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EFFECT OF SPRAYING NANO-AMINO ACIDS AND IRON ON SOME YIELD CHARACTERISTICS AND THE CHEMICAL AND MEDICINAL CONTENT OF TWO CULTIVARES OF FENUGREEK PLANT, *TRIGONELLA FOENUM-GRAECUM* L

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Summary

The experiment was carried out in the nursery of the Agricultural Division of the University of Kufa in Najaf Governorate for the season 2022-2023 to study the effect of spraying nano-amino acids and iron on some yield characteristics and the chemical and medicinal content of two varieties of the fenugreek plant, *Trigonella foenum-graecum* L., the local variety and the Indian variety.

The experiment included eight treatments. It is an interaction between two varieties of fenugreek plant (local A1 and Indian A2) with four spray treatments: T1 control (distilled water), T2 (200 mg. L^{-1}) nano-iron, T3 (2 ml. L^{-1}) nano-amino acids, and T4. (200 mg.L^{-1}+ 2 mL.L^{-1}) nano-iron and nano-amino acids. A factorial experiment was carried out using a completely randomized design (C.R.D) with two factors: varieties and spray treatments, with three replicates. The means were compared according to Duncan's multinomial test at the probability level of 0.05.

Measurements were taken at the end of the growing season. The results showed that foliar spraying of nano-amino acids and iron led to improvement in yield indicators and the chemical and medicinal content of all studied traits compared to control plants. The results of the study also showed that the T3 treatment (nano amino acids) gave the highest rates in most of the traits under study (pod length, number of pods, number of seeds, Choline, Peroxidase and Catalase when interacting with the local variety A1, as it reached 11.03 cm, 54.63 (pod. $Plant^{-1}$), 14.00 (seed. pod^{-1}), 0.44, 1.55, 1.42 (mg. ml^{-1}), respectively. As for the Indian variety A2, it gave the highest rate of the alkaloid Trigonelline when interacting with treatment T3, amounting to 0.79(mg. ml^{-1}).

Keywords: Fenugreek, nano-iron, nano-amino acids, Trigonelline, Choline.

Introduction

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The fenugreek plant (*Trigonella foenum graecumL.*) is a widely known plant and has been used since ancient times for its leaves and seeds (Chauhan et al., 2017). It is a common spice in Indian cuisine. About 260 species of this genus are spread throughout the world (Acharya et al., 2006).

Fenugreek is one of the oldest important medicinal and aromatic plants that belong to the leguminous family(Fabaceae) . Its leaves are used as an herb and its seeds are used as a spice (Zandi et al., 2015). It was called Trigonella from Latin meaning small triangle due to the triangular yellowish-white flower shape (Shashikumar et al., 2018). The fenugreek plant has long pod s and tender leaves that are used in many medicinal preparations (Baliga et al., 2017). The chemical components of seeds, such as saponins, fibres, protein, amino acids, and fatty acids, show noticeable differences between varieties (Taylor et al., 2000).

The fenugreek plant contains chemical compounds, the most important of which are medicinal alkaloids, steroid compounds, and sapogenin. This plant has been used to facilitate childbirth and aid digestion, and it has also been used as a general stimulant to improve food metabolism. Trigonelline is the most important alkaloid resulting from secondary metabolism in the fenugreek plant and has many benefits. Among them is its effectiveness in treating diabetes and reducing cholesterol in the blood, in addition to its use as a plant hormone to treat liver cancer, cervical cancer, and migraines. This compound is produced from niacin, which is one of the nutritional and medical vitamin supplements. Other alkaloids found in the fenugreek plant are choline, gentanin, and carpaine. (Bahmani et al., 2016). Some studies have shown that choline is used to treat Alzheimer's disease, liver and nervous system disorders, and atherosclerosis (Petropoulos, 2002). Fenugreek extracts contain antioxidants such as the enzyme Peroxidase and Catalase, which help protect the body from oxidative stress, in a study conducted by Anuradha and Ravikumar (2001) on diabetic rats in the laboratory.

