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ORIGINAL PAPER

Influence of varying phosphorus fertilization levels on essential oil yield and composition in blue fenugreek (*Trigonella caerulea* (L.) Ser.)*

Hazım Serkan Tenikecier, Ertan Ateş

[1] Department of Field Crops
University of Tekirdağ Namık Kemal, Türkiye

Abstract

Blue fenugreek (*Trigonella caerulea* (L.) Ser.), also known as blue melilot, is an herbaceous annual aromatic plant, which is also used as a forage crop for its green fodder. Notwithstanding the numerous studies conducted on various aromatic and medicinal plant species, there is a notable paucity of research on the impact of fertilization, particularly phosphorus (P), on the dry herb yield, essential oil content, essential oil yield, and composition of blue fenugreek. This two-year field study, conducted in Tekirdağ, eastern Thrace (southeastern Europe), employed a randomized complete block design with three replications to evaluate the effects of different P levels (0 control, 30, 45, 60, 75, and 90 kg P ha^{-1}) on the essential oil yield and composition of blue fenugreek. The highest essential oil yield (1.58 kg ha^{-1}) was recorded at the 90 kg P ha^{-1} application dose. A total of twenty-five volatile oil compounds were identified, comprising 97.21-98.95% of the essential oil and showed four dominating constituents in blue fenugreek, namely phytol (19.97-22.04%), *n*-pentacosane (15.99-18.88%), tiglic aldehyde (13.49-16.07%) and tetradecanoic acid (11.98-14.20%). The highest concentrations of *n*-hexanal (1.20-1.22%), methyl benzoate (0.97-0.98%), L-menthone (0.83%), and heptadecane (0.73-0.80%) were obtained from the control and the 30 kg P ha^{-1} dose. The highest concentration of 1-octadecanol (5.04%), farnesylacetone (3.13%), (*E*)-*β*-damascenone (1.48%), dimethyl tetradecanedioate (2.43%), and (E)- *β* -ionone (1.90%) were found in the control. On the other hand, increasing P levels resulted in an increase in the content of 2-methyl-2-butene (0.89-0.92%), 1-octen-3-ol (0.48-0.50%), (E)-geranylacetone (0.87-0.91%), phytol (22.03-22.04%), tetradecanoic acid (14.19-14.20%) and n-pentacosane (18.81-18.88%). Additionally, principal component analysis (PCA) revealed significant differentiation among P levels. The results demonstrate a clear dose-dependent response, with 90 kg P ha^{-1} maximizing essential oil yield and significantly reshaping the volatile metabolome.

Keywords: blue fenugreek, dry herb yield, essential oil yield, essential oil ~~contents~~ and composition, principal component analyses

Ertan Ateş, Assoc. Prof. Dr., Department of Field Crops, Faculty of Agriculture, Namık Kemal University Tekirdağ, Türkiye, e-mail: ertan_ates@hotmail.com, phone +90 2822502147

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INTRODUCTION

Blue fenugreek (*Trigonella caerulea* (L.) Ser.) belongs to the order Fabales, family Fabaceae, subfamily Faboideae, tribe Trifolieae, subtribe Trigonellinae, genus *Trigonella* L., and subgenus *Trifoliastrum*. The species was first described by Carl Linnaeus (1753) as *Trifolium caeruleum* L. Blue fenugreek, also known as blue melilot *Melilotus caeruleus* (L.) Desr. (Ates 2011, Ateş, Seren 2020, Tenikecier, Ates 2020, Fedosiy et al. 2022), differs morphologically from many species of the genus *Trigonella* by several distinctive morphological characteristics (Ates 2015). It has a taproot system extending approximately 50-100 cm in depth, while the lateral roots remain relatively short. Numerous nodules are formed on the lateral roots. The stem is herbaceous and typically produces 1-5 branches, with a diameter of 3-6 mm. The erect stem is glabrous and can reach a length of 10-125 cm (Ateş, Tenikecier 2022). Blue fenugreek is primarily collected or harvested from natural populations and used as a medicinal and aromatic plant either as fresh herbage or as shade-dried, ground herbage (Arslan et al. 2016). In addition, it is used as a forage crop, particularly in the natural grasslands of the Balkan countries and southeastern Europe, where it occurs abundantly. It is either grazed directly by livestock or harvested for silage production (Tenikecier et al. 2022).

Essential oils are among the most economically important plant secondary metabolites and are widely recognized for their therapeutic and bioactive contributions to many plant species. Their antimicrobial, anti-inflammatory, and antioxidant properties as well as their effects on animal nutrition, rumen fermentation, digestion, and the gut microbiota of swine and poultry are well-documented and continue to be actively researched. Also known as volatile oils, essential oils are mixtures of volatile compounds produced as secondary metabolites (Kumar et al. 2022). In blue fenugreek, oil-secreting glands are mainly found in leaves, pods, and seeds, but also occur in stems and shoots. The oils extracted from these parts are known for their aromatic and volatile nature (Açıkgöz, Kara 2020, Katar et al. 2021, Ateş, Tenikecier 2022), allowing for easy extraction by various methods. Essential oils, usually liquid but sometimes containing solid particles, are complex mixtures of various compounds. Around 3500-4000 essential oils have been identified to date. Factors such as genetics, soil and climate conditions, agricultural practices (Katar et al. 2018), growth stages, drying methods, and distillation techniques all significantly influence essential oil content. In cultivating aromatic plants like blue fenugreek, these factors must be considered to maximize oil yield and composition. Even with ecologically adapted varieties, the yield of herbs, bioactive compounds, and essential oils can be improved by optimizing agronomic practices such as nutrient availability, irrigation, and tillage. Plant nutrition, in particular, plays a key role in biomass production and the phytochemical quality of medicinal and aromatic plants. Adequate macro- and microelement supply enhances plant growth and the biosynthesis of secondary metabolites, especially under nutrient-limited conditions (Nagaveni et al. 2018, Alhasan, Al-Ameri 2021).

