Ann. For. Res. 66(1): 2494-2508, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

EFFECT OF ORGANIC AND BIO-FERTILIZATION AND SPRAYING WITH SOME NANO-ELEMENTS ON THE FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) SEED CONTENT OF THE ACTIVE SUBSTANCE

Nabhan Awad Mohammd Khalaf and Aqeel Najm Abboud Al-Mohammadi
College of Agriculture, Tikrit University, Department of Field Crops, Iraq

Abstract

A field experiment was conducted at the research station of the Field Crops Department - College of Agriculture - Tikrit University, and during the winter season for the years (2018-2019), Factorial experiment was conducted according to the Randomized Complete Block Design (RCBD) according to Split Plots system, and in three replications, The main plot included spraying treatments with nano elements at four levels (without addition, nano cobalt 160 mg.L^{-1}, nano cobalt 3 mg. L^{-1}, nan iron 160 mg.L^{-1} + nano cobalt 3 mg.L^{-1}), As for the secondary plots, they included combinations between biological fertilization levels with (Sinorhizobium meliloti) bacteria at two levels (inoculation, without inoculation) and organic fertilization with decomposed sheep manure at four levels (0, 2, 4, 6) tons. dunam $^{-1}$.The averages were compared using Duncan's multi-range test at (5%) level. The results showed significant differences in the seed content of the active substances (Trigonelline, Diosgenin, Gluthatione) where a result of being affected by the study factors compared to the non-fertilized treatments, as the individual treatments were achieved from foliar fertilization with the two nano elements together (iron 160 mg.L^{-1} + cobalt 3 mg.L^{-1}) and 6 tons of organic fertilizer and bacterial inoculum with Rhizobium in recording the highest averages of active substances (Trigonelline): 177.61ppm, 214.06ppm, 258.54 ppm, (Diosgenin), 147.50 ppm, 172.29 ppm and 202.89 ppm, and (Glutathione) reached 1189.51 ppm, 1472.16 ppm, and 1609.00 ppm) respectively, As for the interaction between the study factors, the combination of the bacterial inoculum and organic fertilization at an average of (6) tons. dunum^{-1} recorded a highly significantly excelled on the seed content of the active substances, reaching (357.14 ppm), (269.96 ppm), and (2163.14 ppm) compared with the non-fertilization treatment. The fertilizer reached (6.19 ppm) (12.13 ppm) (116.18 ppm) for the active substances respectively, Through the results of the study, it is evident that foliar fertilization with chelated micro-elements with nanostructures and bio-fertilization with Rhizobium with the addition of decomposing Organic matters are necessary to create a natural balance in the nutritional status of the fenugreek plant, which is positively reflected in the increase in the seed content of the active substances.

Key words: Fenugreek, Nano fertilization, Bacterial Inoculum (S.meliloti), Organic Fertilizer, Active substances

Introduction

Medicinal plants are considered a source of successful drugs because of the various chemical compounds produced by these plants through secondary metabolism, which have been used in the development of drug synthesis, or as an alternative source of effective and inexpensive drugs for therapeutic chemical compounds. In addition, some of them are an important source of nutrition, so they were used in the therapeutic recommendations for various disease states (Hassan, 2012).

Among the most important of these plants known around the world is the Trigonella foenum-graecum L., which belongs to the Fabaceae family, and this plant has great properties and importance, both in need as a source of medicinal active substances, the most important of which are (Trigonelline, Choline, gentianine, Carpaine) and flavonoids (apigenin, Luteoline, (Quercetin as well as Diosgenin saponins), Yamogenin, Gitogenin, Tigogenin, Neotigogenin) and Mucilage and glycosides, So it is used in the treatment of diabetes and cholesterol in the blood, infections, cancer, oxidation, as an immune stimulant and a source of steroid hormones, or it is used in nutrition as a source of carbohydrates, proteins and vitamins, the most important of which are (A, B1, C), volatile and stable oils, or for some mineral elements such as iron and calcium (Nagananda and others, 2010 and Snehlata and Pyal, 2012 and Moradi Kor and others, 2013),Besides they are used as part of the production of advanced foods such as food stabilizer, agglomerate and emulsifiers and most important of these are their use in the development of healthy foods, nutrients and bakery products (Wani and Kumar, 2018).The growth of the fenugreek plant under the conditions of using modern production techniques leads to the integration of metabolic reactions in the plant and during any period of plant growth can affect the active substance in the plant (Ahmed et al., 2010). and one of the most important modern technologies is the use of nanofertilizers, which are considered one of the reliable sources of nutrients for plants, due to their high stability and the absence of ethylene in their composition and the increase in their percentage on the claw surface, and their high absorption capacity by the plant, which leads to an increase in the average of photosynthesis processes and thus an increase in the average of growth and production of active substances in the plant (Singh et al., 2016).The use of biological fertilizers or biological fertilizers, especially the Rhizobia bacteria, is an important alternative or complementary to chemical fertilizers in providing the plant's needs of nutrients, The use of organic fertilizers improves the structure of the soil and its ability to retain water, improves its biological properties, increases the cation exchange capacity of the soil, increases the absorption of some elements in the soil, and enriches the soil with organic matter, mineral nitrogen, phosphorus, potassium, total carbon, and the amount of carbon dioxide needed by the plant. The use of organic fertilizers is a good step to achieve sustainable agriculture, because it provides the plant with nutrients, introduces the building of organic compounds, and improves the path of vital activities inside the plant, which is positively reflected in plant growth (Zaghloul et al., 2011 and Gayrley et al., 2015). In view of the importance of the fenugreek plant from a medical and nutritional point of view and the lack of studies that dealt with the effect of using iron and cobalt nano fertilizers and bio-organic fertilizers on the growth of the fenugreek plant, and in order to find more appropriate fertilizer applications to improve the growth conditions of the fenugreek and increase its productivity from seeds with a high content of active substances, which are distinguished This study was conducted with the lowest costs and the safest for the agricultural environment and for human health.

