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Effect of Titanium as a Stimulant of Atmospheric Nitrogen Fixation on Faba Bean Plants

Riham M.N. Faiyad and **Mohamed A. El-Sherpiny**

Soil & Water and Environment Research Institute, Agriculture Research Center, Giza, 12619 Egypt

STIMULANTS that enhance atmospheric nitrogen fixation, whether biological or non-biological, can potentially decrease the reliance on industrial nitrogen fertilizers. Biological stimulants involve living organisms, while non-biological stimulants involve naturally occurring substances. Therefore, a field experiment was conducted to assess the impact of using TiO_2 (extracted from the mineral ilmenite, which has the chemical formula $FeTiO_3$) as a catalyst to facilitate the conversion of atmospheric nitrogen into nitrate without the involvement of microorganisms. The tested plant in this investigation was faba bean plants. The primary treatments in the experiment consisted of T_1: Without titanium or rhizobium inoculant (as control$_1$); T_2: Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; T_3: With rhizobium inoculant (as control$_2$) and T_4: Soil injection of titanium at rate of 6 mg L^{-1}. The sub-main factor involved the foliar application of various rates of titanium: F_1: Without titanium (control); F_2: With titanium (3 Ti mg L^{-1}); F_3: With titanium (6 Ti mg L^{-1}); F_4: With titanium (9 Ti mg L^{-1}). The obtained results show that the soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1} (T_2 treatment) was the superior for obtaining the maximum values of nitrogen content in leaves as well as growth performance (*e.g.,* plant height, fresh and dry weights…*etc.*) and productivity (*e.g.,* seed yield, seeds content of protein and carbohydrate…*etc.*) followed by T_3 treatment then T_4 treatment and lately T_1 treatment. Regarding foliar applications, there was a notable and gradual impact of titanium on nitrogen content in leaves, thus all the growth and productivity characteristics that were studied. As the concentration of titanium was increased from 0 mg L^{-1} to 3 and 6 mg L^{-1}, there was a gradual increase observed in these traits, followed by a significant decrease when the concentration of titanium reached 9 mg L^{-1}. Furthermore, it was observed that the values obtained from the control treatment, which did not involve any titanium foliar application, were higher than those found with the plants sprayed with 9 Ti mg L^{-1}, indicating that excessive titanium application have a negative effect on the traits. In general, the obtained results revealed that soaking faba bean seeds before sowing in a solution containing 6 mg Ti L^{-1} with foliar application of solution having a concentration of 6 mg Ti L^{-1} resulted in the highest nitrogen content in leaves, growth performance, and productivity. Finally, the study indicates that TiO_2 has the potential to serve as a cost-effective partial substitute for synthetic nitrogen fertilizers. Nevertheless, it is crucial to bear in mind that the process of non-biological nitrogen fixation on TiO_2 surfaces is still a new and under-explored field of research. More studies are necessary to comprehend the complete potential of TiO_2 as a nitrogen fixation stimulant and to assess its efficacy in diverse crop types and environmental conditions.

Keywords: TiO_2, rhizobium, N fixation, Nitrogen uptake.

1. Introduction

Plants rely on nitrogen as a fundamental element for their growth and development (**Souri and Hatamian 2019**). It plays a pivotal role in chlorophyll, the pigment responsible for photosynthesis that converts sunlight into energy **(Ohyama 2010).** Moreover, nitrogen is an indispensable component of amino acids, the basic building blocks of proteins, and nucleic acids like DNA and RNA **(Leghari *et al.* 2016).** In its absence, plants cannot synthesize these essential molecules, and their growth and reproduction can be impeded **(Gu *et al.* 2018).** Furthermore, plants

*Corresponding author e-mail: m_elsherpiny2010@yahoo.com
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require nitrogen to produce secondary metabolites, including alkaloids and terpenoids, which provide defense against pests and diseases **(Muleta and Aga 2019).** Hence, it is crucial for plants to have sufficient access of nitrogen to thrive and develop optimally (**Sun *et al.* 2020).**

Industrial fertilizers have become the most important source of nitrogen for agriculture in many parts of the world **(Houser and Stuart 2020).** These fertilizers are typically produced from ammonia, which is generated through the Haber-Bosch process, a large-scale industrial method developed in the early 20th century **(Maxwell 2004).** While the use of industrial fertilizers has significantly increased crop yields and helped to feed a growing population, it has also resulted in environmental issues, including waterway pollution and greenhouse gas emissions **(Mulvaney *et al*. 2009).** Additionally, the application of nitrogen synthetic fertilizers may have adverse effects on human health. Nitrate, a byproduct of nitrogen fertilizer use, may cause health issues like methemoglobinemia, or blue baby syndrome **(Powlson *et al.* 2008).** Synthetic nitrogen fertilizers alternative is the utilization of organic and bio fertilizers **(Ijaz *et al.* 2021; Sadvakasova *et al.* 2021**)**.** Other alternatives include cover crops, crop rotation and using nitrogen-fixing plants like legumes. These methods can help to reduce the need for synthetic nitrogen fertilizers and improve soil health **(El-Sherpiny *et al.* 2023).**

