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# FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) RESPONSE TO *AZOTOBACTER* AND ORANGE PEELS IN METABOLISM, GROWTH, AND YIELD TRAITS

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**SUMMARY**

A field experiment commenced in Hindiyah District, Kerbala, Iraq, during the growing season of 2022–2023, to evaluate the effect of *Azotobacter* and orange (*Citrus sinensis* L.) peel treatments on the growth and yield characteristics of fenugreek (*Trigonella foenum-graecum* L.). The experiment had a randomized complete block design with factorial arrangement of two factors and three replications. The first factor included four levels of the *Azotobacter* bacteria (control, 20, 50, and 100 g $L^{-1}$), while the second comprised four concentrations of orange peel extract as a soil amendment (control, 100, 150, and 200 g $L^{-1}$). The treatment with *Azotobacter* (100 g $L^{-1}$) indicated significant effects and provided the highest mean values of the compounds, quercetin, diosgenin and trigonelline (33.20, 36.57, and 37.05 mg $g^{-1}$, respectively). The results also revealed the remarkable effect of the treatment with orange peel (200 g $L^{-1}$), which gave the maximum values of the quercetin, diosgenin, and trigonelline (31.99, 35.73, and 35.91 mg $g^{-1}$, respectively). The interaction effects of the *Azotobacter* and orange peels were also substantial for all the traits of fenugreek (*T. foenum-graecum* L.) under study.

**Keywords:** Fenugreek (*T. foenum-graecum* L.), *Azotobacter*, orange peels, secondary compounds, growth and yield traits

**Key findings:** In fenugreek (*T. foenum-graecum* L.), the treatments with *Azotobacter* (100 g $L^{-1}$), orange peels (200 g $L^{-1}$), and their interactions indicated significant effects to improve the concentrations of secondary metabolism compounds and yield parameters.

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## INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an important species belonging to the leguminous family, Fabaceae. Crucial economic values characterize this herb, such as, rich sources of carbohydrates, proteins, and fibers. In addition, it has medicinal values and a considerable content of quercetin, diosgenin, and trigonelline. Fenugreek also has various therapeutic properties, including anti-inflammatory, regulating blood sugar, lowering cholesterol, and other medical benefits (Zandi *et al.,* 2017).

The cultivation of this herb has begun to increase recently due to its various domestic and medical uses. However, some challenges exist facing fenugreek in the production, such as, water scarcity, low soil nutrients, and temperature fluctuations (Singh *et al*., 2015). These factors can limit the growth and productivity of the fenugreek plant, and thus have a negative impact on its growth and development and its net economic return (Zapotoczny *et al.,* 2016).

One of the most prominent agricultural practices and sustainable methods commonly used to enhance the quantity and quality of the fenugreek crop is the application of biological fertilization system, including the use of microorganisms, such as nitrogen-fixing bacteria *Azotobacter* (Wang *et al.,* 2023). The said bacteria lives freely in the soil and can fix the atmospheric nitrogen and transforms it into a consumable form, benefitting the plants. The said process is a known means of biological nitrogen fixation. Therefore, this process can considerably be an alternative to chemical fertilizers, which contributes to reducing pollution, resulting from excessive chemical fertilization, in addition to enhancing and sustaining the soil properties (Sumbul *et al.,* 2020).

Another agricultural practice used to enhance the productivity of the fenugreek crop is using some plants' organic residues, also called organic fertilization. Orange peels are byproducts easily obtained and provide a rich environment of nutrients necessary for plant growth. Orange peels are a rich source of carbohydrates, vitamins, and some other minerals, such as, phosphorus, potassium, calcium, and magnesium.

These compounds and minerals can improve soil properties and enhance its ability to retain more water and nutrients. These organic products also contribute to increasing microorganisms' activity in the soil and provide an ideal environment for plant growth and increased productivity (Mohsin *et al.,* 2022). Therefore, the presented study sought to determine the combined effect of *Azotobacter* bacteria and orange peels on improving productive and qualitative traits of fenugreek plants.

## MATERIALS AND METHODS

A field experiment, carried out during the crop season of 2022, transpired in the Hindiyah District, Kerbala, Iraq. It aimed to determine the combined effects of *Azotobacter* bacteria and orange peels on the productive and qualitative characteristics of the fenugreek (*Trigonella foenum-graecum* L.). The experimental layout was in a randomized complete block design with factorial arrangement, two factors, and three replications. The first factor included *Azotobacter* bacteria treatments (control, 20, 50, and 100 g $L^{-1}$), while the second factor included four concentrations of orange peels (control, 100, 150, and 200 g $L^{-1}$).

