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ORIGINAL RESEARCH

Application of bio and chemical fertilizers improves yield, and essential oil quantity and quality of Moldavian balm (*Dracocephalum moldavica* L.) intercropped with mung bean (*Vigna radiata* L.)

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Abstract

Intercropping Moldavian balm with mung bean is an ecological approach for improving resource productivity. A field experiment was conducted over two growing seasons (2018 and 2019) to determine the effect of fertilizer application on yield and essential oil (EO) productivity of Moldavian balm intercropped with mung bean. The experiment had a two-factor randomized complete block design (RCBD) with three replicates. The first factor comprised of five cropping patterns: Moldavian balm sole crop (MBs), mung bean sole crop (MGs), one row each of Moldavian balm +mung bean (1MB:1MG), two rows each of Moldavian balm +mung bean (2MB:2MG), and three rows of Moldavian balm +two rows of mung bean (3MB:2MG). The second factor comprised four fertilizer sources: no fertilizer application (C, control), 100% chemical fertilizer (NPK), 50% chemical fertilizer +100% bacterial fertilizer (NPK+BF), and 100% bacterial fertilizer +100% mycorrhizal fungi (BF+MF). The sole crop fertilized with NPK+BF produced the highest seed yields for MG (1189 kg/ha) and MB (7027 kg/ha), while 3MB:2MG fertilized with NPK+BF had the highest nutrient contents. Moldavian balm produced the highest EO content and yield in 2MB:2MG fertilized with NPK+BF. The EO of MB mainly comprised geranyl acetate (30–39%), geranial (20–31%), neral (18–24%), and geraniol (3–8%). In addition, the 3MB:2MG intercropping treatment fertilized with NPK+BF had the highest land equivalent ratio (LER = 1.35). We recommend an intercropping ratio of 2MB:2MG fertilized with NPK+BF is recommended as an alternative and eco-friendly strategy for farmers to improve EO quantity and quality.

KEYWORDS

canopy diameter, essential oil, geranyl acetate, land equivalent ratio, nutrients

1 | INTRODUCTION

Growing consumer interest in herbal-based and organic products, mostly medicinal and aromatic plants (MAPs), has increased the demand in the botanical industry. The extensive use of MAPs in the pharmaceutical, cosmetic, and food industries, among others, requires more research and development efforts in this sector (Ekor, 2014). Moldavian balm, commonly known as dragonhead (*Dracocephalum moldavica* L.) and belonging to the Lamiaceae family, is an annual MAP native to central Asia and Siberia (Mozaffarian, 2013). The Moldavian balm has been used for centuries as a spice, medicinal plant, painkiller, and for its biological effects, including antioxidant (Aprotosoaie et al., 2016; Dastmalchi et al., 2007), antimutagens (Sonboli et al., 2008), antitumor (Chachoyan & Oganesyan, 1996), and antimicrobial activities (Carović-Stanko et al., 2016). Aerial parts of Moldavian balm are an important source of rosmarinic acid, flavonoids, monoterpene glycosides, and essential oil (EO) (Jalaei et al., 2015). Moldavian balm's main EO constituents are geranyl acetate, geraniol, geranial, neral, and neryl acetate (Alaei and Mahna, 2013).

In recent decades, non-judicious long-term application of chemical inputs, including fertilizers, pesticides, and herbicides, has increased environmental pollution (Kumar, Lai, Battaglia, et al., 2019; Kumar, Lai, Kumar, et al., 2019; Battaglia et al., 2021) and negatively affected yield and quality of crops in some cases (Gomiero et al., 2011; Seleiman et al., 2021). The unprincipled use of these inputs has attracted attention and led to the emerging adoption of integrated management in sustainable agriculture systems (Fahad et al., 2021). Obtaining optimum quantity and quality of active ingredients from Moldavian balm requires the implementation of ecological principles and eco-friendly strategies, as intensive chemicals use can have a detrimental impact on them (Amani Machiani et al., 2019; Rezaei-Chiyaneh, Amani Machiani, et al., 2020).

The use of biofertilizers in modern agriculture is an option for reducing chemical input use and maintaining or increasing soil fertility and plant nutrition. In addition to reducing chemical pollution and maintaining biodiversity by avoiding unnecessary and improper nutrient use, biofertilizers can reduce production costs and increase input use efficiencies (Adnan et al., 2020; Rezaei-Chiyaneh, Amirnia, et al., 2020). Biofertilizers can increase crop yield and quality, particularly in sustainable intercropping systems, as they contain beneficial bacteria and fungi that convert essential but inaccessible nutrients to accessible forms during biological degradation (Hafez et al., 2021). Certain soil bacterial species living in the rhizosphere, collectively called plant growth-promoting rhizobacteria, can stimulate plant growth, facilitate the absorption of elements, stabilize atmospheric nitrogen, solubilize minerals such as phosphate, and produce plant hormones such as auxins and gibberellins (Adnan et al., 2020; Tur-Cardona et al., 2018).

The use of soil microorganisms as biofertilizers is an environmentally friendly option for providing nutrients to plants. Arbuscular mycorrhizal fungi (AMF) are a type of biofertilizer that can also increase nutrient absorption, especially phosphorus, through crop root symbiosis. Studies have demonstrated that inoculating plant roots with AMF improves soil water dynamics (Amiri et al., 2015) plant resistance to stress (Begum et al., 2019), and increases protection against pathogens and soil-borne diseases, leading to increased plant growth and productivity (Pirzad & Mohammadzadeh, 2018).

Intercropping combines two or more plant species on the same land for a considerable proportion of their growing periods (Rezaei-Chiyaneh, Battaglia, et al., 2021; Gitari et al., 2020). It is widely practiced for its more efficient use of environmental resources, increased nutrient uptake, reduced incidence of pathogens, weeds, and pests, improved soil fertility through N fixation by legumes, and increased crop quantity and quality (Gitari et al., 2018; Raza et al., 2019; Wang et al., 2020). Intercropping systems can also improve EO quantity and quality of medicinal plants, such as fennel (*Foeniculum vulgare* Mill.), dragonhead (*Dracocephalum moldavica* L.), and sweet basil (*Ocimum basilicum* L.) (Amani Machiani et al., 2019; Rezaei-Chiyaneh, Amani Machiani, et al., 2020).

Mung bean (*Vigna radiata* L.), also known as green-gray ash or moong, is vital short-season, summer-growing legume (Diatta et al., 2020; Liang et al., 2020). Traditionally, mung bean has served as a food crop species, providing additional benefits through its symbiosis with N-fixing bacteria, increased nutrient availability, and decreased need for chemical fertilizer (Gong et al., 2020). Intercropping crops with short growing can improve crop resource use efficiencies (i.e., light, water, nutrients, and land). However, it remains unclear how intercropping Moldavian balm with mung bean uses the available environmental resources and how the crops respond to different sources of fertilizer application. Most biofertilizer studies have been conducted under sole crop cropping systems. To close that knowledge gap, we investigated the effect of different chemical and biofertilizer sources (incorporating bacteria supplying nitrogen, phosphorus, and potassium+mycorrhizal fungi) on the quantitative and qualitative yields of Moldavian balm and mung bean under sole crop and double intercropping systems.

We hypothesized that (i) intercropping will improve the quantity and quality of EO in Moldavian balm compared with sole cropping, (ii) combined application of chemical and bacterial fertilizer under intercropping will be more beneficial for nutrient uptake, root colonization, and crop productivity than sole cropping, and (iii) intercropping will increase the land-use efficiency (LER index) compared with sole cropping.

2 | MATERIALS AND METHODS

2.1 | Research site and experimental design

The experiment was conducted during the 2018 and 2019 growing seasons at the research farm of Urmia Agricultural Education Center, Iran (45°24′ E, 36°57′ N). The site's climatic data were obtained from the Iran Meteorological Organization (https://www.irimo.ir/eng/index.php) (Table 1). The soil had a silty clay (0–30 cm depth) with a pH of 7.8, 1.18% organic C, 0.08% total nitrogen, 9.9 mg/kg available phosphorus, and 192 mg/kg available potassium, 1.8 mg/kg available zinc, 13.9 mg/kg available iron, 12.1 mg/kg available manganese, and 1.1 mg/kg available copper (averaged over two years).