Another approach to reducing the reliance on synthetic fertilizers is the development of new technologies, such as using stimulants that enhance atmospheric nitrogen fixation (non-biologically) **(Doklega *et al*. 2022).** Non-biological nitrogen stimulants involve naturally occurring substances such as titanium dioxide (TiO_2), which is extracted from the mineral ilmenite ($FeTiO_3$) as reported by **Al-Taani (2008)** who stated that this process is known as photocatalytic nitrogen fixation. Many studies confirmed that TiO_2 is a catalyst to facilitate the conversion of atmospheric nitrogen into nitrate without the involvement of microorganisms (**El-Ghamry *et al.* 2018).** Furthermore, titanium assists plants in coping with different environmental stresses, such as salinity, drought, heat, and cold (**Ghazi *et al.* 2021**). It also contributes to enzyme activation and helps to regulate the absorption and distribution of other vital nutrients (**Soliman and El-Sherpiny 2021**). Titanium concentration range in dry plant tissue is between 0.012-0.1, µg kg^{-1} **(Ghazi *et al.* 2022).** Faba bean (*Vicia faba* L.) plant is a crop that requires relatively small amounts of synthetic nitrogen fertilizers as a stimulatory dose (starter dose) because it has the ability to fix nitrogen in its root nodules through a process called biological nitrogen fixation (**El-Sherpiny *et al.* 2023**). As a result, faba bean plant has been chosen as a crop for study because it is expected that the effects of alternative treatments will be more pronounced. In addition to its suitability for research, the Faba bean plant is an important strategic crop **(Farid *et al.* 2021**) due to its nutritional value and economic importance in many regions of the world **(El-Tahlawy *et al.* 2022**), as it is a good source of protein, dietary fiber, and a range of essential vitamins and minerals (**Abdeen and Hefni 2023; El-Mahdy *et al.*,2021).** Therefore, the specific aim of this investigation was to evaluate the effects of using TiO_2 as a catalyst to convert atmospheric nitrogen into nitrate, without relying on microorganisms, and how it affects the growth of faba bean plants.

2. Material and Methods

- Study area

An experiment conducted over two consecutive seasons, 2021/22 and 2022/23, took place at Tag El-Ezz Experimental Farm, Agricultural Research Station (ARS), Egypt with the geographical coordinates of 31°31'47.64" N latitude and 30°56'12.88" E longitude. Table 1 presents the climatic elements at the studied site.

- Soil sampling

Table 2 presents the initial properties of the soil samples collected, which were analyzed using standard procedures. The pipette method was used to identify the particle size distribution (%) (**Gee and Bauder 1986)**, and the soil texture triangle was used to determine the textural class (**Moreno-Maroto Alonso-Azcarate 2022)**. The pH value was determined by means of a pH meter, and the electrical conductivity was measured using an EC-meter (**Sparks *et al.* 2020)**. The amount of organic matter (O.M) was determined through the Walkley and Balck method (**Dewis and Freitas 1970)**. To measure nitrogen, the Kjeldahl method was employed (**Hesse 1971)**, and phosphorus was determined using the spectrophotometric method **(Tandon 2005).** Finally, the flame photometer was used to determine potassium (**Dewis and Freitas 1970)**.

Table 1. Climatic elements in the studied site (means).

Month	Temperature (°C)		Rain (mm)	
	2021/22	2022/23	2021/22	2022/23
October	25.3	26.4	0.01	0.01
November	19.6	20.6	6.50	5.95
December	16.5	15.5	8.30	6.32
January	14.0	14.7	10.6	7.80
February	13.9	14.2	11.0	10.0
March	16.7	17.8	7.40	8.20
April	18.9	20.2	3.20	4.30

Table 2. Properties of the initial soil before sowing (initial soil).

