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Original article

Effect of Fenugreek (*Trigonella foenum-graecum* L.) Extract on Early Growth Parameters of *Phaseolus vulgaris* L.

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Abstract
The effect of fenugreek seed extract on early seedling growth of *Phaseolus vulgaris* L. was studied in the present work. Six concentrations of extract (0% control, 5%, 10%, 20%, 30%, and 40%) were used, and growth parameters were observed daily for 14 days. The results were indicative of a clear concentration-dependent effect. The highest plumule elongation value was observed at 40%, 5.00 cm (a 67% increase compared to the control 2.97cm). Root length was also maximum at 10% concentration (8.00cm), and the maximum number of roots was also observed at 5% concentration (23 roots). Two-way Repeated Measures ANOVA showed significant effects of extract concentration, time, and their interaction ($p < 0.05$), indicating that seedling responses changed over the duration of the experiment. Tukey's HSD post-hoc analysis revealed significant differences between treatments and varying maxima across growth parameters. Fenugreek seed extract contains bioactive chemicals that influence shoot and root growth, suggesting its potential as a natural biostimulant for leguminous crops.
Keywords. Fenugreek, Bean, Biostimulant, Plant Growth, Root Development.

Introduction

The adverse effects of synthetic agrochemicals on the environment, such as soil degradation, water pollution, and Biodiversity disruption, make it challenging to sustain modern agriculture [1]. Therefore, the search for nontoxic, environmentally benign plant growth stimulators is on the rise. Plant-based biostimulants are gaining popularity due to their capacity to enhance plant growth, nutrient availability, and stress tolerance through natural bioactive compounds, rather than direct nutrient delivery [2,3]. Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume with nutritional and medicinal value. The seeds include bioactive substances such as steroidal saponins, phenolic compounds, flavonoids, alkaloids, and free amino acids, which have been shown to have growth-regulating or hormone-like properties [4,5].

According to Hussain et al. (2021) [6], aqueous fenugreek extracts positively influence the growth of several plants, including wheat and tomato. However, the effects of fenugreek vary with concentration; low concentrations may stimulate growth, whereas high concentrations may inhibit it, or vice versa, depending on the plant species [7]. The common bean (*Phaseolus vulgaris* L.) is an important legume that is rich in protein and helps improve soil nitrogen content through a symbiotic process [8]. Seedling establishment impacts plant performance, vigor, and production by determining resource uptake and exposure to stress [9].

Fenugreek seed extract has tremendous potential as a natural biostimulant to enhance early-stage development of bean seedlings, owing to its bioactive composition. However, a comprehensive understanding of the time-dependent and dose-dependent impacts of fenugreek extract on *P. vulgaris* remains scarce. Therefore, the purpose of this study was to investigate the effects of fenugreek seed extract, used in different concentrations, on important early development parameters in *Phaseolus vulgaris* L, particularly plumule elongation, root length, and root number, during a critical 14-day establishing phase. To investigate temporal changes in growth responses, a two-way repeated-measures ANOVA was used. We predicted that fenugreek seed extract would improve seedling growth in a concentration-dependent manner, with different optimum doses for shoot and root development.

Methods

Plant Material and Seed Preparation

Seeds of common bean (*Phaseolus vulgaris* L) were obtained from a recognized source. The seeds were sterilized by immersion in a 2% (v/v) sodium hypochlorite solution for 5 minutes, followed by three thorough rinses with sterile distilled water to remove any remaining sterilant [10].

Extraction of fenugreek seed extracts.

Fenugreek seeds were purchased from a local herbal market, cleaned, and air-dried at room temperature. Fifty grams of dried seeds were ground and extracted with 1000 mL of distilled water using a Soxhlet apparatus. The extraction was carried out at 60°C for 2.5 hours. After cooling to room temperature, 20 mL of the crude extract was protected from light by wrapping in aluminum foil and filtered through Whatman No. 1 filter paper to remove solid debris. The filtrate was stored at 4°C until further use. Conventional procedures for allelochemical recovery are consistent with this extraction method [11].