Phosphorus (P), a macro-element, is an essential nutrient that plants require for growth, flower initiation, maturing of fruit and seed, energy transfer (via ATP), and the biosynthesis of key metabolites, including volatile organic compounds (VOCs). It serves as a structural component of nucleic acids and phospholipids and is integral to the shikimate and methylerythritol phosphate (MEP) pathways, which are pivotal for the synthesis of aromatic amino acids and terpenoids, respectively (Erbaş et al. 2017, Sonmez 2018, Peçanha et al. 2021). It is frequently deficient in agricultural soils across many regions of the world, often serving as a limiting nutrient for plant growth and development. In medicinal and aromatic plants, P plays a vital role in metabolic processes, particularly in the synthesis and assimilation of essential oils (Noh et al. 2017), so P fertilization is important. Although higher application doses of macronutrients such as P and nitrogen (N) can increase essential oil yield, they may also alter the essential oil profile of these plants (Alhasan, Al-Ameri 2021). Along with macronutrients, micronutrients play a very important role in the physiological processes of plants, even though they are needed in lesser quantities. In particular, iron (Fe), zinc (Zn), and manganese (Mn) act as metal components of various enzymes and play a regulatory and structural role in plants. Fe acts as a cofactor for approximately 140 enzymes that catalyze biochemical reactions in plants (Gholinezhad 2017). It plays an important role in the formation of secondary metabolites in plants. Meanwhile, Zn activates many enzymes. Therefore, Zn is a fundamental component of many enzymes and proteins. It affects a variety of processes, including growth hormone production and intermodal length elongation. For example, Gholinezhad (2017) investigated the effects of nano-iron on dill (*Anethum graveolens* L.) and suggested that it may promote growth and increase both the yield and concentration of essential oils. This enhancement is likely attributed to iron's role in chloroplast structure formation, which in turn may stimulate the development of essential oil glands in leaves. Lajayer et al. (2017) studied the effects of Zn on the growth, nutrient availability, and essential oil content and composition of *Mentha pulegium* L. They observed that Zn positively affected these characteristics. Zn induced the formation of pulegone and L-menthone, substances that are essential components of the plant's oil. When applied at an optimal level, Zn enhances photosynthesis, carbon fixation, and glucose production, consequently increasing plant yield and oil content (Kumar et al. 2022).

The influence of P fertilization on essential oil yield and the composition of volatile constituents remains an active area of investigation. Studies have demonstrated that P application can significantly alter the essential oil content, yield, and volatile profiles of various aromatic plant species, including sweet fennel (*Foeniculum vulgare* var. dulce), anise (*Pimpinella anisum* L.), lavandin (*Lavandula* × *intermedia* Emeric ex Loisel. var. Super), coriander

(*Coriandrum sativum* L.), and lavender (*Lavandula angustifolia* Mill.) – Moradi et al. (2011), Meena et al. (2015), Erbaş et al. (2017), Özyazıcı (2020), Peçanha et al. (2021). Although substantial research has been conducted on essential oil-bearing plants, the impact of fertilization, particularly P, on the essential oil yield (EOY) and composition of blue fenugreek remains largely unexplored, with no dedicated studies on this topic. This study aimed to evaluate the response of blue fenugreek to different P fertilization levels under representative field conditions characterized by calcareous soils and conventional fertilizer management, focusing on dry herb yield (DHY), essential oil content (EOC), essential oil yield and, critically, the comprehensive volatile oil profile under the subtropical conditions of the eastern Thrace region. To holistically decipher the complex P-induced shifts in the phytochemical composition, Principal Component Analysis (PCA) was employed to identify the distinct metabolic profiles associated with each treatment. The findings are expected to provide valuable insights for optimizing the cultivation of this aromatic plant under calcareous soil conditions and to provide crucial information for researchers in the fields of human and animal health, food quality, and livestock production.

MATERIALS AND METHODS

Plant material and growth conditions

The study was conducted in Yörük village, located in the eastern Thrace region of Southeastern Europe (40°55′ N, 27°03′ E; 171 m a.s.l.), Türkiye. The experiment was arranged in a randomized complete block design with three replications over two growing seasons (2021-2022 and 2022-2023). Seeds were sown on 12 November 2021 and 26 November 2022. Soil properties and climatic conditions of the experimental site are presented in Table 1 and Figure 1. Climatic data were obtained from the Turkish State Meteorological Service. The experimental soil was characterized by high calcium carbonate content (approximately 8.0% $CaCO_3$) and neutral to slightly alkaline pH (7.31-7.40).

Ateş Mavisi, the first blue fenugreek cultivar registered in the UPOV Plant Variety Database, was hand-sown in 12.5 m^2 plots (10 rows × 5 m) with 0.25 m row spacing at a seeding rate of 30 kg ha^{-1} (Tenikecier, Ates 2020). Soil analysis indicated an average available P content of 9.58 mg kg^{-1} (approximately 19 kg P ha^{-1} on an area basis). According to the classification of Kacar and Katkat (1997), soil P levels below 30 kg ha^{-1} are considered deficient in terms of plant availability. Six P doses (0, 30, 45, 60, 75, and 90 kg ha^{-1}) were applied as triple superphosphate (TSP, 43% P_2O_5) – Tenikecier (2026). P was band-applied at sowing in a placement approximately 5 cm below and 5 cm beside the seed row.

Table 1

Soil properties of the experimental area

Depth (cm)	30	
Years	2021-2022	2022-2023
pH	7.400	7.310
Lime (%, $CaCO_3$)	8.050	7.910
Organic matter (%)	3.570	3.550
Total N (%, method: Kjeldahl)	0.180	0.170
P (mg kg^{-1}, method: Olsen) ¥	9.610	9.550
K (mg kg^{-1}, method: CH_3COONH_4) ¥	445.1	450.2
Ca (mg kg^{-1}, method: CH_3COONH_4) ¥	2616.4	2627.2
Mg (mg kg^{-1}, method: CH_3COONH_4) ¥	118.2	120.0
Fe (mg kg^{-1}, method: DTPA) ¥	14.45	14.57
Cu (mg kg^{-1}, method: DTPA) ¥	1.470	1.420
Zn (mg kg^{-1}, method: DTPA) ¥	3.470	3.500
Mn (mg kg^{-1}, method: DTPA) ¥	20.56	21.00

pH – potential of hydrogen, N – nitrogen, P – phosphorus, K – potassium, Ca – calcium, Mg – magnesium, Fe – iron, Cu – copper, Zn – zinc, Mn – manganese, CH_3COONH_4 – ammonium acetate, DTPA – diethylenetriaminepentaacetic acid, ¥ – available form