Nanomaterials help increase plant productivity and reduce environmental impacts. They include nanoparticles made of metal such as iron or zinc oxide, or organic compounds (Dimkpa and Bindrabin, 2017). Due to its small size, its penetration and spread through the plant’s cell membranes is easy, in addition to its effectiveness in very low concentrations (Butt and Naseer, 2020), which ensures the maximum interaction and efficient use of nutrients by the plant (Mahanta et al., 2019).

Amino acids are biostimulants that are absorbed by the plant and move quickly within it. They work to accelerate the absorption of nutrients from the soil solution, as they have an effect on enzymatic activities, contribute to the process of opening and closing stomata, and have a role in chelating microelements when they are present together to facilitate the process of their entry into the plant (EL-Ghamry et al., 2009). Amino acids contribute to a large number of cellular processes and thus affect a number of functional processes, including plant growth, control of cell pH, and resistance to biotic and abiotic stress (Pratelli and Pilot, 2014), and they participate in making protein and carbohydrates through building chlorophyll and activating the photosynthesis process. It helps in activating many enzymes (Al-Said and Kamal, 2008; Shafeek et al., 2012). In a study conducted by Ajil and Jaafar (2022) on the sweet pepper plant Capsicum annuum L., it was concluded that nano-amino acids had a significant effect on all yield indicators, including the number of fruits, the yield of one plant, and the total yield. In an experiment conducted by Taraseviciene et al. (2021), they found that foliar spraying of amino acids has a significant effect on the total phenol content of different varieties of mint plants, as there was a clear increase in the leaf phenol content that differs according to the varieties, as each variety has a different response from the other varieties to amino acids.

Most of the iron present inside plant cells participates in the process of photosynthesis. It is necessary in the synthesis of chlorophyll, although it is not a component of it. However, it increases its synthesis, increases the synthesis of enzymes, maintains the structure and functions of chloroplasts, and thus increases plant productivity. It is one of the components of ferredoxin proteins and Cytochrome, which participate in the reactions of this process. (Briat et al., 2007; Valizadeh and Milic, 2016). Barker and Stratton (2015) stated that iron participates as a co-enzyme for 140 enzymes, in addition to being part of the components of enzyme antioxidants (catalase and peroxidase) that protect cells from free radicals resulting from oxidation processes. Mohammadzadeh Toutounchi and Amirnia (2016) found that foliar spraying of micronutrients, including iron, on fenugreek plant had a significant effect on plant height, the number of side branches, the number of pods, the number of seeds per pod, and the fresh weight of the shoot. In a study conducted by (Dola et al., 2022) on the soybean plant *Glycine max*L., they found that foliar spraying of nano-iron at a concentration of 200 parts per million led to an increase in seed productivity by 40.12 and 32.60% in drought conditions and good irrigation methods, respectively, compared to untreated plants . Salama et al. (2009) showed that applying iron to the plant led to the activation of enzymes such as peroxidase and catalase in the leaves of the flax plant *Linum usitatissimum*

The study aimed to evaluate the effect of foliar spraying of nano-amino acids and iron and their interaction on some characteristics and indicators of yield and the content of alkaloids and enzymes in the leaves of two varieties of fenugreek plant, the local variety and the Indian variety.

Materials and Methods

The experiment was carried out the Agricultural Division of the University of Kufa in Najaf Governorate for the season 2022-2023, as the seeds were planted during the growing season on the third of October 2022. The seeds of two varieties of the fenugreek plant, *Trigonella foenum graecum* L., were used (the local variety and the Indian variety), which were purchased from reliable agricultural offices in the Najaf Governorate.

Random samples were taken from the experimental soil before planting, and then physical and chemical analyzes were conducted in the Fadak Central Laboratory for Analysis of the disinfected upper threshold, as shown in Table (1). The seeds were planted in cork dishes containing peat moss and then transferred to pots ten days after planting. The diameter of one pot is 22 cm. It contains a mixture of soil taken from the banks of the river with peat moss at a ratio of 3 (soil): 1 (peat moss), and four plants were planted in one pot.