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Experimental Design and Treatment Application

The investigation used a completely randomized design (CRD) with six treatments: 0% (distilled water as the control), 5%, 10%, 20%, 30%, and 40% fenugreek seed extract. Each treatment had three repetitions, with seven seeds per replication. Germination was carried out in 9cm sterile petri dishes lined with two layers of Whatman No. 1 filter paper. The filter paper on each plate was wetted evenly with 5 mL of the corresponding treatment solution. Control plates received an equal amount of distilled water. The seeds were equally distributed over the wet paper, and the plates were sealed with parafilm to keep the humidity within. Plates were cultured in a growth chamber at 25 ± 1°C with a photoperiod of 16/8 hours [10].

Data Collection

Observations were recorded daily for 14 days in accordance with standardized seed germination assessment guidelines [10]. The growth parameters, including plumule length (cm), root length (cm), and number of lateral roots, were measured.

Statistical Analysis

Statistical analyses were performed using SPSS version 26 (IBM Corp.). A two-way repeated measures ANOVA was conducted with extract concentration as the between-subjects factor and time as the within-subjects factor. Sphericity was assessed using Mauchly's test, and post-hoc comparisons were performed using Tukey's HSD test. Statistical significance was set at ($p < 0.05$). All data met the assumptions of normality and homogeneity of variance before analysis. The experimental design and analytic approach followed standard procedures in biological research [12].

Results

The findings of our research demonstrated the effect of various concentrations of fenugreek (*Trigonella foenum-graecum* L.) seed extract on the early seedling growth of common beans (*Phaseolus vulgaris* L.) (Table 1 and Figures 1-3). The growth parameters included plumule length, root length, and root number.

Effect on Plumule Growth

Fenugreek seed extract had a considerable effect on plumule elongation during the experiment (Figure 1, Table 1). All extract treatments enhanced plumule development more than the control (3.00cm) at 14 d, and concentrations (5–30%) showed a similar pattern of growth, with final lengths of 4.50 and 4.70cm. However, the 40% treatment (5.00cm) significantly increased compared to the control and the other concentrations.

Effect on Root Elongation

The concentration of extract had a significant effect on root length (Figure 2). The 10% concentration produced the longest roots (8.00cm), whereas the 5% treatment was the second best. Higher concentrations (20-40%) exhibited only a slight effect on root elongation.

Influence on root proliferation

The 5% concentration induced the highest number of roots (23), followed by the 10% concentration (Figure 3). In contrast, higher extract concentrations suppressed root growth; root production at the 40% treatment was comparable to that of the control.

Table 1. Effect of fenugreek extract concentrations on plumule length, root length, and root number in Phaseolus vulgaris L. plant

Plant species	Fenugreek extract concentrations	Plumule length (cm)	Root length (cm)	Number of roots
Phaseolus vulgaris L.	Control	2.97^{c}± 0.15	6.000^{d}± 0.20	16.00^{c}± 2.00
	5%	4.50^{b}± 0.20	7.00^{b}± 0.20	23.00^{a}± 2.00
	10%	4.50^{b}± 0.20	7.97^{a}± 0.06	20.00^{ab}±1.00
	20%	4.70^{ab} ± 0.20	6.30^{cd}± 0.20	19.00^{bc}±1.00
	30%	4.50^{b}± 0.10	6.50^{c}± 0.10	20.00^{ab}±1.00
	40%	5.00^{a}± 0.20	6.50^{c}± 0.20	16.33^{bc}± 0.58

Values are estimated marginal Means ± SD obtained from two-way repeated measures ANOVA. Different letters indicate significant differences among concentrations according to Tukey's HSD test ($p < 0.05$)