This experiment used a two-factor randomized complete block design with a factorial arrangement of treatments and three replications. The first factor involved five cropping patterns: Moldavian balm sole crop (MBs), mung bean sole crop (MGs), and intercropping one row each of Moldavian balm + mung bean (1MB:1MG), intercropping two rows each of Moldavian balm + mung bean (2MB:2MG), and intercropping three rows of Moldavian balm +two rows of mung bean (3MB:2MG). The second factor comprised four fertilizer sources: 100% chemical fertilizer (NPK), 50% chemical fertilizer +100% bacterial fertilizer [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*)] (NPK+BF), 100% bacterial fertilizer +100% mycorrhizal fungi (*Funneliformis mosseae*) (BF+MF), and an unfertilized control.

TABLE 1 Monthly average temperature, precipitation, and relative humidity in 2018 and 2019 growing seasons in Urmia, Iran

	Average temperature (°C)		Average rainfall (mm)	
	2018	2019	2018	2019
January	3.7	2	17.8	11.4
February	3.2	2.7	67.8	41.8
March	9	4.6	13.2	48.4
April	12.6	12.6	1.9	27.4
May	16.4	13.3	0.8	114.8
June	22	27.9	0	17.2
July	26.2	27.9	0	0
August	27.8	27.1	0	0
September	25	23.4	6.8	0.4
October	15.6	17.9	37.4	4.8
November	11.2	9.7	11	40.9
December	1.8	5	11.2	59.2
Two-year mean	14.5	13.9	13.93	30.52

2.2 | Plant material and cultural management practices

Before cultivation, the seeds of both species were inoculated with a combination of NFB, PSB, and KSB in the form of powder at a rate of 100 g/ha (Zist Fanavar Sabz Company) for a bacterial population of 5×10^8 colony forming units g^{-1}. The bacterial fertilizer powder was mixed with water and uniformly sprayed to cover the whole seed and then seeds were air-dried. At planting, 20 g of inoculum containing ~2200 spores of *F. mosseae* was poured into each planting hole that received the BF+MF treatment.

Each year, the same field plot was conventionally tilled using a chisel plow, disk harrowing, and a leveler. Seeds were planted on July 1, 2018 and 2019, in 40-cm wide rows for both species, with an in-row seed spacing of 20 cm for Moldavian balm and 10 cm for mung bean. The rows were 4 m long for both species. Plots and blocks were separated by a buffer of 1 and 3 m, respectively. The final seeding rate in the pure stand was 12.5 seeds/m^2 for Moldavian balm and 25 seeds/m^2 for mung bean. For the NPK treatment, 100 kg/ha urea (50% at sowing +50% at stem elongation), 150 kg/ha triple superphosphate, and 120 kg/ha potassium sulfate (soil incorporated before sowing), based on soil test results and plant nutrient demands, were applied using the deep strip method. For the NPK+BF treatment, half of the full NPK treatment rates were used at the same timing. Irrigation was applied immediately after planting to facilitate seedling emergence. Subsequent irrigations were applied every seven days, as required, until the end of the growing season. Manual weeding occurred several times during the growing season.

2.3 | Measurements

2.3.1 | Yield and yield components of Moldavian balm and mung bean

Mung bean seeds were harvested on August 12, 2018 and August 15, 2019, when the first pods had fully matured.

Moldavian balm aboveground biomass was harvested at the ground level at 50% flowering on July 14, 2018 and July 11, 2019. At the end of the growing season, we measured seed yield of mung bean and biomass yield of Moldavian balm by harvesting of 4 m^2 per plot (i.e., 2 m length of five central rows) to avoid plot border effects. For mung bean, in addition to determining the dry weight, the seeds of the plants were separated to find out the seed yield. To determine yield components for each plant, ten plants were randomly selected at each plot. Moldavian balm and mung bean samples were air-dried at 25°C in the dark for 12 d. Mung bean samples were then threshed to separate seeds from straw. Other measured traits for Moldavian balm included plant height, lateral branch number, canopy diameter, dry matter yield, EO content and yield, N and P concentrations, and root colonization. Other measured traits for mung bean included plant height, pod number per plant, seed number per pod, 1000-seed weight, seed yield, N and P concentrations, and root colonization.

2.3.2 | Plant nutrient analysis

Moldavian balm leaves and mung bean seeds were used to determine various nutrient concentrations. First, the seeds or leaves were mixed and ground to pass through a 1-mm screen (Weidhuner et al., 2019). To prepare ash for analysis, 10 g of each ground sample was oven-dried at 500°C for 5 h. The Kjeldahl method was used to determine N content (Sáez-Plaza et al., 2013). Sample P concentration was determined using the yellow method, with vanadate–molybdate used as an indicator (Tandon et al., 1968), and measured at 470 nm using a spectrophotometer.

2.3.3 | Essential oil extraction and analysis

Moldavian balm EO was extracted by using the water distillation method. Briefly, 50 g of dry matter (leaf + flower) from each plot was weighed; briefly ground in 500 ml water, before boiling in a Clevenger for 3 h to extract the EO, which was then weighed. The EO content and EO yield were calculated as follows (Amani Machiani et al., 2019):

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

$$\text{EO yield (kg/ha)} = \text{seed yield (kg/ha)} \times \text{EO content}\,(\%).$$

2.3.4 | Essential oil analysis

Gas chromatography–mass spectrometry analysis was undertaken using an Agilent 7890/5975C (Santa Clara, California, USA) GC/MSD. 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 to separate the EO components. The following oven temperature was applied: 3 min at 80°C, before increasing by 8°C/min to 180°C, and held for 10 min at 180°C. Helium was used as the carrier gas at a flow rate of 1 ml/min. The sample was injected (1 μl) in split mode (1:50). The EI mode was 70 Ev. Mass range was set from 40 to 550 m/z. The components were recognized by comparing the calculated Kovats retention indices (RIs), from a mixture of n-alkane series (C8–C30, Supelco, Bellefonte, CA) and mass spectra (Adams, 2007; NIST, 2008). GC-FID analysis was undertaken with an Agilent 7890 A instrument. Separation was performed in an HP-5 capillary column. The analytical conditions were the same as above. Quantification methods were the same as those reported by Morshedloo et al. (2017).

2.3.5 | Root colonization

Root colonization percentage was determined on ten plants per experimental plot. For this purpose, 1 cm root segments were placed in formalin–acetone–alcohol (FAA) solution (I3 ml formalin, 5 ml glacial acetic acid, 200 ml 50% ethanol) for 24 h. The samples were then rinsed with distilled water and cleared in 10% KOH for 1 h at 90°C. Following, roots were placed in 1% hydrochloric acid for 3 min, stained with 0.05% trypan blue, boiled for 45 min, and set in lactoglycerol for 24 h. Root colonization rate was measured as the ratio between the number of root segments containing vesicles, arbuscules or hyphae, and the total number of root segments sampled, expressed as a percentage (Phillips & Hayman, 1970; Wang et al., 2019).

