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Seed Priming *Mellissa officinalis* L. with Potassium Nitrate and Inoculation with Plant Growth Promoting Rhizobacteria at Different Times

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ABSTRACT

Seed priming and seedling inoculation with bioelicitors can enhance growth and phytochemical constituents in medicinal plants. This study was conducted to investigate seed priming with potassium nitrate (at concentrations of 0, 0.25, 0.5, 0.75, and 1%) for various durations, in combination with the inoculation of seedlings derived from seeds primed with rhizobacteria strains (*Pseudomonas fluorescens* and *P. putida*). The treatments were assumed to affect the physiological and metabolic attributes of *Melissa officinalis* L. The findings revealed that the interaction of these treatments had a significant impact on morphological, physiological, photosynthetic pigments, and metabolic traits, including the percentage and composition of essential oils. The most pronounced effects were observed at the 0.25% concentration of potassium nitrate with 72 hours of priming followed by inoculation with *P. putida*. The phenolic content increased to 56.30 mg g-1 of gallic acid per mL of extract in seedlings inoculated with *P. putida* and *P. fluorescens* strains. Maximum antioxidant activity (50.05 mg mL^{-1}) was recorded in seedlings inoculated with *P. fluorescens*, following 12 hours of priming. A notable increase occurred in essential oils (20%) and related compounds, such as carvacrol, thymol, and isomenthone. In conclusion, seed priming and seedling inoculation with Pseudomonas strains had a crucial role in enhancing the primary and secondary metabolites of lemon balm (*Melissa officinalis*) plants.

Introduction

Lemon Balm (*Melissa officinalis* L.) is a perennial medicinal plant belonging to the mint family (Lamiaceae). The essential oils derived from this plant are extensively used in the food, pharmaceutical, and cosmetic industries. The metabolites of lemon balm are also utilized as tranquilizers in the treatment of nerve disorders. However, the seeds of this plant exhibit low vegetative vigor and are challenging to germinate (Omidbaigi, 2010).

The quality of medicinal and aromatic plants is generally assessed based on the quantity of secondary metabolites they produce. According to the literature, various chemical elicitors, bio-stimulants, environmental factors, agronomic techniques, and genetic approaches influence the biosynthetic pathways of secondary metabolites in plant cells (Namdeo, 2007; Rafiee et al., 2016; Yang et al., 2018; Rastegari et al., 2019; Bidabadi and Mehralian, 2020). Investigating the impact of different agronomic practices, including seed

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priming and the application of biofertilizers, on the qualitative performance of plants is considered one of the most critical areas of research in the cultivation of medicinal herbs.

Seed priming has been reported to synchronize seed metabolism by activating specific enzymes, thereby ensuring uniform seedling emergence even under stress conditions. Hydration-based seed priming has demonstrated faster seedling growth compared to non-primed seeds. Seed priming involves hydrating seeds until radicle emergence, followed by drying to return the seeds to their initial moisture level (Lutts et al., 2016). In osmopriming, osmotic solutions with low water potential, such as potassium nitrate (KNO_3), are used to control the amount of water absorbed by the seed (Bewley et al., 2013). KNO_3 is recognized as a chemical agent for breaking seed dormancy according to the International Seed Testing Regulations (International Seed Testing Association, 2018). The positive effect of chemical stimulants like potassium nitrate on seed germination may be attributed to the equilibrium of seed hormonal ratios and the reduction of growth inhibitors such as abscisic acid (ABA) (Pierre-Quatte et al., 2007). During priming with potassium nitrate, the enzyme nitrite reductase delivers nitrate to the embryo, facilitating embryo metabolism and activating certain antioxidant systems (Tulio et al., 2014). Potassium plays a crucial role in various physiological processes, including osmotic regulation, enzymatic activity, cell proliferation, membrane polarity (Elumalai et al., 2002; Kaya et al., 2007), as well as phloem loading and source-to-sink transport (Tohidloo et al., 2018; Mardanluo et al., 2018).

Various bacterial species are vital components of soil ecosystems, contributing to numerous environmental activities. These bacteria participate in nutrient cycling and stabilization, which are essential for crop production (Ahmad et al., 2009; Chandler et al., 2008). They influence plant growth by facilitating nutrient availability in the soil, producing plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, enhancing soil structure, and improving the environmental quality of contaminated soils by separating heavy metals and reducing xenobiotic compounds (Braud et al., 2009; Hayat et al., 2010; Rajkumar et al., 2010; Ahmad and Malik, 2011; Ahemad, 2012). Extensive research has been conducted on beneficial soil bacteria, including the genus *Pseudomonas* (Bakker et al., 2007). Plant growth-promoting rhizobacteria (PGPR) have the potential to maintain ecological stability in soils and enhance plant performance by altering soil physicochemical properties (Ren et al., 2022). PGPRs can be used as biofertilizers to promote sustainable crop production (Lotfi et al., 2022). Furthermore, PGPRs have been widely employed as biotic elicitors to stimulate the production of pharmaceutically active metabolites in various medicinal species, such as *Salvia officinalis* for the elicitation of major volatiles in essential oils (e.g., cis-thujone, camphor, 1,8-cineole) and bioactive molecules (Ghorbanpour et al., 2016), *Tagetes minuta* for the production of monoterpenes and phenolic compounds (Cappellari et al., 2013), and *Pelargonium graveolens* for the accumulation of essential oils and their major constituents (Mishra et al., 2010). Although the potential of PGPR in enhancing plant performance has been extensively studied, there is a lack of information regarding the role of PGPR inoculation in plants raised from primed seeds.

