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Phytochemical and Morphological Features of Moldavian Balm (*Dracocephalum moldavica* L.) and Fenugreek (*Trigonella foenum-graecum* L.) in Intercropping and Pure Stand Cultivation Systems and with Different Fertilizer Sources

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ABSTRACT

When combined with organic and biofertilizer applications, intercropping systems are important in the production of medicinal plants for eliminating or significantly reducing chemical inputs. Also, intercropping is useful for increasing soil fertility, improving plant growth, and enhancing the quality of produce. To determine the effects of different fertilizer sources on the yield and quality parameters of Moldavian balm (MB) while intercropping with fenugreek (FG), two factors were analyzed. The first factor included three cropping patterns: MB pure stand, FG pure stand, and intercropping of two rows of MB with two rows of FG (2FG:2MB). The second factor was the fertilizer source: control plants, 100% chemical fertilizer (NPK), 100% bacterial biofertilizers + 25% synthetic fertilizer (BF+25NPK), 75% humic acid (HA)+ 25% synthetic fertilizer (75HA+25NPK), and 75% vermicompost (V)+ 25% synthetic fertilizer (75V+25NPK). Results showed that the highest basic microbial respiration (0.04 mg CO_2-C g^{-1} day^{-1}), substrate induced respiration (0.27 mg CO_2-C g^{-1} day^{-1}), and the microbial biomass of carbon (1145 mg CO_2-C kg^{-1}) was observed in intercropping 2FG: 2MB, which was fertilized with 75V+25NPK. This treatment also increased the seed yield of FG and dry matter yield of MB by 54% and 80%, respectively, thereby resulting in maximum essential oil (EO) content of MB (0.77%) and oil content of FG (14.2%) . In addition, the intercropping of 2FG:2MB, which was fertilized with 75V+25NPK, improved the EO quality of MB plants by increasing geranyl acetate and geranial concentrations. Also, they improved the oil quality in FG by maximizing unsaturated fatty acids content such as oleic and linoleic acids. Overall, we concluded that intercropping 2MB:2FG fertilized with a fertilizer mix composed of vermicompost (75%) and synthetic NPK fertilizer (25%) can be suggested as an eco-friendly and sustainable system for improving EO, oil content and the quality of FG and MB plants, respectively.

Introduction

Moldavian balm (MB) (*Dracocephalum moldavica* L.) is an annual herbaceous plant in the Lamiaceae family. It is native to Central Asia and has been domesticated in Central and Eastern Europe (Omidbaigi et al., 2009). All the vegetative

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organs in MB contain EO, although concentrations vary across different parts of the plant, with the flowers and stalk having the largest amount of EO (Faridvand et al., 2021). The major EO compounds in the plant, including geranyl acetate, geranial, geraniol, and neral (Golparvar et al., 2016), have antimicrobial and antibacterial properties that are typically used to heal wounds and injuries (Sonboli et al., 2008) and can also be used as a tonic and as an anticonvulsant. Moreover, EO components in MB are utilized to facilitate digestion and reduce symptoms from constipation and heart palpitations (Rezaei-Chiyaneh et al., 2021d).

Fenugreek (FG) (*Trigonella foenum-graecum* L.) is an annual medicinal plant that belongs to the Fabaceae family (Pouryousef et al., 2015). Due to its ability to fix atmospheric nitrogen through symbiosis with soil bacteria, FG is a valuable crop for inclusion in sustainable and organic agricultural systems across the world, especially in smallholder agricultural systems without external N input in developing countries (Diatta et al., 2020). This plant is used to treat a wide range of diseases including diabetes and indigestion, high blood cholesterol, and high blood pressure, among others (El Nasri et al., 2007; Rezaei-Chiyaneh et al., 2021b).

The use of more sustainable agricultural practices has spurred much interest over the last 30 years. Among others, some of the main goals in sustainable agriculture include the reduction in the application from supra-optimum to agronomically optimum fertilizer rates and the promotion in the use of more efficient fertilizer application methods the increased use of bio and organic fertilizers (Adeyemi et al., 2020; Adnan et al., 2020; Ashitha et al., 2021; Battaglia et al., 2021; Fahad et al., 2021). Evidence shows that improper and unbalanced use of synthetic fertilizers in conventional agriculture may lead to soil degradation, reduction in water quality and microbial biomass and its activity in the soil, and reduced quality of crop products, including medicinal plants (Amani Machiani et al., 2018a; Czymmek et al., 2020; Babur et al., 2021; Seleiman et al., 2021; Siri Prieto et al., 2021). Bio and organic fertilizers, on the other hand, are more environmentally friendly and sustainable alternatives to synthetic fertilizers. Organic fertilizers such as humic acid (HA) and vermicompost (V) have the potential to increase crop production and maintain soil fertility in sustainable agriculture systems (Ghaderimokri et al., 2022) through nitrogen fixation, and the release of phosphorus, potassium, and iron ions from their insoluble compounds (Zhaoxiang et al., 2020). Moreover, the different pools of bacteria present in biofertilizers increase the absorption of elements, reduce the incidence of plant diseases, improve soil structure, plant growth, and the quality of the agricultural products, and increase the quantity of these products (Rezaei-Chiyaneh et al., 2020b). Vermicompost has higher levels of nutrients and micronutrients compared to the other organic fertilizers and can act as an organic soil conditioner that is effective in improving the quantitative and qualitative characteristics of medicinal and agricultural plants (Faridvand et al., 2021; Rezaei-Chiyaneh et al., 2021d). Vermicompost has a high level of porosity, good ventilation and drainage capabilities, good water adsorption and retention, and high absorption of water and nutrients (Jami et al. 2020). Humic acid has also been recognized as an environmentally-friendly fertilizer that can improve physical, chemical, and biological parameters of soil quality, and increase the production and quality of agricultural products (Liu et al., 2019).

So far, most studies on fertilizer sources have been based on monoculture conditions. A more sustainable alternative to this is represented by intercropping systems. These systems are characterized by a more efficient use of resources such as radiation, moisture, and nutrients (Raza et al., 2021; Rezaei-Chiyaneh et al., 2021b, d). Specifically, understanding the combined effect of bio-fertilizers on the quantitative and qualitative yield of Moldavian balm intercropped with Fenugreek requires more research. Thus, this study was aimed to evaluate the combined effect of different cropping patterns and fertilizer sources (bacterial fertilizer, vermicompost, humic acid and chemical fertilizer) on agronomic traits related to the quantitative and qualitative yield of MB and FG plants.

Material and Methods

Experimental site and treatment design

The study was conducted as a factorial experiment in a randomized complete block design and with three replications at the research greenhouse of the Higher Education Center of Shahid Bakeri in Miandoab, Iran. The experimental treatments were allocated in pots with dimensions of 80 × 25 × 25 cm, filled with the same amount of soil. Two factors were included in this study: cropping patterns and fertilizer sources. The factor of cropping patterns had three levels: MB as a pure stand; FB as a pure stand; and intercropping pattern of two rows of MB and two rows of FG. The fertilizer factor had five levels, including unfertilized control (C), bacterial bio-fertilizer (BF) {mixture of phosphate-solubilizing bacteria (PSB) *Pantoea agglomerans* + *Pseudomonas putida*, K-solubilizing bacteria (KSB) *Pseudomonas koreensis* + *Pseudomonas vancouverensis*, and N-fixing bacteria (NFB) *Azotobacter vinelandii*)} + 25% chemical fertilizer (BF+25NPK), 75% vermicompost + 25% chemical fertilizer (75V+25NPK), 75% humic acid + 25% chemical fertilizer (75HA+25NPK), and 100% synthetic nitrogen-phosphorus-potassium fertilizer (NPK). As a result, a total of 15 treatments were evaluated in this study. The fertilizer treatments were decided based on a routine soil test taken before treatment allocations (Table 1). Six cultivation rows were utilized in all cases, with a distance of 10 cm between rows. The seeds were planted at 5 cm within the same row, at a depth of 3 cm for FG, and 1 cm for MB. All treatments were irrigated immediately after planting. In subsequent irrigations, on average, irrigation was performed every 3-4 days to maintain adequate soil moisture.