The experiment included 8 treatments, which is an interaction between two varieties of fenugreek plant (local A1 and Indian A2) with four spraying treatments: T1 comparison treatment (distilled water), T2 nano-iron at a concentration of 200 ($mg.L^{-1}$), T3 nano-amino acids at a concentration 2 ($ml.\ L^{-1}$), T4 mixture of nano-iron and nano-amino acids. The nano-iron solution was prepared from the Iranian company Armina Engineering, which was in the form of a black powder, from which 200 mg was dissolved and then added to a liter of distilled water. As for the nano-amino acids, they were prepared from the Turkish company Agri Sciences LTD.STI, where 2 ml were dissolved in a liter of distilled water, and the concentration was twice the concentration recommended by the company.

Table (1): Some physical and chemical of properties the experimental soil before planting

properties	Value	Unit
PH	7.78	-
EC	2.04	$Ds.m^{-1}$
available nitrogen	21.87	$mg.\ kg^{-1}$
phosphorus	37.53	$mg.\ kg^{-1}$
potassium	37.5	$mg.\ kg^{-1}$
sodium	68.75	$mg.\ kg^{-1}$
iron	2.7	$mg.\ kg^{-1}$
sand	872	$g.\ kg^{-1}$
silt	88	$g.\ kg^{-1}$
clay	40	$g.\ kg^{-1}$
soil texture	Sandy mixture	-

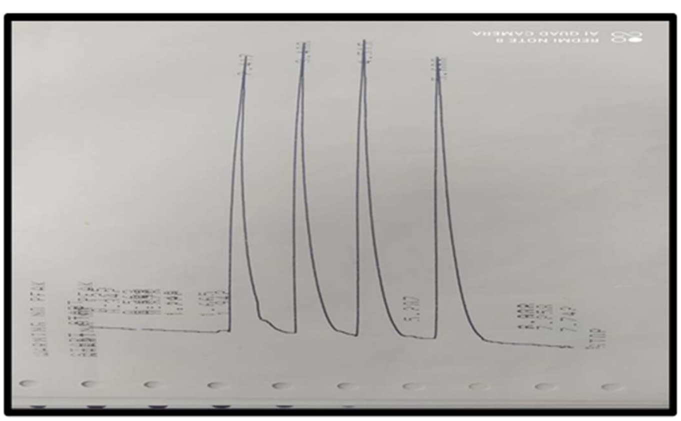
The plants were sprayed with the treatments early in the morning using a hand sprayer until the leaves were completely wet, with the addition of a diffuser, and the comparison treatment was sprayed with distilled water only. The treatments were separated with a piece of plastic to ensure that the spray does not spread and that there is no mixing between adjacent treatments. The spraying process was carried out three times, the first spraying was two months after planting the seeds, with an interval of two weeks between each spraying. Chemical content indicators were measured in mid-February 2023, while yield characteristics and components were taken in May 2023.

The experiment was carried out using a completely randomized design (CRD) in a factorial experiment with two factors: varieties and spray treatments, with three replications. The results were analyzed using the Genestate statistical program, and the experiment data were subjected to the statistical analysis table (Anova), and the means were compared according to the Duncuns Multiple Range Test at the probability level of 0.05. Yield indicators were measured by taking five random plants from each treatment at the end of the experiment to make measurements of the yield and its components. Pod length was measured using a measuring ruler for the plants selected in each treatment, then the average length of the pods was calculated by dividing the total number of pod lengths by the number of pods in the plant. The number of pods was calculated for each plant, and the rate was extracted by dividing the total number of pods by the number of plants. For all treatments, the number of seeds per pod was calculated by dividing the total number of seeds in one plant by the number of pods. The chemical tests were conducted at AL-Rawabi AL-Khadhra Company for Chemical& Environmental Studies & Analysis, Ltd.

A Japanese Shimadzu LC-10 A high-performance liquid chromatography (HPLC) device was used to detect compounds present in plant leaf extracts. The leaf content of the alkaloids Trigonelline and Choline was estimated according to the method of Edeoga et al. (2005). As for the enzymes peroxidase and catalase, they were measured according to the method of Alici and Arabaci (2016), samples were injected into the HPLC device three times. The retention time was determined for standard solutions of Trigonelline , Choline, Peroxidase, and Catalase was determined in Figure 1 and 2.

