plant tissues may promote risk assessment and safe use of it.

**Keywords:** Nitrogen fixation, titanium dioxide, sugar beet and salinity.

### 1. Introduction

Salinity stress remains a main growth limitation factor which affects agricultural production. The increase of salts in the soil profile or in irrigation water increases the osmotic pressure in the area of the roots spreading and so that the plant can resist these

unsuitable conditions in the soil solution, the plant cells raise the internal osmotic pressure of the cytoplasm, and this leads to the plant losing the vital energy necessary for its development and growth, which leads to its weakness and lack of productivity

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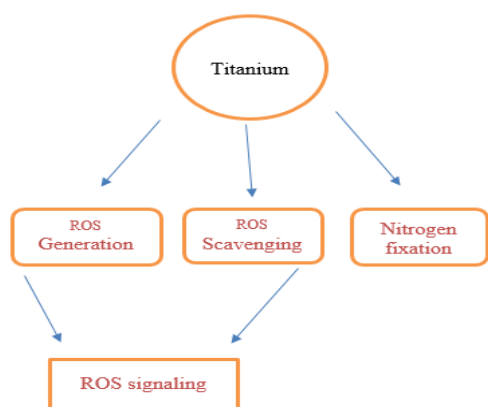
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(El-Hadidi *et al.*, 2020; Zein *et al.*, 2020; Sary, 2021; Abd-Elzaher *et al.*, 2022; Awwad *et al.*, 2022).

There is unprecedented interest in the biological and non-biological atmospheric nitrogen fixation via some elements *e.g.*, titanium, nickel, vanadium *etc.* to reduce the mineral N-fertilizers in the future, especially under salinity conditions. Titanium (Ti) is the ninth most abundant element in the earth's crust (Bain, 1975) and makes up about 0.25% of moles and 0.57% of the weight of the crust of the earth (Tan *et al.*, 2018).

Ti is classified as a useful element for plants (Abdel Latef *et al.*, 2018), which enhances their growth and development (Kushwah *et al.*, 2020). Some researchers confirmed the role of Ti in atmospheric N-fixation (Haghighi *et al.*, 2012; Moll *et al.*, 2016; El-Ghamry *et al.*, 2018). Perhaps the relationship between titanium and iron has a role in highlighting the importance of adding titanium at low concentrations, as titanium encourages the absorption of iron from the soil and titanium replaces iron in the event of a deficiency in the plant as a synergistic relationship (Lyu *et al.*, 2017).



Modulation of sugar beet plant responses (adapted from Abdel Latef *et al.*, 2018).

Sugar beet (*Beta vulgaris* L.) is considered to be a salt-tolerant crop; thus it is a good model for understanding salt acclimation in crops (Farkhondeh *et al.*, 2012; Yassin *et al.*, 2021). Significant progress has been made to understand the positive influences of titanium on plants, including its toxicity aspects, however, not much data is currently available about its potential to be used as a salinity stress-ameliorative in plants.

So, the aim of this study is to assess the Ti element as an element of non-biological atmospheric nitrogen fixation and a regulator of sugar beet plant tolerance to salinity as well as to find out the best addition

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method and proper concentration for sugar beet production.

## 2. Material and Methods

### 2.1. Experimental location

A field trial was carried out during the growing season of 2019/20 at a private farm located at El-Zawya village (31° 10' 41.0" E longitude and 31° 24' 27.0"N latitude), El-Hamoul District, Kafr El-Sheikh governorate, Egypt.

### 2.2. Sources of soil and irrigation water

The characteristics of soil before cultivation were investigated and the results are presented in Table 1. The irrigation water used in this study has EC, pH, SAR values of 4.86 dS m<sup>-1</sup>, 7.70 and 15.5, respectively. Table 2 shows the standard methods used in soil and irrigation water analyses.