Comprehensive studies on the morphophysiological and metabolic mechanisms underlying the effects of potassium nitrate seed priming on plants, in combination with PGPR treatment, have not yet been fully conducted. Therefore, this study was undertaken to investigate the role of seed priming with potassium nitrate at different durations and the inoculation of seedlings derived from primed seeds with rhizobacteria strains (*Pseudomonas fluorescens* and *P. putida*) on the physiological and metabolic attributes of *Melissa officinalis* L.

Material and Methods

Plant materials and bacteria

Lemon balm seeds were procured from Isfahan Pakan Bazr Company, Iran. The initial seed moisture content was determined to be 5.26% on a dry weight basis, measured by grinding the seeds and drying them at 107 °C for 17 hours.

Fourteen isolates of *Pseudomonas* spp. were obtained from the microbial culture collection unit in the Soil Science Department at ValiAsr University of Rafsanjan, Iran. These isolates were originally collected from the plant rhizosphere of the experimental site in Rafsanjan (30° 24' 24" N and 55° 59' 38" E), a region located in southeastern Iran. Following initial screening, two isolates (*Pseudomonas fluorescens* and *Pseudomonas putida*) exhibiting the most efficient growth-promoting attributes (e.g., phosphorus solubility, auxin, siderophore, and ACC-deaminase production) (Table 1) were selected for seedling inoculation as a post-priming treatment in the greenhouse study (Azarmi et al., 2016). The bacterial strains were

routinely cultured on nutrient agar (NA) medium and preserved in Luria Broth (LB) culture medium containing 15% glycerol at -80 °C for long-term storage.

Table 1. Characteristics of *Pseudomonas fluorescens* and *Pseudomonas putida*.

Strain	Phosphorus solubility			Siderophore production	Auxin production	ACC-deaminase activity
	Halo to colony diameter	Liquid medium (mg L^{-1})	Ph of bacterial suspension	Halo to colony diameter	Liquid medium (mg L^{-1})	
Pseudomonas fluorescens	1.61	498	4.01	3.21	5.15	–
Pseudomonas Putida	2.11	474	3.90	2.21	10.1	+

Plant materials, growth conditions, and experimental design

This research was conducted through two experiments, one in the laboratory and the other in the greenhouse of the Faculty of Agriculture and Natural Resources, University of Lorestan, Iran. The first experiment, carried out in 2019, aimed to study the germination traits of the medicinal plant Lemon Balm (*Melissa officinalis* L.) using a factorial experiment based on a completely randomized design with four replicates. Seed germination and seedling growth were studied *in vitro* under the influence of potassium nitrate treatments at five concentrations (0%, 0.25%, 0.5%, 0.75%, and 1.0%) for varying durations (12, 24, 48, and 72 hours). Healthy, uniform seeds were selected and surface-sterilized using sodium hypochlorite (2.6% active chlorine) for 3 minutes, followed by thorough rinsing with deionized water under aseptic conditions prior to priming. Potassium nitrate treatments were applied by exposing the seeds to the respective concentrations in the dark for the specified time durations. Petri dishes were then placed in a germinator under controlled conditions (22 °C, 70% relative humidity, and a light regime of 16:8 hours) (Ruttanaruangboworn et al., 2017).

The number of germinated seeds was recorded daily for 14 consecutive days. At the end of the 14th day, root and shoot length, germination percentage and rate, mean germination time, seed germination index, and seed vigor index were measured.

The second experiment was conducted in the greenhouse to investigate the growth, physiological, and metabolic responses of Lemon Balm plants to the foliar application of selected rhizobacteria strains (*Pseudomonas fluorescens* and *Pseudomonas putida*), applied either individually (without priming) or in combination with priming treatments. Five primed seeds were sown per pot (25 × 30 cm) filled with a mixture of 8.0 kg of homogenized soil, sand, and farmyard manure (1:1:1 ratio) in a completely randomized design (CRD) with three replications (n = 3) for each treatment. Two uniform seedlings were maintained per pot after 2 weeks of sowing. The pots were randomly arranged under greenhouse conditions at 20 ± 2 °C, with a 16:8 hour light-to-dark photoperiod and 68 ± 4% relative humidity. Soil characteristics (texture: sandy-clay; bulk density: 1.16 g cm-3; pH: 6.5; organic C: 1.5 g kg^{-1}; total N: 326 mg kg^{-1}; available P: 18.1 mg kg^{-1}; available K: 245 mg kg-1; EC: 1.3 dS m^{-1}) were determined before the experiment commenced. The PGPR suspensions were diluted with sterile double-distilled water to achieve a final concentration of approximately 108 CFU mL^{-1} (OD620 = 0.1). The freshly prepared suspensions were applied to the foliage of plants using a hand-held sprayer on two occasions: the first when the Lemon Balm seedlings were four weeks old, and the second two weeks after the initial treatment. Approximately 50 mL of the test suspension was used for each treatment (25 mL per application). The control group received an equal volume of distilled water. No agrochemicals were applied during the entire growth cycle. Pots were irrigated with fresh distilled water to maintain approximately 80% of the soil's total water-holding capacity. All plants were harvested after 3 months of growth, during the flowering stage when the quality and quantity of metabolites are typically high, for the estimation of various parameters.

Germination index characteristics

The germination index characteristics, including shoot length, root length, germination percentage, germination rate, vigor index, seed germination index, and mean germination duration, were calculated using established formulas according to the following.