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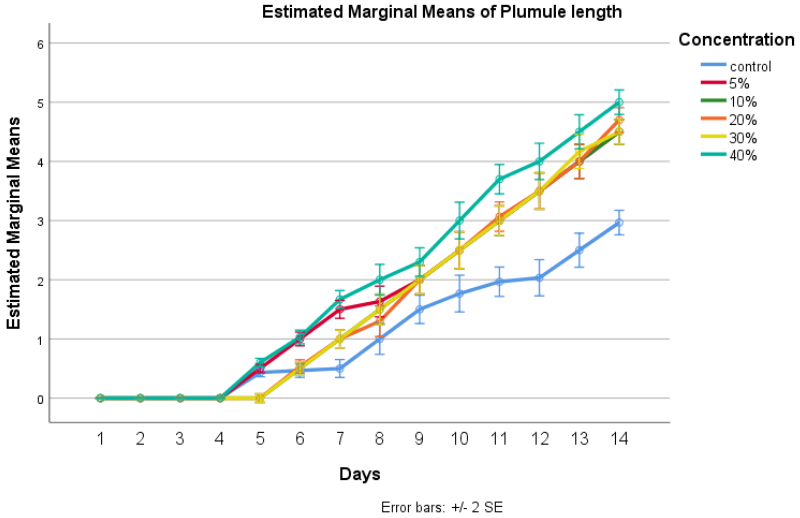

Figure 1. Effect of fenugreek extract concentrations on plumule length (cm) of Phaseolus vulgaris L. seedlings

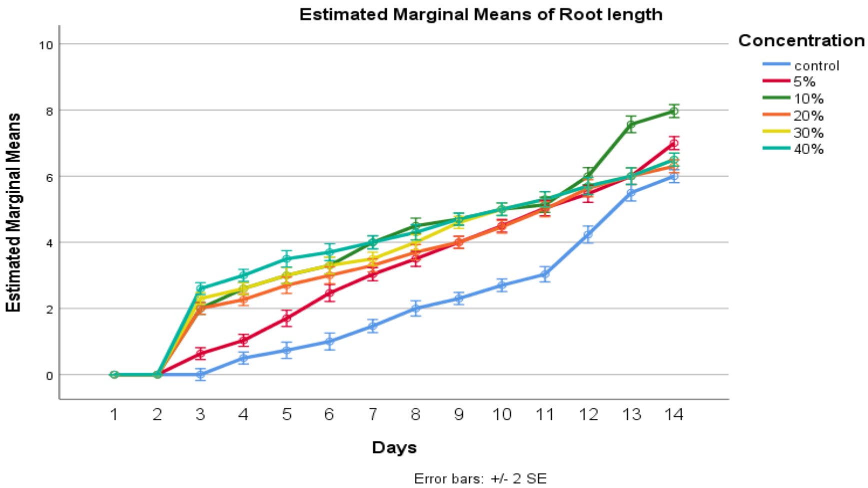

Figure 2. Effect of fenugreek extract concentrations on primary root length (cm) of Phaseolus vulgaris L. seedlings