Band placement was used instead of broadcast application. Inoculation was considered to promote nodulation in blue fenugreek, as it was a new crop in the study area and no native species of its genus were present. Due to the lack of a suitable commercial inoculant, a basal nitrogen dose of 25 kg N ha^{-1} (calcium ammonium nitrate, CAN; 26% N) was applied together with P at sowing. Both fertilizers were applied simultaneously and incorporated at sowing. Nitrogen was also applied at the same dose during the stem elongation stage. In the experiment, although the simultaneous application of CAN with TSP may theoretically enhance Ca-phosphate precipitation due to increased Ca^{2+} activity in the soil solution, this fertilization strategy reflects the standard farmer practice in the Thrace region. The uniform application of CAN across all treatments ensures that any potential Ca-induced effects on P availability remain consistent among treatments, thereby preserving the validity of dose-response comparisons under field conditions. Weed control was performed manually, and no irrigation was applied during either growing season. Manual weeding was conducted at the 5-6 leaf stage and at stem elongation.

Harvesting of plant material and sample preparation procedures

At the full-bloom stage of blue fenugreek (first year 25.05.2022, second year 23.05.2023) – Tenikecier and Ates (2021), fresh herbs were harvested by mowing 3 cm above the ground to avoid contamination (Ates 2015, 2016).

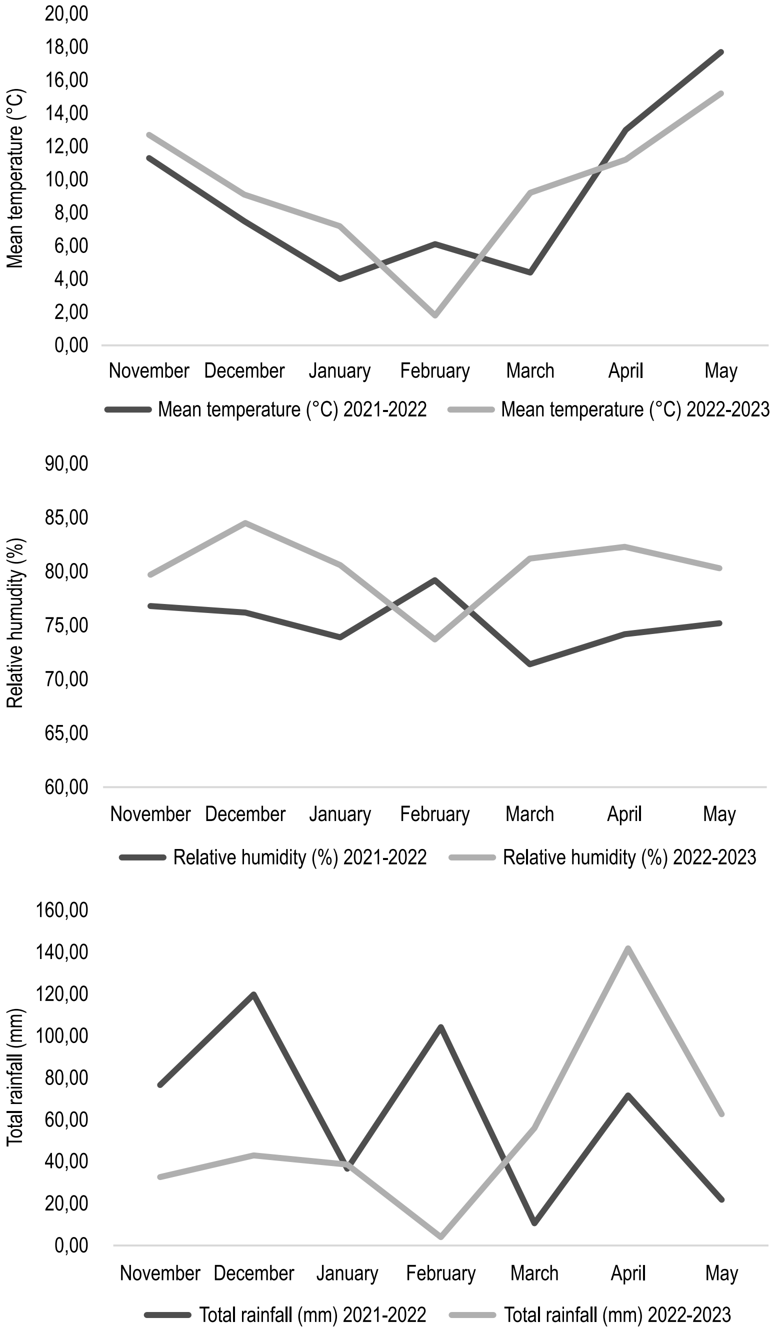

Fig. 1. Climatic conditions during the growing season

This mowing was done by hand on a 2.5 m^2 section of each plot. Each year, one cutting was taken at the same growth stage. Fresh plant samples found to be affected by biotic or abiotic stress factors were excluded from the study and were not utilized in subsequent analyses (Ateş 2025). The remaining samples were then weighed and shade-dried at 25°C for 7 days to minimize the loss of volatile compounds, following the method described by Ates and Tenikecier (2022, 2024). The dried samples were then reweighed to determine dry herb yield (DHY, kg ha^{-1}). Thereafter, the samples were ground to a particle size of ≤1 mm, according to Ates and Tenikecier (2025), and used for subsequent analyses.