2.3.6 | Land equivalent ratio (LER)

The partial LERs of Moldavian balm (LER_{MB}) and mung bean, partial (LER_{MG}), and total LER (LER_T) were calculated as follows (Gitari et al., 2020):

$$LER_{MB} = \left(Y_{MBI}/Y_{MBS}\right) \quad (1)$$

$$LER_{MG} = \left(Y_{MGI}/Y_{MGS}\right) \quad (2)$$

$$LER_T = LER_{MB} + LER_{MG} \quad (3)$$

TABLE 2 Analysis of variance for the effects of year, intercropping, and fertilization on evaluated traits in Moldavian balm

Treatment	Plant height (cm)	Lateral branch number	Canopy diameter (cm)	Dry matter yield (kg/ha)	Essential oil content (%)	Essential oil yield (kg/ha)	Nitrogen (g/kg)	Phosphorus (g/kg)	Root colonization (%)
First year	54.1 ± 7.9 b	10.8 ± 1.6 a	41.0 ± 7.9	5391.1 ± 970 b	0.39 ± 0.06	21.2 ± 4.7	27.9 ± 5.4	27.6 ± 4.6	21.6 ± 3.2
Second year	58.7 ± 9.0 a	10.2 ± 1.9 b	39.4 ± 6.8	5452.0 ± 926 a	0.39 ± 0.07	21.4 ± 4.7	28.4 ± 5.0	27.5 ± 4.5	22.6 ± 3.2
Year (Y)	**	*	NS	*	NS	NS	NS	NS	NS
Intercropping (I)	**	**	**	**	**	**	**	**	**
Fertilization (F)	**	**	**	**	**	**	**	**	**
I × F	**	**	**	**	**	**	**	**	**
Y × I	*	**	NS	NS	NS	NS	**	*	**
Y × F	NS	NS	NS	NS	NS	NS	NS	NS	NS
Y × I × F	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, non-significant; * and ** indicate significant differences at the 5% and 1% probability level, respectively; lower case letters within a column indicate significant differences ($p < 0.05$) between years for a given trait.

where Y_{MBI} or Y_{MBS} is the biomass yield of Moldavian balm either in intercropping or in sole cropping, respectively; and Y_{MBI} or Y_{MBS} is the seed yield of mung bean either in intercropping or in sole cropping, respectively.

2.4 | Statistical analysis

A combined analysis of variance over two years was performed using a mixed linear model with PROC Mixed procedure in SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). The fertilizer sources and cropping treatments were considered fixed effects, and block, year, and interactions were assumed random effects. The means were compared using Duncan's multiple range test at $p < 0.05$.

3 | RESULTS

3.1 | Moldavian balm (MB)

The main effects of intercropping patterns and fertilization sources were significant ($p < 0.01$) for all morphological characteristics, nutrient content, dry matter yield, and EO productivity of MB. The interaction effect of intercropping pattern and fertilizer source was significant for all measured parameters. In addition, plant height, lateral branch number, and dry matter yield were affected by year (Table 2).

Sole cropping of MB fertilized with NPK+BF produced the tallest plants (66.4 cm), while intercropping 3MB:2MG without fertilizer produced the shortest plants (41.1 cm). On average, the three intercropping combinations reduced plant height by 16.3% compared with sole cropping. Averaged across intercropping systems, NPK, NPK+BF, and BF+MF increased plant height by 23%, 29%, and 20%, respectively, relative to the unfertilized control (Figure 1a).

Sole cropping of MB fertilized with NPK+BF produced the most lateral branches (12.9), while the unfertilized 2MB:2MG intercropping system produced the least (7.1). Averaged across intercropping systems, NPK, NPK+BF, and BF+MF increased lateral branch number by 23%, 31%, and 24%, respectively, relative to the unfertilized control (Figure 1b). Overall, the intercropping systems decreased lateral branch number by 20.7% compared with sole cropping (Figure 1b).

Sole cropping of MB fertilized with NPK+BF had the greatest canopy diameter (49.1 cm). On average, intercropping reduced the canopy diameter by 21%, relative to sole cropping, with the smallest canopy diameter under 3MB:2MG without fertilizer (28.1 cm) followed by 1MB:1MG without fertilizer (29.3 cm) and 2MB:2MG

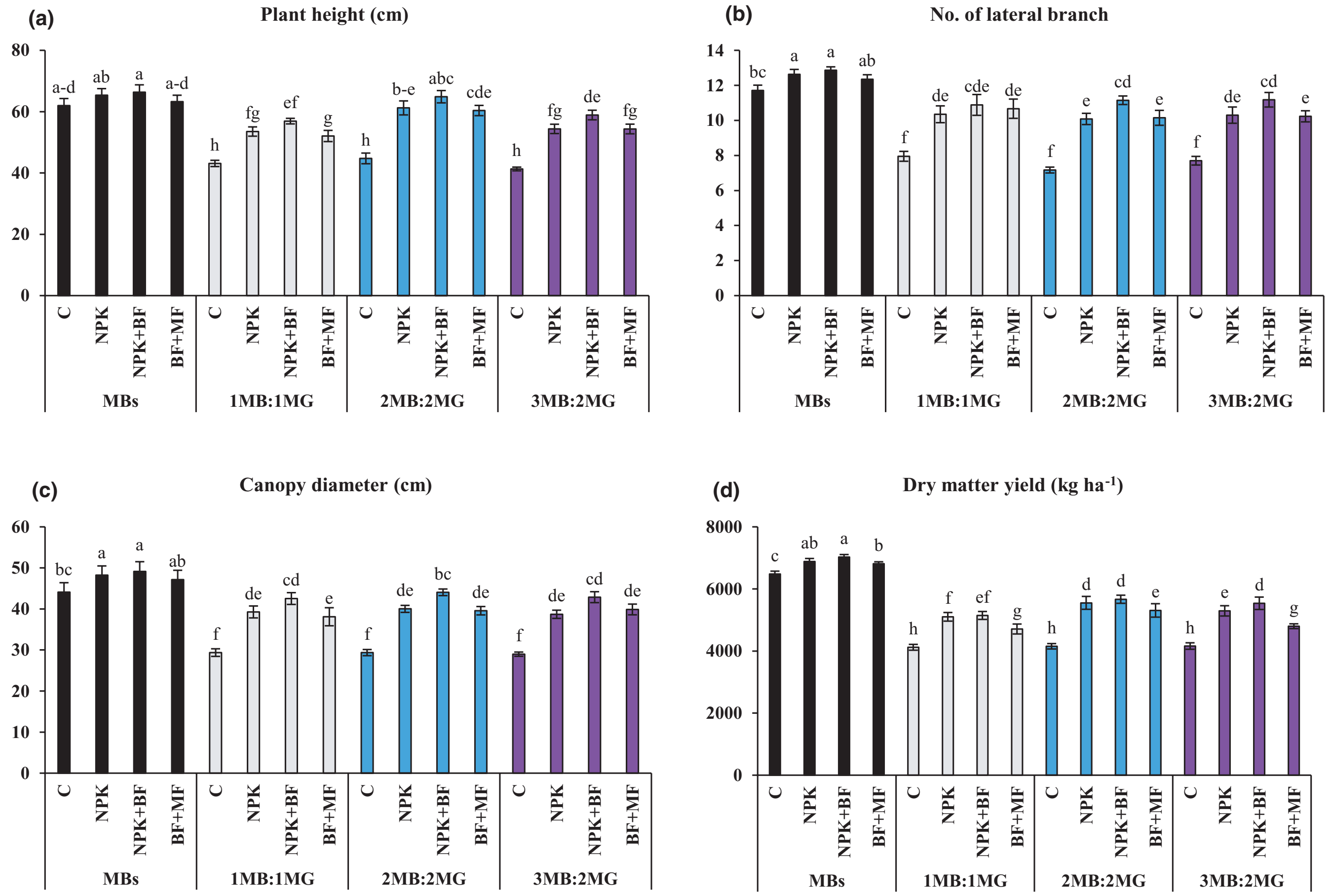

FIGURE 1 Plant height (a), No. of lateral branch (b), canopy diameter (c), and dry matter yield (d) of Moldavian balm under different cropping patterns and fertilizers source. C (control), NPK (chemical fertilizers), BF (bacterial fertilizer), MF (mycorrhizal fungi), MBs (Moldavian balm sole crop). 1MB:1MG, 2MB:2MG and 3MB:2MG indicate the ratios of Moldavian balm and mung bean in cropping pattern. Same letters above bars show non-significant difference at $p < 0.05$ by Duncan test