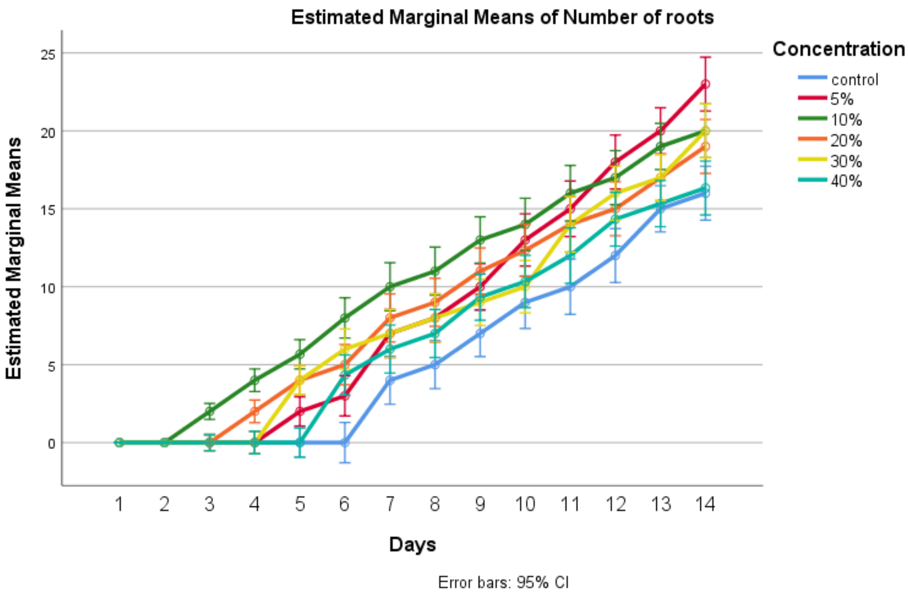

Figure 3. Effect of fenugreek extract concentrations on the number of roots of Phaseolus vulgaris L. seedlings.

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Discussion

This study's results clearly demonstrate a significant, concentration-related influence of aqueous fenugreek seed extract on the early growth of *P. vulgaris* seedlings, with these effects becoming apparent over time. Fenugreek seeds contain bioactive compounds such as phenols and saponins, which influence hormone-like activity in plants [13,5].

Extract concentrations of 5% and 10% significantly promoted root growth, particularly root length and lateral root development. These traits are essential for drought resistance, nutrient uptake, and seedling establishment. The observed stimulatory effect is consistent with the current understanding of bioactive compounds—such as specific phenols and saponins present in fenugreek seeds—which can influence or modulate hormone-like activity in plant tissues [5,13]. These findings agree with those reported by Colla et al. (2017) [14]. These compounds may interact with auxin biosynthesis and polar transport, thereby stimulating pericycle cell division and root primordia initiation—a mechanism also observed with other biostimulants [19,20]. Enhanced root proliferation improves the capacity of seedlings for early resource acquisition—a critical determinant of establishment success. In contrast, the 40% extract concentration suppressed both plumule and root growth relative to lower concentrations, suggesting a threshold-dependent inhibitory response [20]. Hormesis exhibits a biphasic pattern: low doses of a stressor or bioactive chemical stimulate, whereas higher amounts inhibit [7]. Fenugreek secondary metabolites may affect hormonal balance at high doses. *The high solute concentration of the extract may induce mild osmotic stress, reducing water availability and restricting root tissue development [15,16]. Shoot tissues, however, appear more tolerant of such conditions—owing to their distinct structural and regulatory characteristics—and may respond by promoting further elongation [17,18].*

The repeated-measures analysis of variance revealed a significant concentration × time interaction, indicating that the effects are not stable. Seedling responses were not instantaneous but evolved throughout the 14-day observation period, emphasizing that growth is a dynamic trajectory rather than a fixed endpoint. The temporal dimension suggests that fenugreek extract may influence not only the growth rate but also the timing of key developmental transitions

Conclusion

Fenugreek seed extract had a considerable effect as a biostimulant for *Phaseolus vulgaris* L. seedlings, though its impact is complex and varies with specific parameters. Recognizing unique ideal concentrations for root and shoot growth, particularly at 5-10% for roots and 40% for shoots, holds significant importance for agricultural practices. A lower application dose is advisable for nursery production or transplant systems where robust root establishment is paramount. Future studies should focus on identifying the precise active substances responsible for these findings and evaluating their efficacy in actual, real-world settings to transform this promising laboratory breakthrough into a sustainable agricultural method. A limitation of this study is the relatively small sample size (three replicates per treatment with seven seeds). Although significant effects were detected, future studies with larger sample sizes are recommended to confirm these findings.

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Conflicts of Interest

The authors declare no conflicts of interest.

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