Research Article

Relative Effectiveness of Mineral Fortification and Biofertilization on Some Bioactive Constituents of Fenugreek (*Trigonella foenum-graecum* L.) Seeds

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Abstract |Fenugreek (*Trigonella foenum-graecum* L.) is a vital herb with great ethnomedicinal importance. As biofortification of plants with essential minerals, nutrients, and fertilizers can enhance their bio-constitution, this study assessed the effect of acadian marine algal extract, nitrogen, and iron supplementation on the contents of medically bioactive substances of seeds of the fenugreek plant. Field trial was conducted under RCBD with two major factors *i.e.* six combinations of nitrogen and iron (T1 = 0 + 0, T2 = 0 + 50 mg L^{-1}, T3 = 75 kg E^{-1} + 0, T4 = 75 kg E^{-1} + 50 mg L^{-1}, T5 = 150 kg E^{-1} + 0, and T6 = 150 kg E^{-1} + 50 mg L^{-1}) and three concentrations of Acadian marine algae extract (@ zero, 1.0 and 2.0 mL liter^{-1}). The results showed that treatments T5 and T6 were significantly superior to the others and exhibited the highest average concentrations of vaccenic acid (23.49) and palmitic acid (5.10%) in seeds of fenugreek plants as compared to the control treatment. Among algal extract concentrations, application of acadian algal extract @ 1.0 mL liter^{-1} exhibited significantly higher average concentrations of vaccenic acid and formyl histamine in fenugreek seeds (*i.e.* 22.26 and 6.67%, respectively) while application @ 2 mL liter^{-1} significantly enhanced the mean concentrations of palmitic acid (4.67%) and eicosenoic acid (22.14%) in fenugreek seeds. Overall study results advocate the practical implication of these mineral and biofertilizer-based fortification of medicinal plants, including fenugreek, to improve the biological active constitution of their seeds.

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Introduction

Fenugreek (*Trigonella foenum-graecum L.*) is one of the important herbaceous crops that belong to the Leguminosae family. It is an annual herb with ethnomedicinal importance and has been known for its medicinal values since ancient times. It is widely used as a vegetable, spice, and medicinal plant in many countries worldwide. Fenugreek seeds contain many medically effective compounds, including vaccenic acid, palmitic acid, eicosenoic acid, and formyl histamine (Barnes *et al*., 2002; Akbari *et al*., 2019; Visuvanathan *et al*., 2022). Seeds of fenugreek constitute many important medicinal compounds, such as carbohydrates, amino acids, alkaloids, soap steroids, flavones, etc. (Aasim *et al*., 2018; Niknam *et al*., 2021).

Biofortification of plants with essential minerals, nutrients, and fertilizers is one of the prime strategies used to enhance the bioconstitution of these plant products (White and Broadley., 2005). It is necessary to pay attention to fertilizing aromatic and medicinal plant species with the principal nutrients that have a crucial role in improving the growth qualities and that contribute to increasing production, their economic yield and the contents of medically active compounds, as studies have confirmed the importance of major nutrients for plants and their impact on the chemical constitution of the plants (Al-Rubaie, 2011; Jaiswal *et al*., 2022). Micronutrients are also as important as macronutrients, although plants need them in smaller quantities and play a vital role in various biological reactions, and their importance lies in their direct and indirect effect on the activation of various enzymes (Ali, 2012; Ahmed *et al*., 2024).

The priming effect of different minerals, micro and macronutrients, and biofertilizers has been demonstrated on the bioactive compound profiles of various plants, including agricultural and horticultural crops (Sarkar and Rakshit, 2021). Marine algal extracts, for instance, have been used as a bio-stimulant for many physiological functions in plants and usually are rich in nutrients and are characterized by containing auxins and cytokinins and also have many trace elements which exert positive impacts on plant yield and on cell growth, are considered a complement to fertilizers and not a substitute for them (Chojnacka *et al*., 2015; Al-Azzawi *et al*., 2018).