Property and units	Initial soil	Note and references
Sand, %	22.50	Note: The soil samples were taken at a depth of 0-30 cm before planting. The data presented in this table is the combined data over both studied seasons.
Silt, %	29.00	
Clay, %	48.50	
Textural	Clayey	
Available N, mg Kg^{-1}	43.29	
Available P, mg kg^{-1}	8.02	
Available K, mg kg^{-1}	203.14	
EC, dS m^{-1} (Suspension 1:.5)	4.62	
pH (Suspension 1:2.5)	8.00	
Organic matter OM, %	1.15	

- Titanium used and preparing the studied solutions

Titanium dioxide (TiO_2, 59.93 %Ti) was obtained from the Faculty of Agriculture, Mansoura University, Egypt. It was extracted from the mineral ilmenite ($FeTiO_3$). It possesses a molar mass value of 79.866 g/mol, appearance (white solid), density (4.17 g/mL at 25 ° Celsius, lit.), and melting point (1830-3000°Celsius). To create the standard solution, a specific concentration was obtained by dissolving a measured amount of TiO_2 in water, followed by the preparation of varying concentrations according to **El-Ghamry *et al.* (2018); Ghazi *et al.* (2021); Doklega *et al*. (2022)**.

- Tasted plant

Faba bean (*Vicia faba* L.) plant has been chosen as a crop for study because it is expected that the effects of alternative treatments will be more pronounced. Giza 716 seeds, which are an early cultivar, were obtained from ARC, Egypt for planting purposes.

- Experimental design and treatments

A field experiment was conducted under a split plot design with three replicates. The primary treatments in the experiment consisted of **T_1:** Without titanium or rhizobium inoculant (as $control_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as $control_2$) and **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}. The sub-main factor involved the foliar application of various rates of titanium: **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}); **F_4:** With titanium (9 Ti mg L^{-1}). The sub-sub plot size was 14 m^2 (3.5 m × 4 m). A portion of the seeds was treated with Rhizobium inoculum (at a rate of 15 g per 1 kg of seeds) using 40% Arabic gum as a sticker, designated as **T_3** treatment. Another part of the seeds was soaked in a solution with a titanium concentration of 6 mg L^{-1}, designated as **T_2** treatment. For the **T_4** treatment, titanium was directly injected into the soil at a rate of 6 mg L^{-1} after sowing. Additionally, the external application of titanium was done twice, at 45 and 60 days after sowing using a hand sprayer until the saturation point was reached (with a volume of 1300 L ha^{-1}).

- Soil preparation and cultivation

In both studied seasons, the soil was ploughed and tilled to create a fine seedbed. Rocks and other debris were removed from the soil. Calcium superphosphate [$Ca(H_2PO_4)_2$, 15% P_2O_5)] at a rate of 100 kg fed^{-1} was added to the soil before planting. The recommended seed rate (almost 140 kg ha^{-1}) was sown on 10^{th} November. The seeds were sown directly into the soil at a depth of about

3-5 cm, and a spacing of 60 cm among the ridges and 25 cm among the plants at both sides of the ridge. The seeds were covered with soil and then pressed down lightly. After sowing, the field was watered immediately to ensure adequate moisture for seeds germination and the subsequent irrigations were applied as required throughout the growing season. When the plant height became 15 cm, thinning was carried out leaving two plants per hill. After 35 days from sowing, all plots were treated with potassium sulfate (48% K_2O) at a rate of 25 kg K_2O fed^{-1}. After 15 days from sowing, all plots were treated with urea (46.5 % N) at a rate of 15 kg N fed^{-1}. The guidelines provided by the Egyptian Ministry of Agriculture were followed to implement additional recommended agricultural practices for faba beans. The harvest was done on 26th April.

- **Measurements**

Three plants at 70 and 165 days after sowing were randomly sampled from each replicate, along with soil samples at 165 days after sowing, to estimate the characteristics presented in Table 3.

- **Statistical analyses**

The statistical method outlined by **Gomez and Gomez (1984) and CoStat version 6.303 copyright (1998-2004**) was employed to analyze the data. To compare treatment means, the least significant difference (LSD) was utilized at a significance level of 0.05.

Table 3. Methods, formula, and references of measurements.

