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Possibility of Using some Beneficial Elements Stimulating Non-Biological N-Fixation Process As Partial Substitutes of Mineral Nitrogen under Poor Soils.

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ABSTRACT

Due to the environmental hazards of mineral N-fertilizers, finding an effective alternative to it is becoming necessary. So a field experiment was executed in sandy soil condition to evaluate the possibility of using titanium (Ti) and vanadium (V) as a substitute for mineral N-fertilizers. Faba bean was cultivated as an experimental plant based on its significant response to nitrogen fixation process either biological or non-biological. Treatments were different levels of ammonium sulphate (20.5% N) which represented the main plots [100, 75 and 50% of nitrogen recommended dose (NRD) as starter, equivalent to 30, 22.5 and 15 kg N fed^{-1}, respectively] and different levels of Ti and V which represented the sub plots[5.0 and 10.0 mgL^{-1} for both separately in addition to control (plants without Ti and V)].The findings showed that plants treated with both Ti and V at both studied rates under ammonium sulphate fertilizer at rate of 75% of NRD realized plant performance at period of 65 days from sowing and at harvest stage better than that fertilized with ammonium sulphate at rate of 100% of NRD alone without both Ti and V and this may be due to their ability in non-biological N-fixation process taking into consideration that rate of 5 mg L^{-1} for both studied elements was better than rate of 10 mg L^{-1} as well as it can be noticed that Ti was superior compared to V. Generally, it can be concluded that both Ti and V possess a vital role in non-biological N-fixation process.

Keywords: Mineral N-fertilizers, titanium, vanadium and faba bean.

INTRODUCTION

Even though manufactured N-fertilizers are very necessary for all high plants grown on Egyptian soils which suffering from insufficient N in an available form, but the decreasing their added amount without occurring N deficiency symptoms is the major challenge for workers in the field of plant nutrition, where the continued usage of N-fertilizers is environmental harmful through surface and groundwater pollution (El Sherpiny *et al.,* 2021).

Biological nitrogen (N) fixation makes up about 65% of the world's annual N-fixation, and synthetic N-fertilizers, primarily produced through the Haber-Bosch process, account for 25% of the total annual N-fixation. The Haber-Bosch processes possess a relatively high operational cost, operates at relatively high pressures and temperatures as well as depends on non-renewable and depleting sources of energy (Ghazi *et al.,* 2021).Presently; there is unprecedented interest in non-biological N fixation.

Titanium (Ti) is the ninth most abundant element in the earth's crust and makes up about 0.25% by moles and 0.57% by weight of the crust of the earth. Ti is classified as a useful element for plants, which enhances their growth and development. A few researchers confirmed the role of Ti in N- fixation. For example, El-Ghamry *et al.,* (2018) confirmed that the values of available N in the soil after harvest of lettuce plants pronouncedly increased due to titanium element that enhanced N-fixation.

Vanadium (V) belongs to the transition group of metals, along with titanium, where its average content in the earth's crust is 97 mg kg^{-1}. It considers a beneficial element at a low level, where it participates in physiological systems including the normalization of sugar levels and participation in different enzyme systems as an inhibitor and cofactor of oxidation of amines. During plant growth of some plants species, V is considered essential for chlorophyll contents and porphyrin biosynthesis. At high levels of V, the plant's productivity declines. It can be absorbed by plants and regulate their growth and development, although contrasting effects have been reported among species and handling conditions (Taha *et al.,* 2017 and García-Jiménez *et al.,* 2018).There is little published information available on the role of V element in non-biological N- fixation.

Faba bean (*Vicia faba* L.) is one of the oldest legume crops grown in Egypt and belongs to legumes that play a vital role in human nutrition, where it is a rich source of protein, certain minerals and calories. Also, it is sown for feed purposes. It has a significant response to nitrogen fixation process either biological or non-biological (Mady, 2009; Gad *et al.,* 2011 and Youseif *et al.,* 2017).

Therefore, the aim of this investigation was to evaluate the role of both Ti and V elements in non-biological nitrogen fixation with faba bean plants grown on sandy soil because of the importance of faba bean plants as a strategic crop in Egypt.

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MATERIALS AND METHODS

1.Experimental site.

A field trial was carried out at a private farm located at El-Kasasin region, Ismailia governorate, Egypt during winter season of 2019/20.

2.Soil sampling.

Sample of studied sandy soil was taken at depth of 0-30 cm then was analyzed according to Dane and Topp (2020) and Sparks *et al.*, (2020) and its characteristics are presented in Table1.