Materials and Methods

The field experiment for the winter season (4/11/ 2018) was conducted in the fields of the research station of the Field Crops Department of the College of Agriculture at Tikrit University, which is

located in the northern part of Tikrit, the center of Salah al-Din province- Republic of Iraq. Soil service operations were conducted including Tillage and smoothing and the experimental units were divided into dimensions (2 x 2 m), and the soil was analyzed to determine the physical, chemical and biological traits , the details of which are shown in Table (1)

Table (1): Some physical and chemical traits of the experiment soil before cultivation.

traits	Values		Unites
Soil reaction degree (PH)	7.63		/
Electrical conductivity (Ec)	2.49		$DS.m^{-1}$
Cation exchange capacity (CEC)	13.23		Centimol kg-soil
Bulk Density (B.D)	1.37		$g.cm^{-3}$
Organic Matter (O.M)	2.77		g.kg $^{-1}$ soil
Gypsum	38.00		
available nitrogen (N)	17.38		mg.kg $^{-1}$ soil
available phosphorous (P)	5.66		
available Potassium (K)	90.24		
Sulfate (SO4 =)	10.21		Mmol. L $^{-1}$
Calcium (Ca ++)	7.27		
Sodium (Na +)	1.33		
Chloride (CI-)	5.53		
sand	666		g.kg $^{-1}$
Silt	76		
Clay	258		
Soil texture	Sandy Loam		
Microorganism numbers	Before sterilization	after sterilization	
Total fungus numbers	$10^3 \times 0.42$	$10^3 \times 4.23$	CFU gm. $^{-1}$ soil
Total bacterial numbers	$10^4 \times 0.84$	$10^4 \times 7.12$	
Numbers of bacteria (Rhizobium ssp)	$10^4 \times 0.91$	$10^4 \times 1.11$	

Then the drip irrigation pipes were extended along the planting lines, and the number of Emitters and the distance between them were determined according to the distance between the planting lines (30 cm) and the distance between the pit(25 cm).After that, solar sterilization of the soil was conducted by covering the experimental units tightly with transparent polyethylene material and irrigation was conducted every 10 days, and after 60 days of solar sterilization the polyethylene cover was removed,A simple Tillage of the soil was conducted with the addition of organic fertilizer levels according to the treatments with the addition of one batch of superphosphate fertilizer at an average of (30 kg. Dunum-1) and nitrogen fertilizer in the form of urea (46%) at an

average of (10 kg. dunum^{-1}). Use a quarter of the amount and (25) kg.dunum^{-1} potassium and on all experimental units, and the inoculated and unfertilized seeds were sown on 11/4/2020 (Al-Hidwani, 2004).The experiment was conducted according to the Randomized Complete Block Design (RCBD) and according to the Factorial in split plots system, cobalt 3 mg. L^{-1}, (iron 160 + cobalt 3) mg.L $^{-1}$, while the secondary plots included combinations between levels of biological fertilization (without pollination, seed pollination) and organic fertilization levels with decomposing sheep manure (without addition 2, 4, 6) tons.dunum $^{-1}$. Organic fertilizer was added when preparing the soil. As for the bacterial pollen, it was mixed with the seeds when cultivation, while the foliar application of the nano fertilizer was carried out upon the arrival of the plants 4-6 full-grown leaves, with the service operations of the crop being conducted from irrigation and weeding according to the plants need.

Determination of active substances in fenugreek seeds using (HPLC) technology:

An assessment of the active substances (Trigonelline, Diosgenin, and Glutathione) in the harvested fenugreek seeds in the first season was performed in the laboratory of the Department of Environment and Water - Ministry of Science and Technology in Baghdad, using a high-performance liquid chromatography device (HPLC) High-performance liquid. chromatography. Where the amount of (1.20) g was weighed for each sample of the fenugreek seeds representing the treatments and crushed well, and then transferred to volumetric flasks with a capacity of (10) ml, and an amount of (5) ml of (Acetonitrile) was added to it, and in order to ensure its complete dissolution, the samples were placed for a period of time. (10-15) minutes in the ultrasound machine. After that, the solution was diluted to a fixed volume (10) ml with the addition of phosphoric acid at an average of (5) ml, and it was returned again to the ultrasound machine for a period of (20) seconds. Then it was transferred to a centrifuge regulated at (6000 revolutions per minute) and for a period of (5) minutes, the filtrate formed (the top layer of the solution) was taken and transferred to a rotary evaporator device to reduce dehydration. Acetonitrile was added to it with a volume of 3 ml, and then the sample was filtered through the use of filter paper with a pore size of (22) mμ. Under the conditions of separation indicated in Table (2), the readings were recorded depending on the retention time (Rt) of the standard solution and the active substances in the studied samples and according to the method described before (Li et al., 2012).