### Soil samples and plantation

Collected soil samples came from Allahabad, Iraq, with their physicochemical properties analyzed (Table 1). Then, the soil placed in plastic pots (35 cm diameter, 50 cm height) weighed 20 kg per pot. Fifty fenugreek seeds proceeded sowing in each pot.

### Treatments' application

#### *Azotobacter*

After making a one cm cut near the rhizosphere of each plant, the application of the corresponding *Azotobacter* suspension

**Table 1.** Biochemical analysis and composition of the experimental soil.

| Biochemical characters | Unit |
|---|---|
| $NH_4N$ | 28.26 mg kg $^{-1}$ |
| S | 2.47 mm $L^{-1}$ |
| Na | 1.55 mm $L^{-1}$ |
| PH | 7.63 |
| Ca | 4.38 mm $L^{-1}$ |
| Mg | 3.53 mm $L^{-1}$ |
| K | 1.98 mm $L^{-1}$ |
| Cl | 5.36 mm $L^{-1}$ |

continued directly to the roots, performed once during the growing season.

***Orange peel extract***

The extract preparation used a Soxhlet apparatus by extracting 150 g of dried orange peel powder with 300 ml of 70% ethanol for 10 h. Theconcentrated extract utilized a rotary evaporator at 45°C before applying to the soil.

**Data recorded**

Plant growth parameters, including plant height, leaf area, and dry weight for plant, incurred measuring. The calculation of number of pods per plant comprised taking 10 randomly selected plants from each experimental unit and then averaged. Similarly, calculating the number of seeds per pod ensued by counting the total number of seeds from 10 randomly selected pods in each experimental unit, and then averaged. For qualitative characteristics, computing the percentage of oil extracted from 100 g of powdered fenugreek seeds used a Soxhlet apparatus with 150 ml of 90% hexane for 24 h. In the seed content of fenugreek, some active compounds, such as, quercetin, diosgenin, and trigonelline also reached calculations using an HPLC device (Król-Kogus *et al.*, 2018).

**Statistical analysis**

All the recorded data based on various parameters underwent the analysis of variance (ANOVA) as per the randomized complete block design. The least significant difference ($LSD_{0.05}$) test application compared and separated the means (Al-Mohammadi and Al-Mohammadi, 2012). All the analyses processing used the statistical analysis software GenStat12.

**RESULTS AND DISCUSSION**

**Quercetin**

The *Azotobacter* bacteria levels showed significant differences for the quercetin content in fenugreek (*T. foenum-graecum* L.) seeds (Table 2). The highest average quercetin content (33.20 mg $g^{-1}$) resulted with *Azotobacter* bacteria at 100 g $L^{-1}$, compared with the control treatment showing an average of 23.06 mg $g^{-1}$. This may refer to the positive role of the *Azotobacter* bacteria in increasing the nutrient readiness, raising the level of efficiency of vital processes within the plants and, thus, increasing the content of active compounds (Victor *et al.,* 2019).

The results further revealed orange peel treatments showed considerable variances for quercetin content in fenugreek seeds. However, orange peel concentration at 200 g $L^{-1}$ gave the highest average of quercetin (31.99 mg $g^{-1}$), while the comparison treatment reached an average of 24.49 mg $g^{-1}$. This may be due to the positive effects of orange peels with organic materials and mineral elements, as well as, containing vitamins C and E. Thus, this may help encourage the biosynthesis of some secondary metabolic compounds, including quercetin (Zewde and Jembere, 2010).

The interaction of both factors also indicated significant effects on the quercetin content in fenugreek seeds (Table 2).

**Table 2.** Effect of *Azotobacter*, orange peels, and their interactions on the quercetin content in fenugreek seeds.

| *Azotobacter* (g $L^{-1}$) | Orange peels (g $L^{1}$) | | | | Means (mg $g^{-1}$) |
|---|---|---|---|---|---|
| | Control | 100 | 150 | 200 | |
| Control | 20.54 | 22.56 | 23.63 | 25.52 | 23.06 |
| 20 | 22.54 | 25.63 | 27.53 | 30.54 | 26.56 |
| 50 | 26.54 | 29.47 | 31.43 | 34.41 | 30.39 |
| 100 | 28.36 | 31.46 | 35.47 | 37.52 | 33.20 |
| Means (mg $g^{-1}$) | 24.49 | 27.28 | 29.52 | 31.99 | |
| $LSD_{0.05}$ *Azotobacter:* 1.65, Orange peels: 1.65, Interaction: 2.54 | | | | | |