Table 1. Characteristics of studied sandy soil.

Particle size distribution (%)				Texture class	Field capacity	Wilting point	Saturation	Available soil nutrients			EC, dSm^{-1}	pH
C. Sand	F. Sand	Silt	Clay		(%)			N	P	K		
								($mg\ kg^{-1}$)				
71.89	20.0	4.6	3.51	Sandy	17.0	8.5	34.0	12.95	0.35	50.1	0.72	7.82

3.Experimental setup.

A field experiment was executed during the winter season of 2019/20 aiming at evaluating the possibility of using titanium (Ti) and vanadium (V) as a substitute for mineral N-fertilizers. Faba bean was cultivated as an experimental plant based on its significant response to nitrogen fixation process either biological or non-biological. Treatments were different levels of ammonium sulphate (20.5% N) which represented the main plots [100, 75 and 50% of nitrogen recommended dose (NRD) as starter, equivalent to 30, 22.5 and 15 kg N fed^{-1}, respectively] and different levels of Ti and V which represented the sub plots [5.0 and 10.0 mgL^{-1} for both separately, where Ti was added as titanium dioxide (TiO_2), while V was added as vanadium pentoxide (V_2O_5) in addition to control (plants without Ti and V)].

The execution of the experiment was done in a split plot design with three replicates, where the sub plot size was 12.0 m^2 (3.0 m × 4.0 m). Seeds of faba bean (Cv.Giza 843) were gotten from Food Legumes Department, Agri. and Land Rec (MALR), Egypt. The recommended seeds rate (60 kg fed^{-1}) was sown on 10^{th} November at rate of 2 seeds $hill^{-1}$, where all seeds were inoculated with Rhizobium inoculant before cultivation immediately using 40% Arabic gum as a sticker. During soil preparation, the executed trial received organic fertilizer at rate of 10.0 Mg compost fed^{-1} as well as 100 kg calcium superphosphate (15% P_2O_5) fed^{-1}.

Effective N dose was applied under fertigation system at the above-mentioned rates one time in one dose after 15 days from sowing. Also, potassium sulfate (48 % K_2O) at rate of 50 kg K_2O fed^{-1} was applied under fertigation system at period of 65 days from sowing.

Titanium dioxide (TiO_2) and vanadium pentoxide (V_2O_5) were purchased from El-Gamhoria Company, Egypt then Ti and V solutions at studied rates (5.0 and 10.0 mgL^{-1}) were prepared. The addition of both Ti and V was executed after three weeks from sowing and repeated three times with 14 days interval. Other traditional agricultural practices for faba bean production were executed according to the MALR. The irrigation process was done as the faba been plants need using Nile river under drip irrigation system. Both Ti and V were added using fertigation system.

4.Measurements parameters.

At a period of 65 days after sowing, a random sample of seven faba bean plants was taken from each sub plot to measure vegetative growth criteria *i.e.,* plant height (cm), fresh and dry weights (g $plant^{-1}$), leaf area index (LAI) which was determined according to the following formula: LAI = unit leaf area per plant/unit ground area occupied by plant (as mentioned by Watson,1952). As well as chlorophyll content (SPAD value) in addition to chemical constituents in faba bean leaves *i.e.,* N,P,K were determined according to Walinga *et al.,* (2013) at this stage, where the oven-dried faba bean leaves were ground then wet digested by a mixture of sulfuric and perchloric acids (1:1) then N was determined using kjeldahl method and P was determined using spectrophotometer apparatus, while K was determined using flam photometer apparatus).

At harvest stage, the pods yield measurements *i.e.,* pod weight (g), No. of pods $plant^{-1}$, seed yield $plant^{-1}$, weight of 100 seed (g) and seed yield (Mg ha^{-1}) were measured as well as bio constituents and quality of seeds *i.e.,* total carbohydrates, protein content, TDS, fiber and N (%) in seeds were determined according to A.O.A.C (2000), where crude protein percentage was calculated by multiplication of N % in seeds in 6.25. Also, available nitrogen in soil after harvest was determined according to Sparks *et al.,* (2020).