Table (2) shows the conditions and time of separation according to (HPLC) technology for the standard active ingredients and in fenugreek seeds.

D.W : acetonitrile (10 : 90)	**Mobil phase**	**1**
1mL. minute^{-1}	**The speed of flow of the mobile phase**	**2**
3 µmol	**Cell size**	**3**
Phase column C 18 – ODS (25cm * 4.6 mm)	**Column**	**4**
.UV - Vis = 210 nm	**Detection type**	**5**
. م^{0}25	**Separation temperature**	**6**

	25µg .mL^{-1}	Standard compound concentration	7
concentration (ppm)	detention time. Minute^{-1}	Standard active substances	8
10	10.50	Trigonlline	
20	10.57	Diosgenin	
20	6.56	Glutathion	

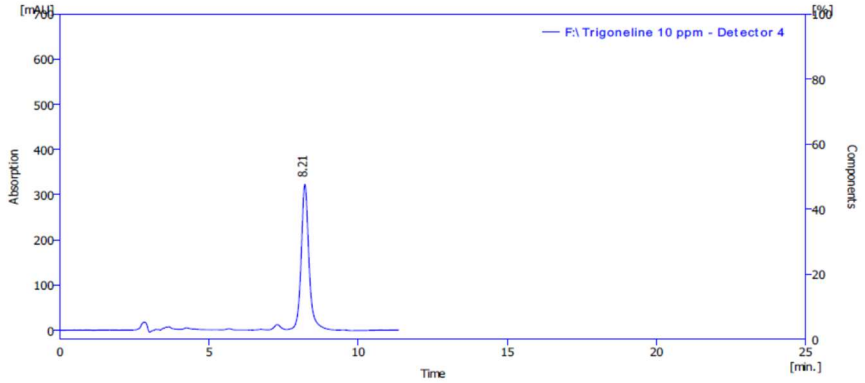

Figure 1. showing the temporal behavior of the active substance Trigonelline using HPLC technology

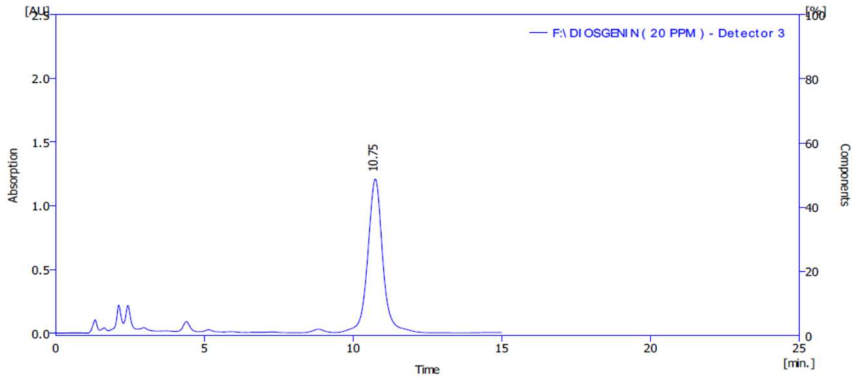

Figure 2. showing the temporal behavior of the active substance Diosgenin using the HPLC .technique

Estimate the active ingredient (glutathione)

Standard solutions - GSH were prepared by dissolving 7- 10 mg of GSH in the appropriate amount of the mobile phase to make a solution containing 1.0 mg. mL^{-1} of GSH after spinning for thirty seconds, The solution is then diluted with the appropriate amount from the mobile phase to create a 2 ml solution and then injected into the HPLC device according to the method (Vijay Kumar et al., 2012).

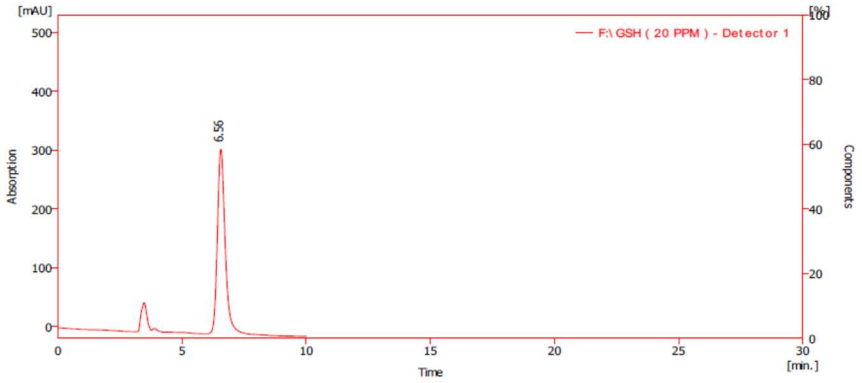

Figure 3. showing the temporal behavior of the active substance Glutathione according to HPLC technique