**Table 3.** Effect of *Azotobacter*, orange peels, and their interactions on the diosgenin content in fenugreek seeds.

| *Azotobacter* (g $L^{-1}$) | Orange peels (g $L^{-1}$) | | | | Means (mg $g^{-1}$) |
|---|---|---|---|---|---|
| | Control | 100 | 150 | 200 | |
| Control | 25.65 | 27.54 | 29.65 | 30.54 | 28.32 |
| 20 | 26.65 | 29.43 | 31.43 | 33.36 | 30.22 |
| 50 | 28.43 | 33.54 | 35.65 | 36.36 | 33.50 |
| 100 | 29.35 | 35.64 | 38.63 | 42.65 | 36.57 |
| Means (mg $g^{-1}$) | 27.97 | 31.54 | 33.84 | 35.73 | |
| $LSD_{0.05}$ *Azotobacter*: 1.54, Orange peels: 1.54, Interaction: 2.54 | | | | | |

However, the supreme quercetin content (37.52 mg $g^{-1}$) appeared with the interaction of *Azotobacter* bacteria (100 g $L^{-1}$) and orange peel extract concentration (200 g $L^{-1}$), while the lowest interaction effects (20.54 mg $g^{-1}$) resulted in the control treatment of both factors.

**Diosgenin**

For diosgenin content, the *Azotobacter* levels revealed significant variations in fenugreek seeds (Table 3). Application of *Azotobacter* noticeably increased the diosgenin content, with the highest diosgenin concentration (36.57 mg $g^{-1}$) observed with *Azotobacter* at 100 g $L^{-1}$, compared with the control treatment (28.32 mg $g^{-1}$). The increase in diosgenin content in fenugreek seeds may be due to the positive role of *Azotobacter* bacteria in enhancing nutrient readiness to dissolve insoluble phosphorus molecules, allowing the plant to absorb them and, thus, boosting the activity of growth. These development processes contribute to increasing the synthesis of hormones and enzymes responsible for the synthesis of active substances (Sharma *et al.,* 2011).

The results also showed remarkable differences among the orange peel treatments for diosgenin content in fenugreek seeds (Table 3). The highest average diosgenin content (35.73 mg $g^{-1}$) occurred with the orange peel concentration 200 g $L^{-1}$, while the control treatment gave the lowest average, amounting to 27.97 mg $g^{-1}$. This effect was likely due to the presence of beneficial compounds in orange peels, such as flavonoids and vitamins, which can serve as activators of diosgenin biosynthesis (Tsai, 2008).

The interactions of *Azotobacter* bacteria and orange peel extract also provided significant effect on diosgenin biosynthesis (Table 3). The utmost interaction reached a diosgenin content of 42.65 mg $g^{-1}$ by adding *Azotobacter* bacteria (100 g $L^{-1}$) and orange peel extract concentration (200 g $L^{-1}$). Meanwhile, the control treatments of both factors were apparently with the least diosgenin content (25.65 mg $g^{-1}$) in fenugreek seeds.

**Trigonelline**

Regarding the *Azotobacter* bacteria treatments, substantial differences were evident for trigonelline content in fenugreek seeds (Table 4). However, the highest trigonelline average (37.05 mg $g^{-1}$) recorded was with the *Azotobacter* bacteria at 100 g $L^{-1}$, compared to the control treatment, which gave the least average (28.02 mg $g^{-1}$). This increase could be attributable to the improved nutrient availability enhanced by the action of *Azotobacter* bacteria, which had a positive effect on increasing trigonelline (Martyniuk and Martyniuk, 2003).

Orange peel extract also considerably increased the trigonelline content (Table 4), and the maximum trigonelline concentration (35.91 mg $g^{-1}$) emerged with the orange peel extract (200 g $L^{-1}$), compared with the control treatment (29.03 mg $g^{-1}$). The antioxidant properties of orange peel extract may have enhanced by protecting the plant cells from oxidative damage and promoting overall plant health, positively affecting an increase in trigonelline (Boyd, 2011).

The combined application of *Azotobacter* and orange peel extract resulted in significant effects on the trigonelline content in fenugreek seeds (Table 4). The highest trigonelline content (41.63 mg $g^{-1}$) were evident with the application of *Azotobacter* (100 g $L^{-1}$) and orange peel extract (200 g $L^{-1}$), while the lowest interaction (26.75 mg $g^{-1}$) manifested with the control treatment of both factors.