4. Statistical analysis.

It was executed using CoStat (Version 6.303, CoHort, USA, 1998–2004) according to **Gomez and Gomez, (1984).**

RESULTS AND DISCUSSION

1. Performance after 65 days from sowing and at harvest stage.

It is clear that the impact of nitrogen fertilization, as well as titanium and vanadium elements and their interactions on the performance of faba bean plants at a period of 65 days from sowing expressed in plant height (cm), fresh and dry weights (g $plant^{-1}$), leaf area index (LAI), chlorophyll content (SPAD value) and chemical constituents in faba bean leaves i.e., N, P, K (%) (Table 2) as well as the performance of faba bean plants at harvest stage expressed in pod weight (g), No. of pods $plant^{-1}$, seed yield $plant^{-1}$, the weight of 100 seed (g) and seed yield (Mg ha^{-1}) and bio constituents and quality of seeds i.e., total carbohydrates, protein content, TDS, fiber and N (%) (Table 3), was significant during the winter growing season of 2019/20.

Table 2. Effect of nitrogen fertilization as well as titanium and vanadium elements on performance of faba bean plants at a period of 65 days from sowing.

Treatments		Growth criteria				Chemical constituents in leaves			
		Plant height (cm)	Fresh weight	Dry weight	Leaf area index (LAI)	*Chl*, (SPAD, reading)	N	P	K
			(g plant^{-1})				(%)		
Nitrogen fertilization									
100% of NRD		114.22a	122.42a	21.83a	4.86a	44.16a	4.51a	0.390a	2.77a
75% of NRD		109.09b	113.05b	19.64b	4.57b	43.07b	4.19b	0.365b	2.62b
50% of NRD		99.59c	94.90c	15.83c	4.06c	41.12c	3.65c	0.331c	2.34c
LSD at 5%		0.18	0.16	0.24	0.05	0.29	0.04	0.006	0.02
Stimulants of N fixation									
Tap water		102.86d	100.80e	16.86e	4.21e	41.56d	3.84e	0.335e	2.42e
Ti at rete of 5.0 mg L^{-1}		111.45a	117.24a	20.58a	4.72a	43.60a	4.34a	0.378a	2.69a
Ti at rete of 10.0 mg L^{-1}		109.57b	113.69b	20.01b	4.61b	43.21ab	4.22b	0.373b	2.64b
V at rete of 5.0 mg L^{-1}		107.79c	110.79c	19.34c	4.51c	42.93bc	4.12c	0.366c	2.60c
V at rete of 10.0 mg L^{-1}		106.49c	108.09d	18.70d	4.43d	42.62c	4.05d	0.358d	2.55d
LSD at 5%		1.60	0.47	0.26	0.07	0.46	0.05	0.004	0.03
Interaction									
100 % of NRD	Tap water	106.62gh	108.03i	18.47h	4.40gh	42.49hij	4.06hi	0.356e	2.54i
	Ti at rete of 5.0 mg L^{-1}	118.80a	130.07a	23.41a	5.10a	45.10a	4.78a	0.409a	2.90a
	Ti at rete of 10.0 mg L^{-1}	116.91ab	127.56b	23.07a	5.01ab	44.71ab	4.68b	0.402ab	2.85b
	V at rete of 5.0 mg L^{-1}	115.15bc	124.60c	22.40b	4.93bc	44.39abc	4.57c	0.396b	2.81c
	V at rete of 10.0 mg L^{-1}	113.63cd	121.82d	21.79c	4.87cd	44.09bcd	4.47d	0.387c	2.78d
75 % of NRD	Tap water	104.95hi	105.29j	17.80i	4.32hi	42.20hij	3.98i	0.330gh	2.48j
	Ti at rete of 5.0 mg L^{-1}	112.25de	119.17e	21.13d	4.80de	43.77cde	4.39e	0.381c	2.72e
	Ti at rete of 10.0 mg L^{-1}	110.68ef	116.37f	20.42e	4.70e	43.39def	4.27f	0.379cd	2.68f
	V at rete of 5.0 mg L^{-1}	109.31fg	113.53g	19.73f	4.59f	43.14efg	4.17g	0.372d	2.65g
	V at rete of 10.0 mg L^{-1}	108.25fg	110.87h	19.12g	4.46g	42.87fgh	4.13gh	0.362e	2.58h
50 % of NRD	Tap water	97.02l	89.07o	14.32n	3.90l	40.00m	3.49m	0.318i	2.24o
	Ti at rete of 5.0 mg L^{-1}	103.29ij	102.48k	17.19j	4.26i	41.91ijk	3.86j	0.345f	2.44k
	Ti at rete of 10.0 mg L^{-1}	101.12jk	97.15l	16.54k	4.13j	41.53jkl	3.73k	0.338fg	2.38l
	V at rete of 5.0 mg L^{-1}	98.91kl	94.24m	15.88l	4.03jk	41.25kl	3.62l	0.331gh	2.34m
	V at rete of 10.0 mg L^{-1}	97.60l	91.58n	15.20m	3.97kl	40.89l	3.55lm	0.325hi	2.31n
LSD at 5%		2.71	0.80	0.45	0.11	0.80	0.08	0.008	0.008