Diagnostic and estimation method using (HPLC) device:

After the standard solutions of the active compounds (Trigonelline, Diosgenin, and Glutathione) are prepared in solvents prepared for this purpose and under a special degree, as well as for the solutions of the seed samples to be evaluated for the above active compounds, and after that an amount of the solution is injected, the compounds Triaconylene, Diosgenin and Clutathione are diagnosed. For study samples based on standard forms. The concentration of compounds was calculated by comparing the results of the quantification of the compounds present in the samples of the samples under study for both the detention time and the unknown beam area of the models with the detention time and area of the curves of the known standard models. The concentration of the compounds was calculated in the model using the following equation: -

compound concentration in the sample=$\frac{Composite\ package\ space}{space\ of\ package\ standard\ sample}\times$ Standard sample concentration x number of dilutions

Using the SAS program, the data averages were analyzed using the RCBD factor only and for one repeater. The means of the studied traits were compared according to the (Duncan) test method, which has a multiple range and at the level (5%) (Al-Rawi and Khalaf Allah, 2000).

1- Trigonelline seed content:

The results in Table (3) showed that the foliar spraying with Nano elements on the fenugreek plant had a significant effect in increasing the seed content of the active substance (Trigonelline) compared to the non-fertilized treatments. The treatment (160 iron + 3 cobalt) mg.L^{-1} gave the highest average for the trait (177.61 ppm), with an increase of (72.96%), followed by the treatment of iron 160 and cobalt 3 mg. L^{-1} with an average of 158.27 and 149.39, which did not differ from each other compared to the control treatment, which recorded the lowest average for the trait was (102.69 ppm).The biological fertilization treatment with *S. meliloti* bacteria also had a significant effect on increasing the seed content of this substance, which recorded the highest average of the material reaching 258.54 ppm, with an increase of 629.31%, while the non-inoculated treatment with bacteria recorded the lowest average of 35.45ppm. The treatment of organic fertilization also achieved a significant effect in this trait, where the treatment gave 6 tons.dunum^{-1} organic fertilizer with the highest average for the characteristic (pmm214.06), with an increase of 135.96%, while the treatment gave the lowest average for the characteristic reached (90.72 ppm).As for the

combination of the two treatments, their effects were also significant in increasing the seed content of (Trigonelline). The combination between spraying the treatment (iron + cobalt) with the inoculation treatment with *S. meliloti* gave the highest average for the material, reaching (310.84 ppm) with a difference. An increase of 1036.11% over the comparison treatment, which showed the lowest average for the trait of 27.36 ppm.

Table (3) the effect of organic, bio and nano minerals fertilization with chelated iron and cobalt nano, and the interaction between them on the fenugreek seed content of(Trigonelline. ppm)

Bio fertilizer	Organic fertilizer tons. Dunum	Fertilizer levels				The interaction between fertilizer (bio x organic)	The effect of biofertilizer
		0	Fe (160) mg. L^{-1}	Co(3) mg. L^{-1}	Fe (160)+ Co(3) mg. L^{-1}		
Without inoculum	0	--	--	--	--	g 6.19	b 35.45
	2	--	--	--	--	g 19.24	
	4	--	--	--	--	f 45.38	
	6	--	--	--	--	e 70.98	
inoculum	0	--	--	--	--	d 175.25	a 258.54
	2	--	--	--	--	c 211.90	
	4	--	--	--	--	b 289.86	
	6	--	--	--	--	a 357.14	
Effect of chelated nano fertilizer		c 102.69	b 158.27	b 149.39	a 177.61	The effect of organic fertilizer ton. $Dunum^{-1}$	
interaction between Nano x bio fertilizer	Without inoculum	d 27.36	d 36.54	d 33.51	d 44.38		
	inoculum	c 178.03	b 280.00	b 265.28	a 310.84		
interaction between Nano x organic	0	i 60.16	gh 99.37	gh97.03	g 106.34	d 90.72	
	2	hi 77.25	fg 123.21	fg 120.25	ef 141.56	c 115.57	
	4	g 110.94	cd185.59	d 171.73	bc 202.22	b 167.62	
	6	c 162.42	b 224.91	b 208.57	a 260.33	a 214.06	

2- Diosgenin seed content (Diosgenin.ppm):