Parameters	Methods and formula	References
	At flowering stage (70 days from sowing)	
Plant height (cm), fresh and dry weights (g plant^{-1})	Manually and visually	**Hacıseferoğulları *et al.* (2003)**
Nitrogen uptake	N-uptake = N concentration x dry weight /100	**Li *et al*. (2003)**
Chlorophyll content, SPAD value	SPAD reading(SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera, Osaka, Japan)	**Castelli *et al*. (1996)**
Digested plant samples	Mixed of $HClO_4 + H_2SO_4$	**Peterburgski (1968)**
N, P, K%	Micro-Kjeldahl, Spectrophotometrically and Flame photometer, respectively	**Walinga *et al.* (2013)**
	At harvest (165 days from sowing)	
No. of pods plant^{-1}, pod weight (g), and seed yield (Mg ha^{-1})	Manually and visually	**López-Bellido *et al.* (2005)**
Total dissolved solids (TDS), %	Hand refractometer	
Protein percentage, %	Micro-Kjeldahl Protein % = (N) × 6.24.	**A.O.A.C (2000)**
Total percentage, %	Carbohydrates	
Available soil N, P, K, mg kg^{-1}	Micro-Kjeldahl, Spectrophotometrically and Flame photometer, respectively	**Dewis and Freitas (1970); Hesse (1971)**

3. Results

- **Nitrogen uptake in plant tissues and soil available nitrogen**

Fig.1 presents the results of the study on the impact of titanium and rhizobium on the mean values of nitrogen uptake in leaves of faba bean plants after 70 days of sowing, conducted over two seasons, 2021/2022 and 2022/2023. While Fig 2 illustrates the impact of titanium and rhizobium on the mean values of soil available N at harvest stage (combined data over both seasons).

Fig.1 shows that the soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1} (**T_2** treatment) caused the maximum mean values of nitrogen uptake in leaves followed by T_3 treatment (with rhizobium inoculant, as control2) then T4 treatment (Soil injection of titanium at rate of 6 mg L^{-1}) and lately T_1 treatment (without titanium or rhizobium inoculant, as control1). Regarding foliar applications, there was a notable and gradual impact of titanium on nitrogen uptake in leaves. As the concentration of titanium was increased from 0 mg L^{-1} to 3 and 6 mg L^{-1}, there was a gradual increase observed in nitrogen uptake in leaves, followed by a significant decrease when the concentration of titanium reached 9 mg L^{-1}. Furthermore, it was observed that the mean values of N-uptake under the control treatment, which did not involve any titanium foliar application, were higher than those found with the plants sprayed with 9 Ti mg L^{-1}, indicating that excessive titanium application have a negative effect. In general, the obtained results revealed that soaking faba bean seeds before sowing in a solution containing 6 mg Ti L^{-1} with foliar application of solution having a concentration of 6 mg Ti L^{-1} resulted in the maximum mean values of nitrogen uptake in leaves.

According to Fig. 2, all titanium treatments tested **(T_2, T_3, T_4)** resulted in increased levels of available nitrogen in the soil after harvest, compared to the control treatment **(T_1).** Specifically, injecting titanium into the soil at a rate of 6 mg L^{-1} (T_4 treatment) led to the highest N values (mg kg^{-1}), followed by the T_3 treatment (which involved using a rhizobium inoculant), then the $\mathbf{T_2}$ treatment (which involved soaking seeds in a solution with a titanium concentration of 6 mg L^{-1}) and, finally, the control treatment **(T_1)** with no titanium or rhizobium inoculant. It was unclear from the data whether the foliar application of titanium had any effect on the available nitrogen levels in the soil. In general, it appears that all titanium treatments may have improved the process of N-fixation, as evidenced by the mean values of N-uptake and soil available N at the harvest stage. Overall, these findings suggest that TiO_2 has the potential to be a viable alternative to synthetic nitrogen fertilizers.

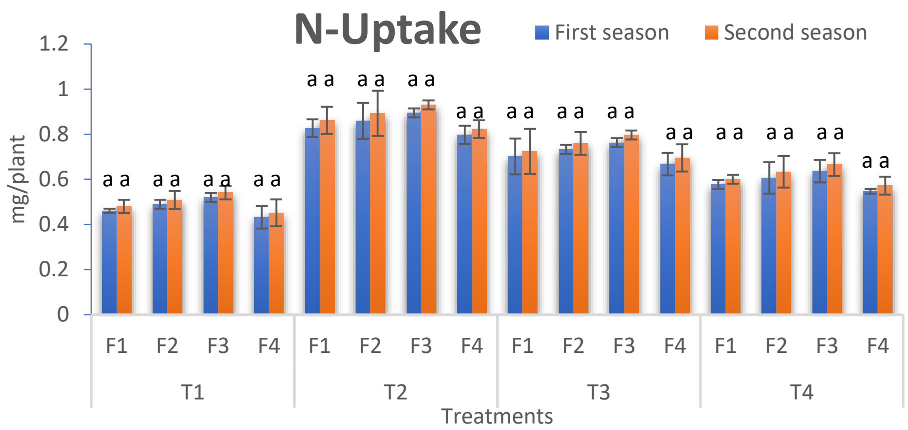

Fig. 1. Effect of studied treatments on N uptake by plants after 70 days of sowing.