Equation (1) $GP = 100(NG/NT)$

Equation (2) $RS = \sum \frac{Si}{Di}$

Equation (3) $VI = (Ls \times PG)/100$

Equation (4) $GI = \sum \frac{n}{d}$

Equation (5) $MGT = \sum Dn / \sum n$

Equation (1) represents the germination percentage, where GP denotes the germination percentage, NG is the number of germinated seeds, and NT is the total number of seeds (Maguire, 1962).
Equation (2) calculates the germination rate, where RS represents the germination rate, S is the number of germinated seeds on the i-th day, and Di is the number of days after the start of the experiment (Jalili Marandi, 2008).
Equation (3) is used to determine the seed vigor index (VI), where VI represents the vigor index and LS is the length of the seedling (Abdulbaki and Anderson, 1973).
Equation (4) calculates the seed germination index, where n indicates the number of germinated seeds and d represents the number of days since the beginning of germination.
Equation (5) is used to determine the mean germination time (MGT), where MGT is the mean germination time (in days), n is the number of seeds that have germinated on day D, and D is the number of days since the beginning of germination (Salehzade et al., 2009).

Morphological traits

To assess morphological traits, we measured plant height, the number of main branches and sub-branches, and the fresh weight of the shoots and roots using a digital scale with an accuracy of 0.01 g. To determine the dry weight of the shoots and roots, collected samples were placed in a shaded, well-ventilated room at a controlled temperature for one week. Once fully dried, the dry weight of the shoots and roots was measured.

Measurement of relative water content (RWC)

To determine the relative water content of randomly collected leaves, fresh weight was measured immediately. The leaves were then submerged in distilled water for 24 hours at room temperature. After 24 hours, the saturated weight of the leaves was recorded by gently blotting them dry with tissue paper. Finally, the leaves were dried in an oven at 75 °C for 48 hours, after which their dry weight was measured. The relative water content of the leaves was calculated using the following formula (Ferrat et al., 1999):

$$RWC = \frac{FW-DW}{TW-DW} \times 100$$

Where FW is the fresh weight, DW is dry weight, and TW is the turgescence weight of the leaf.

Measurement of electrolyte leakage

To measure electrolyte leakage, 0.5 g of the leaves of each pot (each replicate) were prepared in a circular disc. The samples were stored in distilled water for 24 h at room temperature. After 24 h, the electrical conductivity of each sample was measured using an EC meter (Lutron) (EC_1). To measure the total electrolyte leakage due to cell death, Falcon tubes were placed in a Ben Murray machine at 95 °C for 90 min. After cooling the tubes, the electrical conductivity of each sample was measured again (EC2). Finally, the electrolyte leakage (%) was calculated using the following equation (Karlidag et al., 2009):

$$\text{Electrolyte leakage} = \frac{EC1}{EC2} \times 100$$

Photosynthetic pigments (chlorophyll and carotenoids)

The photosynthetic pigments were extracted according to the Lichtenthaler method (1987). Absorptions at 663, 645, and 480 nm wavelengths were read by a spectrophotometer and relevant pigments were measured using the following formulas (Lichtenthaler, 1987):

$$\text{Chl a (mg mL}^{-1}) = [(12.25\ A663) - (2.79\ A646)] \times V/W$$

$$\text{Chl b (mg mL}^{-1}) = [(21.51\ A646) - (5.1\ A663)] \times V/W$$

$$\text{Chl T (mg mL}^{-1}) = [\text{Chl a} + \text{chl b}] \times V/W$$

$$\text{Car(mg mL}^{-1}) = [(1000\ A470 - 1.8\ \text{chl a} - 85.02\ \text{chlb})/198] \times V/W$$

A = absorption wavelength spectrophotometer, Chl = chlorophyll, Car = carotenoid

Preparation of alcoholic extract

One g of the dried leaf sample was completely powdered and placed in an ultrasonic bath with 10 mL of 80% methanol for 30 min. Then, the samples were centrifuged for 5 min at 14000 rpm. The supernatant was separated and the extract was stored in a dark glass (impermeable to air and light) and placed in a refrigerator at 4 °C (Chang et al., 2002).

Total flavonoid contents

The aluminum chloride colorimetric method was employed to quantify the flavonoid content in the samples (Hang et al., 2002). Specifically, 2 mL of the diluted plant extract was mixed with 2 mL of

2% aluminum chloride solution in a test tube. The mixture was then vortexed at room temperature for 15 minutes. The absorbance of the resulting compound was measured at a wavelength of 415 nm using a spectrophotometer. A standard curve was generated using quercetin solutions ($y = 0.023x + 0.4323$, $R^2 = 0.97$).

Total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu reagent. A 400 µL aliquot of the diluted extract was combined with 2 mL of Folin-Ciocalteu reagent (diluted 1:10) and then mixed with an aqueous solution of sodium carbonate (Na_2CO_3) at a concentration of 75 g L^{-1}. The mixture was allowed to react at room temperature for 30 minutes. The absorbance was measured at 765 nm using a spectrophotometer against a blank. A standard curve was established using various concentrations of gallic acid in methanol ($R^2 = 0.99$, $y = 0.0064x + 0.0984$). The results were expressed in milligrams of gallic acid equivalent per gram of dry weight (Singleton et al., 1965).