Table 1. Physical and chemical properties of the soil routine analysis and the vermicompost utilized in the pot study.

	Texture	pH	EC (dS m^{-1})	Organic matter (%)	Total N (%)	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})
Soil	Silty clay	7.79	0.92	0.82	0.09	11.24	189
Vermicompost	-	8.45	3.91	7.75	3.02	28.90	310.00

Before sowing, a batch of seeds of both species was inoculated with the combination of NFB, PSB, and KSB in the form of powder at a rate of 100 g ha^{-1} for a bacterial population of 5×10^8 colony forming units g^{-1}. Briefly, the contents of the packets were first mixed with water and then sprayed on the seeds to form a completely uniform coating on the seed surface. Then, the seeds were dried for 24 h in the shade and sown in the pots. Based on the recommendations of the bio-fertilizer manufacturer (Zist Fanavar Sabz Company, Iran), the bio-fertilizer solution was used as the irrigation fertilizer at stem elongation, at the beginning of flowering. The vermicompost treatment was applied in the amount of 15% of the pot volume (150 g) and was then thoroughly mixed with the soil in the pots. The synthetic fertilizer treatment was determined according to the results of the soil test (Table 1). Here, 180 mg kg^{-1} of soil in the form of triple superphosphate fertilizer and potassium sulfate, and 150 mg kg^{-1} of nitrogen soil in the form of urea fertilizer were applied. All the triple superphosphate and potassium sulfate fertilizers and one-third of the urea fertilizer were applied before planting. After planting, the remaining two-thirds of the urea fertilizer were used in halves at the stem elongation and then at the flowering stage. For the HA treatment, a total application rate of 200 mg of the HA powder was utilized for each pot. One-third of this amount was dissolved in water and applied at planting, while the remaining two-thirds were added to the water and used in halves to irrigate pots at the stem elongation and later at the flowering stages. Weeds were regularly removed by hand across the whole growing period.

Measurements

At the maturity stage (when about 75% of the pods were yellowed), 10 FB plants were randomly harvested from each pot for determination of yield-component traits, including plant height, number of pods per plant, pod length, number of seeds per pod, 1000-seed weight, seed yield, and oil yield. At the time of the 50% flowering of MB, 10 plants were randomly selected from each pot for determination of plant height, flowering stem length, canopy diameter, number of lateral branches, dry matter yield, and EO content and EO yield.

MB Air-dried plant material (leaves + flowers) samples (50 g) from Moldavian balm were hydro-distilled in a Clevenger-type apparatus with 500 mL of deionized water for 3 h (Rezaei-Chiyaneh et al., 2020a). Extracted EOs were dried using anhydrous sodium sulfate and transferred to an amber glass bottle, capped, and stored at 4 °C until Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

The EO content and yield of MB samples were determined using the following formulas (Amani Machiani et al., 2018b):

$$\text{EO content (\%)} = \frac{\text{Extracted EO (\%)}}{\text{50 g of Moldavian Balm ground dry matter}} \times 100$$

$$\text{EO yield (g plant}^{-1}) = \text{EO content (\%)} \times \text{dry matter yield (g plant}^{-1})$$

Moldavian Balm essential oil analysis

Gas chromatography-mass spectrometry analysis was performed with an Agilent 7890/5975A GC/MSD. For the separation of essential oil components, an HP-5 MS capillary column (5% phenyl methyl polysiloxane, 30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used. The oven operated for 3 min at 80°C, subsequently at 8°C min^{-1} to 180°C, and held for 10 min at 180°C. Helium was used as carrier gas at a flow rate of 1 ml min^{-1}. The sample was injected (1 μL) in split mode (ratio, 1:50). Electron impact mode was 70 Ev. The components were recognized by comparing the calculated Kovats retention indices (RIs), calculated for a mixture of n-alkane series, and finally obtaining the mass spectra (Adams, 2007). A gas chromatography–flame ionization detection analysis was done with an Agilent 7890 A instrument. The separation was performed in an HP-5 capillary column. The analytical conditions were the same as above. Details regarding quantification methods can be found in Morshedloo et al. (2017) and Amani Machiani et al. (2019).

Fenugreek oil isolation and analysis

Seeds of FG were pulverized to pass a 1-mm screen (Weidhuner et al., 2019) to facilitate the extraction of their oil content. Briefly, 5 g of ground seed was extracted in 300 mL of n-hexane in a Soxhlet apparatus. After 6 h of extraction, the solvent was removed from the oil by rotary evaporation. The extracted oil was stored in amber glass bottles and refrigerated until analysis (Fotohi Chiyaneh et al., 2022).
The oil content was calculated as:

$$\text{Oil content (\%)} = \frac{\text{Extracted oil (g)}}{\text{5 g fenugreek seed}} \times 100$$

In addition, the oil yield of fenugreek was calculated as oil content (%) × seed yield (g $plant^{-1}$).

Extraction of oil

Fatty acids were converted to fatty acid methyl esters (FAMEs) to make them volatile. They were analyzed by GC-FID. For this purpose, 0.1 g of oil was mixed with 1.5 ml of hexane and 0.2 ml of 2N methanolic KOH. They were then vortexed for 5 sec and centrifuged at 2500 rpm for 1 min. The upper layer was taken and kept at 4 °C for analysis.
An Agilent 6890N, GC apparatus (Wilmington, DE, USA) equipped with an FID detector, was utilized for sample analysis. For FAME separations, an HP-88 capillary column (88% - Cyanopropy) aryl-polysiloxane, 100 m length, 0.25 mm i.d., 0.2 μm film thickness) (Agilent) was used. The oven temperature was programmed as follows: 5 min at 140°C, subsequently 4°C min^{-1} to 240°C, and then the temperature was held for 15 min at 240°C. The carrier gas was nitrogen, and flow rates were 1.0 mL.min^{-1} and 45 mL.min^{-1}, respectively. Temperatures of the injection port and detector were set at 260˚C and 280˚C, respectively. The injector was set in a split mode (split ratio of 1:30). The ChemStation software was used to acquire and process data. For the identification of fatty acids, a FAME mixture (Supelco 37 Component FAME Mix Bellefonte, PA, USA) was used (Rezaei-Chiyaneh et al. 2020b).

Soil biological activity and enzyme activities

To determine the effect of experimental treatments on soil properties, six random samples were collected from each plot (0-20 cm depth, rhizosphere) at field capacity moisture, near the end of the growing season. Soil samples were thoroughly mixed. A composite sample of 500 g was taken and stored at 4°C for further analysis. The soil microbial respiration and substrate-induced respiration were measured according to Anderson and Domsch (1993) and Alef and Nannipieri (1995), respectively, and microbial biomass carbon was measured using the fumigation-extraction method (Jenkinson and Powelson, 1976). Enzyme activities, including alkaline phosphatase, acid phosphatase, and urease were measured according to Kandeler and Gerber (1988) and Tabatabai and Bremner (1969).

Statistical analysis

Analysis of variance was performed using PROC Mixed procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The fertilizers application and cropping ratio were considered as having fixed effects, whereas blocks were considered random. Mean comparisons for each trait were performed using Duncan's multiple range test ($P < 0.05$).