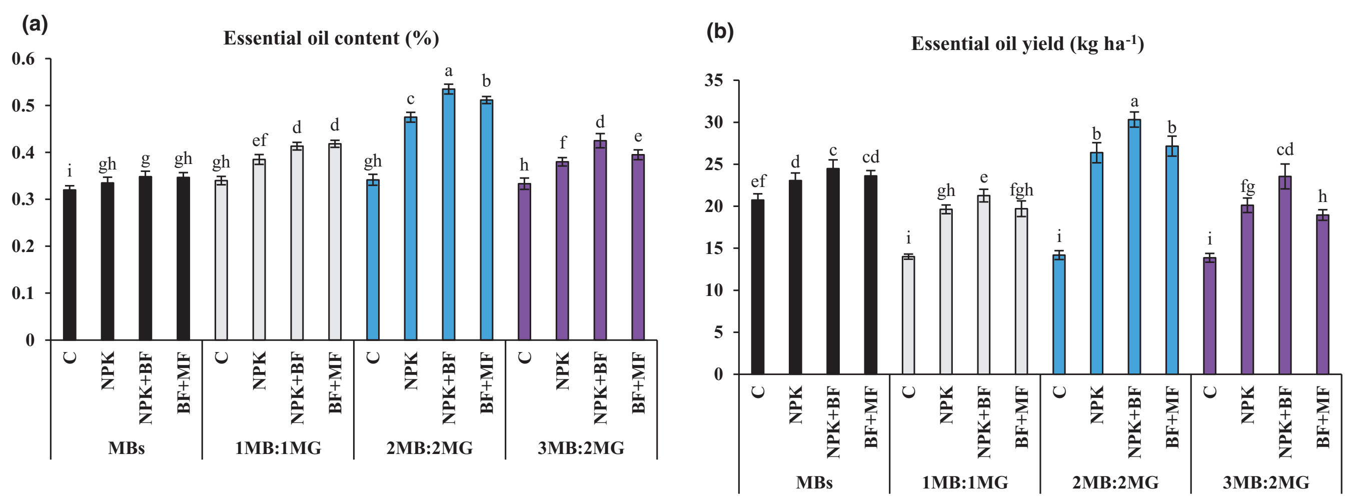

FIGURE 2 Essential oil content (a) and essential oil yield (b) of Moldavian balm under different cropping patterns and fertilizer sources. C (control), NPK (chemical fertilizer), BF (bacterial fertilizer), MF (mycorrhizal fungi), MBs (Moldavian balm sole crop). 1MB:1M, 2MB:2M, and 3MB:2M indicate the ratios of Moldavian balm (MB) and mung bean (MG) in the intercropping pattern. Same letters above bars show non-significant difference at $p < 0.05$ by Duncan test

without fertilizer (29.4 cm) (Figure 1c). Averaged across intercropping systems, NPK, NPK+BF, and BF+MF increased the canopy diameter of MB by 24%, 33%, and 23%, respectively, relative to the unfertilized control (Figure 1c).

Sole cropping of MB fertilized with NPK+BF produced the most dry matter yield (7027 kg/ha), while 1MB:1MG without fertilizer produced the lowest (4120 kg/ha). The 1MB:1MG, 2MB:2MG, and 3MB:2MG intercropping treatments had 30%, 24%, and 27% lower dry matter yields sole cropping. Averaged across intercropping systems, NPK, NPK+BF, and BF+MF increased dry matter yield by 21%, 24%, and 14%, respectively, relative to the unfertilized control (Figure 1d).

The intercropping systems and fertilizer treatments significantly increased EO content of MB, with 2MB:2MG with NPK+BF producing the highest EO content (0.53%), and sole cropping without fertilizer producing the lowest (0.32%). On average, the 1MB:1MG, 2MB:2MG, and 3MB:2MG intercropping treatments increased EO content by 15%, 38%, and 12%, respectively, relative to sole cropping. Averaged across intercropping systems, NPK, NPK+BF, and BF+MF increased EO content by 19%, 30%, and 27%, relative to the unfertilized control (Figure 2a).

The 2MB:2MG intercropping system fertilized with NPK+BF produced the maximum EO yield (30.3 kg/ha), while 3MB:2MG without fertilizer produced the lowest (13.9 kg/ha). The 2MB:2MG intercropping treatment had about 7% higher EO yield than sole cropping. Moreover, the application of NPK, NPK+BF, and BF+MF increased EO yield by 42%, 59%, and 42%, relative to the unfertilized control (Figure 2b).

The GC-MS-based analysis detected 18 EO components (90.7–99.68% of total composition) in MB dry matter (Table 4), with the major components being geranyl acetate (30–39%), geranial (20–31%), neral (18–24%), and geraniol (3–8%). The 2MB:2MG intercropping system fertilized with NPK+BF had the highest geranyl acetate, geranial, and neral contents, MB sole cropping without fertilizer had the lowest. Overall, intercropping produced higher contents of EO components than sole cropping (Table 3). Bio and chemical fertilizers significantly improved most EO compounds, regardless of the cropping ratio, relative to the unfertilized control (Table 2). The relative contents of geranyl acetate, geranial, neral, and geraniol under intercropping increased by 4.61%, 16.46%, 8.04%, and 18.16%, respectively, compared with sole cropping (Table 3).

3.2 | Mung bean (MG)

The main effects of intercropping patterns and fertilization sources were significant ($p < 0.01$) for all measured parameters of MG. The interaction intercropping pattern and fertilizer source were significant for plant height, pod number per plant, and seed yield of MG. However, the year effect was not significant for any of the measured traits (Table 4).

The 2MB:2MG intercropping system fertilized with NPK+BF produced the tallest plants (59 cm), while sole cropping without fertilizer produced the shortest plants (43 cm). On average, intercropping increased plant height of MG by 13.3%, relative to the sole cropping. Averaged across intercropping systems, application of NPK, NPK+BF, and BF+MF increased plant height by 11%, 14%, and 5%, respectively, relative to the unfertilized control (Figure 3a).

Sole cropping of MG produced the most seeds per pod, while 1MB:1MG produced the least (Figure 4a). Seed number per pod did not significantly differ for the three intercropping combinations. Overall, intercropping decreased seed number per pod by 17%, relative to sole cropping (Figure 4a). Averaged across intercropping systems, NPK+BF produced the most seeds per pod, while the control produced the least. The application of NPK, NPK+BF, and BF+MF increased seed number per pod by 10%, 16%, and 9%, respectively, relative to the unfertilized control (Figure 4b).

Sole cropping of MG produced the most pods per plant, more so when supplied with NPK+BF (37.8), followed by NPK (37.2), while 1MB:1MG without fertilizer produced the least. Pod number per plant did not significantly differ among the three intercropping combinations. Averaged across fertilizer treatments, intercropping decreased pod numbers per plant by 13%, relative to sole cropping. Averaged across all fertilizer treatments, NPK, NPK+BF, and BF+MF increased pod numbers per plant by 26%, 35%, and 23%, respectively, relative to the unfertilized control (Figure 3b).

Sole cropping of MG produced the highest 1000-seed weight (4.96 g), while 3MB:2MG produced the lowest (4.19 g). Intercropping reduced 1000-seed weight by 14%, relative to sole cropping (Figure 4c). The application of NPK+BF produced the greatest 1000-seed weight for MG, relative to the other fertilizer treatments. Averaged across intercropping systems, NPK, NPK+BF, and BF+MF enhanced 1000-seed weight by 4%, 7%, and 4%, respectively, relative to the unfertilized control (Figure 4d).

Intercropping produced lower seed yields of MG than sole cropping. Sole cropping with NPK+BF produced the highest seed yield (1189 kg/ha), while 1MB:1MG without fertilizer produced the lowest (466 kg/ha). Averaged across fertilizer treatments, the 1MB:1MG, 2MB:2MG, and 3MB:2MG reduced seed yield of MG by 48%, 40%, and 36%, relative to sole cropping. Averaged across intercropping systems, NPK, NPK+BF, and BF+MF increased seed yield by 29, 34, and 27%, respectively, relative to the unfertilized control (Figure 3c).