T_1: Without titanium or rhizobium inoculant (as $control_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as $control_2$) ; **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}; **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}) and **F_4:** With titanium (9 Ti mg L^{-1})

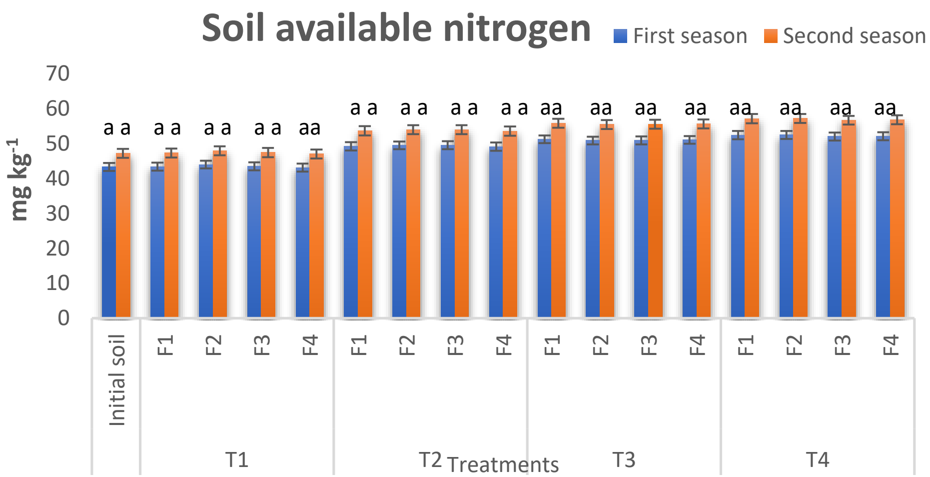

Fig. 2. The impact of titanium and rhizobium on soil available N values at harvest stage

T_1: Without titanium or rhizobium inoculant (as $control_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as $control_2$) ; **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}; **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}) and **F_4:** With titanium (9 Ti mg L^{-1})

- **Performance and productivity**

The data presented in Tables 4, 5, 6 and 7 indicate the significant effects of the treatments on various growth criteria and productivity of faba bean plants. Plant height (cm), fresh and dry weights (g plant^{-1}), and chlorophyll content (SPAD value) after 70 days from sowing were significantly affected by the studied treatments during both seasons of 2021/22 and 2022/23. Moreover, the chemical constituents in leaves, such as N, P, and K (%), were significantly affected by the studied treatments during the same period in both seasons. The treatments also had a significant impact on pod yield measurements, including the number of pods plant^{-1}, pod weight (g), and seeds yield (Mg ha^{-1}) as well as seed bio-constituents, such as protein, carbohydrates, and TDS % after 165 days from sowing faba bean plants during both seasons.

Table 4. The impact of titanium and rhizobium on faba bean growth criteria and chlorophyll level after 70 days of sowing, across the seasons of 2021/2022 and 2022/2023.

Treatments	Plant height, cm		Fresh weight, g plant^{-1}		Dry weight, g plant^{-1}		Chlorophyll, SPAD value	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Main factor								
T_1	62.17d	65.59d	98.04d	99.21d	13.56d	13.84d	43.42d	44.43d
T_2	80.66a	84.88a	123.08a	124.65s	20.28a	20.68a	47.03a	47.95a
T_3	74.63b	78.25b	115.01b	116.97b	18.10b	18.47b	46.03b	46.91b
T_4	68.63c	72.60c	106.34c	107.72c	15.88c	16.20c	44.85c	45.70c
LSD at 5%	**0.37**	**0.39**	**1.56**	**0.38**	**0.15**	**0.19**	**0.64**	**0.63**
Sub main factor (foliar applications)								
F_1	70.74c	74.17c	109.59c	110.96c	16.68c	17.01c	45.08bc	46.00b
F_2	72.32b	76.19b	111.78b	113.34b	17.24b	17.60b	45.51ab	46.34ab
F_3	73.86a	77.92a	113.79a	115.48a	17.81a	18.15a	45.82a	46.83a
F_4	69.17d	73.03d	107.31d	108.77d	16.09d	16.41d	44.91c	45.82b
LSD at 5%	**0.40**	**0.42**	**1.24**	**0.39**	**0.10**	**0.24**	**0.52**	**0.53**
Interaction (TxF)								
LSD at 5%	**0.81**	**0.83**	**2.50**	**0.78**	**0.19**	**0.47**	**1.05**	**1.05**

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

T_1: Without titanium or rhizobium inoculant (as control$_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as control$_2$) ; **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}; **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}) and **F_4:** With titanium (9 Ti mg L^{-1})

Table 5. The impact of titanium and rhizobium on chemical constituents in leaves of faba bean plant after 70 days of sowing, across the seasons of 2021/2022 and 2022/2023.