The N-fertilizer treatments significantly affected the values of all aforementioned traits, where the rate of 100% of NRD was the most superior followed by 75% and 50% of NRD. The improvement of faba bean performance at both studied stages with an increasing rate of NRD may be attributed to the role of the N element as a major component of chlorophyll which is an important component in the photosynthesis process as well as N is the main component of amino acids which are the building blocks of proteins that without it, plants wither then die (Haynes , 2012).

Regarding individual effect of titanium and vanadium elements, the data of the same Tables show that both studied elements were beneficial compared to control treatment (plants without them), where the plants treated with both Ti and V at both studied rates realized plant performance at periods of 65 days from sowing and at harvest stage better than that of corresponding plants grown without both Ti and V and this may be due to their ability in non-biological N-fixation process taking into consideration that rate of 5.0 mg L^{-1} for both studied elements was better than rate of 10 mg L^{-1} and this may be due to appearing their toxicity at high concentrations (10 mg L^{-1}) as well as it can be noticed that Ti was superior compared to V. On other words, the sequence order of studied elements treatments from the most effective to the less was as follows;

Ti at rate of 10.0 mg L^{-1} > Ti at rate of 5.0 mg L^{-1} > V at rate of 10.0 mg L^{-1} > V at rate of 5.0 mg L^{-1} > control (without Ti and V).

The significant role of both Ti and V at rate of 5.0 mg Ti L^{-1} may be attributed to their clear role in N-fixation process as reported by Al-Taani, (2008) who stated occurring non-biological N-fixation owing to TiO_2 in NO_3 form. Beside El-Ghamry *et al*., (2018) and Ghazi *et al*., (2021) who confirmed that Ti is considered a beneficial element at low concentration only and its toxicity appears at high concentration. On the other hand, García-Jiménez *et al.,* (2018) reported that V led to regulate the growth of plants, although contrasting impacts have been found among plants species.

Concerning the interaction effect among the studied treatments, the data of the same Tables showed that the highest values of all studied parameters either at period of 65 days from sowing or at harvest stage were recorded when plants received ammonium sulphate fertilizer at rate of 100 % of NRD and Ti element at 5.0 mgL^{-1}. On the other hand, the plants treated with both Ti and V at both studied rates under ammonium sulphate fertilizer at rate of 75% of NRD realized plant performance at period of 65 days from sowing and at harvest stage better than that fertilized with ammonium sulphate at rate of 100% of NRD alone without both Ti and V.

Table 3. Effect of nitrogen fertilization as well as titanium and vanadium elements on yield measurements and seeds quality of faba bean plants at harvest stage.