**Plant height**

The outcomes showed notable differences among the *Azotobacter* bacteria levels for plant height in fenugreek plants (Table 5). The taller plants (38.73 cm) were visible with the application of *Azotobacter* bacteria at 100 g $L^{-1}$, compared with the control treatment, showing the least average for the plant height (30.54 cm). This increase in plant height could refer to the positive role of *Azotobacter* in promoting plant growth through the production of plant hormones, i.e., IAA, gibberellin, and cytokinin. These hormones stimulate the various physiological processes, leading to an increased plant height in crop plants (Raj and Tabassum, 2017).

The orange peel treatments also showed meaningful variations for plant height in fenugreek plants (Table 5). The topmost plant height (36.78 cm) was evident with the orange peel extract at 200 g $L^{-1}$, versus the control treatment, which gave the lowest average (31.7 cm). This may be due to the increased efficiency of the roots' absorption of nutrients positively affected by adding orange peels, enriching soil fertility and improving its properties. These processes lead to an increased transfer of nutrients from the soil to the plants, in turn, reflecting an elevated plant height (Zewde and Jembere, 2010).

For plant height in fenugreek, the interactions of *Azotobacter* bacteria and orange peel extract also exhibited significant interaction effects (Table 5). The maximum plant height (42.87 cm) appeared by adding *Azotobacter* bacteria (100 g $L^{-1}$) and orange peel extract concentration (200 g $L^{-1}$), while interactions of both control treatments recorded with the least plant height (28.87 cm) in fenugreek.

**Pods per plant**

The results indicated noteworthy diversities among the treatments of adding *Azotobacter* bacteria's different levels for the number of pods in fenugreek (Table 6). The exceeding level of the *Azotobacter* bacteria (100 g $L^{-1}$) gave the highest average (21.55 pods $plant^{-1}$), while the control treatment provided the lowest average (14.48 pods $plant^{-1}$). *Azotobacter* bacteria convert nutrients into absorbable forms and, thus, work to raise the level of absorption and transfer of nutrients to the pods and seeds formation called as sink (Tripathi *et al.,* 2014).

The findings also indicated significant differences among the orange peel treatments for the number of pods (Table 6). The maximum average of pods per plant (20.91 pods $plant^{-1}$) resulted in the orange peel extract concentration of 200 g $L^{-1}$, compared with the control treatment, which had the lowest average for the said trait (15.41 pods

plant$^{-1}$). This may be due to the positive role of orange peel extracts, providing a nutrient-rich environment of potassium, calcium, magnesium, and other compounds directly increasing biological processes efficiency, causing an increased number of pods per plant (Wafik *et al.,* 2015).

The results showed significant interaction effects of the combined application of both study factors in fenugreek (Table 6). The highest number of pods per plant (25.65 pods plant$^{-1}$) emerged by adding the *Azotobacter* bacteria (100 g L$^{-1}$) with orange peel extract (200 g L$^{-1}$). Meanwhile, the lowest interaction effects (12.65 pods plant$^{-1}$) resulted in the control treatment for both factors.

**Seeds per pod**

For seeds per pod, the *Azotobacter* bacteria levels enunciated notable differences in fenugreek (Table 7). The *Azotobacter* bacteria (100 g L$^{-1}$) treatment excelled with the highest rate (18.90 seeds pod$^{-1}$), while the lowest rate was evident in the control treatment (10.68 seeds pod$^{-1}$). This may be because *Azotobacter* bacteria are vital in the readiness and stabilization of nutrients, especially nitrogen,

**Table 4.** Effect of *Azotobacter*, orange peels, and their interactions on the trigonelline content in the fenugreek seeds.

| *Azotobacter* (g L$^{-1}$) | Orange peels (g L$^{-1}$) | | | | Means (mg g$^{-1}$) |
|---|---|---|---|---|---|
| | Control | 100 | 150 | 200 | |
| Control | 26.75 | 27.43 | 28.45 | 29.43 | 28.02 |
| 20 | 28.41 | 30.52 | 32.95 | 34.73 | 31.65 |
| 50 | 29.54 | 33.57 | 36.63 | 37.83 | 34.39 |
| 100 | 31.43 | 35.62 | 39.52 | 41.63 | 37.05 |
| Means (mg g$^{-1}$) | 29.03 | 31.79 | 35.66 | 35.91 | |
| LSD$_{0.05}$ *Azotobacter*: 1.49, Orange peels: 1.49, Interaction: 3.62 | | | | | |