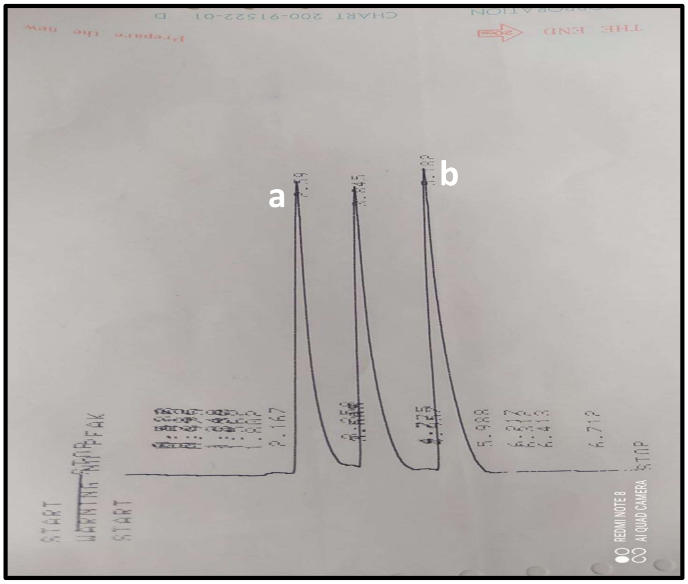
The concentration of each compound was calculated through the following equation:

Sample concentration (µg.ml-1)=(sample area)/(standard solution area) x standard solution concentration x number of dilutions.



Detention time for Choline b - Detention time for Trigonelline -a

Detention time for Alkaloids 1- Figure



a- Detention time for Catalase b- Detention time for Peroxidase

Figure 2- Detention time for Enzyme

Results

Table (2) shows that the Indian variety (A2) was significantly superior to the local variety (A1) in terms of pod length, as it gave an average of 10.32 (cm). It is clear in the same table that treatment T3 was significantly superior to the rest of the treatments, giving an average of 10.71 (cm). The significant effect of the interaction was clear in the aforementioned trait, as it exceeded the interaction between the local variety (A1) and the treatment T3, as it gave 11.03 (cm**).**

Table (2) Effect of cultivar, spraying of nano-amino acids ,iron and their interaction on Pod length (cm).

Cultivars	Treatments				Means
	T1	T2	T3	T4	
A1	8.40 c	9.86 b	11.03 a	9.96 b	9.81 b

*

A2	10.13 ab	10.00 b	10.40 ab	10.76 ab	10.32 a
Means	9.26 c	9.93 b	10.71 a	10.36 ab	

Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05)

The results in Table (3) indicate that the local variety (A1) was significantly superior, as it gave the highest average number of pods, amounting to 39.82(pod. Plant^{-1}), while the Indian variety (A2) gave the lowest rate, amounting to 16.91(pod. Plant^{-1}). The results in the same table indicated that there was a significant effect of the spray treatments in the same mentioned characteristic, the T3 treatment outperformed all the treatments and gave a rate of 37.56 (pod. Plant^{-1}). As for the overlap treatments, they also had a significant effect on the number of pods per plant. The highest rate was when the local variety (A1) was overlapped with the T3 treatment, which amounted to 54.63 (pod. Plant^{-1}).

The results in Table (4) show that there is no significant effect of the variety on the average number of seeds per pod. As for the spraying treatments, treatment T3 was significantly superior to the comparison treatment T1, giving 13.11 (seed. pod^{-1}), while the comparison treatment gave 10.50 (seed. pod^{-1}). As for the interaction, it had a clear significant effect, as the interaction between the local variety (A1) and treatment T3 was significantly superior to the rest of the treatments, as it gave an average of 14.00 (seed. pod^{-1}).

Table (3) Effect of cultivar, spraying of nano-amino acids ,iron and their interaction on Pod number. (pod. Plant^{-1}).