Treatments		Yield measurements					Quality and bio chemical constituents				
		Pod weight, g	No. of pods plant^{-1}	Seed yield plant^{-1}	Weight of 100 seed, g	Seed yield, Mg ha^{-1}	Carbo-hydrates	Protein	TDS	Fiber	N
							(%)				
Nitrogen fertilization											
100% of NRD		21.77a	20.87a	78.65a	86.85a	2.17a	53.28a	24.22a	3.80a	11.11a	3.87a
75% of NRD		20.26b	18.27b	72.41b	83.20b	2.00b	52.15b	22.90b	3.62b	10.95b	3.66b
50% of NRD		17.62c	15.40c	62.14c	78.21c	1.71c	49.81c	20.30c	3.20c	10.71c	3.25c
LSD at 5%		0.21	2.25	0.24	0.15	0.05	0.41	0.23	0.07	0.08	0.04
Stimulants of N fixation											
Tap water		18.38e	16.22c	65.22e	79.67e	1.80e	50.48c	21.06d	3.34e	10.77b	3.37d
Ti at rete of 5.0 mg L^{-1}		20.94a	19.56a	75.32a	84.72a	2.07a	52.43a	23.40a	3.69a	11.02a	3.74a
Ti at rete of 10.0 mg L^{-1}		20.47b	19.11ab	73.45b	84.01b	2.02b	52.30a	23.11a	3.62b	10.98a	3.70a
V at rete of 5.0 mg L^{-1}		20.03c	18.22ab	71.57c	83.09c	1.97c	51.93ab	22.63b	3.56c	10.95a	3.62b
V at rete of 10.0 mg L^{-1}		19.58d	17.78b	69.77d	82.29d	1.92d	51.59b	22.17c	3.49d	10.89ab	3.55c
LSD at 5%		0.23	1.50	0.28	0.36	0.03	0.67	0.30	0.04	0.15	0.05
Interaction											
	Tap water	19.46i	17.33d-g	69.50i	81.60i	1.92fg	51.50fgh	22.23g	3.53f	10.87d-g	3.56g
100 %	Ti at rete of 5.0 mg L^{-1}	23.05a	23.00a	83.70a	89.49a	2.30a	54.28a	25.29a	3.98a	11.22a	4.05a
of	Ti at rete of 10.0 mg L^{-1}	22.56b	22.33a	81.85b	88.59b	2.26ab	53.92ab	24.88ab	3.90b	11.18ab	3.98ab
NRD	V at rete of 5.0 mg L^{-1}	22.12c	21.00ab	79.99c	87.68c	2.21bc	53.51abc	24.50bc	3.83c	11.16abc	3.92bc
	V at rete of 10.0 mg L^{-1}	21.65d	20.67abc	78.19d	86.90d	2.16cd	53.19ad	24.19cd	3.77cd	11.10a-d	3.87cd
	Tap water	19.01j	17.00d-g	67.64i	80.79j	1.87g	51.17ghi	21.58h	3.45g	10.84d-h	3.45h
75 %	Ti at rete of 5.0 mg L^{-1}	21.20e	19.33bcd	76.41e	85.09e	2.11d	52.91be	23.73de	3.73de	11.05a-d	3.80de
of	Ti at rete of 10.0 mg L^{-1}	20.79f	19.00bcd	74.56f	84.30f	2.05e	52.59cf	23.50ef	3.68e	11.00a-e	3.76ef
NRD	V at rete of 5.0 mg L^{-1}	20.36g	18.33cde	72.63g	83.29g	2.00e	52.21dg	23.13f	3.66e	10.96b-f	3.70f
	V at rete of 10.0 mg L^{-1}	19.94h	17.67def	70.82h	82.54h	1.94f	51.86efg	22.58g	3.57f	10.91c-g	3.61g
	Tap water	16.69o	14.33h	58.51o	76.62n	1.61j	48.76k	19.38k	3.05k	10.60h	3.10k
50 %	Ti at rete of 5.0 mg L^{-1}	18.56k	16.33e-h	65.86k	79.58k	1.81h	50.12ij	21.17hi	3.35h	10.79e-h	3.39hi
of	Ti at rete of 10.0 mg L^{-1}	18.05l	16.00e-h	63.93l	79.13k	1.75i	50.39hij	20.94i	3.29h	10.76e-h	3.35i
NRD	V at rete of 5.0 mg L^{-1}	17.62m	15.33fgh	62.09m	78.29l	1.71i	50.06ij	20.25j	3.20i	10.72fgh	3.24j
	V at rete of 10.0 mg L^{-1}	17.16n	15.00gh	60.31n	77.42m	1.65j	49.73jk	19.75jk	3.13j	10.66gh	3.16jk
LSD at 5%		0.40	2.60	0.49	0.62	0.05	1.15	0.52	0.07	0.26	0.08

2. Available nitrogen in soil at harvest stage.

Fig 1 shows the effect of nitrogen fertilization as well as titanium and vanadium elements on the values of soil available nitrogen (mg kg^{-1}) after harvest of faba bean plants.

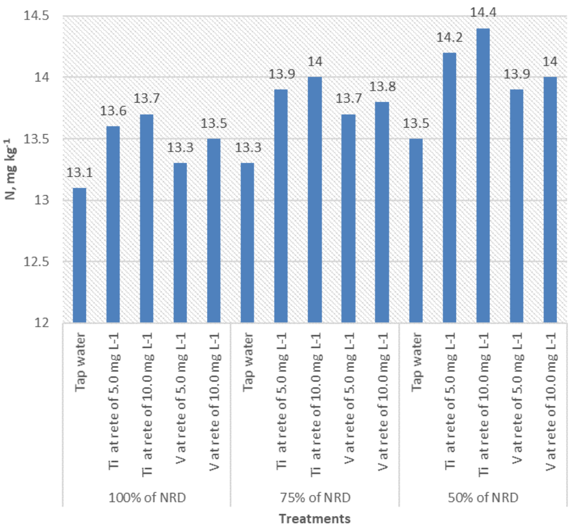

Fig 1. Effect of nitrogen fertilization as well as titanium and vanadium elements on soil available N after harvest.