Essential oil extraction, analysis and determining its components

For the essential oil analysis, a quantity of 150 g of a ground dry herb sample was utilized for each P dose within each replicate. The samples were extracted by hydro distillation in a Neo-Clevenger apparatus for 6 h according to the standard procedure described in EP (2023) to determine the EOC (%). The EOY (kg ha^{-1}) was estimated according to the following equation:

$$EOY= DHY \times EOC.$$

The essential oils were subsequently dried over anhydrous sodium sulfate (Na_2SO_4) and stored at -20°C under cryogenic conditions (Wang et al. 2018) until analysis by gas chromatography-mass spectrometry (GC-MS). To facilitate the analysis of their constituents, the oils were diluted 1:100 with *n*-hexane ($CH_3(CH_2)_4CH_3$, ReagentPlus®) – Karayel (2022). A 1 µL portion of the sample was injected into an Agilent 6890N-5973 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a HP-5ms (5%-phenyl)-methylpolysiloxane capillary column (30.0 m × 0.25 mm × 0.25 µm) and coupled with a mass selective detector (MSD) operating in electron impact (EI) mode, where the essential oil components were identified. Helium (He) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The injector temp. was set to 270°C, and the oven temperature program was as follows: initial temp. 60°C for 5 min, ramped to 120°C at 4°C min^{-1}, followed by a 5-minute hold, then increased to 170°C at 3°C min^{-1} and held for 2 min, and finally increased to 280°C at 10°C min^{-1}. The detector temp. was set to 280°C. The analysis was performed in triplicate. Mass spectrometric analysis was conducted under the following conditions: scan range of 15-500 amu in total ion current (TIC) mode; injection port temp. maintained at 250°C; initial column oven temp. set to 60°C; ion source temp. at 230°C; nitrogen (N_3) as the carrier gas with a flow rate of 1.6 mL min^{-1}; electron ionization (EI) at 70 eV; quadrupole temp. at 150°C; and a mass scan range of m/z 40-400. The solution of standard alkanes mixtures (C6-C32) was also analyzed using the above conditions. Anhydrous sodium sulfate (Na_2SO_4), used for drying the essential oils, was procured from SAFC®, and all other analytical-grade reagents were obtained from Sigma-Aldrich®.

Compound identification was carried out by comparing the retention indices (RIs), calculated according to the Kovats method using a homologous series of *n*-alkanes (C6-C32), with those available in established reference libraries, including the NIST 23 database (Gaithersburg, MD, USA), the Wiley 9th edition mass spectral library, and published literature (Adams 2012). The odor description followed the methodology of Yang et al. (2008), and the percentage of the oil constituents was calculated from the GC peak areas (Moradi et al. 2011, Aghamirzaei et al. 2024).

Statistical analyses

The two-year mean data for DHY, EOC, EOY, and volatile oil constituents were statistically analyzed using TARİST software (version 4.0). Since no significant differences were found between the years (P>0.05), the data were pooled, and treatment means were compared using the least significant difference (LSD) test at a significance level of P<0.01 (Steel et al. 1996). Analysis of variance (ANOVA) was employed to determine the significance of treatment effects on individual variables, while PCA was performed in the *R* statistical environment (version 4.3.1) using the FactoMineR package, version 2.8 (Lê et al. 2008) to examine multivariate patterns and visualize the distinct phytochemical profiles associated with different P fertilization levels.

RESULTS AND DISCUSSION

The effect of P application on DHY

The P application exerted a significant influence on the DHY of blue fenugreek (P<0.01) – Figure 2. Contrary to conventional expectations, the maximum DHY (1213.3 kg ha^{-1}) was obtained from the control treatment, whereas the minimum yield (843.3 kg ha^{-1}) was recorded at a P application dose of 45 kg ha^{-1}. P is universally acknowledged as a vital macronutrient crucial for energy transfer, photosynthesis, and overall plant development, with its deficiency typically leading to diminished biomass production (Erbaş et al. 2017, Peng, Ng 2022). However, its fertilization in our soil with confirmed P deficiency (Olsen P ≈9.5 mg kg^{-1}) did not produce the expected increase in biomass. This unexpected response suggests that the initial hypothesis, focused predominantly on P deficiency, overlooked the complexity of pre-existing nutrient interactions in our specific soil conditions. The pronounced yield reduction at the moderate P dose can be attributed to P-induced nutrient disorders. The underlying mechanism likely involves P-Zn antagonism, which impaired the plant's ability to utilize the marginal soil Zn (DTPA-Zn ≈3.5 mg kg^{-1}). This was compounded by competitive inhibition of Mg uptake, given the soil's significant cation imbalance (Ca/Mg ratio ≈22:1) and low level of exchangeable Mg (≈120 mg kg^{-1}).

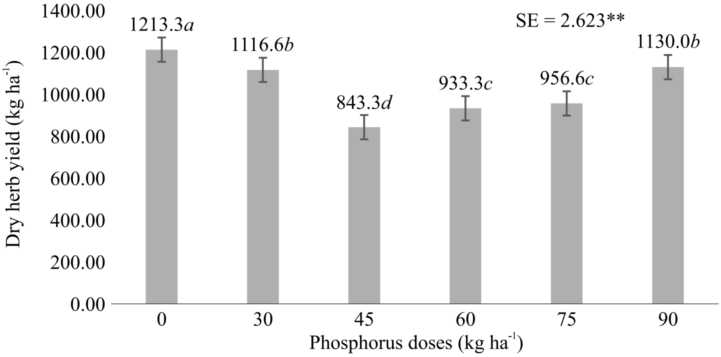

Fig. 2. Effect of increasing P doses on dry herb yield of blue fenugreek
Different letters over the bars indicate statistically significant differences between the experimental groups (** P<0.01, LSD).
Error bars on each column indicate standard deviation from three replicates; SE – standard error

The concurrent limitation of Mg and Zn likely disrupted primary metabolic processes, thereby restraining vegetative growth. The partial yield recovery at higher P application doses (60-90 kg ha^{-1}) can be interpreted through the mass-action principle, where meeting the plant's fundamental P requirement partially offset the other nutrient limitations. However, the failure of even the highest P doses to surpass the control yield demonstrates that the ultimate constraint on biomass was not the initial P deficiency, but rather a P-induced disruption of cationic and micronutrient homeostasis. Consequently, for maximizing DHY in soils with our specific profile – characterized by P deficiency, marginal Zn, and high Ca/Mg ratio – an integrated nutrient management strategy combining low-to-moderate P application with Mg supplementation and preventive Zn chelation is recommended. The present results find support in some, but not all, previous studies. Fedosiy et al. (2022) reported a DHY range of 0.6 to 1.2 t ha^{-1} for blue fenugreek, with which our findings are consistent. Similarly, Ateş and Seren (2020) documented DHY between 2534.8 and 3142.4 kg ha^{-1} at different growth stages. However, our results contrast with other reports. Said-Al Ahl et al. (2024) observed a significant increase in the DHY of *Satureja montana* L. with N and P fertilization. Furthermore, Jiang et al. (2017) emphasized that low available P adversely affects biomass and plant growth, while Benetoli da Silva et al. (2020) and Peng and Ng (2022) identified optimal P ranges of 80-120 kg ha^{-1} and up to 200 kg ha^{-1} for maximizing biomass in *Salvia hispanica* L. and *Vitex negundo* Linn., respectively. These discrepancies highlight the context-specific nature of P fertilization responses, influenced by species-specific requirements and distinct soil nutrient profiles.