**Table 1. Characteristics of initial soil.**

Particle size distribution (%)		
Sand	Silt	Clay
21.89	24.6	53.51
Texture class is Clay		
Hydro physical measurements		
Field capacity	Wilting point (%)	Saturation
45.18	22.50	90.36
Chemical analyses		
EC, dSm <sup>-1</sup>	pH	O.M, %
6.25	7.82	1.12
Available nutrients, mg kg <sup>-1</sup>		
N	P	K
47.00	8.99	225.1

### 2.3. Experimental setup

A field trial, as an exploratory experiment, was executed in a randomized complete block design (RCBD) to highlight the role of Ti element in non-biological atmospheric nitrogen fixation and regulating sugar beet plant tolerance to salinity conditions (soil EC value =6.25 dSm<sup>-1</sup> & irrigation water EC value =4.86 dSm<sup>-1</sup>). The treatments were as follows;

T<sub>1</sub>: Without Ti (0.0 mg L<sup>-1</sup>); T<sub>2</sub>: Adding Ti as foliar application (5.0 mg L<sup>-1</sup>); T<sub>3</sub>: Adding Ti as foliar application (10.0 mg L<sup>-1</sup>); T<sub>4</sub>: Adding Ti as foliar application (15.0 mg L<sup>-1</sup>); T<sub>5</sub>: Adding Ti as soil

injection (5.0 mg L<sup>-1</sup>); T<sub>6</sub>: Adding Ti as soil injection (10.0 mg L<sup>-1</sup>); T<sub>7</sub>: Adding Ti as soil injection (15.0 mg L<sup>-1</sup>); T<sub>8</sub>: Combination of both methods (Soil + foliar) [5.0 mg L<sup>-1</sup>, (2.5+2.5 for each method)]; T<sub>9</sub>: Combination of both methods (Soil + foliar) [10.0 mg L<sup>-1</sup>, (5.0+5.0 for each method)]; and T<sub>10</sub>: Combination of both methods (Soil + foliar) (15.0 mg L<sup>-1</sup>, (7.5+7.5 for each method)].

The experimental unit was 10.5 m<sup>2</sup> (3 m × 3.5 m). Seeds of suger beet (*Beta vulgaris* "(Cv Pleno)" were obtained from the Ministry of Agricultural and Soil Reclamation (MASR), Egypt. Planting took place, following rice pn the 15<sup>th</sup> of October, on one side of the ridge with a distance of 25.0 cm among sugar beet plants, at a rate of 3-4 balls/hill. The thinning process was done at a period of 45 days from sowing to ensure one plant hill<sup>-1</sup> with ten plants for each replicate.

*The schematic diagram illustrating the distribution of titanium treatments as related to the layout of the trial*

<b>Treatments</b>	T <sub>1</sub> : Without Ti (0.0 mg L <sup>-1</sup> )	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
	T <sub>2</sub> : Adding Ti as foliar application (5.0 mg L <sup>-1</sup> )	R <sub>2</sub>	R <sub>3</sub>	R <sub>1</sub>
	T <sub>3</sub> : Adding Ti as foliar application (10.0 mg L <sup>-1</sup> )	R <sub>3</sub>	R <sub>2</sub>	R <sub>1</sub>
	T <sub>4</sub> : Adding Ti as foliar application (15.0 mg L <sup>-1</sup> )	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
	T <sub>5</sub> : Adding Ti as soil injection (5.0 mg L <sup>-1</sup> )	R <sub>2</sub>	R <sub>3</sub>	R <sub>1</sub>
	T <sub>6</sub> : Adding Ti as soil injection (10.0 mg L <sup>-1</sup> )	R <sub>3</sub>	R <sub>2</sub>	R <sub>1</sub>
	T <sub>7</sub> : Adding Ti as soil injection (15.0 mg L <sup>-1</sup> );	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
	T <sub>8</sub> : Combination of both methods (Soil + foliar) (5.0 mg L <sup>-1</sup> )	R <sub>2</sub>	R <sub>3</sub>	R <sub>1</sub>
	T <sub>9</sub> : Combination of both methods (Soil + foliar) (10.0 mg L <sup>-1</sup> )	R <sub>3</sub>	R <sub>2</sub>	R <sub>1</sub>
	T <sub>10</sub> : Combination of both methods (Soil + foliar) (15.0 mg L <sup>-1</sup> ).	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>

Compost (16.8 Mg ha<sup>-1</sup>) and calcium superphosphate (240 kg ha<sup>-1</sup>, 15% P<sub>2</sub>O<sub>5</sub>) was added to the experimental area during soil preparation (before sowing). Ammonium nitrate (26 %N) was added at a rate of 192.0 kg N ha<sup>-1</sup> at two equal doses (the 1<sup>st</sup> was immediately after thinning and the 2<sup>nd</sup> was a month later). With the first dose of N-fertilizer, the K fertilization (potassium sulfate, 48 % K<sub>2</sub>O) was applied at a rate of 120.0 kg ha<sup>-1</sup>.The form of titanium used in this research work was titanium dioxide (TiO<sub>2</sub>) which was purchased from El-Gamhoria Company, Egypt. Then the standard solution was prepared with a known concentration by dissolving a known mass of the compound in the solvent, then preparing the different concentrations. The following schematic diagram illustrates the properties of TiO<sub>2</sub>.