One of the extracts of marine algae is Acadian, which refers to a seaweed extract product derived from brown seaweed *Ascophyllum nodosum*. It is being used as a nutritional supplement for plants to enhance their productivity and environmental stress resistance (Gardner *et al*., 2009; Kumari *et al*., 2023). It is a rich source of primary and secondary nutrients and carbohydrates, stimulating the quality and productivity of plants, enhancing the production of enzymes, sugars and plant growth hormones, proteins, and fats, and increasing the effectiveness of antioxidants. This field experiment was aimed to determine the impact of acadian marine algal extract, nitrogen, and iron applications on the medically bioactive constituents of the fenugreek plant and to determine the best treatment for the production of medically active compounds.

Materials and Methods

Experimental treatments

This research work was performed in the experimental area of the Department of Vocational Education, Ibn Al-Bitar Vocational Preparatory School, Directorate of Education, Holy Karbala, Iraq, during the winter season 2024–2023. The prime objective of the study was to evaluate the effect of supplementation by acadian marine algal extract, nitrogen, and iron on some medically effective compounds of fenugreek plant seeds. The study involved two major independent variables. The first one included six nitrogen concentrations, which were directly added to seed seedbed combined with foliar application of six different concentrations of iron sprayed on the plants. Nitrogen and iron treatments were as follows; T1 = 0 + 0, T2 = 0 + 50 mg L^{-1}, T3 = 75 kg E^{-1} + 0, T4 = 75 kg E^{-1} + 50 mg L^{-1}, T5 = 150 kg E^{-1} + 0, and T6 = 150 kg E^{-1} + 50 mg L^{-1} . The second factor was the foliar application of three different concentrations of water-soluble Acadian marine algae extract (*i.e.*, spraying with distilled water only, spraying the plants with marine algae @ 1 ml $liter^{-1,}$ and spraying the plants with marine algae @ 2 ml $liter^{-1}$). The experimental design was according to RCBD and each treatment was replicated three times.

After seed bed preparation through routine agronomic practices, the experimental area was divided into plot size of 6 m^2 (3 × 2 m), and the seeds of Indian variety of fenugreek were planted at a depth of 3 cm with plant to plant and line to line distance of 20 and 50 cm, respectively. The harvesting of the plants was done at the full maturity stage of the crop. The seed contents of the bioactive substances (*i.e.*, concentrations of vaccenic acid, palmitic acid, eicosenoic acid, and formyl histamine) were determined using gas-chromatography-cum-mass spectrometer (GC-MS) (Model: A7820; Agilent Technologies, Santa Clara, CA) according to the protocol described by Meyer (2016).

Statistical analyses of the data regarding the effect of nutritional or fertilizer treatments on the concentrations of medically bioactive constituents of the seeds of fenugreek plants was done using Statistical 8.1. Factorial analysis of variance (ANOVA) was executed to assess the impact of treatments on the parameters studied using the standard level of significance (α = 0.05).

Results and Discussion

Effect of treatments on vaccenic acid contents of fenugreek seeds

Results shown in Table 1 exhibit a significant (P ≤ 0.05) effect of the applications of nitrogen and iron fertilizers or nutrients and foliar sprays of acadian marine algal extract on the concentration of vaccenic acid contents of the fenugreek seeds. However, the interaction of both of these factors did not exhibit a significant effect on the vaccenic acid contents of the seeds.

Table 1: *Effect of nitrogen, iron, and marine algae extract and their interaction on vaccenic acid contents (%) of fenugreek (Trigonella foenum-graecum L.) seeds.*

Nitrogen and Iron (N + Fe) Treatments	Acadian marine algae (g L^{-1})			Mean
	0	1	2	
T1	15.29	17.81	15.65	16.25
T2	19.24	24.85	25.69	23.26
T3	16.36	23.34	18.19	19.30
T4	15.39	23.84	15.99	18.41
T5	21.88	24.91	23.68	23.49
T6	16.16	18.80	16.31	17.09
Mean	17.39	22.26	19.25	-
LSD *0.05*	N + Fe	Acadian	Acadian × N + Fe	
	5.069	2.956	NS	