Effect of P application on EOC and EOY

The P application exerted a statistically significant influence on both EOC and EOY of blue fenugreek (P<0.01, Figure 3).

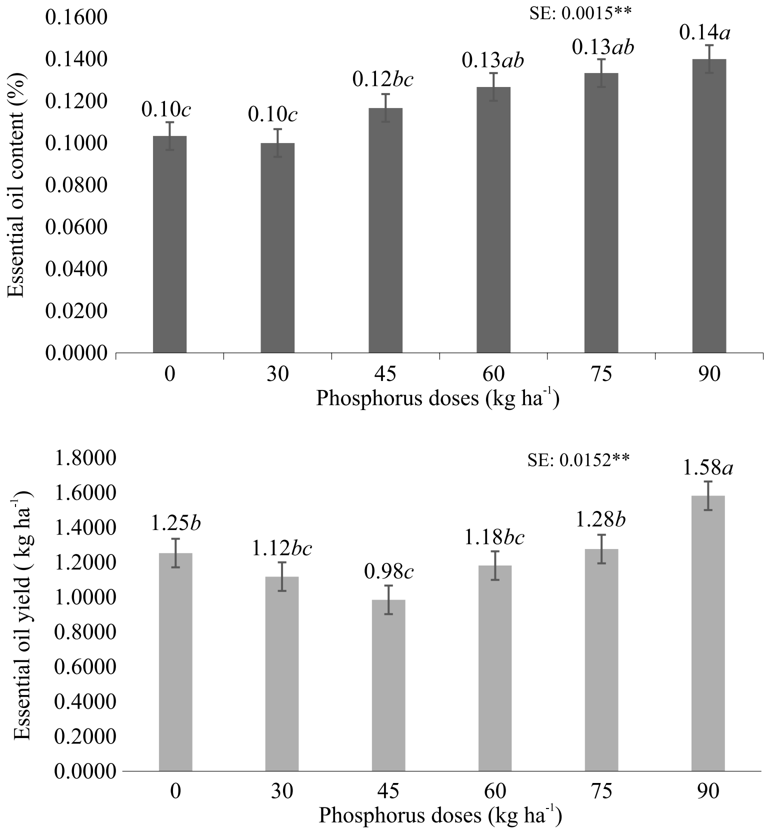

Fig. 3. Effect of increasing P doses on essential oil content and essential oil yield of blue fenugreek.
Different letters over the bars indicate statistically significant differences between the experimental groups (** $P<0.01$, LSD).
Error bars on each column indicate standard deviation from three replicates; SE – standard error

A distinct increasing trend in EOC was observed with rising P application doses, particularly when compared to the control and 30 kg P ha^{-1} treatment groups. The maximum EOC (0.14%) was achieved at the 90 kg P ha^{-1} application rate, followed closely by 75 and 60 kg P ha^{-1} (both 0.13%), and then 45 kg P ha^{-1} (0.12%). EOY values ranged from 0.98 to 1.58 kg ha^{-1} across the treatments, with the peak EOY (1.58 kg ha^{-1}) also recorded at the 90 kg P ha^{-1} rate. This pattern demonstrates a clear, dose-dependent response of essential oil production to P fertilization. In contrast to its negative impact on biomass yield, P fertilization consistently enhanced secondary metabolism, significantly boosting both EOC and EOY. The attainment of the highest EOY at the 90 kg P ha^{-1} level strongly suggests that adequate P availability directly stimulates the biosynthesis of volatile compounds. It is well-established that fertilization management is a critical factor affecting essential oil production in aromatic plants. The influence of nutrient inputs on plant metabolic pathways – and consequently on the biosynthesis of secondary metabolites like essential oils – is modulated by various environmental and edaphic factors, including soil mineral availability, soil type, climatic conditions, and species-specific physiological responses. Therefore, the observed variations in EOC and EOY under different P treatments reflect the complex interaction between nutrient management and the specific growth environment (Rioba et al. 2015).

The literature presents divergent findings regarding the impact of P application on EOY and EOC (Sonmez 2018, Özyazıcı 2020, Said-Al Ahl et al. 2024). Our results align with previous studies reporting that increased P fertilization can enhance essential oil production in species such as *Origanum majorana* L., *Lavandula x intermedia* Emeric ex Loisel. var. Super, and *Vitex negundo* Linn. (Trivino, Johnson 2000, Erbaş et al. 2017, Peng and Ng 2022).

However, the EOY levels recorded in our study were lower than the 194.1 to 268.1 kg ha^{-1} reported by Sonmez (2018) for *Pimpinella anisum* L., supporting our findings, Alharbi et al. (2019) applied four P doses (0, 12, 24, and 36 kg ha^{-1}) to *Petroselinum crispum* L. and found that higher P levels significantly improved EOY and increased the concentrations of key volatile components, including β-myrcene, 1,3,8-*p*-menthatriene, myristicin, and β-phellandrene. These consistent results reinforce the conclusion that P application can markedly alter both the quantity and composition of essential oils. The EOC values obtained in our study under increasing P doses were higher than the range of 0.09% to 0.11% reported for blue fenugreek dry herb by Tenikecier et al. (2022).

Influence of varying P doses on the composition of volatile oil constituents

Analysis of blue fenugreek essential oil revealed twenty-five volatile compounds, representing 97.21-98.95% of the total essential oil content (Table 2). Four major constituents were identified at concentrations exceeding 11% (Table 3): phytol (19.97-22.04%), n-pentacosane (15.99-18.88%), tiglic aldehyde (13.49-16.07%), and tetradecanoic acid (11.98-14.20%). Additionally, six compounds – ethyl linoleate, dimethyl tetradecanedioate, farnesylacetone, 13-hexyloxacyclotridec-10-en-2-one, heptacosane, and 1-octadecanol – were detected at concentrations above 1.41%. P application significantly altered the relative proportions of essential oil constituents at the 1% significance level (Table 3). Increasing P doses resulted in elevated concentrations of 2-methyl-2-butene, 1-octen-3-ol, (E)-geranylacetone, phytol, tetradecanoic acid, and n-pentacosane.