The results presented in the contents of Table (4) indicate that the foliar spraying with Nano elements on the fenugreek plant had a significant effect in increasing the seed content of the active substance (Diosgenin.ppm) compared to the non-fertilized treatments, where the treatment (iron 160 + 3 cobalt) gave mg.L^{-1}, the highest average for trait was (147.50 ppm), with an increase of (72.79)%, followed by the treatment of iron 160 and cobalt 3 mg. L^{-1} averaged 132.47 ppm and 122.08 ppm which did not differ from each other. While the control treatment recorded the lowest average for the trait, which was 85.31 ppm. The biological fertilization treatment with *S. meliloti* bacteria also had a significant effect on increasing the seed content of this substance, which recorded the highest average of the material reaching 202.89 ppm, with an increase of (397.40)%, while the treatment of non-inoculated bacteria recorded the lowest average of 40.79 ppm. The organic fertilization treatments achieved a significant effect in this trait, where the treatment 6 tons.dunum^{-1} organic fertilizer excelled and giving the highest average of 172.29 ppm, with an increasing difference of (123.70)%, while the control treatment recorded the lowest average of this substance, which amounted to 77.02. ppm. As for the combination of the two treatments, their effects were also significant in increasing the seed content of Diosgenin ppm. The combination between spraying the treatment (iron + cobalt) and the inoculation with Reusobia bacteria (S. meliloti) gave the highest average for the substance, reaching 246.91 ppm, with an increasing difference of 645.30% compared to the comparison treatment that showed the lowest average of the trait of 33.19 ppm. The combination of spraying (160 iron + 3 cobalt) mg. L^{-1}) with the addition of 6 tons.dunum^{-1} recorded a significant effect on the average of this substance, with the highest average for this characteristic reaching 209.64 ppm, with an increased difference of (339.68%) compared to the treatment the lowest average for this trait was 47.68 ppm. While the combination between the biological fertilization treatment with S. meliloti bacteria and the treatment with the addition of 6 tons. dunum^{-1} organic fertilizer recorded a significant superiority by giving the highest average of the substance (269.96 ppm) and a difference of (2125.56%) compared to the comparison treatment that recorded the lowest average for the substance reached 12.13 ppm.

Table (4) the effect of organic, bio and nanominerals fertilization with chelated iron and cobalt nano, and the interaction between them on the fenugreek seed content of (Diosgenin .pmm).

Bio fertilizer	Organic fertilizer tons. Dunum	Fertilizer levels				The interaction between fertilizer (bio x organic)	The effect of biofertilizer
		0	Fe (160) mg. L^{-1}	Co(3) mg. L^{-1}	Fe (160)+ Co(3) mg. L^{-1}		
Without inoculum	0	--	--	--	--	g 12.13	b 40.79
	2	--	--	--	--	g 28.27	
	4	--	--	--	--	f 48.15	
	6	--	--	--	--	e 74.62	
inoculum	0	--	--	--	--	d 141.90	a 202.89

	2	--	--	--	--	c 181.33	
	4	--	--	--	--	b 218.36	
	6	--	--	--	--	a 269.96	
Effect of chelated nano fertilizer		c 85.31	b 132.47	b 122.08	a 147.50		
interaction between Nano x bio fertilizer	**Without inoculum**	d 33.19	d 43.35	d 38.55	d 48.08	**The effect of organic fertilizer ton. Dunum^{-1}**	
	inoculum	c 137.44	b 221.59	b 205.61	a 246.91		
interaction between Nano x organic	**0**	i 47.68	fgh84.45	ghi 73.93	fgh102.02	d 77.02	
	2	hi 72.00	de120.17	ef105.90	de121.12	c 104.80	
	4	fgh 83.57	bc 153.83	cd138.41	bc157.21	b 133.25	
	6	cd 138.01	b 171.42	b 170.08	a 209.64	a 172.29	

2- Glutathione seed content (Glutathione.ppm):

The results presented in Table (5) showed that foliar spraying with chelated metallic Nano elements on the fenugreek plant had a significant effect in increasing the seed content of the active substance (Glutathione. ppm) compared to the non-fertilized treatments. The biological fertilization treatment with S. meliloti bacteria also had a significant effect on increasing the seed content of this substance, which recorded the highest average of the material reaching 1609.00 ppm, with an increase of 260.30%, while the non-inoculated treatment with bacteria recorded the lowest average of 446.57 ppm. The treatment of organic fertilization also achieved a significant effect in this aspect, where the treatment 6 tons. Dunum-1 organic fertilizer gave the highest average of pmm 1472.16, with an increase of 65.32%, while the non-fertilized treatment gave the lowest average for the material, which amounted to 890.51 ppm. As for the combination of the two treatments, their effects were also significant in increasing the seed content of (Glutathione.ppm), the combination between spraying the treatment (160 iron + 3 cobalt) mg.L^{-1} with the treatment of inoculation with S. meliloti bacteria was higher an average for the material was 1857.24 ppm, with a difference of 403.75% increase on the control treatment, which gave a lower average of the trait amounted to 368.68 ppm.The combination of spraying (160 iron + 3 cobalt mg. L $^{-1}$) with the addition of 6 tons.dunum^{-1} recorded a significant effect on the average of this substance, with the highest average amounting to 1773.74 ppm, with a difference of 240.79% more than the lowest control treatment the average for this article was 520.48 ppm. While the

combination between the biological fertilization treatment with S. meliloti bacteria and the treatment with the addition of 6 tons. dunum^{-1} organic fertilizer recorded a significantly excelled by giving the highest average of the substance (2163.17 ppm), with an increase of 1761.91% compared to the control treatment, which recorded the lowest average for the substance, which amounted to 116.18. ppm.