Nitrogen and iron treatments: T1 = 0 + 0, T2 = 0 + 50 mg L^{-1}, T3 = 75 kg E^{-1} + 0, T4 = 75 kg E^{-1} + 50 mg L^{-1}, T5 = 150 kg E^{-1} + 0, and T6 = 150 kg E^{-1} + 50 mg L^{-1}

The coefficients of adding nitrogen and iron combinations showed a significant impact on the concentration of vaccenic acid in the seeds of the fenugreek plant, as the treatment T5 significantly surpassed all other treatments and exhibited the highest (23.49%) average vaccenic acid content, followed by treatment T2 (23.26%). In comparison, the control treatment T1 exhibited the lowest vaccenic acid average (16.25%), followed by treatment T6 (17.09%) (Table 1).

Regarding the effect of acadian marine algal treatments, a significant (P ≤ 0.05) effect of spraying different concentrations of marine algae extract on the vaccenic acid content of fenugreek seeds has been observed. The concentration of 1.0 g liter^{-1} exhibited the highest average of vaccenic acid (22.26%), with an increase of 28.01% compared to the no-addition of algal extract, exhibiting the lowest average of 17.39% of vaccenic acid.

Effect of fertilizer and nutrient supplementation on seed palmitic acid contents

A similar trend was observed regarding the effect of treatments on seed palmitic acid contents. A significant (P ≤ 0.05) effect of the supplementation of nitrogen and iron combination coefficients, as well as a significant effect of spraying different concentrations of acadian marine algal extract, was recorded on the palmitic acid content of the seeds of fenugreek. However, the interaction of both these factors, *i.e.*, different combinations of nitrogen and iron supplements and spraying fenugreek plants with acadian marine algae extract, exerted no considerable impact on the content of palmitic acid in fenugreek plant seeds (Table 2).

Table 2: *Effect of nitrogen, iron, and marine algae extract and their interaction on palmitic acid contents (%) of fenugreek (Trigonella foenum-graecum L.) seeds.*

Nitrogen and iron (N + Fe) treatments	Acadian marine algae (g L^{-1})			Mean
	0	1	2	
T1	3.08	3.27	3.19	3.18
T2	3.20	3.62	3.55	3.46
T3	3.54	3.88	4.93	4.11
T4	4.98	4.97	5.01	4.98
T5	3.72	4.07	5.07	4.29
T6	4.68	4.96	5.67	5.10
Mean	3.87	4.13	4.67	-
LSD *0.05*	N + Fe	Acadian	Acadian × N + Fe	
	0.815	0.379	NS	

Nitrogen and iron treatments: T1 = 0 + 0, T2 = 0 + 50 mg L^{-1}, T3 = 75 kg E^{-1} + 0, T4 = 75 kg E^{-1} + 50 mg L^{-1}, T5 = 150 kg E^{-1} + 0, and T6 = 150 kg E^{-1} + 50 mg L^{-1}

Regarding mineral fortification of fenugreek plants, treatment T6 outperformed the rest of the treatments and showed the highest palmitic acid average (5.10%), followed by treatment T4 (4.98%) and T5 (4.29%), with an increase of 60.52% as compared to the control treatment T1 that exhibited the lowest average of seed palmitic acid content (3.18%), followed by T2 (3.46%). Among marine algal treatments, the concentration of 2.0 g liter^{-1} showed the maximum average concentration of palmitic acid in fenugreek seeds (4.67%), followed by 1.0 g liter^{-1} (4.13%), while the non-addition treatment (control) gave the lowest average of seed palmitic acid content (3.87%). There was an increase of 18.16% of seed palmitic acid contents by adding 2.0 g liter^{-1} of acadian marine algal extract (Table 2).