Conversely, higher P levels reduced concentrations of *n*-hexanal, methyl benzoate, L-menthone, heptadecane, 1-octadecanol, farnesylacetone, (E)-β-damascenone, dimethyl tetradecanedioate, (E)-β-ionone, and tiglic aldehyde.

Table 2

The essential oil components of blue fenugreek and odor description of compounds

No	RI[a]	Compound name	Odor description[d]	Identification[e]
1	514	2-methyl-2-buteneb	petroleum-like	MS, RI
2	800	*n*-hexana[l]c	green, grassy	MS, RI
3	985	tiglic aldehyde[c]	green, fruity	MS, RI
4	986	1-octen-3-ol[b]	mushroom	MS, RI
5	1049	phenylacetaldehyde[c]	sweet, rose	MS, RI
6	1160	L-menthon[e]c	minty	MS, RI
7	1191	methyl benzoat[e]c	fruity, sweet	MS, RI
8	1317	(E,E)-2,4-decadienal[b]	fatty	MS, RI
9	1409	(*E*)-β-damasceno[n]eb	floral, fruity	MS, RI
10	1416	dodecanal[b]	soapy	MS, RI
11	1460	(E)-geranylacetone[b]	fruity-floral, rosy, green magnolia	MS, RI
12	1469	(E)-β-ionone[b]	woody	MS, RI
13	1512	3,4-dimethyl-5-pentylidene-2(5H)-furanone[b]	spicy-herbal to mint-like	MS, RI
14	1701	heptadecane[b]	fuel-like	MS, RI
15	1714	pentadecanal-[b]	fresh waxy	MS, RI
16	1774	tetradecanoic acid[b]	waxy-oily	MS, RI
17	1920	farnesylacetone[b]	fruity	MS, RI
18	1927	methyl palmitate[b]	waxy	MS, RI
19	2055	dimethyl tetradecanedioate[b]	NDA	MS, RI
20	2070	13-hexyloxacyclotridec-10-en-2-one[b]	NDA	MS, RI
21	2081	1-octadecanol[b]	faint	MS, RI
22	2122	phyto[l]b	faint floral	MS, RI
23	2162	ethyl linoleate[b]	floral	MS, RI
24	2500	*n*-pentacosan[e]b	waxy	MS, RI
25	2700	heptacosane[b]	NDA	MS, RI

[a] Retention index based on HP-5ms column using a series of n-hydrocarbons. [b] Previously identified from blue fenugreek dry herb using GC-MS (Tenikecier at al. 2022). [c] Previously identified from blue fenugreek dry herb using GC-MS (Ayvazyan et al. 2023). [d] The description of odor was determined by the method reported by Yang et al. (2008). [e] MS, by comparison of the mass spectrum with the NIST/Wiley mass spectral library; RI, by comparison of RI with those from the literature. NDA – no data available

The control and 30 kg P ha^{-1} treatments yielded the highest concentrations of *n*-hexanal (1.20-1.22%), methyl benzoate (0.97-0.98%), L-menthone (0.83%), and heptadecane (0.73-0.80%). Maximum concentrations of 1-octadecanol (5.04%), farnesylacetone (3.13%), (E)-β-damascenone (1.48%), dimethyl tetradecanedioate (2.43%), and (E)-β-ionone (1.90%) were observed in control treatments.

Table 3

Volatile compound profile changes with increasing P doses

Volatile compounds (%)	P doses (kg ha $^{-1}$)							
	0	30	45	60	75	90	means	standard error
2-Methyl-2-butene	0.67*c*	0.77*b*	0.89*a*	0.90*a*	0.92*a*	0.89*a*	0.69	2.04E-02**
Dimethyl tetradecanedioate	2.43*a*	2.14*c*	2.17*bc*	2.20*bc*	2.21*b*	2.20*bc*	2.22	1.63E-02**
1-Octen-3-ol	0.33*b*	0.37*b*	0.48*a*	0.50*a*	0.49*a*	0.50*a*	0.44	2.00E-02**
Heptadecane	0.80*a*	0.73*a*	0.58*b*	0.60*b*	0.60*b*	0.60*b*	0.65	1.60E-02**
Dodecanal	0.57*a*	0.43*b*	0.53*a*	0.58*a*	0.59*a*	0.61*a*	0.55	1.56E-02**
(E,E)-2,4-Decadienal	0.58*b*	0.58*b*	0.66*a*	0.60*b*	0.60*b*	0.59*b*	0.60	1.09E-02**
(E)-β-Damascenone	1.48*a*	1.27*c*	1.33*b*	1.30*bc*	1.29*bc*	1.29*bc*	1.33	1.03E-02**
(E)-Geranylacetone	0.73*b*	0.87*a*	0.88*a*	0.89*a*	0.91*a*	0.90*a*	0.86	1.36E-02**
(E)-β-Ionone	1.90*a*	1.68*b*	1.72*b*	1.70*b*	1.70*b*	1.70*b*	1.73	0.98E-02**
Pentadecanal	1.18*a*	1.13*ab*	1.04*c*	1.10*bc*	1.11*ab*	1.09*bc*	1.11	1.49E-02**
Phenylacetaldehyde	1.07*ab*	1.09*a*	1.04*bc*	1.04*bc*	1.05*abc*	1.03*c*	1.05	1.23E-02**
Tiglic aldehyde	15.30*b*	16.07*a*	15.17*c*	13.50*d*	13.49*d*	13.51*d*	14.51	2.48E-02**
3,4-Dimethyl-5-pentylidene-2(5H)-furanone	1.39*b*	1.47*a*	1.40*b*	1.40*b*	1.41*b*	1.40*b*	1.41	0.89E-02**
Methyl palmitate	1.00*b*	1.12*a*	0.99*b*	1.01*b*	1.00*b*	1.05*ab*	1.03	1.92E-02**
Farnesylacetone	3.13*a*	3.06*b*	3.02*b*	2.02*c*	2.00*c*	2.03*c*	2.54	1.69E-02**
n-Pentacosane	16.37*c*	15.99*d*	16.09*b*	18.81*a*	18.88*a*	18.81*a*	17.49	4.21E-02**
13-Hexyloxacyclotridec-10-en-2-one	3.00*b*	2.94*c*	3.01*ab*	3.00*b*	3.06*a*	3.03*ab*	3.01	1.26E-02**
Ethyl linoleate	2.08*ab*	1.99*c*	2.03*bc*	2.10*a*	2.10*a*	2.08*ab*	2.06	1.19E-02**
1-Octadecanol	5.04*a*	4.27*b*	4.45*b*	3.91*c*	3.89*c*	3.90*c*	4.24	5.48E-02**
Phytol	19.97*c*	20.13*b*	20.13*b*	22.04*a*	22.04*a*	22.03*a*	21.06	1.14E-02**
Heptacosane	4.06*c*	4.15*b*	4.24*a*	3.20*d*	3.19*d*	3.18*d*	3.67	0.79E-02**
Tetradecanoic acid	11.98*c*	12.58*b*	12.52*b*	14.20*a*	14.19*a*	14.19*a*	13.28	2.73E-02**
n-Hexanal	1.22*a*	1.20*a*	1.09*b*	0.97*c*	0.96*c*	0.96*c*	1.07	0.49E-02**
Methyl benzoate	0.98*a*	0.97*a*	0.81*b*	0.65*c*	0.65*c*	0.64*c*	0.78	0.53E-02**
L-Menthone	0.83*a*	0.83*a*	0.74*b*	0.53*c*	0.53*c*	0.52*c*	0.66	1.28E-02**