Antioxidant activity by DPPH

The antioxidant activity of the sample extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method as described by Burits and Bucar (2000). Briefly, 2 mL of the methanolic extract was mixed with 1 mL of 0.2 mM DPPH methanolic solution. The absorbance was measured at 517 nm against a blank. The percentage of inhibition by the DPPH free radical was calculated to evaluate the antioxidant activity according to the following equation:

$$R\% = AC\text{-}AS/AC \times 100$$

Where, AC is the absorbance value of the blank (at t = 0 min), and AS is the absorbance of the test solution (at t = 30 min). The IC50 concentration is the extract volume (µg mL^{-1}) that provides 50% inhibition of the DPPH free radicals (Dapkevicius et al., 1998).

Gas chromatography/mass spectrometry (GC/MS) analysis

The analysis of essential oil constituents was conducted using an Agilent 6890 Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 µm) and a flame ionization detector (FID). The column temperature was initially set at 60 °C and then gradually increased to 120 °C at a rate of 3 °C min-1. Subsequently, the temperature was further raised to 300 °C at a rate of 15 °C min-1 and held at 300 °C for 5 minutes. The gas chromatograph was coupled with an HP 5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA). The mass spectrometer was equipped with an ion-trap analyzer, operating at a scan rate of 1508 amu s-1 with an electron multiplier voltage of 1350 V. Scanning was performed at an ionization voltage of 70 eV over the mass range of 40 to 550 m/z. The total runtime for each sample was approximately 72 minutes.

The constituents of the essential oil samples were identified by comparing their mass spectra with those stored in the Wiley NBS75 K.L. library and obtained from the National Institute of Standards and Technology (NIST), based on retention indices (RI) relative to n-alkanes (C5-C24) under the same chromatographic conditions. For quantification, normalized peak area abundances were calculated without applying correction factors (Adams, 2001).

Statistical analysis

Statistical analysis was performed using SAS software version 9.1. Duncan's multiple range test was employed to compare mean values, while charts were generated using Microsoft Excel 2013.

Results

Effect of priming with potassium nitrate on germination indices

Based on the results of analysis of variance (Table 2), the effects of priming with potassium nitrate were highly significant on germination percentage, germination rate, seedling vigor index, and mean root length ($p \leq 0.01$). Also, the effects were significant on shoot length ($p \leq 0.05$). According to Table 3, the highest germination percentage was related to the interaction effect of 0.25% potassium nitrate concentration with 12 h of priming (56.25%), and the lowest value was related to the 1% potassium nitrate concentration with 12 h of priming (30%). Also, the highest germination rate related to the control treatment and 72 h of priming, and the concentration of 1% potassium nitrate in 48 h of priming caused the lowest germination rate.

The highest seed germination index related to the interaction of potassium nitrate with a concentration of 0.25% and in 12 h of priming. The lowest value of this trait was obtained when using a concentration of 0.75% of potassium nitrate with 12 h of priming.

Table 2. Analysis of variance of germination traits of lemon balm with seed priming by potassium nitrate at different concentrations and times.

S.O. V	df	Mean Squares (MS)						
		Germination percentage	Germination rate	Mean germination time	Seed germination index	Vigor index	Shoot length	Root length
T	3	268.69^{**}	6.27^{**}	2.12^{**}	46.56^{**}	0.32^{**}	0.33^{**}	1.42^{**}
K	4	212.93^{**}	2.39^{**}	0.73^{ns}	39.6^{**}	0.26^{**}	0.182^{**}	0.015^{ns}
K × T	12	150.24^{**}	1.18^{**}	0.4^{ns}	10.25^{ns}	0.22^{**}	0.06^{*}	0.144^{**}
error		64.73	0.25	0.44	8.95	0.07	0.03	0.041
c.v (%)		18.76	14.99	19.85	20.4	19.36	10.40	13.10

**: Significantly at 1% level, *: Significantly at the level of 5%, and ^{ns}: no statistical significance. T: priming with potassium nitrate at different times, K: Different concentrations of potassium nitrate.

Table 3. Mean comparisons of germination traits of lemon balm with seed priming by potassium nitrate at different concentrations and times.

Time	KNO_3	Germination percentage (%)	Germination rate (Seed per d)	Vigor index	Shoot length (cm)	Root length (cm)
12	0	40^{b-e}	3.34^{c-e}	1.30^{b-f}	1.47^{e-g}	1.73^{b-d}
	0.25	56.25^{a}	3.57^{b-d}	1.99^{a}	1.45^{e-g}	2.1^{a}
	0.5	43.75^{a-e}	4.23^{a-c}	1.60^{a-e}	1.7^{a-f}	1.66^{b-e}
	0.75	43.33^{a-e}	4.36^{ab}	1.04^{f}	1.52^{c-g}	1.92^{ab}
	1	30^{e}	3.89^{a-d}	1.71^{a-c}	1.49^{d-g}	2.13^{a}
24	0	35^{de}	2.15^{f}	1.19^{c-f}	1.75^{a-e}	1.66^{b-e}
	0.25	46.25^{a-d}	3.37^{c-e}	1.61^{a-d}	1.94^{a}	1.29^{e-g}
	0.5	45^{a-e}	3.53^{b-e}	1.27^{b-e}	1.84^{a-c}	1.62^{b-f}
	0.75	36.25^{c-e}	3.05^{d-f}	1.33^{b-f}	1.81^{a-d}	1.45^{c-g}
	1	33.33^{de}	2.61^{ef}	1.08^{ef}	1.78^{a-e}	1.41^{d-g}
48	0	46.66^{a-d}	2.15^{f}	1.38^{b-f}	1.4^{fg}	1.45^{c-g}
	0.25	45^{a-e}	3.53^{b-e}	1.50^{a-f}	1.59^{b-g}	1.78^{a-c}
	0.5	53.33^{ab}	3.14^{de}	1.76^{ab}	1.63^{a-f}	1.62^{b-f}
	0.75	51.66^{a-c}	3.02d^{ef}	1.61^{a-d}	1.67^{a-f}	1.36^{d-g}
	1	42.83^{a-e}	2.18^{f}	1.23^{c-f}	1.72^{a-f}	1.3^{d-g}
72	0	48.33^{a-d}	4.62^{a}	1.28^{b-f}	1.28^{g}	1.24^{fg}
	0.25	38.75^{b-e}	4.21^{a-c}	1.12^{d-f}	1.78^{a-e}	1.12^{g}
	0.5	41.66^{a-e}	4.20^{a-c}	1.47^{b-f}	1.72^{a-f}	1.33^{e-g}
	0.75	36.25^{c-e}	3.17^{de}	1.18^{d-f}	1.89^{ab}	1.27^{e-g}
	1	43.75^{a-e}	2.79^{ef}	1.40^{b-f}	1.83^{a-c}	1.36^{d-g}