Impact of nutritional supplementation on eicosenoic acid contents of fenugreek seeds

Table 3 showed no significant (P ≥ 0.05) effect of the nitrogen and iron supplementation on the eicosenoic acid content of fenugreek seeds, although the application of different concentrations of acadian marine algal extract exerted a significant effect (P ≤ 0.05) on seed eicosenoic acid contents. Moreover, the interaction of both factors (nitrogen and iron application and marine algal supplementation) exerted no significant effect on the concentration of eicosenoic acid in fenugreek plant seeds.

Table 3: *Effect of nitrogen, iron, and marine algae extract and their interaction on eicosenoic acid contents (%) of fenugreek (Trigonella foenum-graecum L.) seeds.*

Nitrogen and iron (N + Fe) treatments	Acadian marine algae (g L^{-1})			Mean
	0	1	2	
T1	17.33	18.17	17.81	
T2	20.50	21.85	24.34	
T3	18.80	20.50	19.44	
T4	17.94	20.92	24.66	
T5	17.57	18.54	22.55	
T6	19.29	21.85	24.02	
Mean	18.57	20.30	22.14	
LSD *0.05*	N + Fe	Acadian	Acadian × N + Fe	
	NS	2.474	NS	

Nitrogen and iron treatments: T1 = 0 + 0, T2 = 0 + 50 mg L^{-1}, T3 = 75 kg E^{-1} + 0, T4 = 75 kg E^{-1} + 50 mg L^{-1}, T5 = 150 kg E^{-1} + 0, and T6 = 150 kg E^{-1} + 50 mg L^{-1}

Treatment T2 and T1 of nitrogen and iron supplementation exhibited maximum (22.23%) and minimum (17.77%) of seed contents of eicosenoic acid without any significant difference. Regarding treatment-wise comparison, foliar application of highest concentration of marine algal extract (2.0 g liter^{-1}) exhibited a significant effect and produced an average of 22.14% eicosenoic acid in the fenugreek seeds with an increase of about 19.22% as compared to no addition of algal extract, that gave the lowest average (18.57%) of eicosenoic acid contents in fenugreek seeds (Table 3).

Effect of nitrogen, iron, and marine algal extract on formyl histamine contents of fenugreek seeds

Data analysis showed no significant (P ≥ 0.05) effect of the application of nitrogen and iron fertilizers and a significant (P ≤ 0.05) effect of different concentrations of acadian marine algal extract and their interaction on the concentration of formyl histamine in fenugreek plant seeds (Table 4).

Table 4: *Effect of nitrogen, iron, and marine algae extract and their interaction on formyl histamine contents (%) of fenugreek (Trigonella foenum-graecum L.) seeds.*

Nitrogen and iron (N + Fe) treatments	Acadian marine algae (g L^{-1})			Mean
	0	1	2	
T1	4.15	4.18	5.23	4.52
T2	4.96	6.32	6.42	5.90
T3	5.36	6.79	5.67	5.94
T4	4.61	7.78	5.55	5.98
T5	5.12	7.01	5.43	5.85
T6	4.81	7.92	7.81	6.85
Mean	4.83	6.67	6.02	-
LSD *0.05*	N + Fe	Acadian	Acadian × N + Fe	
	NS	0.529	1.713	

Nitrogen and iron treatments: T1 = 0 + 0, T2 = 0 + 50 mg L^{-1}, T3 = 75 kg E^{-1} + 0, T4 = 75 kg E^{-1} + 50 mg L^{-1}, T5 = 150 kg E^{-1} + 0, and T6 = 150 kg E^{-1} + 50 mg L^{-1}

According to treatment-wise comparison, it was found that the concentration of 1.0 g liter^{-1} of marine algal extract gave the highest average value (6.67%) of formyl histamine contents in the seeds of fenugreek plants, followed by the treatment with 2.0 g liter^{-1} of marine algal extract (6.02%) with an increase of about 38.10% as compared to control treatment with any addition of algal extract that exhibited the lowest average concentration (4.83%) of formyl histamine in the seeds of the fenugreek plant. Regarding the interaction effect of both factors, results indicated highest average concentration of formyl histamine contents (7.92%) in the seeds of fenugreek plants was achieved when the plants were treated with a nitrogen-iron fertilizer combination of 150 kg E^{-1} + 50 mg L^{-1} and were sprayed with 1.0 g liter^{-1} of marine algal extract (Table 4).