Fig show that the soil available N after faba bean plants harvest pronouncedly increased over that before sowing and this may be owing to the role of faba bean roots activity resulting from studied treatments in raising the soil acidity, which in turn increases the nitrogen availability.

The available N content in the soil that received 100% of NRD was more than the soil that received 75 % of NRD which came in the second-order, while the less available N content was recorded with the soil received 50 % of NRD and this may be due to the plants received 100% of NRD absorbed more N from the soil as a result of improving plants status and this caused to reduce the residues of available N in the soil.

Also, usage of Ti and V clearly increased available soil N compared to the corresponding soil without the addition of them and this may be attributed to their ability in stimulating non-biological N-fixation, where the highest value of N residue in the soil at harvest was recorded with the addition of Ti at rate of 10 mg L^{-1} followed by Ti at rate of 5.0 mg L^{-1} then V at rate of 10 mg L^{-1} and lately V at rate of 5.0 mg L^{-1}. On the other hand, the soil untreated with both Ti and V possessed the lowest value of available N content at harvest stage.

These results are in harmony with those obtained by El-Ghamry *et al.*, (2018) and Ghazi *et al.*, (2021).

CONCLUSION

This investigation confirms that both titanium and vanadium have a vital role in non-biological nitrogen fixation with faba bean plants grown on poor soil such as sandy soil, where they can fix atmospheric N. On the other hand, these elements are beneficial at a low concentration

(5.0 mg L^{-1}) more than at a high concentration (10.0 mg L^{-1}) and this due to appearing their toxicity at high concentrations. Moreover, titanium is superior in process of non-biological nitrogen fixation compared to vanadium.

Generally, it can be concluded that application of both titanium and vanadium may be a good substitute for mineral N-fertilizers in sustainable development.

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إمكانية استخدام بعض العناصر المفيدة المحفزة لعملية التثبيت الغير حيوي للنيتروجين كبدائل جزئية للنيتروجين المعدني في الأراضي الفقيرة.

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نظرًا للمخاطر البيئية للأسمدة المعدنية النيتروجينية، أصبح من الضروري إيجاد بديل فعال لها. لذلك تم تنفيذ تجربة حقلية بأرض رملية لتقييم إمكانية استخدام التيتانيوم والفناديوم كبديل للأسمدة المعدنية النيتروجينية. تمت زراعة الفول البلدي كنبات تجريبي بسبب استجابته المعنوية لعملية تثبيت النيتروجين سواء كان تثبيت حيوي او غير حيوي. كانت المعاملات المدروسة عبارة عن مستويات مختلفة من كبريتات الأمونيوم (20.5٪ نيتروجين) كقطع رئيسية [100، 75 و50٪ من جرعة النيتروجين الموصي بها، بما يعادل 30، 22.5 و15 كجم نتروجين الفدان-1، على التوالي] وإضافة مستويات مختلفة من التيتانيوم والفناديوم (5.0 و10.0 مجم لتر -1 لكلاهما على حدة) بالإضافة إلى الكنترول (نبات نامي بدون التيتانيوم والفناديوم) كقطع منشقة. أظهرت النتائج أن النباتات التي تم معاملتها بكل من التيتانيوم والفناديوم بكلا المعدلين المدروسين كمعاملة مشتركة مع سماد كبريتات الأمونيوم بمعدل 75٪ من الموصي به تجعل أداء النبات في فترات 65 يومًا من الزراعة وكذلك عند مرحلة الحصاد أفضل من اداء تلك التي تم تسميدها بكبريتات الأمونيوم بمعدل 100٪ من الموصي به فقط بدون كل من التيتانيوم والفناديوم وقد يكون هذا بسبب قدرتهما في عملية تثبيت النيتروجين الغير حيوي مع الأخذ في الاعتبار أن معدل 5 مجم لتر -1 لكلا العنصرين المدروسين كان أفضل من معدل 10 مجم لتر -1 بالإضافة إلى أنه يمكن ملاحظة أن التيتانيوم كان متفوقًا عن الفناديوم. بشكل عام، يمكن استنتاج أن كلا من التيتانيوم والفناديوم لهما دور حيوي في عملية تثبيت النيتروجين الغير حيوية.