Chemical formula >>>>>	TiO <sub>2</sub> , 59.93 %Ti
TiO <sub>2</sub> extracted from	the mineral ilmenite (FeTiO <sub>3</sub> )
Molar mass >>>>>>>>>	79.866 g/mol
Concentration range in dry plant tissue >>>>>>>>>	0.012-0.1, µg kg <sup>-1</sup>
Appearance >>>>>>>>>	White solid
Odor >>>>>>>>>>>>>>>>>>>	Odorless
Density >>>>>>>>>>>>>>>>>>>	4.17 g/mL at 25 °C (lit.)
Melting Point >>>>>>>>>>>>>>>>>>>	1830-3000°C

Ti solutions were prepared at the investigated levels, where their additions were executed immediately after thinning and repeated 4.0 times at 14.0 days intervals (either as foliar application or as soil injection). The agricultural drainage water under the surface irrigation regime was used in the irrigation process as the sugar beet plants needed. The other traditional agricultural practices e.g., boron, molybdenum and potassium fertilization (as foliar application) were done depending on the MASR for sugar beet production.

**2.4. Measurements**

At a maturity stage (180 days from planting), samples of sugar beet plants were taken and carefully uprooted for determining top and root fresh weights (g plant<sup>-1</sup> and Mg ha<sup>-1</sup>) as well as root length and diameter (cm) in addition to other the criteria as shown in Table 3. Juice quality and its chemical characteristics were determined depending on the procedures of the sugar beet laboratory of one of the factories in Egypt.

**2.5. Soil and Plant analyses**

Chemical analyses and hydro-physical measurements were determined in the soil prior to sugar beet cultivation. Particle size distribution was determined using pipette method. Saturation, field capacity and wilting limits were determined. EC value was determined in saturated soil paste extract using EC-meter, while pH value was determined by pH meter. Organic carbon was determined as described by Walkly and Balck method. Available nitrogen was determined by Micro-Kjeldahl method. Available potassium was determined using flame photometer. Available phosphorus was spectrophotometrically determined (Table 2).

For plant samples, juice quality and its chemical characteristics i.e., T. nitrogen, T. phosphorus, T. Potassium, T. sodium, alfa amino nitrogen (α- amino-N %), sucrose percentage, total dissolved solids percentage (TDS), impurities (α-amino N, Na and K

contents in juice), purity percentage, sugar loss percentage, sugar recovery (S.R.), recoverable sugar yield, quality index and sugar loss yield were determined (Table 3).

## 2.6. Statistical analyses

It was implemented according to Gomez and Gomez, (1984). Treatment means were compared by using the

least significant difference (LSD) at 0.05 level of probability. All statistical analysis was performed using the analysis of variance technique by means of CoStat computer software package (Version 6.303, CoHort, USA, 1998–2004).

**Table 2. The standard analytical techniques for analyzing soil and irrigation waters.**

Parameters	References	Notes
<b>Soil analyses</b>		
<b>Particle size distribution</b>	Dane and Topp (2020)	
<b>Chemical analyses</b>	Sparks <i>et al.</i> , (2020)	Initial soil sample was taken at depth of 0-30 cm.
<b>Hydro physical measurements</b>	Richards, (1954)	
<b>Irrigation water analyses</b>		
<b>pH</b>	Richards, (1954)	Potentiometry, pH meter
<b>EC, dS m<sup>-1</sup></b>	Richards, (1954)	Conductometry, EC meter
<b>SAR</b>	Richards, (1954)	<b>SAR:</b> Sodium adsorption ratio was calculated using the following formula $SAR = Na / \sqrt{(Ca^{+2} + Mg^{+2}) / 2}$ Water class is severe salty according to <b>Ayers and Westcot (1994)</b> .