Results

Phytochemical and Morphological features of Fenugreek (FG)

All traits of FG, except for plant height, were strongly affected by the interaction of cropping patterns × fertilizer sources. Also, the plant height of FG was affected by the main effects of both cropping patterns and fertilization source (Table 2).

Table 2. Analysis of variance for Fenugreek traits across cropping patterns, fertilization sources, and the interaction between both factors.

	Treatments	Plant height	Pods per plant	Pod length	Seeds per pod	1000-Seed weight	Seed yield	Fixed oil	Oil yield
Fenugreek	Replication	0.984^{ns}	0.188^{ns}	0.054^{ns}	0.182^{ns}	0.44**	0.002^{ns}	0.138^{ns}	0.00003^{ns}
	Cropping pattern (CP)	392.929**	22.499**	18.724**	12.936**	25.789**	1.614**	67.99**	0.005**
	Fertilization (F)	63.702**	13.832**	8.231**	8.086**	3.182**	0.136**	7.836**	0.003**
	CP × F	0.786^{ns}	1.318**	0.955**	0.720**	0.262*	0.02**	0.308*	0.0002*
	Error	0.613	0.19	0.153	0.146	0.076	0.003	0.1	0.00004
	C.V.(%)	2.40	2.45	4.14	4.46	2.93	6.71	2.88	7.26

NS, *, and ** indicated non-significant and significant differences at 5%, and significant differences at 1% probability level, respectively.

Plant height

The tallest FG plants occurred from NPK fertilization (35.4 cm) and BF+25NPK (34.8 cm), while the lowest plant height (27.2 cm) was observed with the unfertilized control. Compared to the control, the application of NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK increased plant height by 30%, 28%, 22%, and 18%, respectively (Fig. 1A). Also, when FG was intercropped with MB, the plant height of intercropped FG plants decreased by 20% in comparison with FG plants in pure stands (Table 4).

Number of pods per plant

The highest number of pods (20.1) per plant was observed in the pure stand fertilized with the 75V+25NPK, and the lowest (13.6) in the intercropping system of 2FG:2MB without fertilization. Compared to the average of the unfertilized control, in both cropping patterns of NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK, the number of pods per plant increased by 22, 16, 26, and 16%, respectively. Within each cropping pattern, 75V+25NPK resulted in the highest number of pods per plant. The number of pods per plant of FG in intercropping decreased by 9% in comparison with the FG pure stand (Fig. 1B).

Pod length

The longest pods of FG were observed in the pure stand, fertilized with NPK and 75V+25NPK. Compared with the unfertilized control, the application of NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK enhanced the number of pods per plant by 39, 22, 35, and 22%, respectively. The shortest pod length was recorded in the unfertilized control by intercropping. By the average value of all intercropping treatments, the pod length of FG decreased by 15% in comparison with the pod length across the pure stand treatments (Fig. 1C).

Seeds per pod

Similar to pod length, the highest number of seeds per pod in FG plants occurred in the pure stand fertilized with 75V+25NPK. (10.2), and NPK (9.9), whereas the lowest occurred in the unfertilized control by intercropping (5.7). The number of seeds per pod increased by 39, 22, 41, and 19 in FG plants fertilized with NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK. In addition, the average pod length of FG in the pure stand was about 16% higher than that of plants in the intercropping system (Fig. 1D).

Thousand Seed weight (TSW)

The average TSW of FG was 23% higher across pure stand treatments, compared with intercropping treatments. The highest TSW was achieved in the pure stand of FG plants, fertilized with NPK (11.8 g) and 75V+25NPK (11.0 g). The unfertilized control by intercropping resulted in the lowest TSW (7.3 g). When compared with the average unfertilized control for both pure and intercropping systems, the application of NPK, BF+25NPK, 75V75+25NPK, and 75HA+25NPK increased the TSW by 25, 10, 20, and 10%, respectively (Fig. 2A).

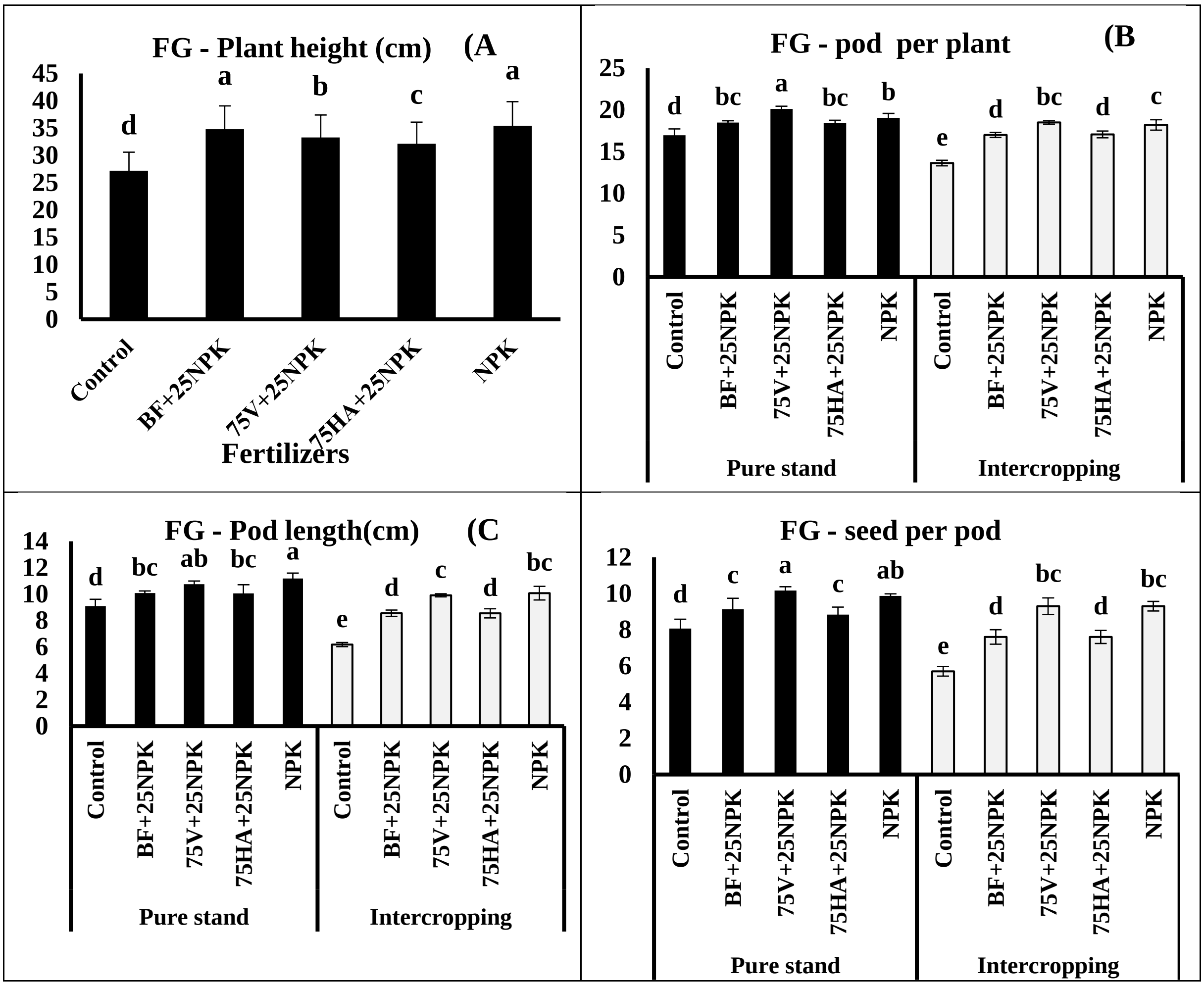

Fig. 1. Plant height (A), the number of pods per plant (B), pod length (C), and the number of Fenugreek seeds per pod (D) (FG) as affected by different cropping patterns and fertilizer sources in the Fenugreek pure stand and the intercropping system of Fenugreek and Moldavian balm (2FG:2MB) for the control, BF+25NPK (bacterial fertilizer + 25% NPK synthetic fertilizer), 75V+25NPK (75% vermicompost + 25% NPK), 75HA+25NPK (75% humic acid + 25% NPK), and NPK fertilizer treatments. The same letters above the bars indicate significant differences at the $p < 0.05$ level.