TABLE 3 Proportion of Moldavian balm constituents using different cropping patterns and fertilizer sources (average over two years)

		Cropping pattern[a]							
Components	**RI[b]**	**MBs**	**MBs +NPK**	**MBs +NPK+BF**	**MB$_s$ +BF+MF**	**1MB:1MG + C**	**1MB:1MG + NPK**	**1MB:1MG + NPK+BF**	**1MB:1MG + BF+MF**
Decane	998	0.11	0.09	0.07	0.13	0.07	–	0.06	0.04
Linalool	1099	0.4	0.19	0.17	0.35	0.09	0.21	0.09	0.12
Cis chrysanthenol	1164	0.35	0.58	0.54	0.63	1.29	0.54	0.46	0.32
Menthol	1182	1.39	0.86	0.8	1.01	1.54	–	0.71	0.59
Neral	1243	**17.83**	**19.97**	**20.84**	**20.01**	**20.33**	**21.54**	**22.88**	**22.52**
Geraniol	1254	**4.06**	**4.6**	**4.02**	**4.46**	**4.09**	**4.5**	**5.39**	**5.81**
Geranial	1270	**20.19**	**27.67**	**27.82**	**27.19**	**23.41**	**28.55**	**26.47**	**28.51**
Trans-anethole	1288	1.64	0.5	0.14	0.14	0.88	0.63	0.21	0.04
Carvacrol	1300	0.99	0.24	0.08		0.14	0.13	0.06	
Methyl geranate	1322	1.5	0.2	0.14	0.31	0.06	0.93	0.07	0.04
Neryl acetate	1363	4.31	5.86	5.11	6.39	2.33	3.16	2.56	2.74
Geranyl acetate	1382	**30.04**	**35.55**	**36.97**	**33.72**	**32.33**	**37.67**	**38.58**	**34.91**
Beta-elemene	13.96	1.5	0.17	0.17	0.22	0.04	0.12	0.07	–
trans-Caryophyllene	1424	1.9	0.12	0.07	0.18	0.95	0.19	0.06	0.01
Germacrene-D	1486	1.87	0.28	0.44	0.36	0.1	0.36	0.09	0.02
Spathulenol	1584	1.93	0.19	0.21	0.16	1.10	0.15	–	–
Caryophyllene oxide	1590	1.10	–	–	0.38	0.91	0.46	0.05	0.03
Alpha-Cadinol	1658	1.85	–	–	0.88	0.97	0.54	0.03	0.01
Total identified (%)	**92.96**	**97.07**	**97.59**	**96.52**	**90.63**	**99.68**	**97.84**	**95.71**	**90.7**

[a]MBs (Moldavian balm sole crop), C (control), CF (chemical fertilizer), BF (bacterial fertilizer), MF (mycorrhizal fungi), 1MB:1 M, 2MB:2 M, and 3MB:2 M are the ratios of Moldavian balm and mung bean in the intercropping pattern; main components are in bold.

[b]RI, linear retention indices on DB-5 MS column, experimentally determined using homologue series of *n*-alkanes.

TABLE 4 Analysis of variance for the effects of year, intercropping and fertilization on evaluated traits in mung bean

Treatments	**Plant height (cm)**	**Pod number per plant**	**Seed number per pod**	**1000-seed weight (g)**	**Seed yield (kg/ha)**	**Nitrogen (g/kg)**	**Phosphorus (g/kg)**	**Root colonization (%)**
First year	50.0 ± 4.5	29.2 ± 5.9	9.06 ± 1.4	4.42 ± 0.34	800.2 ± 245	3.06 ± 0.44	2.54 ± 0.33	18.2 ± 2.6
Second year	50.6 ± 5.0	28.6 ± 5.7	8.54 ± 1.3	4.45 ± 0.35	776.8 ± 223	3.05 ± 0.43	2.58 ± 0.33	19.5 ± 2.7
Year (Y)	NS	NS	NS	NS	NS	NS	NS	NS
Intercropping (I)	**	**	**	**	**	**	**	**
Fertilization (F)	**	**	**	**	**	**	**	**
I × F	**	**	NS	NS	**	**	**	**
Y × I	**	*	**	NS	*	NS	NS	NS
Y × F	NS	NS	NS	NS	NS	NS	NS	NS
Y × I × F	NS	NS	NS	NS	NS	NS	NS	NS

NS, non-significant; * and ** indicate significant differences at the 5% and 1% probability level, respectively; lower case letters within a column indicate significant differences ($p < 0.05$) between years for a given trait.

2MB:2MG + C	2MB:2MG + NPK	2MB:2MG + NPK+BF	2MB:2MG + BF+MF	3MB:2MG + C	3MB:2MG + NPK	3MB:2MG + NPK+BF	3MB:2MG + BF+MF	Average
0.16	–	0.05	0.05	–	0.12	0.09	0.09	0.08
0.32	–	0.09	0.18	0.16	0.21	0.19	0.19	0.18
1.27	–	0.04	0.54	0.59	0.6	0.71	0.72	0.61
1.8	0.15	0.03	0.84	0.86	0.89	0.96	0.02	0.83
21.04	**22.59**	**23.99**	**21.69**	**17.98**	**21.58**	**18.91**	**21.59**	20.96
4.31	**4.75**	**3.41**	**8.01**	**5.09**	**5.92**	**6.32**	**5.14**	4.99
24.15	**28.25**	**31.09**	**28.1**	**25.17**	**28.15**	**29.2**	**30.54**	27.15
0.13	–	0.05	0.05	0.14	0.27	0.14	0.09	0.34
0.19	–			0.66				0.22
0.27	–	0.01	0.11	0.55	0.15	0.41	0.09	0.32
3.28	3.15	1.11	2.64	3.21	3.15	3.29	2.92	3.38
30.69	**37.37**	**38.84**	**36.23**	**32.11**	**35.5**	**36.94**	**37.56**	35.5
0.16	0.01	0.11	0.09	–	–	–	0.18	0.24
0.23	0.04	0.04	0.07	0.23	0.15	0.19	0.06	0.28
0.54	0.09	0.01	0.29	0.49	0.48	0.42	0.31	0.38
0.22	0.03	0.05	0.12	0.42	–	0.09	–	0.26
0.95	0.01	0.09	0.09	0.69	0.54	0.3	0.14	0.41
0.99	0.02	–	–	0.25	–	–	–	0.61
96.46	**99.01**	**99.1**	**88.6**	**97.71**	**98.16**	**99.64**	**96.42**	

3.3 | Root colonization in Moldavian balm and mung bean

The 3MB:2MG intercropping treatment fertilized with BF+MF had the most root colonization for Moldavian balm (69%) and mung bean (79%), while sole cropping with NPK had the lowest (Figure 5a,b). Averaged across fertilizer treatments, intercropping increased root colonization in Moldavian balm by 50% and mung bean by 52%, relative to the pure stand.

3.4 | Nutrient concentrations in Moldavian balm and mung bean

The N and P contents in both species were affected by cropping pattern, fertilizer source, and their interaction (Tables 2 and 4). Intercropping with fertilizer application had higher N and P contents in MG seeds and MB leaves than those in the corresponding sole cropping without fertilizer. The 3MB:2MG intercropping treatment fertilized with NPK+BF had the highest N and P contents for both species, while sole cropping without fertilizer had the lowest N and P contents for MG and MB (Figure 6a–d). Overall, intercropping increased N and P contents of MG by 19% and 16% and MB by 34% and 16%, respectively, relative to sole cropping.

3.5 | Land equivalent ratio (LER)

The intercropping treatments had total LER values >1. The 3MB:2MG intercropping treatment fertilized with NPK or NPK+BF had the highest partial LERs for MB (0.68) and MG (0.69), respectively (Figure 7). The 3MB:2MG intercropping treatment fertilized with NPK+BF had the highest total LER (1.35), while 1MB:1MG without fertilizer had the lowest (1.04) (Figure 7).