Table (5) the effect of organic, bio and nanominerals fertilization with chelated iron and cobalt nano, and the interaction between them on the fenugreek seed content of(Gluthathione .pmm

Bio fertilizer	Organic fertilizer tons. Dunum	Fertilizer levels				The interaction between fertilizer (bio x organic)	The effect of biofertilizer
		0	Fe (160) mg. L^{-1}	Co(3) mg. L^{-1}	Fe (160)+ Co(3) mg. L^{-1}		
Without inoculum	0	--	--	--	--	g 116.18	b 446.57
	2	--	--	--	--	f 357.83	
	4	--	--	--	--	f 531.11	
	6	--	--	--	--	e 781.17	
inoculum	0	--	--	--	--	d 1194.28	a 1609.00
	2	--	--	--	--	c 1423.19	
	4	--	--	--	--	b 1655.37	
	6	--	--	--	--	a 2163.14	
Effect of chelated nano fertilizer		b783.72	a1105.90	a1032.01	a1189.51	The effect of organic fertilizer ton. Dunum^{-1}	
interaction between Nano x bio fertilizer	Without inoculum	c368.68	c469.30	c426.53	c521.78		
	inoculum	b1198.76	a1742.50	a1637.48	a1857.24		
interaction between Nano x organic	0	g520.48	efg698.77	efg663.80	efg737.87	655.23	
	2	fg642.30	cdef972.24	def956.80	cde990.72	890.51	
	4	efg731.07	cd1194.65	cd1191.53	bcd1255.71	1093.24	
	6	bcd1241.03	ab1557.96	bc1315.91	a1773.74	1472.16	

The increase in seed content of active substances (Trigonelline, Diosgenin, Glutathione) may explain the positive role that the study factors and their levels of mineral fertilization with nano iron and cobalt , bio fertilization with (S.meliloti) and organic fertilization with decomposed sheep manure in improving growth Plant and the activity of physiological activities that occur inside the plant, The nano iron is characterized by its small size compared to the pores of the cell wall, which allows the plant to absorb it easily and faster and participate in the activation of many enzymatic systems that enter into the synthesis of their proteins such as enzymes of electron transport in the steps of respiration and energy production (catalase, cytochrome oxidase, cytochrome) (Jonce, 2012) and enzymes. Associated with stimulation of the biosynthesis pathway for chlorophyll synthesis, protein building, and root meristem growth (Connorton et al., 2017), and stimulate the electron transport system during the process of photophosphorylation in the second photosystem that occurs in plastids at the level of the Thylakoid to enter into the synthesis of the (ferredoxin) protein, and stimulate the bacterial enzymatic system (S. meliloti) by entering the composition of the nitrogenase enzyme responsible for the reduction of biologically inert atmospheric nitrogen It is supplied to the plant in the form of ammonia, where it is considered one of the components of building the pigment (Legoheamoglobin) in the root nodes (Brear et al., 2013 and Hassan and Saadeddin, 2018).In addition to the important biological role of nano cobalt in activating plant metabolism and growth processes by inhibiting the biological pathway for ethylene synthesis that causes plant aging in addition to reducing the activity of the enzyme (peroxidase) that breaks down the auxin molecule (IAA) (Minz et al., 2018), and the main role of cobalt in the success of the atmospheric nitrogen fixation process by participating in the formation of cobamine and Colpermin coenzyme complexes which participate with other enzymes such as (Mwthylmlloonyl Co-isomerase, glutamate mutase, Glycerol dehydrates, dioldehydeotase, Ethanlamine deaminase, B-L-Molecule transporter). Hydrogen (H) during the reduction of atmospheric nitrogen to ammonia, in addition to its participation in the formation of the protein (Legheamoglobin) responsible for protecting the enzyme nitrogenase from oxidative processes in the root ganglia (Lui, 1998 and Havlin et al., 2014). The overall positive biological effects of the two elements, iron and cobalt, may have a great role in building the effective enzymatic and hormonal system of the plant and activating many physiological processes such as photosynthesis, respiration, and cell division, which leads to the growth of an ideal vegetative system for the plant, which is positively reflected in the accumulation of secondary metabolites and their migration to places of storage in the seeds These results are consistent with a study that they conducted (Kadim and Yaseen, 2018, and Yassin, 2020, and Gad and Abdel moez, 2015). . While the increase in the accumulation of active compounds (Trigonelline, Diosgenin, Glutathione) when treated with biological fertilization with bacteria (S. meliloti) may be due to the activation of the entire metabolic processes that occur in the plant through the process of atmospheric nitrogen fixation and the provision of the nitrogen component in a form that can be used by the plant, which is an essential element in building the enzymatic and hormonal system, the formation of chlorophyll and the protein content of the plant, which leads to stimulating plant growth thanks to the stimulation of the photosynthesis process and the accumulation of its products from the organic materials necessary for growth, in addition