Non-identical letters indicate a significant difference at the level of 5% among the means based on Duncan's test.

Morphological traits

The results of analysis of variance showed that the interaction of priming with potassium nitrate at different concentrations and times, along with inoculation of plant growth-promoting rhizobacteria, marked a significant effect on all morphological traits estimated in this experiment, except plant height ($p \leq 0.01$). This contrast also showed a significant effect on shoot fresh weight ($p \leq 0.05$) (Table 4).

The mean comparisons revealed that the interaction of *Pseudomonas fluorescens* bacteria with 1% potassium nitrate concentration and 24 hours of priming significantly increased the number of main stems (32.5) compared to the control (no potassium nitrate), where 12 and 48 hours of priming with *Pseudomonas putida* inoculation resulted in the statistically lowest number of main stems (10.5). A similarly significant increase in the number of side stems (22) was observed with the interaction of priming with potassium nitrate at different concentrations compared to the control (no potassium nitrate) with 12 hours of priming and

P. fluorescens inoculation. The control (no potassium nitrate) with 48 hours of priming with *P. putida* showed the highest number of side stems (3.5), which was the lowest among the traits measured (Table 5).

Table 4. Analysis of variance of morphological traits influenced by priming with potassium nitrate at different concentrations and times with inoculation of *Pseudomonas fluorescens* and *Pseudomonas putida* on lemon balm.

		Mean Squares (MS)						
S.O. V	df	Bush height	Number of main stems	Number of side stems	Root fresh weight	Root dry weight	Stem fresh weight	Stem dry weight
T	3	3.84^{ns}	90.2**	54.03*	1974.35**	54.50**	577.0**	89.73**
K	4	57.64**	280.08**	44.11**	445.11**	33.85**	227.66^{ns}	65.96**
B	2	26.86^{ns}	82.70**	55.26**	5307.74**	155.87**	1244.31**	122.10**
T × K	4	61.53**	93.94**	11.78*	2232.97**	78.97**	673.18**	142.26**
T × B	6	14.99^{ns}	90.70**	79.72**	2137.85**	62.07**	54.79**	66.86**
K × B	4	12.90^{ns}	42.21**	58.63**	1660.64**	3.99^{ns}	99.83^{ns}	71.32**
K × T × B	4	16.59^{ns}	64.77**	109.25**	2843.73**	56.38**	552.0**	98.57**
Test error		16.17	5.81	4.04	48.35	2.34	155.64	3.37
Coefficient of variation c.v (%)		18.55	12.19	21.81	11.96	18.08	28.42	14.39

**: Significantly at 1% level, *: Significantly at the level of 5%, and ^{ns}: no statistical significance. T: priming with potassium nitrate at different times, K: Different concentrations of potassium nitrate. B: *Pseudomonas fluorescens* and *Pseudomonas putida*.

The interaction of priming with different concentrations of potassium nitrate and varying durations, along with the inoculation of plant growth-promoting rhizobacteria, revealed that the highest root fresh weight (144.43 g) was observed in the control (no potassium nitrate) after 24 hours of priming with *Pseudomonas putida*. In contrast, the lowest root fresh weight (9.68 g) was recorded with 0.5% potassium nitrate concentration after 12 hours of priming with *Pseudomonas fluorescens*.

For root dry weight, the interaction of priming with varying potassium nitrate concentrations and times, along with rhizobacterial inoculation, showed that the control (no potassium nitrate) after 24 hours of priming with *P. putida* inoculation had the highest dry weight. There was no statistically significant difference when 1% potassium nitrate was used at the same priming duration with *P. putida*. However, the control (no potassium nitrate) after 24 hours of priming with *P. fluorescens* inoculation resulted in the lowest root dry weight (Table 5).

Stem fresh and dry weight

As shown in the mean squares (Table 5), the highest fresh stem weight (80.33 g) was found in the control (no potassium nitrate) after 24 hours of priming with *P. putida*, and also in the control after 48 hours of priming with the same bacterium. Additionally, the mean squares (Table 5) indicated that the highest stem dry weight (28.58 g) occurred with 0.25% potassium nitrate and *P. putida* inoculation after 72 hours of priming. The lowest stem dry weight (4.14 g) was observed in the control (no potassium nitrate) after 24 hours of priming with *P. fluorescens* inoculation.