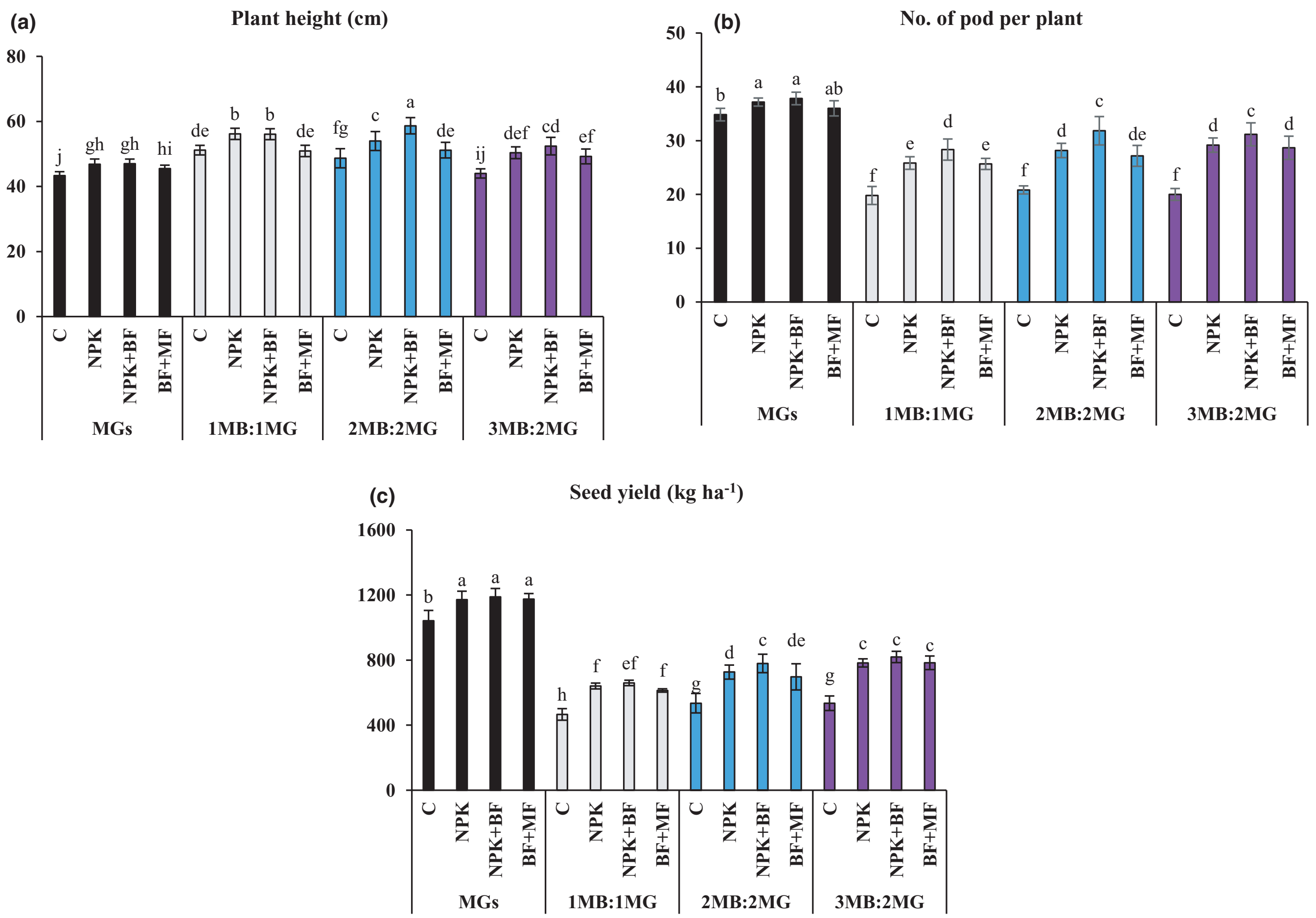

FIGURE 3 Plant height (a), pod number per plant (b), and seed yield (c) of mung bean under different cropping patterns and fertilizer sources. C (control), NPK (chemical fertilizer), BF (bacterial fertilizer), MF (mycorrhizal fungi), MGs (mung bean sole crop). 1MB:1M, 2MB:2M, and 3MB:2M indicate the ratios of Moldavian balm (MB) and mung bean (MG) in the intercropping pattern. Same letters above bars show non-significant difference at $p < 0.05$ by Duncan test

4 | DISCUSSION

4.1 | Yield and yield components

This study revealed that root colonization, nutrient content, agronomic variables, and yield of MB, and MG improved in response to fertilizer application, particularly to the application of NPK+BF. Intercropping increased plant height of MG, which could be due to better light utilization and an increased synthesis of gibberellin in stems and stem nodes (Wang et al., 2021). In line with our second hypothesis, intercropping enhanced root colonization and nutrient uptake in both species, especially with BF+MF fertilization. This could be attributed to the increased availability of legume-fixed nitrogen and organic matter, which positively affects soil microbial communities, especially AMF (Wahbi et al., 2016). Moreover, increased nutrient uptake in intercropped MG and MB could be due to the result of an increased enzyme activity and root exudation, and better use of available resources when compared with the sole cropping comparisons. In a previous study conducted in a similar environment, Rezaei-Chiyaneh, Jalilian, et al., (2021) reported an increased in the root colonization and nutrient uptake in intercropping systems where legumes where included, which the authors attributed to the beneficial impacts of increased N fixation by the legumes components.

Compared with sole cropping, intercropping decreased yield and yield components in both plant species, mainly as a result of the lower plant densities and higher interspecific competition for environmental resources when more than one species coexists together and compete for available resources in the same environment (Gao et al., 2020). Amani Machiani et al. (2018b) reported that sole cropping of peppermint (*Mentha piperita* L.) and soybean (*Glycine max* L.) produced higher biomass yields than intercropping of both species. However, the lower yields for each individual species under intercropping do not equate to a lower overall productivity (Rezaei-Chiyaneh et al., 2021). In this regard, LER is a better indicator of productivity level when comparing intercropping and sole cropping