Cultivars	Treatments				Means
	T1	T2	T3	T4	
A1	31.00 c	32.86 c	54.63 a	40.80 b	39.82 a
A2	12.30 e	15.93 de	20.50 d	18.93 d	16.91 b

*

Means	21.65 d	24.40 c	37.56 a	29.86 b	

Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05)

Table (4) Effect of cultivar, spraying of nano-amino acids ,iron and their interaction on seeds number. (seed.pod $^{-1}$).

*

Cultivars	Treatments				Means
	T1	T2	T3	T4	
A1	9.53 d	12.36 bc	14.00 a	12.46 bc	12.09 a
A2	11.46 c	12.96 ab	12.23 bc	12.63 b	12.32 a
Means	10.50 b	12.66 a	13.11 a	12.55 a	

Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05)

Table (5) shows that the variety had a significant effect on the Trigonelline content of the leaves, as the Indian variety (A2) was significantly superior to the local variety (A1), giving the highest rate of 0.63 (mg.ml^{-1}), while the local variety gave the lowest rate of 0.51. (mg.ml^{-1}).

Likewise, treatment T3 was significantly superior to all other treatments, giving a rate of 0.70 (mg.ml^{-1}) As for the interference treatments, they also had a significant effect on the content of the leaves of the aforementioned substance. The treatment of the Indian variety (A2) with treatment T3 gave the highest rate of 0.79 (mg.ml^{-1}), and the lowest rate was when the local variety (A1) was mixed with the comparison treatment T1 (spraying distilled water), which amounted to 0.41 (mg. ml^{-1}), an increase of 92.68%.

Table (5) effect of the cultivar, the spraying of nano- amino acids and iron, and their interaction on the average content of the leaves of the alkaloid Trigonelline (mg. ml^{-1})

*

Cultivars	Treatments				Means
	T1	T2	T3	T4	
A1	0.41 d	0.48 cd	0.61 bc	0.55 bc	0.51 b
A2	0.52 bcd	0.58 bc	0.79 a	0.63 b	0.63 a
Means	0.46 c	0.53 b	0.70 a	0.59 b	

Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level ($P<0.05$)

Table (6) shows that the local variety (A1) was significantly superior to the Indian variety (A2) in terms of the leaf content of the alkaloid Choline, with the highest rate amounting to 0.35 ($mg.ml^{-1}$), while the Indian variety had the lowest rate of 0.26 ($mg.ml^{-1}$). As for spraying treatments, treatment T3 outperformed the comparison treatment T1 with the highest rate of 0.37 ($mg.ml^{-1}$). which did not differ significantly from treatments T2 and T4. As for the interference treatments, the interaction treatment of the local variety (A1) with treatment T3 was significantly superior at the highest rate of 0.44 ($mg.ml^{-1}$) compared to the treatment of the interference of the Indian variety with the comparison treatment T1, which gave the lowest rate of 0.18 ($mg.ml^{-1}$) and by An increase of 144.44%

Table (6) effect of the cultivar, the spraying of nano- amino acids and iron, and their interaction on the average content of the leaves of the alkaloid Choline ($mg. ml^{-1}$)

Cultivars	Treatments				Means
	T1	T2	T3	T4	
A1	0.27 b	0.33 ab	0.44 a	0.37 ab	0.35 a

*

A2	0.18 c	0.28 b	0.30 b	0.29 b	0.26 b
Means	0.22 b	0.30 a	0.37 a	0.33 a	

Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05)

Table (7 and 8) shows that the local variety (A1) was significantly superior to the highest rate of 1.23 and 0.98 (mg. ml^{-1}), respectively, over the Indian variety (A2), which gave the lowest rate of 0.98 and 0.76 (mg. ml^{-1}) respectively. As for the spraying treatments, they also gave significant differences, as treatment T3 was significantly superior to all treatments, giving the highest rate of 1.39 and 1.20 (mg. ml^{-1}) respectively, compared to the comparison treatment T1 (spraying distilled water), which gave the lowest rate of 0.76 and 0.69. (mg. ml^{-1}) respectively. The results of the tables show the significant effect of the interference treatments, as the interaction treatment of the local variety (A1) with the T3 treatment gave the highest value, amounting to 1.55 and 1.42 (mg. ml^{-1}) respectively.