Physiological traits

In this study, the chlorophyll index was measured two months and four months after culture, along with photosynthetic pigments, electrolyte leakage, and relative water content. The results of the analysis of variance and mean squares for these traits are presented in Tables 6 and 7. The analysis of variance table indicated that photosynthetic pigments and the chlorophyll index, four months after culture, were significantly affected (p≤0.01) by the interaction of priming with different concentrations of potassium nitrate and varying durations, along with the inoculation of plant growth-promoting rhizobacteria.

Table 5. Mean comparisons of morphological traits influenced by priming with potassium nitrate at different concentrations and times with inoculation of *Pseudomonas fluorescens* and *Pseudomonas putida* of lemon balm.

Time	KNO_3	Inoculation with plant growth promoting rhizobacteria	Number of main stems	Number of side stems	Stem fresh weight (g)	Stem dry weight (g)	Root fresh weight (g)	Root dry weight (g)
12	0	Control	21.63^{d-g}	7.27^{f-j}	38.17^{d-h}	15.46^{cd-f}	49.61^{f-h}	8.76^{c-f}
		P. putida	10.50^{i}	6^{h-j}	26.52^{f-h}	7.77^{k-m}	22.52^{i}	4.05^{ij}
		P. fluorescens	14.60^{j-l}	22^{a}	26.91^{f-h}	5.56^{mn}	41.57^{h}	6.01^{f-j}
	0.5	*P. putida*	22^{jkl}	12.5^{cd}	45.23^{c-h}	18.66^{c}	75.34^{d}	8.17^{c-g}
		P. fluorescens	18.25^{hi}	6.75^{f-j}	36.99^{d-h}	9.30^{i-l}	9.68^{j}	8.7^{c-g}
	1	*P. putida*	26.66^{bc}	12.5^{cd}	45.34^{c-h}	11.49^{g-j}	59.20^{ef}	7.06^{d-h}
		P. fluorescens	21.50^{d-g}	9.25^{d-h}	43.04^{c-h}	13^{e-h}	45.41^{gh}	6.83^{e-i}
24	0	Control	15^{i-k}	10.25^{d-f}	35.83^{d-h}	9.16^{i-l}	54.96^{f-h}	8.99^{c-e}
		P. putida	16^{h-k}	17.5^{b}	80.33^{a}	23.21^{b}	144.43^{a}	21.49^{a}
		P. fluorescens	13.25^{j-l}	6.50^{f-j}	34.28^{d-h}	4.31^{b}	27.05^{i}	3.29^{j}
	0.25	*P. putida*	16.83^{h-j}	12.50^{cd}	51.72^{b-f}	10.67^{h-k}	54.95^{f-h}	8.08^{c-g}
		P. fluorescens	17.44^{gh-j}	5.05^{ij}	34.67^{d-h}	9.33^{i-l}	49.40^{f-h}	4.43^{h-j}
	1	*P. putida*	23^{c-e}	7^{fg-j}	57.76^{a-d}	12.45^{f-i}	92.66^{b}	20.8^{a}
		P. fluorescens	32.5^{a}	7^{fg-j}	51.09^{b-h}	17.58^{cd}	69.62^{de}	8.78^{d-f}
48	0	Control	23^{c-e}	8.50^{e-i}	28.56^{fgh}	10.56^{h-k}	55.80^{fg}	4.33^{h-j}
		P. putida	11.06^{i}	3.50^{j}	23.38^{h}	7.031^{mn}	29.29^{i}	4.37^{h-j}
		P. fluorescens	13.16^{j-l}	8.50^{e-i}	42.33^{c-h}	10.26^{h-l}	43.65^{gh}	5.97^{f-j}
	0.25	*P. putida*	12^{kl}	10^{de-g}	44.64^{c-h}	10.53^{h-k}	49.21^{f-h}	9.92^{cd}
		P. fluorescens	17.55^{g-j}	5.10^{ij}	43.52^{c-h}	12.23^{f-j}	59.49^{ef}	5.63^{c}
	0.75	*P. putida*	25.5^{b-d}	5.75^{h-j}	56.56^{b-e}	16.45^{cd}	70.97^{de}	13.93^{b}
		P. fluorescens	22.5^{c-f}	14.25^{bc}	40.28^{c-h}	14.90^{d-f}	69.64^{de}	6.69^{e-i}
72	0	Control	20.16^{e-h}	6.50^{fg-j}	50.14^{b-g}	16.17^{c-e}	76.87^{cd}	10.4^{c}
		P. putida	24.50^{b-e}	9.50^{d-h}	64.45^{a-c}	17.75^{cd}	87.46^{bc}	10.12^{c}
		P. fluorescens	25.75^{b-d}	5^{ij}	40.92^{c-h}	10.63^{h-k}	46.78^{f-h}	6.45^{e-i}
	0.25	*P. putida*	28^{b}	11.5^{cde}	71.98^{ab}	28.58^{a}	96.62^{b}	14.3^{b}
		P. fluorescens	18^{fg-i}	6.27^{g-j}	39.09^{c-h}	14.67^{d-g}	49.62^{f-h}	8.49^{c-g}
	0.75	*P. putida*	28.5^{b}	14.5^{bc}	43.06^{cd-h}	10.87^{h-k}	50.78^{f-h}	6^{fg-j}
		P. fluorescens	15.04^{i-l}	6.99^{g-j}	32.09^{e-h}	8.84^{j-l}	45.15^{f-h}	4.98^{h-j}

Non-identical letters indicated a significant difference ($p \leq 0.05$) among the means based on Duncan's test.