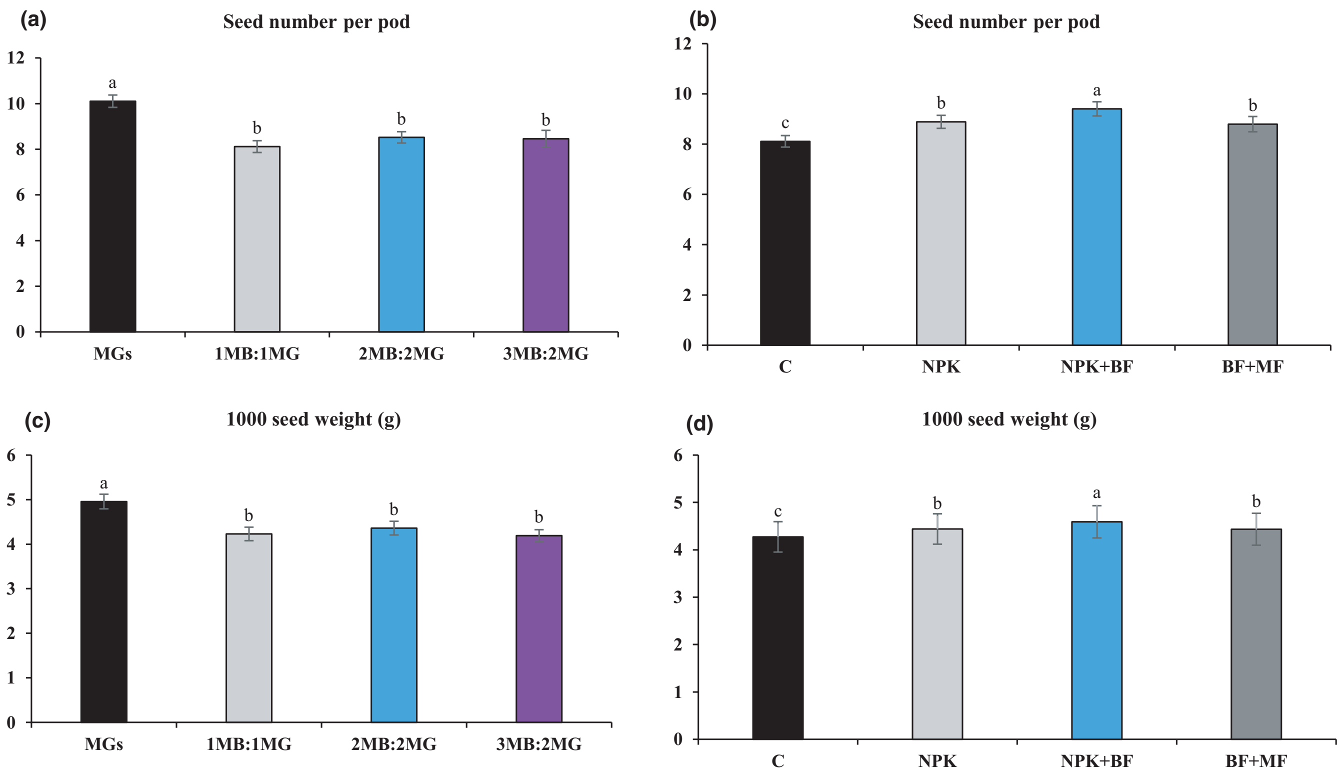

FIGURE 4 Seed number per pod (a, b) and 1000 seed (c, d) weight of mung bean under different cropping patterns and fertilizers source C (control), NPK (chemical fertilizers), BF (bacterial fertilizer), MF (mycorrhizal fungi), MGps (mung bean sole crop). 1MB:1M, 2MB:2M, and 3MB:2M indicate the ratios of Moldavian balm (MB) and mung bean (MG) in the intercropping pattern. Same letters above bars show non-significant difference at $p < 0.05$ by Duncan test

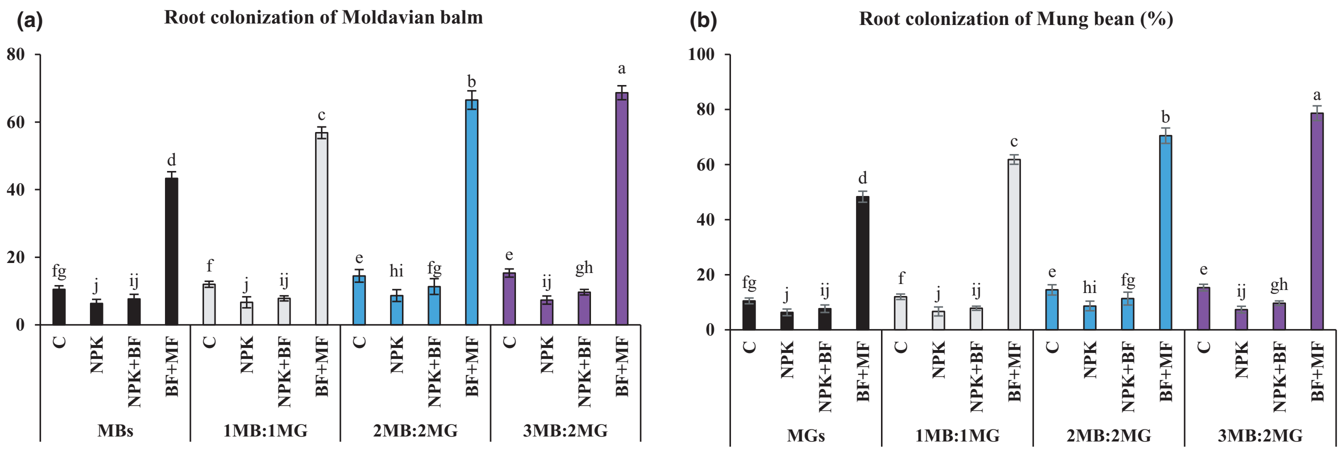

FIGURE 5 Root colonization of Moldavian balm (a) and mung bean (b) under different cropping patterns and fertilizer sources. C (control), NPK (chemical fertilizer), BF (bacterial fertilizer), MF (mycorrhizal fungi), MBs (Moldavian balm sole crop), MGs (mung bean sole crop). 1MB:1M, 2MB:2M, and 3MB:2M indicate the ratios of Moldavian balm (MB) and mung bean (MG) in the intercropping pattern. Same letters above bars show non-significant differences at $p < 0.05$ by Duncan test

systems. In our study, all intercropping treatments had LER values >1, indicating a higher overall productivity when the species were intercropped compared with the yields of MB and MG under sole cropping (Figure 7). Since the overall yield of the cropping system was optimized in terms of LER, the two crops could be intercropped without expecting yield penalties from a system standpoint.

Plant growth is influenced by several factors, including soil nutrient availability, temperature, and water and light availability. Accessibility to macronutrients (e.g., N, P, and K) and micronutrients is important for physiological and biochemical activities in plants (Gitari et al., 2018). In our study, the integrative application of NPK and BF increased plant height and some yield components in