Seed yield

The highest seed yield (1.272 g plant^{-1}) was measured in the FG pure stand fertilized with 75V+25NPK, followed by the application of NPK, 75HA+25NPK, and BF+25NPK (1.056, 0.961, and 0.956 g plant^{-1}, respectively). The lowest FG seed yield occurred with the intercropping of 2FG:2MB without fertilization, BF+25NPK, and 75HA+25NPK (0.459, 0.554, and 0.554 g plant-1, respectively). On average, seed productivity of FG in intercropping was reduced by 43% when compared with the pure stand. Also, FG seed yield increased by 38, 21, 54, and 21% with NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK, respectively (Fig. 2B).

Oil content

The highest oil content of FG (14.2%) was achieved when FG was intercropped with MB and fertilized with 75V+25NPK, followed by intercropping with BF+25NPK (13.0%) and NPK (12.5%). Overall, the lowest FG oil content occurred with the control under pure stand conditions (7.8%). The oil content of FG was 32% higher when FG was intercropped with MB, compared to FG, grown as a pure stand. Finally, the application of NPK, BF+25NPK, 75V75+25NPK, and 75HA+25NPK enhanced oil content by 20, 24, 33, and 14%, respectively, when compared with the unfertilized control (Fig. 2C).

Oil yield

The maximum oil yield of FG (0.133 g plant^{-1}) was measured in the pure stand fertilized with 75V+25NPK, followed by NPK application (0.122 g plant^{-1}), although both values were not

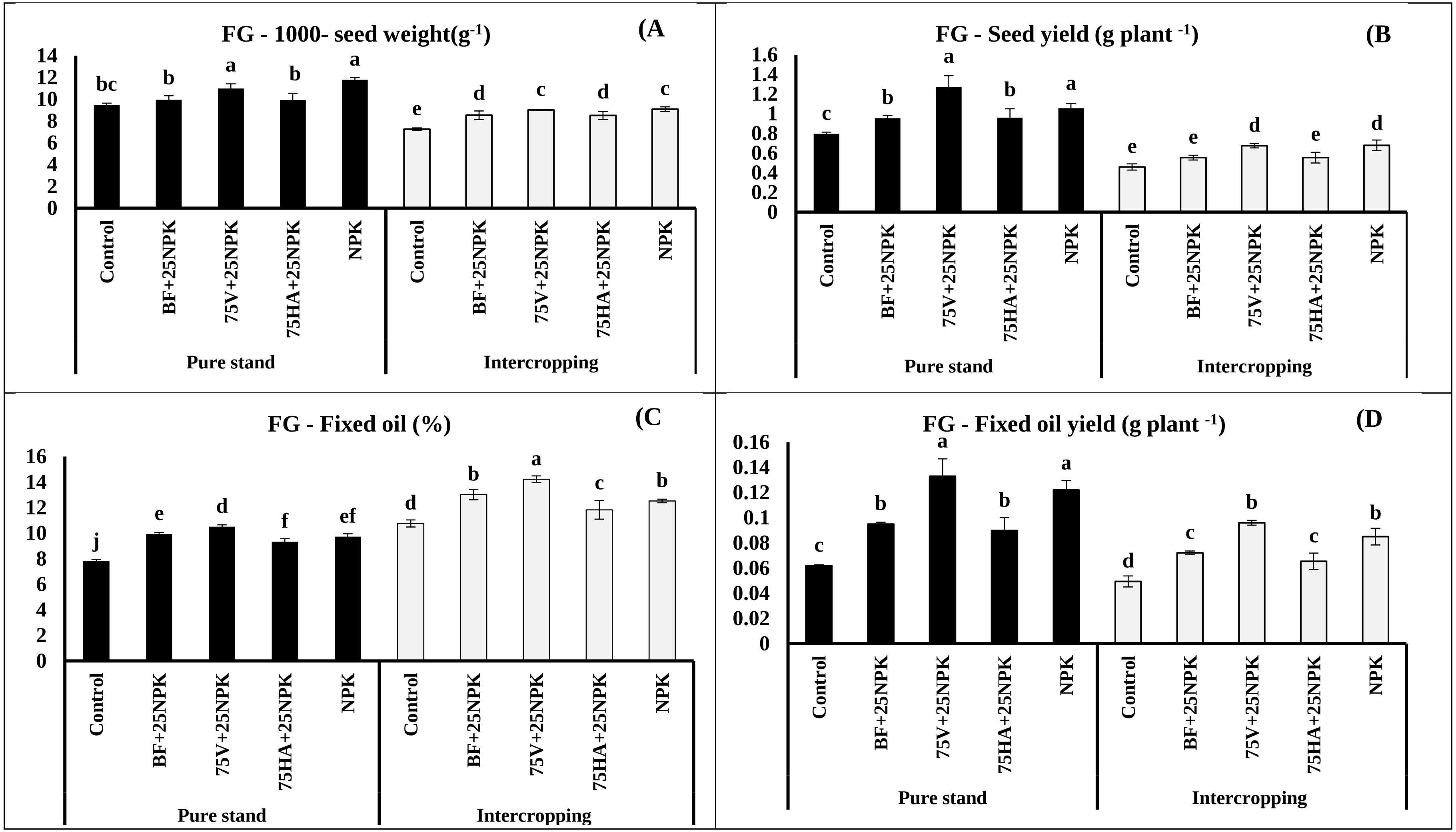

Fig. 2. 1000-seed weight (A), seed yield (B), fixed oil content (C), and fixed oil yield (D) of fenugreek (FG) as affected by different cropping patterns and fertilizer sources in the fenugreek pure stand and the intercropping system of fenugreek and Moldavian balm (2FG:2MB) for the control, BF+25NPK (bacterial fertilizer + 25% NPK synthetic fertilizer), 75V+25NPK (75% vermicompost + 25% NPK), 75HA+25NPK (75% humic acid + 25% NPK), and NPK fertilizer sources. The same letters above the bars indicated significant differences at the $p < 0.05$ level.

significantly different. Within each cropping pattern, the control treatment resulted in the lowest oil yield (0.062 and 0.049 g plant-1 under pure and intercropping conditions, respectively). On average, the oil yield of FG, intercropped with MB, was reduced by 43%, compared with that of the FG in the pure stand. On average, the oil yield of FG was 67, 33, 83, and 33% greater in FG plants fertilized with NPK, BF+25NPK, 75V75+25NPK, and 75HA+25NPK, respectively (Fig. 2D).

Phytochemical and Morphological features of Moldavian Balm (MB)

The analysis of variance showed that all measured parameters in MB were affected by the main effects of cropping patterns and fertilization sources. However, canopy diameter, number of lateral branches, dry matter yield, EO content, and EO yield were also significantly affected by the interaction of cropping patterns × fertilizer sources (Table 3).