to its positive effect on the growth of roots and the increase in the area of their spread in the soil, and their hormonal secretions. Vegetation (Auxin, gibberellins, cytokinins) in the root nodes, all of this may be positively reflected in the production of secondary metabolites and their transfer to centers of storage in the seeds. These results agree with(Pawar and others, 2014, Singh and Patel, 2016 and Zaghloul, 2019) and (Tamimi, 2009 and Menbari et al., 2017). While the increase in the seed content of the active substances (Trigonelline, Diosgenin, Glutathione) through treatment with organic degradation residues of sheep manure may explain the improvement that occurred in the conditions of root growth by improving the natural properties of the soil (porosity, ability to retain water, The cohesion of silt and clay soil groups and Soil fertility traits (enrichment of the soil with some elements necessary for growth) and its vital activity, This is positively reflected in the formation of a large root and vegetative growth, which activates many of the plant's physiological processes, such as the absorption of nutrients and water, stimulation of the process of photosynthesis and respiration, and the release of energy particles. These results agree with Ciancio et al. 2014 and Golijan and Murkovic, 2018) and are consistent with their findings (Al Zahwan et al., 2013).

Conclusions:

1 - The fenugreek plant showed a positive response to spraying with chelated nano micronutrients at the concentrations that were sprayed, and the best of these treatments was spraying with both elements of iron and cobalt together at a concentration of 016 and 3 $mg.L^{-1}$ respectively, which significantly affected the seed content of the active ingredient.

2- Biological fertilization with (Sinorhzobium meliloti) bacterial inoculum of fenugreek seeds showed a highly significant effect in increasing the seed content of active substances.

3- The results of the study showed that organic fertilization using different levels of decomposing sheep waste has an important role in improving the conditions of fenugreek growth, and that the best of these levels is the treatment at an average of 6 tons. $dunam^{-1}$

4- The combinations between the study factors had a significant effect in increasing the studied traits, and the best of these combinations is the combination between biological fertilization and organic fertilization treatments at an average of 6 $tons.dunum^{-1}$, which gave the highest values in these materials, by providing balanced nutritional conditions for the plant by providing the necessary elements for growth in a way that made the plants grow better than non-fertilized treatments.

References

1. Al-Tamimi, Jamil Yassin Ali. (2010). The effect of bacterial pollination and cobalt on growth, yield, and chemical properties of Trigonella foenum-graecum L. Tikrit Journal of Pure Sciences. 15 (2), 228-238.

2. Hassan, Ahmed Yassin, Saad Eddin, Shurooq Muhammad Kazim. (2018). The effect of spraying with zinc and iron on the seed yield and the active substance Trigonelline in the seeds of fenugreek (Trigonella Foenum-graecum L). Kirkuk University Journal of Agricultural Sciences. 9 (2), 137--143.

3. Alrawi, Mahmoud Khashi and Abdulaziz Muhammad Khalaf Allah. (2000). Design and analysis of agricultural experiments. faculty of Agriculture. University of Al Mosul. Iraq.
4. Zaghloul, Rashid Abdel-Fattah (2019) Applied Microbiology. Faculty of Agriculture - Benha University - Republic of Egypt.
5. Zaghloul, Abdullah Qasim Abdullah. Meshaal, Hassan Rashwan Abdel-Hadi, (2011). Field crops. Ministry of Education – Egypt
6. Zahwan, Thamer Abdullah. Al-Kartani, Abdul Karim Oreibi and Al-Fahd, Moaz Abdel-Wahab. (2013). The effect of chemical, organic and biological fertilization on some growth, yield and active substances of Pimpinella anisum L. in gypsum soil. Tikrit Journal of Pure Sciences, Volume 18 (4), p (89-106).
7. Gayerly, Hilal, Hafez, Amira, Al-Zoubi, Muhammad Manhal, Ramadan, Yahya, Shibli, Khaled, Al-Katto, Ali, and Fatoum, Mayada. (2015). The effect of organic and green fertilizers on some soil properties and on the productivity of some crops in gypsum soil. Jordanian Journal of Agricultural Sciences. 173 (3693), 1- 15.
8. Al-Hidwani, Ahmed Khaled Yahya (2004). The effect of fertilization with some nutrients on the quantitative and qualitative characteristics of some medically active compounds in the seeds of two varieties of fenugreek, Trigonella foenum-graecum. PhD thesis, College of Agriculture, University of Baghdad, Ministry of Higher Education and Scientific Research, Republic of Iraq.
9. Yassin, Abdul Latif Yassin (2020) The effect of spraying with nan iron, bio-fertilizer and phosphate on some growth, yield and active substance of the fenugreek plant (Trigonella foenum-graecum L). PhD thesis - College of Agriculture - University of Tikrit - Ministry of Higher Education and Scientific Research - Republic of Iraq.