**Table 5.** Effect of *Azotobacter*, orange peels, and their interactions on the plant height in fenugreek plants.

| *Azotobacter* (g L$^{-1}$) | Orange peels (g L$^{-1}$) | | | | Means (cm) |
|---|---|---|---|---|---|
| | Control | 100 | 150 | 200 | |
| Control | 28.87 | 29.96 | 30.56 | 32.76 | 30.54 |
| 20 | 30.43 | 31.76 | 32.87 | 33.72 | 32.20 |
| 50 | 32.74 | 33.56 | 35.83 | 37.64 | 34.94 |
| 100 | 34.76 | 36.65 | 40.65 | 42.87 | 38.73 |
| Means (cm) | 31.7 | 32.98 | 34.98 | 36.78 | |
| LSD$_{0.05}$ *Azotobacter*: 2.87, Orange peels: 2.87, Interaction: 3.65 | | | | | |

**Table 6.** Effect of *Azotobacter*, orange peels and their interactions on the pods per plant in fenugreek plants.

| *Azotobacter* (g L$^{-1}$) | Orange peels (g L$^{-1}$) | | | | Means (pods plant$^{-1}$) |
|---|---|---|---|---|---|
| | Control | 100 | 150 | 200 | |
| Control | 12.65 | 13.87 | 14.65 | 16.76 | 14.48 |
| 20 | 14.87 | 15.87 | 16.47 | 18.65 | 16.47 |
| 50 | 16.45 | 18.75 | 19.67 | 22.56 | 19.36 |
| 100 | 17.65 | 20.46 | 22.45 | 25.65 | 21.55 |
| Means (pods plant$^{-1}$) | 15.41 | 17.24 | 18.31 | 20.91 | |
| LSD$_{0.05}$ *Azotobacter*: 1.65, Orange peels: 1.65, Interaction: 2.56 | | | | | |

**Table 7.** Effect of *Azotobacter*, orange peels, and their interactions on the seeds per pod in fenugreek plants.

| *Azotobacter* (g $L^{-1}$) | Orange peels (g $L^{-1}$) | | | | Means (seeds $pod^{-1}$) |
|---|---|---|---|---|---|
| | Control | 100 | 150 | 200 | |
| Control | 8.65 | 9.56 | 11.76 | 12.75 | 10.68 |
| 20 | 9.54 | 10.75 | 13.76 | 15.64 | 12.42 |
| 50 | 11.65 | 13.65 | 16.76 | 18.54 | 15.15 |
| 100 | 13.65 | 16.74 | 20.54 | 24.65 | 18.90 |
| Means (seeds $pod^{-1}$) | 10.87 | 12.68 | 15.71 | 17.90 | |
| $LSD_{0.05}$ *Azotobacter*: 1.84, Orange peels: 1.84, Interaction: 2.39 | | | | | |

which contributes to enhancing the growth and development processes in crop plants, and, thus, reflecting increased yield components (Rezaei-Chiyaneh *et al.,* 2021).

Orange peel treatments also exhibited meaningful differences in the number of seeds per pod in fenugreek (Table 7). The orange peel concentration of 200 g $L^{-1}$ exceeded by giving the maximum number of seeds per pod (17.90 seeds $pod^{-1}$), compared with the control treatment giving the lowest rate (10.87 seeds $pod^{-1}$). This could refer to orange peel extract containing a group of organic compounds and nutrients working to enhance the root growth and stimulate the photosynthesis process in crop plants. It may also contain nutrients, such as, potassium and magnesium, contributing to increasing plant growth and development and, eventually, yield (Boyd, 2011).

The outcomes also revealed remarkable interaction effects for seeds per pod between the combined application of *Azotobacter* bacteria and orange peels in fenugreek (Table 7). The most number of seeds per pod (24.65 seeds $pods^{-1}$) resulted with adding of *Azotobacter* bacteria (100 g $L^{-1}$) and orange peel extract (200 g $L^{-1}$). Meanwhile, the least interaction effects (8.65 seed $pods^{-1}$) were evident in the comparison treatments for both factors.

## CONCLUSIONS

The results concluded the combined application of *Azotobacter* bacteria and orange peels' extract positively affected in improving the growth, seed yield, and seeds' biochemical components in fenugreek.

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