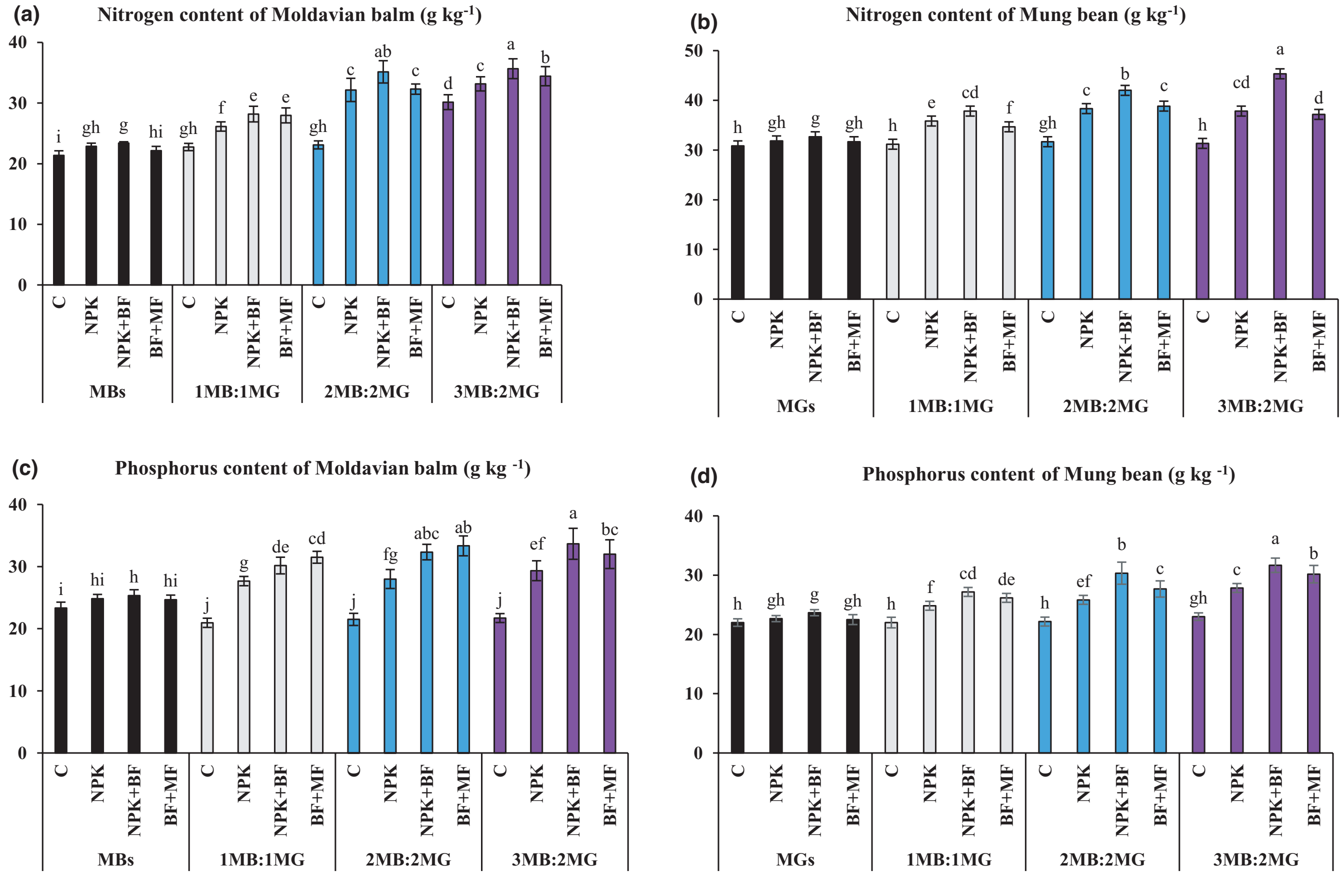

FIGURE 6 Nitrogen and phosphorus contents in Moldavian balm and mung bean under different cropping patterns and fertilizer sources. C (control), NPK (chemical fertilizer), BF (bacterial fertilizer), MF (mycorrhizal fungi), MBs (Moldavian balm sole crop), MGs (mung bean sole crop). 1MB:1M, 2MB:2M, and 3MB:2M indicate the ratios of Moldavian balm (MB) and mung bean (MG) in the intercropping pattern. Same letters above bars show non-significant difference at $p < 0.05$ by Duncan test

both species due to the timely supply of nutrients, confirming our hypothesis that nutrient uptake affects crop productivity. Plant performance and productivity under these conditions could be attributed to N-fixing and P- and K-solubilizing bacteria increasing nutrient availability to plants, which increases growth parameters, such as plant height and photosynthetic rate (Nasar et al., 2021). As hypothesized, different fertilizer sources increased yield in both species. Similarly, in other studies, the integrative application of chemical fertilizer and BF enhanced plant growth characteristics, nutrient uptake, and chlorophyll content in oil palm (*Elaeis guineensis*) (Ajeng et al., 2020), and the application of plant growth-promoting bacteria (PGPR) enhanced seed yield of intercropped common bean and fennel (Rezaei-Chiyaneh, Amirnia, et al., 2020).

4.2 | Essential oil content and yield

As hypothesized, intercropping and application of NPK+BF increased EO productivity in MB. Rehman and Asif Hanif (2016) noted that EO content in MAPs is related to EO gland cell numbers and sizes, which enhance nutrient availability. Moreover, increasing EO productivity in MB intercropping could be related to direct or indirect transition of N fixed by legume species, enhancing plant access to nutrients, especially N, and increasing the EO content (Amani Machiani et al., 2018a). The gradual release of micro- and macronutrients through the integrative application of NPK+BF enhanced nutrient availability in MB and increased EO content in MB by improving plant growth conditions (Amooaghaie & Golmohammadi, 2017). Similarly, Rezaei-Chiyaneh, Amani Machiani, et al., (2020) reported that sweet basil intercropped with common bean had higher EO percentages in the first (27%) and second (32%) harvests than sole cropping; when fertilized with vermicompost, EO productivity in the first and second harvests increased by 14% and 18%, respectively, relative to sole cropping, due to the gradual release of nutrients and increased nutrient availability for plants. In this study, 2MB:2MG fertilized with NPK+BF produced the highest EO yield of MB, which could be due to the higher EO content and herbal yield than other treatments. Therefore, our results confirm that

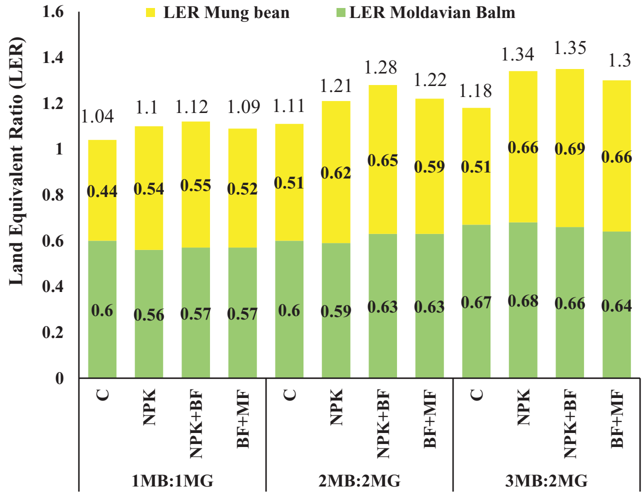

FIGURE 7 Land equivalent ratio (LER) values under different cropping patterns and fertilizer sources. C (control), NPK (chemical fertilizer), BF (bacterial fertilizer), MF (mycorrhizal fungi). 1MB:1M, 2MB:2M, and 3MB:2 M indicate the ratios of Moldavian balm (MB) and mung bean (MG) in the intercropping pattern

fertilized intercropping systems can increase EO quality and production in MB due to increased nutrient uptake efficiency.

4.3 | Essential oil composition

Among other attributes, the qualitative value of MAP plants largely depends on their EO composition and, according to our results, changes in the row ratio of crops could significantly affect the EO compositions and yield quality. The major constituents of Moldavian balm EO were geranyl acetate, geranial, and neral. Intercropping increased the content of these constituents, especially in the 2MB:2MG treatment fertilized with NPK+BF. The increasing EO content with fertilizer application, especially biofertilizer, shows the important role of nutrients, especially nitrogen, in developing and dividing new cells containing EOs, EO channels, and glandular trichomes (Amani Machiani et al., 2018a). The contents of precursor EO compounds, including ATP, acetyl-CoA, and NADPH, also depend on nutrient availability (Ormeno & Fernandez, 2012). The increased and balanced nutrient availability with biofertilizer and chemical fertilizer application enhanced crop resource use efficiency in the intercropping system, with a positive effect on EO composition. Intercropping with MB, especially as 2MB:2MG fertilized with NPK+BF, increased EO composition due to positive interactions with MG. Similarly, Rezaei-Chiyaneh, Amirnia, et al., (2021) reported that black cumin (*Nigella sativa* L.) and fenugreek (*Trigonella foenum-graecum* L.) intercropping with bacteria and mycorrhizal fungi inoculation improved EO content and enhanced EO composition of black cumin, including thymol, p-cymene, geranyl acetate, trans-caryophyllene, and borneol.

4.4 | Land equivalent ratio (LER)

Intercropping significantly decreased the partial productivity of MB and MG, relative to sole cropping. As per our three hypotheses, all intercropping system had values LER >1, indicating that intercropping has advantages over sole cropping. Sole cropping required 4–35% more area to achieve the same yields as intercropping. This advantage is attributed to the increased environmental use efficiency of both species and improved spatial, temporal, and chemical complementarity in intercropping (Raza et al., 2020). A higher LER in intercropping systems has been reported for sweet basil/common bean (Rezaei-Chiyaneh, Amani Machiani, et al., 2020), potato/common bean/lablab (Gitari et al., 2020), fennel/dragonhead/common bean (Amani Machiani et al., 2019), and barley/pea (Chapagain & Riseman, 2014).

5 | CONCLUSION

Integrative biofertilizer and chemical fertilizer application in intercropping systems significantly increased crop growth and development, and high-quality EO content of Moldavian balm, mainly due to enhanced nutrient uptake. Overall, sole cropping produced the highest yields of Moldavian balm and mung bean, while intercropping increased the EO content of Moldavian balm, on average, by 21.66% compared with sole cropping. In addition, intercropping two rows of mung bean + two rows of Moldavian balm (2MB:2MG) fertilized with NPK+BF produced the highest amounts of EO compounds (geranyl acetate, geranial, and neral). The land equivalent ratios in all intercropping patterns, especially those fertilized with NPK+BF, were higher than sole cropping. Thus, we recommend that farmers adopt 2MB:2MG intercropping combined with NPK+BF fertilization as an alternative strategy as an eco-friendly and alternative strategy for increasing EO productivity and composition of Moldavian balm. This is particularly important for small-scale farmers to generate more income, reduce chemical fertilizer use, increase food security, and move toward a more sustainable cropping system.

CONFLICT OF INTEREST

None declared.

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