Different letters in a row indicate statistically significant differences between the experimental groups (** P<0.01)

The 30 kg P ha^{-1} application produced the highest levels of tiglic aldehyde (16.07%), 3,4-dimethyl-5-pentylidene-2(5H)-furanone (1.47%), methyl palmitate (1.12%), and phenylacetaldehyde (1.09%). Minimum concentrations of 2-methyl-2-butene (0.67%), (E)-geranylacetone (0.73%), phytol (19.97%), and tetradecanoic acid (11.98%) occurred in control plants, while the lowest n-pentacosane (15.99%), dodecanal (0.43%), ethyl linoleate (1.99%), and 13-hexyloxacyclotridec-10-en-2-one (2.94%) were recorded at 30 kg P ha^{-1}. The 45 kg P ha^{-1} treatment yielded peak concentrations of (E,E)-2,4-decadienal (0.66%) and heptacosane (4.24%), but the lowest pentadecanal content (1.04%). These compositional shifts can be attributed to P-mediated regulation of terpenoid biosynthesis. Since P constitutes an essential component of acetyl-coenzyme A – the key substrate in terpenoid biosynthesis (Yang et al. 2012, Peng, Ng 2022) – increased P availability likely enhances terpenoid production, consequently elevating essential oil yield. The four dominant compounds identified in this study – phytol, *n*-pentacosane, tiglic aldehyde, and tetradecanoic acid – contribute significantly to blue fenugreek's characteristic aroma. While tiglic aldehyde was previously reported by Ayvazyan et al. (2023), these specific compounds were not documented by Ney (1986). Our identification of 25 compounds exceeds the 21 reported by Tenikecier et al. (2022), expanding the known volatile profile of this species. The most notable effect of P fertilization was the substantial modification of blue fenugreek's volatile metabolome. This aligns with Bistgani et al. (2018), who demonstrated fertilizer-induced alterations in essential oil aromatic profiles, and Alhasan and Al-Ameri (2021), who reported P-mediated enhancements in both oil yield and composition.

Particularly significant were P-induced increases in *n*-pentacosane, a component of Apis queen pheromones, and phytol, a common floral volatile. These compounds may function as pollinator attractants (Wright, Schiestl 2009), potentially explaining the observed attraction of honeybees to drying samples. Conversely, reduced tiglic aldehyde at higher P doses, a compound with demonstrated antifungal activity (Anonymous 2025), might influence plant-pathogen interactions and allelopathic functions. The elevated tetradecanoic acid concentrations under P fertilization carry implications for animal nutrition. As a saturated fatty acid crucial in energy metabolism and immune function, its enhancement in forage could improve livestock product quality. For tetradecanoic acid optimization, we can recommend 60 kg P ha^{-1} application in blue fenugreek cultivated for grazing systems.

PCA analysis of treatment effects

While ANOVA identified statistically significant effects of P doses on individual measured variables, it does not elucidate the interrelationships between these variables or the comprehensive multivariate patterns induced by the treatments. The PCA was therefore employed as a complementary multivariate technique to visualize the complex, multi-dimensional relationships among all measured parameters simultaneously. This approach enabled the identification of groups of volatile compounds and agronomic traits that responded similarly to specific P levels, thereby characterizing the distinct phytochemical profiles associated with each treatment. The PCA results effectively differentiated the P treatments, revealing clear separation in phytochemical composition (Figure 4). The first principal component (PCA A) exhibited strong positive loadings for parameters including EOC, EOY, tiglic aldehyde, 1-octen-3-ol, (E)-geranylacetone, dimethyl tetradecanedioate, and (E)-β-ionone, while showing negative correlations with DHY, 2-methyl-2-butene, phenylacetaldehyde, L-menthone, methyl benzoate, 3,4-dimethyl-5-pentylidene-2(5H)-furanone, heptadecane, and 1-octadecanol. Subsequent components further refined these relationships. The second component (PCA B) revealed positive associations for DHY, EOC, EOY, phenylacetaldehyde,