Table 3. Analysis of variance for Moldavian Balm traits across cropping patterns, fertilization sources, and the interaction between both factors.

	Treatments	Plant height	Flowering stem length	Canopy diameter	Lateral branches	Dry matter	Essential oil content	Essential oil yield
Moldavian Balm	Replication	4.359^{ns}	0.013^{ns}	0.862^{*}	0.228^{ns}	0.001^{ns}	0.00046^{ns}	0.000005^{ns}
	CP	374.392^{**}	12.714^{**}	76.800^{**}	34.176^{**}	17.972^{**}	0.186^{**}	0.0002^{**}
	F	113.439^{**}	8.999^{**}	54.388^{**}	18.243^{**}	16.872^{**}	0.065^{**}	0.002^{**}
	CP × F	5.261^{ns}	0.067^{ns}	0.752^{*}	0.572^{*}	0.488^{**}	0.002^{**}	0.00004^{**}
	Error	5.926	0.181	0.241	0.196	0.056	0.0004	0.000004
	C.V. (%)	4.9	5.32	2.38	3.09	2.95	3.77	4.65

NS, *, and ** indicated non-significant, significant difference at 5%, and significant difference at 1% probability level, respectively.

Plant height

Among the fertilizer sources, the tallest MB plants were measured when using the 75V+25NPK (53.25 cm) and the NPK sources (52.01 cm), while the shortest plants (42.55 cm) corresponded to the unfertilized control. Compared to the control, plant height in MB increased by 22, 18, 25, and 16% with the application of NPK, BF+25NPK, 75V7+25NPK and 75HA+25NPK, respectively (Fig. 3A). Moreover, the plant height of MB in intercropping decreased by 20% in comparison with the pure stand (Table 4).

Flowering stem length

The application of NKP fertilizer resulted in the maximum (9.68 cm) flowering stem length. When no fertilizer was applied (i.e. control), the minimum flowering stem length was observed (6.51 cm). Compared to the control, the application of NPK, BF+25NPK, 75V7+25NPK, and 75HA+25NPK increased the flowering stem length by 49, 13, 34, and 19%, respectively (Fig. 3B). Finally, when intercropped with FG, the flowering stem length of MB decreased by 15%, compared to the plants of pure stands (Table 4

Table 4. Plant height of fenugreek (FG), plant height, and flowering stem length of Moldavian Balm (MB) in pure stands of each species when intercropped at the ratio of 2FG:2MB.

Cropping pattern	FG plant height (cm)	MB plant height (cm)	MB flowering stem length (cm)
Pure stand	36.2 ± 3.43 a	53.2 ± 4.65 a	8.7 ± 1.26 a
Intercropping	29.0 ± 2.74 b	46.1 ± 4.52 b	7.3 ± 1.1 b

Canopy diameter

The application of 75V+25NPK and NPK in pure stands caused a maximum increase in the canopy diameter of MB. Within each cropping pattern, the control resulted in the smallest canopy diameter. On average, and compared to the unfertilized control, the application of NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK enhanced the canopy diameter of MB by 39, 16, 41, and 18%, respectively (Fig. 3C).

Number of lateral branches

The application of BF+25NPK and NPK in pure stands maximized the number of lateral branches of MB (16.6 and 16.0, respectively). Within each cropping pattern, the control group resulted in the lowest number of lateral branches. On average, and compared to the unfertilized control, the application of NPK, BF+25NPK, 75V+25NPK and 75HA+25NPK resulted in 34, 37, 26, and 22% more lateral branches, respectively. In addition, when MB was intercropped with FG, the number of lateral branches of MB decreased by 13% in comparison with plants of the pure stand (among the fertilizer sources) (Fig. 3D).

Dry matter yield (DMY)

Maximum dry matter yield was obtained when 75V+25NPK and NPK were applied in pure stands (10.14 and 9.89 g plant^{-1}, respectively). For each cropping system, the control treatment resulted in the lowest DMY under both pure stands and intercropping (5.90 and 4.78 g plant^{-1}, respectively). The application of NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK increased the DMY in Moldavian Balm by 73, 48, 80, and 49%, respectively. On an average of the fertilizer sources, the DMY of MB in intercropping was 18% lower than that of MB in the pure stands (Fig. 4A).

Essential oil (EO) content

The maximum EO content of MB (0.77%) was achieved in intercropping with 2FG:2MB, when fertilized with 75V+25NPK. Similar to the case of DMY, in each cropping system, the unfertilized control produced the lowest EO content under both pure stands and intercropping (0.33 and 0.43%, respectively). Averaged across the cropping patterns, the application of NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK enhanced the EO content of MB by 66, 32, 71, and 37% when compared to the control, respectively. On the other hand, across the fertilizer sources, the EO content of MB in intercropping was 38% higher than that obtained under the pure MB stands (Fig. 4B).

Essential oil yield

On average, the EO yield of MB in intercropping was 24% higher than that observed in pure stand conditions. The highest EO yield (0.0663 g plant-1) was achieved when the fertilizer combination of 75V+25NPK was applied in intercropping conditions. Similar to the cases of DMY and EO content, within each cropping system, the control treatment produced the lowest essential EO yield in both pure stands (0.0194 g plant^{-1}) and intercropping (0.0205 g plant^{-1}). The application of NPK, BF+25NPK, 75V+25NPK, and 75HA+25NPK increased the EO yield of MB by 200, 100, 250, and 100%, compared with the unfertilized control, respectively (Fig. 4C).

Oil constituents

The GC analysis showed that the main fatty acids (FAs) of FG were linoleic acid (35.16-40.0%), oleic acid (19.5-25.8%), linolenic acid (16.0-19.0), and palmitic acid (10.1-12.3%). The maximum content of oleic and linoleic acids in FG occurred by intercropping and using 75V+25NPK, while the lowest content corresponded to the unfertilized control in the pure stand. Conversely, the maximum content of saturated FAs, including palmitic and stearic acid and unsaturated linolenic acid occurred with the unfertilized control in the pure stands (Table 5).

Essential oil compositions

Based on the chemical analysis performed by GC-MS and GC-FID, the main EO components of MB were geranyl acetate (28.1-35.9%), geranial (17.9-21.1%), geraniol (12.1-16.9%) and neral (12.8-15.5%). The maximum contents of geranyl acetate and geranial were measured in the intercropping system when using 75V+25NPK. The highest geraniol content in MB occurred when NPK was applied in intercropping. The unfertilized control in the pure stands produced the lowest contents of geranyl acetate, geranial, and geraniol (Table 6).

Soil biological activity

Soil biological indicators, including basic microbial respiration, substrate-induced respiration, and microbial biomass carbon were significantly affected by the main effects of cropping patterns and fertilizer sources. However, the interaction of cropping patterns × fertilizer sources was also significant in the three parameters (Table 7) as analyzed herein (Fig. 5).
The highest values of basic microbial respiration (0.04033 mg CO2-C g^{-1} day^{-1}), substrate-induced respiration (0.27267 mg CO2-C g^{-1} day^{-1}), and microbial biomass carbon (1145 mg CO_2-C kg^{-1}) were observed in the intercropping system fertilized with 75V+25NPK in all cases.

The lowest values for these three parameters were recorded in the MB pure stand, fertilized with NPK. When averaged across the fertilizer sources, intercropping enhanced substrate-induced respiration and microbial biomass carbon by 14 and 33%, compared with FG pure stands, and by 26 and 46% when compared to the MB pure stands, respectively (Fig. 5A, B, and C).