**Table 3. Juice quality and its chemical analysis of sugar beet plants.**

Parameters	Methods	References
Digested plant samples	Mixed H <sub>2</sub> SO <sub>4</sub> + HClO <sub>4</sub> method	Jackson, (1973)
T. Nitrogen, %	Micro-Kjeldahl	
T. Phosphorus, %	spectrophotometrically	Walinga <i>et al.</i> , (2013).
T. Potassium, %		
T. Sodium, %	Flame photometer	
Alfa amino nitrogen ( $\alpha$ - amino-N %)	Fluorometric OPA-method	Cooke and Scott, (1993)
Sucrose percentage	Saccharometer	Le-Docte, (1927).
Total dissolved solids percentage (TDS)	Hand refractometer	A.O.A.C. (1995).
Impurities ( $\alpha$ -amino N, Na and K contents in juice)	Automated Analyzer	Cooke and Scott, (1993)
Purity percentage	$Purity \% = \{ (Sucrose \% - Sugar\ loss\ \%) / Sucrose\ \% \times 100 \}$ .	Carruthers and Oldfield, (1961)
Sugar loss percentage	$Sugar\ loss\ percentage = 0.29 + 0.343 (K+Na) + 0.094 \alpha\text{-amino-N}$ .	Harvey and Dutton (1993),
Sugar recovery (S.R.)	$Sugar\ recovery\ (\%) = sucrose\ \% - Sugar\ loss\ \%$	
Recoverable sugar yield	$Recoverable\ sugar\ yield\ (ton\ fed^{-1}) = root\ yield\ (ton\ fed^{-1}) \times sugar\ recovery\ \%$	
Quality index	$Quality\ index\ \% = (Sugar\ recovery\ \% \times 100) / Sucrose\ \%$	Cooke and Scott, (1993)
Sugar loss yield	$Sugar\ loss\ yield\ (ton\ fed^{-1}) = Root\ yield\ (ton\ fed^{-1}) \times Sugar\ loss\ \%$	

## 3. Results and Discussion

Sugar beet performance, root yield and juice quality at a maturity stage. Tables 4, 5 and Figs from 1 to 7 illustrate most of the factors affecting sugar beet

production depending on instructions from the sugar beet laboratories of the factories in Egypt.

Table 4 shows the effect of titanium application (via foliar application method and soil injection method as well as via combination of both methods) at different rates on some growth criteria *i.e.*, top fresh weight, root fresh weight, root length, diameter and yield as well as yield of sugar at a maturity stage (after 180 days from planting) during growing season of 2019/20. The superior treatment, which achieved the highest values of all aforementioned traits, was T<sub>8</sub> treatment. When comparing the treatments, it can be noticed that the plants that received Ti either as a foliar application or as soil injection possessed performance better than the other Ti levels under both solely and interaction treatments. Thus, it can be said that applying Ti at low concentrations (5.0 mg Ti L<sup>-1</sup>) is beneficial to plants and this may be due to its vital role in non-biological nitrogen fixation. Also, the gradual decrease in the performance associated with raising Ti levels more than 5 mg L<sup>-1</sup> may be due to the appearance of Ti toxicity on plants as mentioned by Al-Taani, (2008) and El-Ghamry *et al.*, (2018) who confirmed occurring non-biological N-fixation due to TiO<sub>2</sub> in NO<sub>3</sub> form as well as they confirmed occurring toxicity due to Ti addition at high concentration. Also, overall, it can be noticed that applying Ti as foliar and soil injection together (as a combined method) was the most effective Ti application method followed by the foliar application and the soil injection, respectively.

This may be due to the high efficiency of the application-combined method. In other words, the foliar application method could reduce the lag time between applying the Ti element and its absorption by the sugar beet plant in addition the soil injection method may be stimulated N-fixation in soil (Wang *et al.*, 2012). Generally, the findings illustrate that the difference due to the studied treatments was significant, where the sequence order of the evaluated Ti treatments from the most effective to the less was as follows; T<sub>8</sub>>T<sub>2</sub>>T<sub>3</sub>>T<sub>9</sub>>T<sub>5</sub>> T<sub>6</sub>>T<sub>1</sub>> T<sub>7</sub>>T<sub>4</sub>>T<sub>10</sub>.

Table 5 shows the effect of titanium application (via foliar application method and soil injection method as well as via combination of both methods) at different rates on N, P, K, Na and  $\alpha$ -amino N of sugar beet plants at the maturity stage (after 180 days from planting) during growing season of 2019/20. From Table 5, it can be noticed that Ti treatments at rates of 5.0 or 10.0 mg L<sup>-1</sup> significantly increased the values of N & P contents (%) and impurities such as K, Na and  $\alpha$ -amino N (%) compared to control treatment. This trend was achieved under all studied application methods.