According to the mean comparisons table, the highest chlorophyll index (39.95% SPAD readings) was observed with the interaction of 0.75% potassium nitrate and 48 hours of priming with *Pseudomonas putida* bacteria, measured four months after culture. No statistically significant difference was observed compared to the control (no potassium nitrate) with 72 hours of priming with this bacterium. Conversely, the lowest chlorophyll index was recorded in the control with *P. putida* bacteria during 48 hours of priming. There was no statistically significant difference when using 1% potassium nitrate with 24 hours of priming with *P. putida* bacteria.

Chlorophyll a

The interaction of priming with various concentrations of potassium nitrate and different durations, combined with inoculation of plant growth-promoting rhizobacteria, showed that 0.25% potassium nitrate with 72 hours of priming and *P. putida* bacteria yielded the highest chlorophyll a value (440.7 µg g-1 leaf fresh weight). In contrast, 0.5% potassium nitrate with

12 hours of priming and *Pseudomonas fluorescens* bacteria resulted in the lowest chlorophyll a content (Table 7).

Table 6. Analysis of variance of physiological traits under the influence of priming with potassium nitrate at different concentrations and durations with inoculation of Pseudomonas fluorescens and Pseudomonas putida of lemon balm.

		Mean Squares (MS)							
S.O. V	df	Chlorophyll index (two months after culture)	Chlorophyll index (four months after culture)	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids	Electrolyte leakage	Relative water content
T	3	29.25^{ns}	31.40**	19307.35**	239.9^{ns}	29022.58**	20316.54**	108.56^{ns}	158.98^{ns}
K	4	43.70^{ns}	16.77*	3177.51*	441.86*	5974.99**	8689.98**	32.30^{ns}	101.96^{ns}
B	2	78.81*	14.66^{ns}	25995.47**	1324.18**	38409.16**	14466.06**	14.34^{ns}	5.92^{ns}
T × k	4	20.28^{ns}	55.70**	47587.07**	2061.39**	77367.62**	6176.72**	101.62^{ns}	162.28^{ns}
T × B	6	29.53^{ns}	5.88^{ns}	26205.09**	1154.63**	3944.07**	1514.35**	92.82^{ns}	67.73^{ns}
k × B	4	23.62^{ns}	7.04^{ns}	32899.43**	2382.05**	50852.6**	12005.68**	109.29^{ns}	167.194^{ns}
k × B × T	4	20.52^{ns}	41.77**	34985.43**	4357.42**	60743.5**	11786.61**	49.75^{ns}	149.91^{ns}
Test error		23.84	6.54	966.06	134.13	1504.76	511.04	61.35	100.78
Coefficient of variation c.v (%)		12.69	7.63	21.9	23.23	19.56	14.92	8.72	16.39

**: Significantly at 1% level, *: Significantly at the level of 5%, and ^{ns}: no statistical significance. T: priming with potassium nitrate at different times, K: Different concentrations of potassium nitrate, B: *Pseudomonas fluorescens* and *Pseudomonas putida*.

Chlorophyll b

According to the mean comparisons in Table 7, the highest chlorophyll b content (11.114 µg g-1 leaf fresh weight) was observed with 0.25% potassium nitrate during 72 hours of priming with *P. putida* bacteria. Conversely, 0.75% potassium nitrate with 48 hours of priming resulted in the lowest chlorophyll b content (8.79 µg g-1 leaf fresh weight) when using *P. fluorescens* bacteria and 1% potassium nitrate. The highest total chlorophyll content (554.82 µg g-1 leaf fresh weight) was found with 0.25% potassium nitrate during 72 hours of priming with *P. putida* bacteria, while the lowest total chlorophyll content (35.07 µg g-1 leaf fresh weight) was recorded with 12 hours of priming with *P. fluorescens* bacteria at 0.5% and 1% potassium nitrate.

Carotenoids

Table 7 indicates that the highest carotenoid content (226.2 µg g-1 leaf fresh weight) was observed with 0.25% potassium nitrate during 12 hours of priming in the control (non-inoculated). This value showed no significant difference compared to *P. fluorescens* bacteria during 48 hours of priming with 0.75% and 0.25% potassium nitrate. A less significant difference was noted with the control (no bacterial inoculation and no potassium nitrate) during 24 hours of priming. The minimum carotenoid content (26.31 µg g-1 leaf fresh weight) was observed with 1% potassium nitrate during 12 hours of priming with *P. fluorescens* bacteria.

Metabolic traits

In this study, metabolic traits such as phenols, flavonoids, and antioxidant activity were measured. The results of the variance analysis (Table 8) indicated that the interaction of priming with different concentrations of potassium nitrate and varying times, combined with inoculation of plant growth-promoting rhizobacteria, had a significant effect on flavonoids and phenols ($p \leq 0.01$). Additionally, plant growth-promoting rhizobacteria significantly influenced antioxidant activity ($p \leq 0.01$).

Table 7. Mean comparisons of physiological traits under the influence of priming with potassium nitrate at different concentrations and durations with inoculation of *Pseudomonas fluorescens* and *Pseudomonas putida* of lemon balm.