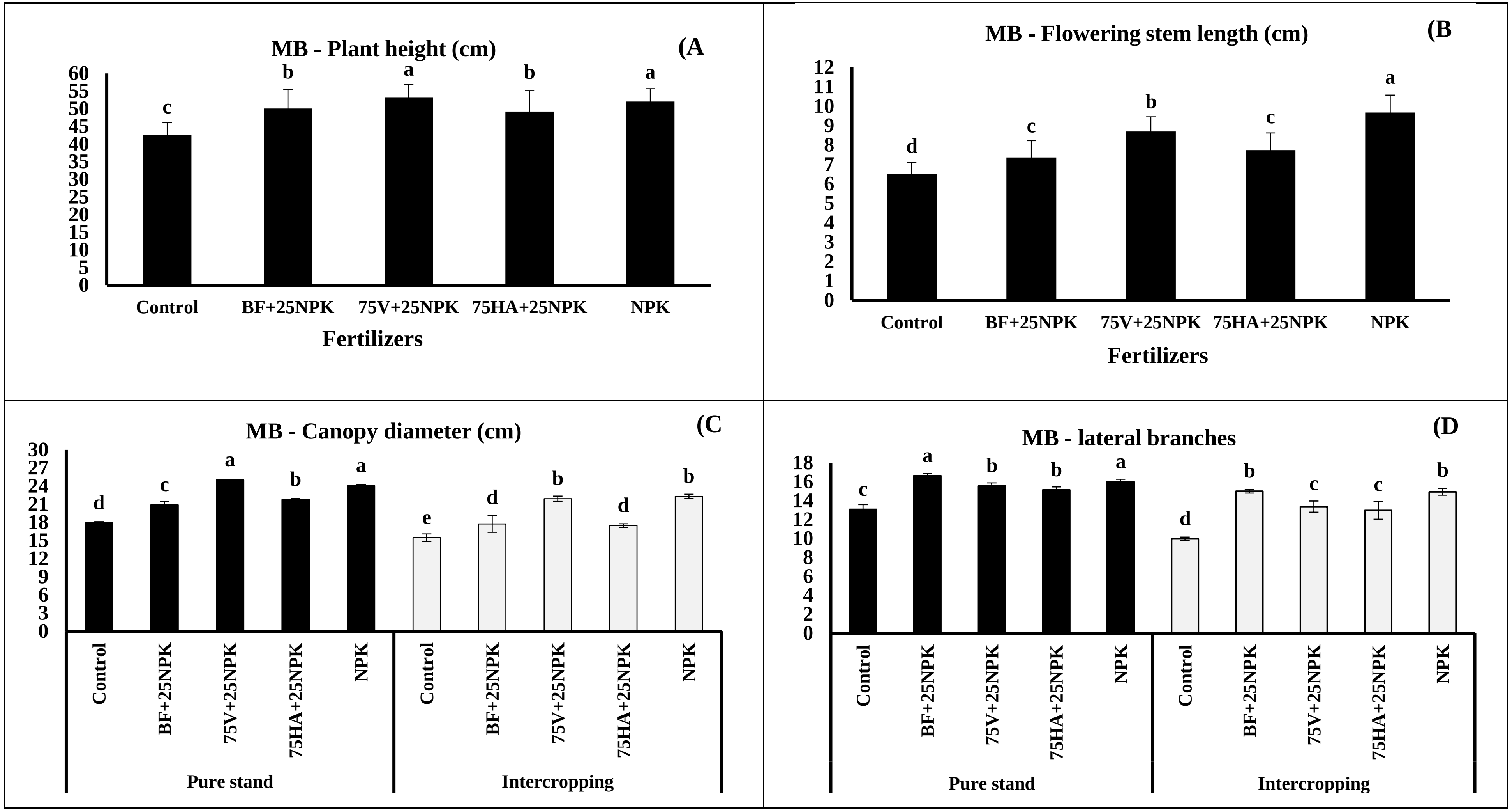

Fig. 3. Plant height (A), flowering stem length (B), canopy diameter (C), and the number of lateral branches (D) of Moldavian balm (MB) as affected by different cropping patterns and fertilizer sources in the fenugreek pure stand and the intercropping system of fenugreek and Moldavian balm (2FG:2MB) for the control, BF+25NPK (bacterial fertilizer + 25% NPK synthetic fertilizer), 75V+25NPK (75% vermicompost + 25% NPK), 75HA+25NPK (75% humic acid + 25% NPK), and NPK fertilizer sources. The same letters above bars indicate significant differences at the $p < 0.05$ level.

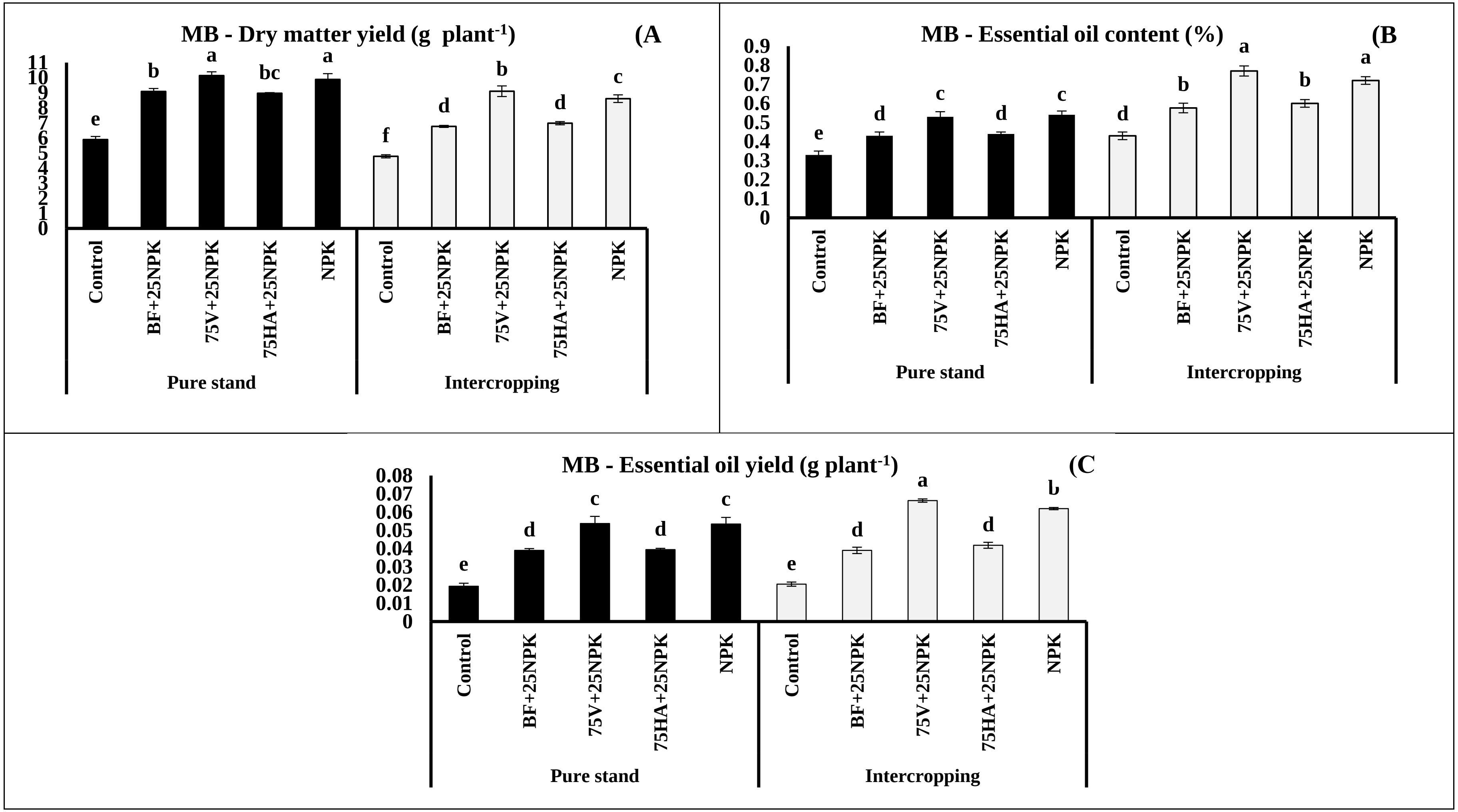

Fig. 4. Dry matter yield per plant (A), essential oil content (B), and essential oil yield (C) of Moldavian balm (MB) across different cropping patterns and fertilizer sources in the fenugreek pure stand and the intercropping system of fenugreek and Moldavian balm (2FG:2MB). The same letters above bars indicate significant differences at the $p < 0.05$ level.