Figure 1 and Table 6 illustrate the juice quality traits like sucrose (%) & TDS (%) & sugar recovery (%) &

purity (%) & impurity (%) & sugar loss (%) & recoverable sugar yield (%) & sugar loss yield (Mg fed<sup>-1</sup>) and quality index (%) at a maturity stage (180 days from planting) during growing season of 2019/20. It is quite obvious that titanium addition was beneficial for sugar beet plants at the rate of 5.0 mg Ti L<sup>-1</sup> (the lowest concentration) while this positive effect decreased at application rate of 10.0 mg L<sup>-1</sup> and exerted toxic effect juice quality at an application rate of 15.0 mg Ti L<sup>-1</sup>, respectively (at the highest concentrations) regardless of the additional method. In other words, the values of all juice quality characteristics, except both purity and quality index (%) which took another trend, increased significantly with raising Ti level from 0.0 to 5.00 mg Ti L<sup>-1</sup> then decreased significantly as the Ti rate increased to 10.00 and exhibited toxicity at 15.00 mg Ti L<sup>-1</sup>, where the lowest values were noticed. On the contrary, it could be found that the values of both purity and quality index (%) increased as Ti rate increased regardless of the additional method. These results may be due to the ability of titanium to fix the non-biological atmospheric nitrogen as some scientists have mentioned *i.e.*, Al-Taani, (2008), El-Ghamry *et al.*, (2018) and Ghazi *et al.*, (2021). Generally, titanium, at low concentration (5.0 mg Ti L<sup>-1</sup>), positively affected the growth and performance may via improving chlorophyll production, increasing the efficiency of the photosynthesis process or increasing enzyme activity and absorbing nutrients from the soil. Also, it can be said that titanium also might make sugar beet more resistant to salinity stress conditions.

On the other hand, application of titanium at a rate of 15.00 mg Ti L<sup>-1</sup> had a toxic effect on sugar beet plants compared to control treatment for most of the studied factors which affects sugar beet production, where the excessive titanium is well known to exert genotoxic effects on plants.

It is also worth noting that when compare with the reference control, T<sub>10</sub> is still higher than the reference control. Abdel Latef *et al.*, (2018) reported that the excessive titanium might cause the formation of agglomerates which can reduce the availability of free titanium to the plants. The mechanism of titanium toxicity to plant tissues at the studied high concentrations (15.00 mg Ti L<sup>-1</sup>) can be outlined through formation of the reactive oxygen Species (ROS) which might be produced following the induction of electron-hole pairs; leading to plant cell damage and lipid peroxidation Generally, a better understanding of titanium toxicity in plant tissues may promote risk assessment and safe use of it (Hou *et al.*, 2019).

**Table 4. Effect of titanium application (via foliar application method and soil injection method as well as via combination of both methods) at different rates on some growth criteria and root yield of sugar beet plants at a maturity stage (after 180 days from planting).**

Treatments	Top fresh weight (g plant <sup>-1</sup> )	Top fresh weight (Mg fed <sup>-1</sup> )	Root fresh weight (g plant <sup>-1</sup> )	Root length (cm)	Root diameter (cm)	Root yield (Mg fed <sup>-1</sup> )	Sugar yield (Mg fed <sup>-1</sup> )
T <sub>1</sub>	285.00g	5.97g	1087.33g	30.07e	12.23e	22.78g	4.20g
T <sub>2</sub>	363.00b	7.61b	1274.33b	33.60b	14.27ab	26.70b	5.42b
T <sub>3</sub>	348.67c	7.31c	1239.00c	32.97bc	13.77bc	25.96c	5.18c
T <sub>4</sub>	249.00i	5.22i	995.00i	28.57g	11.57fg	20.85i	3.69i
T <sub>5</sub>	316.00e	6.59e	1160.33e	31.40d	13.03d	24.31e	4.66e
T <sub>6</sub>	303.67f	6.36f	1123.33f	30.80d	12.43e	23.53f	4.42f
T <sub>7</sub>	262.67h	5.50h	1043.33h	29.30f	11.90ef	21.87h	3.95h
T <sub>8</sub>	379.67a	7.96a	1314.67a	34.33a	14.73a	27.55a	5.71a
T <sub>9</sub>	333.00d	6.98d	1201.00d	32.27c	13.47cd	25.16d	4.90d
T <sub>10</sub>	229.00j	4.80j	953.00j	27.73h	11.23g	19.97j	3.44j
<b>LSD at 5%</b>	<b>7.32</b>	<b>0.14</b>	<b>7.67</b>	<b>0.73</b>	<b>0.57</b>	<b>0.16</b>	<b>0.10</b>

Means within a row followed by a different letter (s) are statistically different at a 0.05% level.