1. Ahmed, M. A., Ibrahim, O. M., & Elham, A. B. (2010). Effect of bio and mineral phosphorus fertilizer on the growth, productivity and nutritional value of fenugreek (Trigonella foenum graecum L.) in Newly Cultivated Land. Research Journal of Agriculture and Biological Sciences, 6(3), 339-48.
2. Brear, E. M., Day, D. A., and Smith, P. M. C. (2013). Iron: an essential micronutrient for the legume-rhizobium symbiosis. Frontiers in plant science, 4, 359.
3. Ciancio, N. R., Ceretta, C. A., Lourenzi, C. R., Ferreira, P. A. Trentin, G., Lorensini, F., and Brunetto, G. (2014). Crop response to organic fertilization with supplementary mineral nitrogen. Revista Brasileira de Ciência do Solo, 38(3), 912-922.
4. Connorton, J. M., Balk, J., and Rodríguez-Celma, J. (2017). Iron homeostasis in plants–a brief overview. Metallomics, 9(7), 813-823.
5. Gad, N., and Abdel-Moez, M. R. (2015). Effect of cobalt on growth and yield of fenugreek plants. International Journal of ChemTech Research, 8(11), 85-92.
6. Golijan, J., and Marković, D. (2018). Importance of using compost and bacterial biofuels in the organic production of medicinal and aromatic plants. АГРОЗНАЊЕ, 19(3), 211-219.

7. Hassan, Bassam. (2012). Medicinal Plants (Importance and Uses). Pharmaceutica Analytica Acta. 03. 10.4172/2153-2435.1000e139.
8. Havlin, J.L. et al., 2014. Soil Fertility and Fertilizers 8th Ed. Prentice Hall, Inc. Upper Saddle River, NJ
9. Jonse,j.benton. 2012. Plant nutritio and soil fertility manual . second edition . International Standard Book Number-13: 978-1-4398-1610-3
10. Kadim, A. A. Y. A. M. (2018). Impact of nano chelated iron, GA3 and organic fertilizer (Acadian) in moringa leaves content of α-Tocopherol and phytosterols. Research Journal of Pharmacy and Technology, 11(5), 1840-1846.
11. Li, P., Mou, Y., Lu, S., Sun, W., Lou, J., Yin, C., & Zhou, L. (2012). Quantitative determination of diosgenin in Dioscorea zingiberensis cell cultures by microplate-spectrophotometry and high-performance liquid chromatography. African journal of pharmacy and pharmacology, 6(15), 1186-1193.
12. Liu, J. (1998). Cobalt: physiological effects and uptake mechanisms in plants/by Juhong Liu ()Doctoral dissertation.
13. Menbari, S.,Alizadeh Salteh,S.,Bolandnazar, S.,and Sarikhani,M.R. (2017). Evaluation of the effects of biological and chemical fertilizers on some physiological and growth characteristics of fenugreek (Trigonella foenum-graecum). Journal of Agricultural Science and Sustainable Production, 27(4), 153-169.
14. Minz, A., Sinha, A. K., Kumar, R., Kumar, B., Deep, K. P., and Kumar, S. B. (2018). A review on importance of cobalt in crop growth and production. Int J Curr Microbiol App Sci, 7, 2978-2984.
15. Moradi kor, Zahra AND Zadeh ,Jalal Bayati (2013). Fenugreek (Trigonella foenum-graecum L.) as a valuable medicinal plant. International Journal of Advanced Biological and Biomedical Research, 1(8), 922-931.
16. Nagananda, G. S., Das, A., Bhattacharya, S., and Kalpana, T. (2010). In vitro Studies on the Effects of Biofertilizers(Azotobacter and Rhizobium) on Seed Germination and Development of Trigonella foenum-graecum L. using a Novel Glass Marble containing Liquid Medium. International Journal of Botany, 6(4), 394-403.
17. Pawar, V. A., Pawar, P. R., Bhosale, A. M., and Chavan, S. V. (2014). Effect of Rhizobium on seed germination and growth of plants. Journal of Academia and Industrial Research, 3(2), 84-88.
18. Singh, M. D. (2017). Nano-fertilizers is a new way to increase nutrients use efficiency in crop production. International Journal of Agriculture Sciences, ISSN, 0975-3710.
19. Snehlata, H. S., and Payal, D. R. (2012). Fenugreek (Trigonella foenum-graecum L.): an overview. Int J Curr Pharm Rev Res, 2(4), 169-87.
20. Soundari, M., T. Vaithiyanathan, and P. Sundaramoorthy. "Response of fenu-greek (Trigonella foenum-graecum L.) to Rhizobium inoculation." International Letters of Natural Sciences 6 (2015).

21. Vijaykumar, S., Daniel, w., and Werner,j.(2012). Development and Validation of a Novel RP-HPLC Method for the Analysis of Reduced Glutathione. Department of Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH 44272, USA.
22. Wani, S. A., and Kumar, P. (2018). Fenugreek: A review on its nutraceutical properties and utilization in various food products. Journal of the Saudi Society of Agricultural Sciences, 17(2), 97-106.
23. Yaseen, A.A. and Kadim, S.M. (2017). Response of some anatomical properties and growth of calotropis procera (AIT) R. Br to method adition of nano-iron, zinc and organic fertilizer growth. Journal of Global Pharma Technology. 9. 457-468.