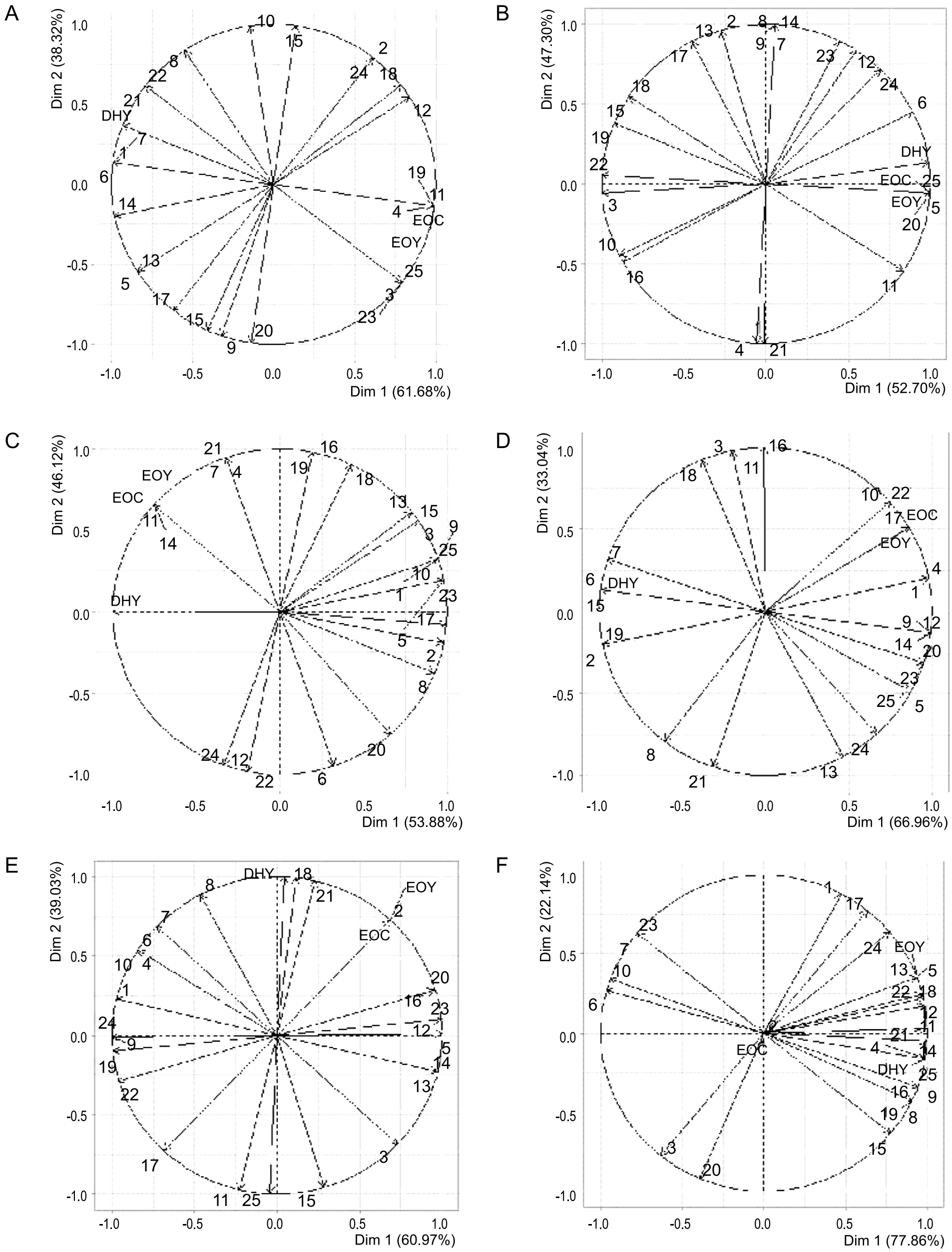

Fig. 4. Principal component analysis of the dry herb yield (DHY), essential oil yield (EOY), essential oil content (EOC) and twenty-five essential oil compounds (for essential oil compound numbers from 1 to 25, see Table 2) of blue fenugreek at six P doses: 0 kg P ha^{-1} (A), 30 kg P ha^{-1} (B), 45 kg P ha^{-1}(C), 60 kg P ha^{-1} (D), 75 kg P ha^{-1} (E), and 90 kg P ha^{-1} (F).
The lines starting from the center of the biplots indicate negative or positive associations between different variables, and their proximity indicates the degree of correlation with a particular treatment

L-menthone, and 13-hexyloxacyclotridec-10-en-2-one under the 30 kg P ha^{-1} treatment. In contrast, the third component (PCA C) demonstrated a negative loading for DHY at 45 kg P ha^{-1}. The fourth component (PCA D) was characterized by negative loadings for DHY, *n*-hexanal, L-menthone, methyl benzoate, pentadecanal, and dimethyl tetradecanedioate. Higher P applications induced distinct metabolic shifts, with the 75 kg P ha^{-1} treatment showing positive loadings for phenylacetaldehyde, (E)-β-ionone, 3,4-dimethyl--5-pentylidene-2(5H)-furanone, heptadecane, 13-hexyloxacyclo-tridec-10--en-2-one, and heptacosane. Conversely, the 90 kg P ha^{-1} application resulted in negative loadings for L-menthone, methyl benzoate, and dodecanal.

The PCA results statistically confirm significant differentiation among P treatments, demonstrating that each application dose induces a unique phytochemical signature. This multivariate pattern substantiates the conclusion that P availability serves as a major regulatory factor shaping the metabolic composition of blue fenugreek. These findings align with Wang et al. (2021), who reported significant correlations between P levels and both soluble protein content and secondary metabolite concentrations in *Allium tuberosum*. Furthermore, our results are consistent with previous studies employing PCA to identify key factors influencing plant secondary metabolites (Al-Nemi et al. 2022, Rafiq et al. 2023).

CONCLUSIONS

This study demonstrates that P fertilization in blue fenugreek cultivation results in a clear dose-dependent response under calcareous soil conditions of the Thrace region. Although a portion of the applied P was likely immobilized due to reactions with Ca^{2+} and Mg^{2+} ions, this process occurred uniformly across all treatments, allowing valid comparison of relative yield responses among P doses. Under these conditions, the highest P dose (90 kg ha^{-1}) resulted in maximum DHY and EOY, indicating that higher application doses may be required to overcome the strong P-buffering capacity of calcareous soils. Therefore, appropriate P management should consider soil chemical constraints to improve nutrient use efficiency. For regions with similar soil characteristics, integrated nutrient management practices and improved P placement strategies (e.g., band application or split applications) may enhance fertilizer efficiency. Further research is needed to evaluate long-term soil P dynamics and optimize fertilization strategies for sustainable blue fenugreek production. Regardless of the agronomic goal, caution is warranted when using dried blue fenugreek as a fodder substitute due to its significant potential to alter the aroma of meat, poultry, and dairy products.

Author contributions

H.S.T., E.A. – conceptualization, E.A. – writing, H.S.T., E.A. – review & editing, H.S.T., E.A. – methodology, H.S.T., E.A. – visualization, writing – original draft preparation. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare

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