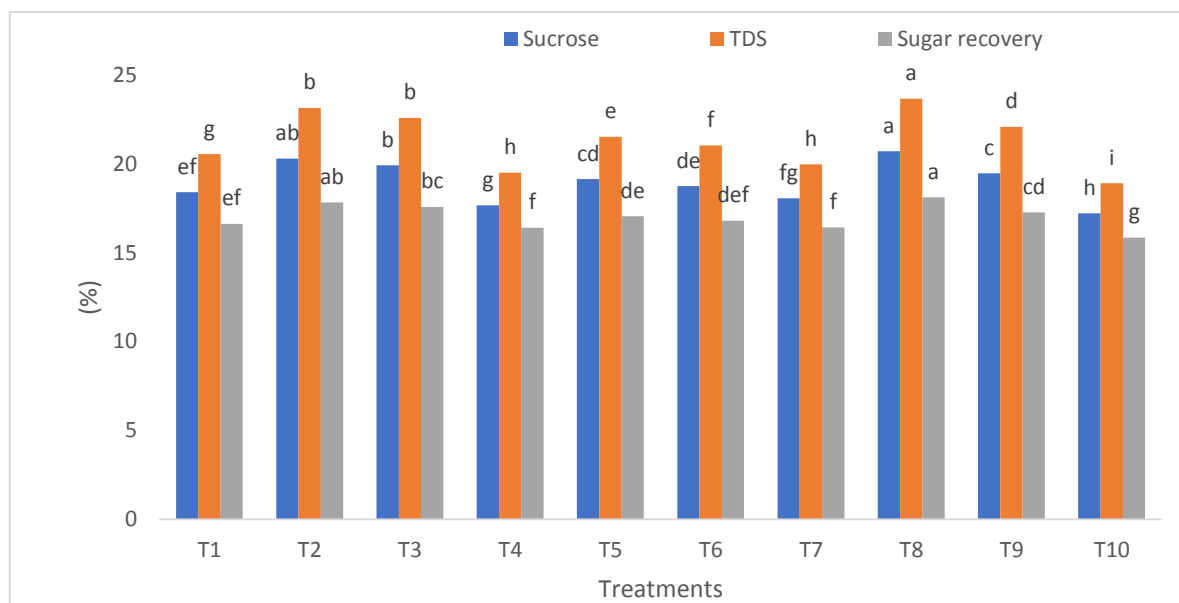
T<sub>1</sub>: Without Ti (0.0 mg L<sup>-1</sup>); T<sub>2</sub>: Adding Ti as foliar application (5.0 mg L<sup>-1</sup>); T<sub>3</sub>: Adding Ti as foliar application (10.0 mg L<sup>-1</sup>); T<sub>4</sub>: Adding Ti as foliar application (15.0 mg L<sup>-1</sup>); T<sub>5</sub>: Adding Ti as soil injection (5.0 mg L<sup>-1</sup>); T<sub>6</sub>: Adding Ti as soil injection (10.0 mg L<sup>-1</sup>); T<sub>7</sub>: Adding Ti as soil injection (15.0 mg L<sup>-1</sup>); T<sub>8</sub>: Combination of both methods (Soil + foliar) (5.0 mg L<sup>-1</sup>); T<sub>9</sub>: Combination of both methods (Soil + foliar) (10.0 mg L<sup>-1</sup>) and T<sub>10</sub>: Combination of both methods (Soil + foliar) (15.0 mg L<sup>-1</sup>).