Table 5. Percentage of fenugreek oil constituents across different cropping patterns and fertilizer sources.

	Pure stand					Intercropping				
Components	**Control**	**BF+25NPK**	**75V+25NPK**	**75HA+25NPK**	**NPK**	**Control**	**BF+25NPK**	**75V+25NPK**	**75HA+25NPK**	**NPK**
Myristic Acid	0.3	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.3
Palmitic acid	12.3	11.4	11.2	11.1	11.4	12.0	10.8	10.1	11.3	11.3
Margaric acid	0.3	0.2	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.3
Linoleic acid	35.1	39.6	38.0	38.7	38.8	36.8	38.9	40.0	38.1	37.9
Oleic acid	19.5	20.4	21.0	21.3	21.0	22.3	23.9	25.8	23.0	23.5
Linolenic acid	19.0	17.7	16.0	16.4	17.9	18.2	17.3	16.5	17.4	18.0
Stearic acid	7.9	5.2	5.6	5.6	5.9	6.0	5.9	5.0	4.8	5.2
Total (%)	94.3	94.6	92.3	93.4	95.5	95.8	97.2	97.8	94.9	96.4

Control, BF+25NPK (bacterial fertilizer + 25% NPK synthetic fertilizer), 75V+25NPK (75% vermicompost + 25% NPK), 75HA+25NPK (75% humic acid + 25% NPK), and NPK fertilizer sources in fenugreek pure stands and when intercropped with Moldavian balm.

Table 6. Essential oil composition (%) of Moldavian balm in different cropping patterns and with different fertilizer sources.

		Pure stand					Intercropping				
	RI	**Control**	**BF+25NPK**	**75V+25NPK**	**75HA+25NPK**	**NPK**	**Control**	**BF+25NPK**	**75V+25NPK**	**75HA+25NPK**	**NPK**
p-Cymene	1025	4.9	2.3	2.1	3.4	5.8	3.4	2.4	1.9	2.8	1.3
Linalool	1099	0.1	0.1	0.9	0.9	0.8	2.0	1.0	0.9	1.1	1.2
Menthol	1182	0.8	1.1	1.7	0.6	0.4	0.7	1.1	0.9	1.1	1.2
Neral	1243	13.7	14.0	14.1	14.6	14.8	13.3	15.5	15.2	12.8	14.8
Geraniol	1254	12.1	13.6	12.5	13.4	14.5	13.1	13.9	15.1	16.8	16.9
Geranial	1270	17.9	19.8	20.2	18.5	18.6	18.7	21.0	21.1	18.9	20.5
trans -anethole	1288	0.6	1.0	0.8	0.5	0.7	1.8	0.7	1.0	1.0	0.8
Carvacrol	1300	2.8	0.9	1.6	1.8	0.8	1.7	1.0	0.9	1.1	0.9
Methyl Geranate	1322	0.5	1.4	0.9	0.8	1.0	1.9	0.4	0.3	1.7	0.6
Neryl acetate	1363	4.5	2.2	2.3	2.5	2.9	2.9	4.5	2.4	4.4	4.1
Geranyl acetate	1382	28.7	30.6	32.6	30.9	31.4	28.1	30.2	35.9	33.9	30.9
trans-Caryophyllene	1424	1.9	0.9	1.1	0.6	1.1	1.0	0.4	0.7	0.7	0.7
Germacrene-D	1486	1.6	1.0	1.9	1.0	1.3	1.0	1.3	0.9	1.1	1.9
Spathulenol	1584	1.7	1.4	0.9	0.6	0.3	0.4	0.3	0.7	0.4	0.9
Caryophyllene oxide	1590	2.0	2.8	1.4	0.7	-	0.8	0.9	0.8	0.5	0.7
α-.-Cadinol	1658	0.9	0.8	1.3	1.6	2.8	3.4	2.9	1.0	0.8	1.3
Total (%)		94.7	93.7	96.1	92.3	97.1	94.2	97.5	99.5	99.1	98.6

RI, linear retention indices on DB-5 MS column, experimentally determined using homolog series of n-alkanes, control, BF+25NPK (bacterial fertilizer + 25% NPK synthetic fertilizer), 75V+25NPK (75% vermicompost + 25% NPK), 75HA+25NPK (75% humic acid + 25% NPK), and NPK fertilizer sources under fenugreek pure stands and when intercropped with Moldavian balm.