The biocomposition of plant seeds can be improved by the supplementation of different nutrients at different stages (Malhi *et al*., 2013). Particularly, enhancing the seed contents of medicinally important bioactive constituents is one of the prime objectives of research on medicinal plants (Sławińska and Ola, 2022). This research work was performed to determine the effect of fortification of fenugreek plants with nitrogen, iron and supplemented with foliar application of different concentrations of acadian marine algal extract on the average contents of different medically important constituents of fenugreek seeds.

It is clear from the results that the edaphic application of nitrogen, and foliar application of iron supplements, and the foliar application of different concentrations of acadian marine algal extract exerted a significant impact on the amount or concentration of some medically active substances of the seeds for the fenugreek plant. Particularly, the soil application of 150 kg E^{-1} of nitrogen fertilizer and 50 mg L^{-1} foliar application of iron supplement and application of acadian algal extract @ 1.0 or 2.0 g L^{-1} significantly enhanced most of the studied parameters of fenugreek seeds. This might be because nitrogen plays a vital role in promoting medically effective compounds in plant parts, including their seeds. Nitrogen fertilization contributes to an increased production of secondary substances and metabolites associated with the formation of medicinal compounds in different medicinal plant species, including fenugreek (Al-Husseini and Al-Mahdi, 1990; Singh *et al*., 2018; Hao *et al*., 2024).

The increased concentration of active substances in the plants is associated with their biofortification with different macro and micronutrients, which increase the accumulation of processed substances, including secondary compounds and their downstream transfer (Malka *et al*., 2022). These nutrients also positively affect the production of secondary compounds within the plants (Al-Khamas, 2023). Hussain *et al*. (2013) and Al-Rikabi (2014) indicated that fenugreek plants respond positively to nitrogen fertilizer, leading to an enhancement of some bioactive substances in the plant, including plant seeds.

Moreover, increase in active substances within the plants can be credited to iron's role as an enzymatic companion of many biological reactions that occur within the plant tissues, which contribute to the formation of different by-products that enter into the formation of active substances in medicinal plants (Al-Halbousi and Mahidi, 2015; Al-Issawi and Mahdi, 2016; Zhou *et al*., 2022).

Similarly, our results regarding the increased amount of all four medically bioactive constituents of fenugreek seeds induced by the foliar supplementation of acadian marine algal corroborate with the findings of previous studies showing positive and priming effects of this biofertilizer on growth and performance of different plant species including fenugreek (Tuhy *et al*., 2020; Battah *et al*., 2021). The increased concentration of active compounds in plants by the addition of marine algae extracts would be because it catalyzes many metabolic processes within plant cells (El-Gamal, 2010; Sabry and Tawfik, 2020; Ali *et al*., 2021). Studies have confirmed that the use of marine algal extract exerts a positive effect on many medicinal plant species (Battah *et al*., 2021; Kadhim and Athfua, 2023; Al-Ealayawi and Al-Dulaimy, 2023).

Conclusions and Recommendations

The overall conclusion based on the study results is that the supplementation of fenugreek plants with nitrogen and iron fertilizers and the application of marine algal-based biofertilizer can significantly enhance the concentration of medically important bioconstituents of fenugreek seeds. These results advocate the considering practical implication of these mineral and biofertilizer based fortification of medicinal plants to improve the bioactive compounds profile of the plant products.

Novelty Statement

Assess the effects of Acadian marine algal extract, nitrogen, and iron on fenugreek's medicinal components and determine the optimal treatment for producing medically active compounds of fenugreek.

Author's Contribution

Ahmed Najm Al-Mosawy and Zahraa Mohsen Al Assafi: Contributed to methodology, experiment data analysis, discussed the results, wrote, reviewed, and finalized the manuscript.
Both author have read and agreed to the published version of the manuscript.

Conflict of interest
There is no conflict of interest among the authors regarding the publication of this research work.

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