**Table 5. Effect of titanium application (via foliar application method and soil injection method as well as via combination of both methods) at different rates on N, P, K, Na and α-amino N of sugar beet plants at the maturity stage (after 180 days from planting).**

Treatments	N	P	K (%)	Na	αN
T <sub>1</sub>	1.52g	0.229g	2.63g	0.96g	2.86g
T <sub>2</sub>	1.94b	0.296b	3.56b	1.66b	4.10b
T <sub>3</sub>	1.84c	0.282c	3.40c	1.53c	3.87c
T <sub>4</sub>	1.32i	0.196i	2.27i	0.64i	2.39i
T <sub>5</sub>	1.71e	0.258e	3.04e	1.23e	3.40e
T <sub>6</sub>	1.61f	0.244f	2.84f	1.12f	3.15f
T <sub>7</sub>	1.45h	0.213h	2.46h	0.79h	2.62h
T <sub>8</sub>	2.02a	0.308a	3.74a	1.78a	4.35a
T <sub>9</sub>	1.78d	0.271d	3.22d	1.37d	3.62d
T <sub>10</sub>	1.17j	0.181j	2.06j	0.51j	2.11j
<b>LSD at 5%</b>	<b>0.06</b>	<b>0.006</b>	<b>0.07</b>	<b>0.05</b>	<b>0.08</b>

Means within a row followed by a different letter (s) are statistically different at a 0.05% level.

T<sub>1</sub>: Without Ti (0.0 mg L<sup>-1</sup>); T<sub>2</sub>: Adding Ti as foliar application (5.0 mg L<sup>-1</sup>); T<sub>3</sub>: Adding Ti as foliar application (10.0 mg L<sup>-1</sup>); T<sub>4</sub>: Adding Ti as foliar application (15.0 mg L<sup>-1</sup>); T<sub>5</sub>: Adding Ti as soil injection (5.0 mg L<sup>-1</sup>); T<sub>6</sub>: Adding Ti as soil injection (10.0 mg L<sup>-1</sup>); T<sub>7</sub>: Adding Ti as soil injection (15.0 mg L<sup>-1</sup>); T<sub>8</sub>: Combination of both methods (Soil + foliar) (5.0 mg L<sup>-1</sup>); T<sub>9</sub>: Combination of both methods (Soil + foliar) (10.0 mg L<sup>-1</sup>) and T<sub>10</sub>: Combination of both methods (Soil + foliar) (15.0 mg L<sup>-1</sup>).



**Fig. 1.** Effect of titanium application (via foliar application method and soil injection method as well as via combination of both methods) at different rates on quality of sugar beet plants *i.e.*, sucrose (%) & TDS (%) and sugar recovery (%) at the maturity stage (after 180 days from planting).

**T<sub>1</sub>**: Without Ti (0.0 mg L<sup>-1</sup>); **T<sub>2</sub>**: Adding Ti as foliar application (5.0 mg L<sup>-1</sup>); **T<sub>3</sub>**: Adding Ti as foliar application (10.0 mg L<sup>-1</sup>); **T<sub>4</sub>**: Adding Ti as foliar application (15.0 mg L<sup>-1</sup>); **T<sub>5</sub>**: Adding Ti as soil injection (5.0 mg L<sup>-1</sup>); **T<sub>6</sub>**: Adding Ti as soil injection (10.0 mg L<sup>-1</sup>); **T<sub>7</sub>**: Adding Ti as soil injection (15.0 mg L<sup>-1</sup>); **T<sub>8</sub>**: Combination of both methods (Soil + foliar) (5.0 mg L<sup>-1</sup>); **T<sub>9</sub>**: Combination of both methods (Soil + foliar) (10.0 mg L<sup>-1</sup>) and **T<sub>10</sub>**: Combination of both methods (Soil + foliar) (15.0 mg L<sup>-1</sup>).

**Table 6.** Effect of titanium application (via foliar application method and soil injection method as well as via combination of both methods) at different rates on quality of sugar beet plants *i.e.*, purity (%), impurity (%), sugar loss (%), recoverable sugar yield (%), sugar loss yield (Mg fed<sup>-1</sup>) at the maturity stage (after 180 days from planting).

Treatments	Purity, %	Impurity, %	Recoverable sugar yield, Mg fed <sup>-1</sup>	Quality index, %
<b>T<sub>1</sub></b>	89.59ab	6.45g	3.79g	90.28cd
<b>T<sub>2</sub></b>	87.71b	9.32b	4.77b	87.86g
<b>T<sub>3</sub></b>	88.20b	8.80c	4.56c	88.23fg
<b>T<sub>4</sub></b>	90.64a	5.30i	3.42i	92.78a
<b>T<sub>5</sub></b>	88.92ab	7.66e	4.15e	89.17def
<b>T<sub>6</sub></b>	89.10ab	7.11f	3.96f	89.66cde
<b>T<sub>7</sub></b>	90.46a	5.86h	3.59h	90.89bc
<b>T<sub>8</sub></b>	87.53b	9.87a	4.99a	87.48g
<b>T<sub>9</sub></b>	88.24b	8.22d	4.35d	88.66efg
<b>T<sub>10</sub></b>	91.02a	4.68j	3.17j	92.03ab
<b>LSD at 5%</b>	<b>N.S</b>	<b>0.14</b>	<b>0.11</b>	<b>1.28</b>

Means within a row followed by a different letter (s) are statistically different at a 0.05% level.