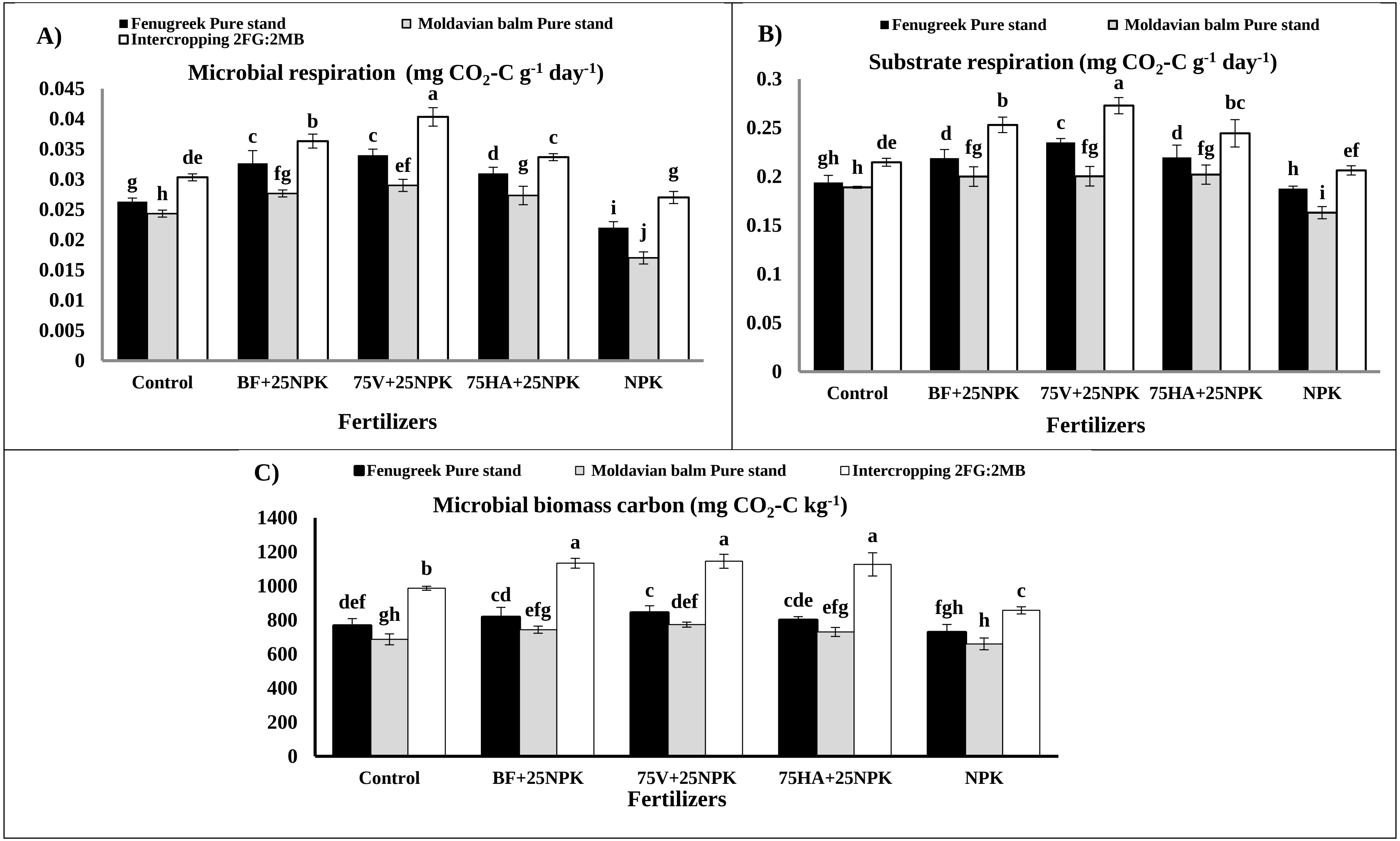

Fig. 5. Basic microbial respiration (A), substrate-induced respiration (B), and microbial biomass carbon (C) of fenugreek and Moldavian balm, as affected by different fertilizer source and cropping patterns. The same letters above bars indicate significant differences at the $p < 0.05$ level.

Table 7. Analysis of variance on soil biological activity in Moldavian balm.

Treatments	Basic microbial respiration	Substrate induced respiration	Microbial biomass carbon
Replication	0.000006**	0.001**	265.689ns
Cropping pattern (CP)	0.0003**	0.008**	454699.356**
Fertilization (F)	0.0002**	0.004**	45582.356**
CP × F	4.506**	0.00024**	7058.522**
Error	0.0000009	0.000035	1454.26
C.V. (%)	3.16	2.82	4.47

NS, *, and ** indicated non-significant, significant difference at 5%, and significant difference at 1% probability level, respectively.

Discussion

Recently, there has been an increase in research interests relating to the use of plant-based medicines across the world. Developing sustainable and eco-friendly strategies that include the use of beneficial soil microorganisms can play an important role in improving the quantity and quality of medicinal and aromatic plants. Soil microbial activity, as an indicator of soil quality and ecosystem processes, is broadly recognized as one of the main factors for increased soil fertility and nutrient availability for plants (Rezaei-Chiyaneh et al., 2021c). In this study, our results showed that soil microbial activity was greatly enhanced under intercropping (2FG:2MB), fertilized with 75V+25NPK. In intercropping patterns, different root structures from different plant species produce a variety of rhizosphere exudates that exert a strong rhizosphere effect leading to increased species distribution, abundance, and diversity of microorganisms in the soil (Gao et al., 2019).

Nyawade et al. (2019) reported that potato/lima bean (*Phaseolus lunatus*) and potato/dolichos (*Lablab purpureus*) intercropping increased the soil microbial respiration by 20–34% and the microbial biomass by 15–38%. In addition, the increase in soil microbial activity, following vermicompost application, may be a result of greater amounts of soil organic matter and improvements in chemical as well as biochemical parameters of the soil (Pathma and Sakthivel, 2012). Moreover, vermicompost application reportedly increased the concentration of root growth stimulators in the soil which, in turn, enhanced plant root exudates for a positive effect on soil microbial activity (Rezaei-Chiyaneh et al., 2021a).

Our results showed that morphological traits such as plant height and other yield components of FG and MB had higher numerical values in the pure stands. The decrease in yield and yield components in intercropping patterns could be explained by a higher intra-specific competition for environmental resources and by lower plant densities when intercropping, compared to the pure stands (Namazi et al., 2022). In intercropping (2MB:2FG), the occupied area of each plant was half in comparison with the pure stand. Therefore, the partial yield of each plant decreased, compared with the productivity of yield in the pure stands.

The integrative application of 75V+25NPK enhanced plant growth characteristics and productivity of both plant species. This can be explained by the positive role of vermicompost in increasing the activity of beneficial microorganisms and improving soil structure, physical and biological activities, increasing water retention capacity. These lead to greater nutrient availability and more uptake by the plants, as well as greater plant productivity (Rekha et al., 2018). Compared with the application of synthetic fertilizers, which typically result in large amounts of nutrients that are quickly unavailable to plants, accompanied by a slower nutrient release after vermicompost application. These can improve overall soil nutrient availability at different plant stages, thereby increasing plant productivity. Similar to our results, Jami et al. (2020) reported that the application of 24,000 kg ha-1 vermicompost enhanced leaf dry weight of saffron (Crocus sativus L.) by almost 42%, compared to the untreated control.

We found that the application of 75V+25NPK in intercropping maximized the oil content of FG. Also, under these conditions, the oil quality improved in comparison with pure stand conditions without fertilizer application by enhancing the unsaturated FAs profile. The ratio of unsaturated FAs to saturated FAs is one of the important indexes that determine oil quality (Marro et al. 2020). Briefly, the oil quality and stability increase when the concentration of unsaturated FAs increases. In intercropping patterns, with the integrative application of vermicompost and synthetic fertilizer, a higher soil microbial activity usually enhances the nutrient availability for both intercropped species, which may lead to greater photosynthesis rates and a higher supply of carbon resources, as well as other essential precursor components of FAs, such as ATP and NADPH (Shu-tian et al., 2018).

In MB, the EO content also improved maximally by intercropping (2MB:2FG), fertilized with 75V+25NPK. Also, the main EO constituents of MB, including geranyl acetate and geranial, were increased under these conditions and, as a result, the EO quality increased. In medicinal and aromatic plants, volatile organic compounds originate from three categories including phenolic compounds, fatty acid derivatives, and isoprenoids (Lara et al., 2020). EOs are final terpenoid products and are formed by a large group of enzymes known as terpene synthases. The activity of these enzymes increases as nutrient availability increases in the soil and for plants, especially N and P. Therefore, the improved EO content and quality under these conditions could be explained by the higher environmental use efficiency of plants in intercropping systems with higher plant nutrient availability, as a result of vermicompost application (Rehman et al., 2016).

In addition, the oil yield of FG was maximized under the effect of pure stands (monoculture), fertilized with 75V+25NPK. Therefore, the higher oil yield in the above-mentioned treatments could be attributed to the higher seed productivity in pure-stand conditions, compared with the intercropping pattern. In contrast, the higher EO yield of MB was observed when intercropping (2MB:2FG), fertilized with V+25% NPK, which resulted from a higher EO content in this treatment.

Conclusions

Intercropping (2MB:2FG) and using 75V+25NPK as a combination of fertilizers caused maximum enhancements in soil microbial activity and plant growth. Additionally, this treatment improved the oil quality of fenugreek by enhancing its unsaturated fatty acid profile, and also by increasing the essential oil quality of Moldavian balm. This resulted from an increase in the abundance of geranyl acetate and geranial. Based on the results of this study, we concluded that intercropping (2MB:2FG) and using a fertilizer mixture composed of vermicompost (75%) and synthetic NPK fertilizer (25%) could suitably improve the quantity and quality of Moldavian balm and fenugreek.

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