Treatments	N,%		P,%		K,%	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Main factor						
T_1	3.51d	3.58d	0.343d	0.350d	2.09d	2.11d
T_2	4.16a	4.24a	0.454a	0.462a	2.92a	2.96a
T_3	3.96b	4.03b	0.414b	0.420b	2.70b	2.74b
T_4	3.72c	3.81c	0.378c	0.387c	2.41c	2.45c
LSD at 5%	**0.04**	**0.04**	**0.003**	**0.003**	**0.02**	**0.02**
Sub main factor (foliar applications)						
F_1	3.81c	3.88b	0.393c	0.400c	2.50c	2.53c
F_2	3.86b	3.93b	0.402b	0.409b	2.58b	2.61b
F_3	3.92a	4.01a	0.412a	0.421a	2.63a	2.67a
F_4	3.76c	3.83c	0.383d	0.390d	2.42d	2.44d
LSD at 5%	**0.05**	**0.05**	**0.002**	**0.002**	**0.03**	**0.01**
Interaction (TxF)						
LSD at 5%	**0.10**	**0.10**	**0.005**	**0.005**	**0.07**	**0.03**

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

T_1: Without titanium or rhizobium inoculant (as control$_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as control$_2$) ; **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}; **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}) and **F_4:** With titanium (9 Ti mg L^{-1})

Table 6. The impact of titanium and rhizobium on yield and its components of faba bean plant after 165 days of sowing, across the seasons of 2021/2022 and 2022/2023.

Treatments	No. of pods plant^{-1}		Pod weight, g		Seed yield, Mg ha^{-1}	
	1st	2nd	1st	2nd	1st	2nd
			Main factor			
T_1	18.00d	18.00d	16.53d	16.83d	2.414d	2.453d
T_2	29.92a	29.92a	22.33a	22.68a	4.118a	4.186a
T_3	26.25b	26.25b	20.46b	20.91b	3.662b	3.725b
T_4	21.50c	21.33c	18.55c	18.96c	2.966c	3.005c
LSD at 5%	**1.93**	**1.59**	**0.27**	**0.15**	**0.012**	**0.012**
			Sub main factor (foliar applications)			
F_1	23.08b	23.08b	19.22c	19.66c	3.230c	3.278c
F_2	24.67a	24.50a	19.74b	20.11b	3.365b	3.418b
F_3	25.42a	25.50a	20.19a	20.57a	3.485a	3.542a
F_4	22.50b	22.42b	18.72d	19.04d	3.082d	3.134d
5%	**0.78**	**1.09**	**0.22**	**0.12**	**0.018**	**0.02**
			Interaction (TxF)			
LSD at 5%	**1.55**	**2.19**	**0.44**	**0.23**	**0.036**	**0.038**

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

T_1: Without titanium or rhizobium inoculant (as control$_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as control$_2$) ; **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}; **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}) and **F_4:** With titanium (9 Ti mg L^{-1})

Table 7. The impact of titanium and rhizobium on bio constituents of faba bean seeds after 165 days of sowing, across the seasons of 2021/2022 and 2022/2023.

Treatments	Protein, %		Carbohydrates, %		TDS, %	
	1st	2nd	1st	2nd	1st	2nd
			Main factor			
T_1	15.72d	15.90d	53.75d	54.76d	2.98d	3.06d
T_2	20.63a	20.88a	55.80a	56.89a	4.07a	4.12a
T_3	19.04b	19.35b	55.15b	56.29b	3.72b	3.78b
T_4	17.47c	17.70c	54.49c	55.50c	3.35c	3.40c
LSD at 5%	**0.26**	**0.26**	**0.41**	**0.38**	**0.03**	**0.11**
			Sub main factor (foliar applications)			
F_1	17.97c	18.19c	54.69bc	55.74bc	3.49c	3.54b
F_2	18.43b	18.69b	54.89ab	56.05ab	3.57b	3.61b
F_3	18.86a	19.13a	55.08a	56.16a	3.65a	3.76a
F_4	17.60d	17.83d	54.53c	55.48c	3.40d	3.44c
LSD at 5%	**0.21**	**0.21**	**0.33**	**0.34**	**0.05**	**0.08**
			Interaction (TxF)			
LSD at 5%	**0.41**	**0.41**	**0.66**	**0.68**	**0.09**	**0.15**