As for nano-iron T2 and the mixture, we notice from the results that they have a significant effect on all the traits under study compared to the control plant group T1.

Table (7) effect of the cultivar, the spraying of nano- amino acids and iron, and their interaction on the average content of the leaves of the Peroxidase enzyme (mg. ml^{-1})

Cultivars	Treatments				Means
	T1	T2	T3	T4	
A1	0.81 ef	1.14 bc	1.55 a	1.45 a	1.23 a
A2	0.72 f	0.97 ed	1.23 b	1.03 cd	0.98 b
Means	0.76 d	1.05 c	1.39 a	1.24 b	

*** Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05)**

Table (8) effect of the cultivar, the spraying of nano- amino acids and iron, and their interaction on the average content of the leaves of the Catalase enzyme (mg. ml^{-1})

*

Cultivars	Treatments				Means
	T1	T2	T3	T4	
A1	0.76 cd	0.80 c	1.42 a	0.97 b	0.98 a
A2	0.62 d	0.75 cd	0.98 b	0.71 cd	0.76 b
Means	0.69 c	0.77 b	1.20 a	0.84 b	

Means followed by the same letter do not differ by Duncuns Multiple Test at a probability level (P<0.05)

Discussion

The results in Tables (2, 3, 4) showed that there are significant differences between the two varieties, and this may be due to their genetic differences and the geographical regions from which they were brought (Kizil, 2003). Amino acids have led to an increase in vegetative growth, as they are easily absorbed by the leaves and led to the formation of protein and an increase in the total chlorophyll content, thus improving the productivity and quality of the crop. (Neri et al., 2001). Likewise, amino acids are responsible for cell division and the production of some natural growth hormones, including IAA and GA3, which leads to an increase in yield characteristics. (Baqir et al., 2019).

The reason for the increase in yield indicators due to foliar spraying of nano-amino acids and iron may be that the absorption of these elements or nutrients is better than if they were in the soil because they do not move easily in alkaline soil. Therefore, these substances have many physical and chemical roles that contribute to increasing plant growth in general. (Hanjagi and Singh, 2017). As for the effect of nano-iron, its use can help increase plant growth, as small, highly soluble compounds are absorbed faster by the plant, and this leads to meeting the plant's nutrient needs (Mohamadipoor et al., 2013) and thus helps increase crop growth. This is consistent with what was found by Zafar et al. (2023) when iron was used as a foliar spray on the *Vigna radiate* plant**.**

As for tables (5, 6, 7, 8), the results also indicated that there are significant differences between the varieties, and this is due to genetic and environmental differences between the varieties. The effect of nano-amino acids on the content of chemical compounds in leaves in general may be due to the fact that these acids have a role in raising the efficiency of metabolic processes and antioxidants by increasing the proportion of enzymes in plant tissues. Amino acids are involved in the manufacture and stimulation of carbohyd rates, proteins, and the process of photosynthesis through Its role in building chlorophyll and encouraging the work of many enzymes that are related to controlling the plant in harsh conditions and pressures, stimulating it biologically and chemically, and thus developing the growth standards of the plant (Thomas et al., 2009; Ghazal and Al-Nussairaw 2022) . Amino acids are involved in the synthesis of alkaloids, meaning they represent the beginnings for their manufacture, and also depend on the nitrogen provided by the amino acids (Dey et al., 2020). This explains the increase in the leaves' content of the alkaloids Trigonelline and Choline due to the effect of foliar spraying of nano-amino acids. Nano- iron also has an effective and necessary role in many important enzymes, including catalase, peroxidase, and cytochrome oxidase. The participation of iron in these compounds is of particular importance in oxidation reactions, as it is important in transferring electrons through oxidation and reduction reactions, which is one of the important roles in cell metabolism (Yasin, 2001; Focus, 2003).

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