**T<sub>1</sub>**: Without Ti (0.0 mg L<sup>-1</sup>); **T<sub>2</sub>**: Adding Ti as foliar application (5.0 mg L<sup>-1</sup>); **T<sub>3</sub>**: Adding Ti as foliar application (10.0 mg L<sup>-1</sup>); **T<sub>4</sub>**: Adding Ti as foliar application (15.0 mg L<sup>-1</sup>); **T<sub>5</sub>**: Adding Ti as soil injection (5.0 mg L<sup>-1</sup>); **T<sub>6</sub>**: Adding Ti as soil injection (10.0 mg L<sup>-1</sup>); **T<sub>7</sub>**: Adding Ti as soil injection (15.0 mg L<sup>-1</sup>); **T<sub>8</sub>**: Combination of both methods (Soil + foliar) (5.0 mg L<sup>-1</sup>); **T<sub>9</sub>**: Combination of both methods (Soil + foliar) (10.0 mg L<sup>-1</sup>) and **T<sub>10</sub>**: Combination of both methods (Soil + foliar) (15.0 mg L<sup>-1</sup>).

Our findings are in harmony with those of **Ghazi *et al.*, (2021)** who reported that the best performance of sugar beet was found in plants treated by Ti at rate of 5.00 mg L<sup>-1</sup>, but this performance decreased as the Ti addition rate increased. Beside **El-Ghamry *et al.*, (2018)** proved that titanium was beneficial at relatively higher concentrations (25.0 mg L<sup>-1</sup>) due to its role in the N-fixation process, while its toxicity began to appear with increasing its addition rate.

From the same Tables, it turns out that application of Ti to sugar beet plants as foliar spraying application and soil injection together (combination of both methods) was the most efficient then the foliar application method (alone) and the soil injection method (alone), respectively. The superiority of the combined application method compared to other studied methods may be attributed to the foliar application could reduce the lag time between addition and plant uptake. Moreover, the soil injection might stimulate N-fixation in soil, and thereby the combination of them was the most efficient (**Wang *et al.*, 2012**). Our findings are in accordance with those of **Ghazi *et al.*, (2021)** who reported that Ti addition to sugar beet as a combined method between foliar application and soil injection was the most effective procedure for... than using either foliar application or soil injection solely. Beside **Abdel Latef *et al.*, (2018)** concluded that Ti improved faba bean growth and performance under salinity conditions at rate of 0.01% (nTiO<sub>2</sub>) as foliar application.

#### 4. Conclusion

The obtained results indicate that applying Ti at a low concentration *i.e.*, 5.0 mg L<sup>-1</sup> either as foliar application or as soil injection or through both methods together to sugar beet plants is beneficial, but its toxicity started to appear at the high concentrations *i.e.*, 10 and 15 mg L<sup>-1</sup>. Also, it can be concluded that the combined addition method of Ti (foliar plus soil) at rate of 5.0 mg L<sup>-1</sup> was the most effective treatment. Generally, the obtained results also indicate possibility of owning Ti a vital role in non-biological nitrogen fixation under salinity circumstances. The toxicity of the titanium began to appear at the studied high concentrations (10.0 and 15.0 mg L<sup>-1</sup>). Generally, a better understanding of titanium toxicity in plant tissues may promote risk assessment and safe use of it. Also, this investigation highlights the need to precisely optimize the working rates of titanium according to the plant species and application method as well as plant life stage. Therefore, the results can serve as a very good starting point for

the development of titanium fertilizers for non-biological atmospheric nitrogen fixation and mitigating the negative influences of salinity under field conditions. Perhaps with more studies using modern techniques from advanced scientific devices, titanium can be considered one of the essential nutrients for plants in the next few years.

#### Conflicts of interest

Authors have declared that no competing interests exist.

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