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

T_1: Without titanium or rhizobium inoculant (as control$_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as control$_2$) ; **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}; **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}) and **F_4:** With titanium (9 Ti mg L^{-1})

The obtained results show that the soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1} (**T_2** treatment) was the superior for obtaining the maximum values of growth performance and productivity followed by **T_3** treatment (with rhizobium inoculant, as control$_2$) then **T_4** treatment (Soil injection of titanium at rate of 6 mg L^{-1}) and lately **T_1** treatment (without titanium or rhizobium inoculant, as control$_1$). Regarding foliar applications, there was a notable and gradual impact of titanium on the growth and productivity characteristics that were studied. As the concentration of titanium was increased from 0 mg L^{-1} to 3 and 6 mg L^{-1}, there was a gradual increase observed in these traits, followed by a significant decrease when the concentration of titanium reached 9 mg L^{-1}. Furthermore, it was observed that the values obtained from the control treatment, which did not involve any titanium foliar application, were higher than those found with the plants sprayed with 9 Ti mg L^{-1}, indicating that excessive titanium application have a negative effect on the traits. In general, the obtained results revealed that soaking faba bean seeds before sowing in a solution containing 6 mg Ti L^{-1} with foliar application of solution having a concentration of 6 mg Ti L^{-1} resulted in the highest growth performance and productivity.

- Soil available P and K after harvest

The impact of titanium and rhizobium treatments on the mean values of soil available P and K (mg kg^{-1}) at the harvest stage (combined data over both seasons) is presented in Table 8. The results indicate that the values of both P and K (mg kg^{-1}) at the harvest stage were higher for all treatments compared to the initial soil values before sowing. **T_1** treatment (Without titanium or rhizobium inoculant) caused the highest values of P, K (mg kg^{-1}), followed by **T_4** (soil injection of titanium at a rate of 6 mg L^{-1}), and then **T_3** (with rhizobium inoculant). The lowest values were observed with **T_2** (soaking seeds in a solution with a titanium concentration of 6 mg L^{-1}). This suggests that the best treatment for achieving optimal performance and productivity may also result in a decrease in the mean values of both P and K (mg kg^{-1}). This could be due to the effectiveness of the superior treatment (**T_2**) in enhancing the ability of faba bean roots to absorb these elements from the soil, leading to a reduction in soil stocks of these nutrients. Concerning the foliar applications, **F_3** treatment caused the lowest values of P, K (mg kg^{-1}) under each main treatment. By the same logic, it can be inferred that the application of Ti via foliar spraying at a rate of 6 mg L^{-1} was the most effective treatment, resulting in an improvement in the status of faba bean plants and a decrease in the residues of P and K in the soil compared to the control treatment (with maximum values) and other treatments **(F_1, F_2, F_4).**

Table 8. The impact of titanium and rhizobium on soil available P and K values at harvest stage (combined data over both seasons).

Treatments		P, mg kg^{-1}	K, mg kg^{-1}
T_1	**F_1**	10.75	236.41
	F_2	10.64	234.49
	F_3	10.57	232.48
	F_4	10.84	239.88
T_2	**F_1**	9.50	229.87
	F_2	9.37	228.39
	F_3	9.30	226.79
	F_4	9.62	231.18
T_3	**F_1**	9.93	223.55
	F_2	9.81	221.70
	F_3	9.72	220.33
	F_4	10.02	225.26
T_4	**F_1**	10.32	218.69
	F_2	10.20	217.78
	F_3	10.12	216.32
	F_4	10.42	219.46

T_1: Without titanium or rhizobium inoculant (as $control_1$); **T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}; **T_3:** With rhizobium inoculant (as $control_2$) ; **T_4:** Soil injection of titanium at rate of 6 mg L^{-1}; **F_1:** Without titanium (control); **F_2:** With titanium (3 Ti mg L^{-1}); **F_3:** With titanium (6 Ti mg L^{-1}) and **F_4:** With titanium (9 Ti mg L^{-1}).

4. Discussion

In general, the superiority of titanium-treated faba bean plants over untreated plants can be attributed to the favorable impact of titanium on plant growth and development **(Doklega *et al*. 2022).**. As a micronutrient, titanium plays a crucial role in various physiological processes, including nutrient uptake, photosynthesis and stress tolerance **(Moll *et al.* 2016).** By promoting root development, improving nutrient uptake efficiency, and increasing chlorophyll content, titanium treatments can enhance plant growth and yield. Additionally, titanium can enhance faba bean plant resistance to biotic and abiotic stresses such as disease, drought, and high salinity, resulting in improved plant performance and yield (**Soliman and El-Sherpiny 2021).** Therefore, the application of titanium treatments can offer a significant advantage in faba bean production by enhancing plant growth performance, yield, and quality. Moreover, there is some evidence to suggest that titanium may play a role in the non-biological fixation of atmospheric nitrogen to nitrate on TiO_2 surfaces. In this process, TiO_2 acts as a catalyst to facilitate the conversion of atmospheric nitrogen into nitrate through a series of reactions involving intermediate nitrogen oxide species. When titanium dioxide (TiO_2) is exposed to ultraviolet (UV) light, it undergoes a photochemical reaction that can lead to various effects and applications **(Kozlova *et al.* 2021).** In the case of TiO_2, it has a relatively wide bandgap energy, which means it absorbs UV light and reflects or transmits most of the visible light. When UV light with energy higher than the bandgap energy of TiO2 is incident upon it, electron-hole pairs (excitons) are generated. Electrons from the valence band are excited to the conduction band, leaving behind positively charged holes in the valence band. These electron-hole pairs in titanium dioxide can participate in various photochemical reactions **(Wu**

***et al.* 2022).** The excited electrons and holes can react with adsorbed molecules on the surface of titanium dioxide such as nitrogen, leading to chemical transformations. The results are in harmony with those of **Al-Taani (2008)** who demonstrated the occurrence of this process due to TiO_2, while **El-Ghamry *et al.* (2018)** found that low concentrations of Ti addition could be beneficial for the N-fixation process, but high concentrations could lead to toxicity. It is possible that the soaking and injection of titanium treatments could have led to atmospheric nitrogen fixation, particularly when compared to rhizobium treatments known for their ability in biological nitrogen fixation. However, further research would be required to confirm this and to fully understand the potential mechanisms underlying any observed effects of titanium treatments on nitrogen fixation. It is also worth noting that biological nitrogen fixation through the action of nitrogen-fixing bacteria like rhizobium remains an important and effective means of increasing soil fertility and improving plant growth, particularly in leguminous crops like faba beans.

The superior performance of the soaking treatment (**T_2:** Soaking seeds before sowing in a solution having a titanium concentration of 6 mg L^{-1}) could potentially be attributed to several factors. Soaking seeds in a solution containing titanium may increase the availability of the micronutrient to the developing plant, thereby promoting growth and yield. Additionally, soaking may help to overcome any potential seed dormancy or germination barriers, allowing for more rapid and uniform seedling emergence.The use of a low concentration of titanium in the soaking solution may also be beneficial, as excessive concentrations of micronutrients can sometimes have toxic effects on plants. Further research would be required to fully understand the underlying mechanisms of any observed effects and to confirm the cause of the superior performance of the soaking treatment. It is possible that foliar applications of titanium may lead to the accumulation of the micronutrient in faba bean plant tissues, which could potentially provide a defense against environmental stress. This trend seems to be observed at low concentrations of titanium (3 and 6 mg L^{-1}). However, at high concentrations (9 mg L^{-1}), the application of titanium appears to have a toxic effect on faba bean plants. It is important to note that the specific effects of titanium on plant growth and development can vary depending on a range of factors, including plant species, application method, and concentration of the micronutrient. Further research would be required to fully understand the optimal conditions for the use of titanium as a micronutrient supplement in agriculture. The findings are in agreement with those of **Ghazi *et al.* (2021) and (2022).**

4. Conclusion

In conclusion, the study highlights the potential of TiO_2 as a substitute for synthetic nitrogen fertilizers. The obtained results revealed that soaking faba bean seeds before sowing in a solution containing 6 mg Ti L^{-1} with foliar application of solution having a concentration of 6 mg Ti L^{-1} resulted in the highest nitrogen content in leaves, growth performance, and productivity. Moreover, it is important to note that excessive titanium application can have a negative impact on these traits.

Generally, the use of TiO_2 as a cost-effective alternative to synthetic nitrogen fertilizers holds promise for sustainable agriculture. Therefore, it is recommended that farmers and researchers consider the use of TiO_2 as a non-biological stimulant to enhance atmospheric nitrogen fixation, but caution should be taken to avoid excessive titanium application, which may have negative effects on the traits. Further studies are needed to explore the long-term effects of using TiO_2 and to determine the optimal concentrations and application methods for different crops and environmental conditions.

Conflicts of interest

Authors have declared that no competing interests exist.

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