Time	KNO_3	Inoculation Plant Growth Promoting Rhizobacteria	Chlorophyll a (µgn g^{-1} LFW)	Chlorophyll b (µg g^{-1} LFW)	Total chlorophyll (µg g^{-1} LFW)	Carotenoid (µg g^{-1} LFW)	Chlorophyll index (four months after culture
12	0	Control	256.29^{c-e}	74.94^{bc}	331.24^{cd}	154.49^{d-h}	32.63^{c-g}
		P. putida	60.85^{l-n}	19.76^{j-m}	88.41^{l-o}	42.53^{ij}	31.20^{d-g}
		P. fluorescens	47.45^{mn}	29.53^{i-m}	70.32^{l-o}	124.14^{gh}	33.03^{b-f}
	0.5	*P. putida*	112.4^{jkl}	65.12^{b-d}	233.93^{e-h}	182.58^{a-f}	36.1^{a-d}
		P. fluorescens	24.74^{n}	11.22^{lm}	35.96^{o}	79.04^{i}	33.3^{b-f}
	1	*P. putida*	187.28^{f-h}	56.77^{b-g}	244.06^{e-h}	54.84^{ij}	37.96^{ab}
		P. fluorescens	26.27^{mn}	8.79^{m}	35.07^{o}	26.31^{j}	35.3^{a-e}
24	0	Control	320.9^{b}	75.22^{bc}	408.47^{b}	216.3^{a}	32.33^{c-h}
		P. putida	172.06^{f-i}	63.94^{b-e}	238.28^{e-h}	209.31^{ab}	35.26^{a-e}
		P. fluorescens	79.26^{k-n}	41.9^{e-i}	139.35^{i-l}	118.42^{h}	30.30^{e-g}
	0.25	*P. putida*	210.43^{d-f}	56.07^{b-g}	272.83^{de}	187.97^{a-e}	31.8^{c-g}
		P. fluorescens	57.73^{lmn}	40.88^{f-j}	178.35^{g-k}	161.85^{c-g}	30.5^{e-g}
	1	*P. putida*	38.74^{mn}	19.91^{j-m}	56.45^{m-o}	118.58^{h}	27.96^{g}
		P. fluorescens	165.07^{f-j}	69.42^{b-d}	226.80^{e-h}	203.46^{a-c}	34^{b-f}
48	0	Control	70.44^{k-n}	41.17^{fgh}	111.62^{k-n}	198.84^{a-c}	31.43^{c-g}
		P. putida	160.4^{f-j}	52.19^{d-h}	251.62^{e-g}	153.18^{e-h}	27.7^{g}
		P. fluorescens	44.09^{mn}	14.83^{lm}	46.79^{no}	62.38^{ij}	30.1^{fg}
	0.25	*P. putida*	57.82^{l-n}	32.49^{h-l}	82.85^{l-o}	162.36^{c-g}	33^{b-f}
		P. fluorescens	85.54^{k-m}	75.92^{b}	127.98^{j-m}	214.81^{a}	33.3^{b-f}
	0.75	*P. putida*	120.67^{i-k}	50.29^{d-i}	170.96^{h-k}	142.52^{f-h}	39.95^{a}
		P. fluorescens	281.52^{bc}	100.4^{a}	381.92^{bc}	211.87^{a}	34.33^{b-f}
72	0	Control	148.54^{g-j}	48.05^{d-h}	196.60^{fg-j}	196.89^{a-d}	34.13^{b-f}
		P. putida	203.01^{e-g}	53.38^{c-h}	267.38^{d-f}	166.93^{b-f}	39.55^{a}
		P. fluorescens	260.42^{cd}	62.88^{b-f}	333.44^{cd}	177.85^{a-f}	33.33^{b-f}
	0.25	*P. putida*	440.7^{a}	114.11^{a}	554.82^{a}	216.22^{a}	36.53^{a-c}
		P. fluorescens	157.99^{fg-j}	37.62^{g-k}	211.48^{f-h}	182.43^{a-f}	33.65^{b-f}
	0.75	*P. putida*	39.74^{mn}	17.64^{c-m}	52.54^{no}	71.53^{i}	34.46^{b-f}
		P. fluorescens	141.73^{h-j}	61.04^{b-f}	202.78^{e-i}	204.05^{a-c}	34.4^{b-f}

Non-identical letters indicate a significant difference at the level of 5% among the means based on Duncan's test.

Table 8. Analysis of variance of metabolic traits under the influence of priming with potassium nitrate at different concentrations and durations with inoculation of *Pseudomonas fluorescens* and *Pseudomonas putida* of lemon balm.

		Mean Squares		
S.O. V	**df**	**Phenol**	**Flavonoids**	**Antioxidants**
T	3	878.72**	3.06**	33.43^{ns}
K	4	361.81**	7.97**	66.24^{ns}
B	2	590.12**	10.67**	1024.68**
T × k	4	147.63^{ns}	1.91**	20.59^{ns}
T × B	6	290.15**	1.54**	22.37^{ns}
k × B	4	168.55^{ns}	0.82**	98.70^{ns}
k × B × T	4	123.93^{ns}	4.80**	50.78^{ns}
Test error		79.60	0.16	125.18
Coefficient of variation c.v (%)		23.47	22.94	24.93

**: Significantly at 1% level, *: Significantly at the level of 5%, and ^{ns}: no statistical significance. T: priming with potassium nitrate at different times, k: Different concentrations of potassium nitrate, B: *Pseudomonas fluorescens* and *Pseudomonas putida*.

The effect of interaction between priming with potassium nitrate at different times and inoculation with plant growth-promoting rhizobacteria was studied on the phenol quantity of the herbal medicinal plant of lemon balm. The *P. Fluorescence* and *P. Putida* bacteria for 12 h priming had the highest amount of this trait (56.31 mg GAE g^{-1} DW) as compared to other treatments that showed non-significant differences in this contrast (Fig. 1).

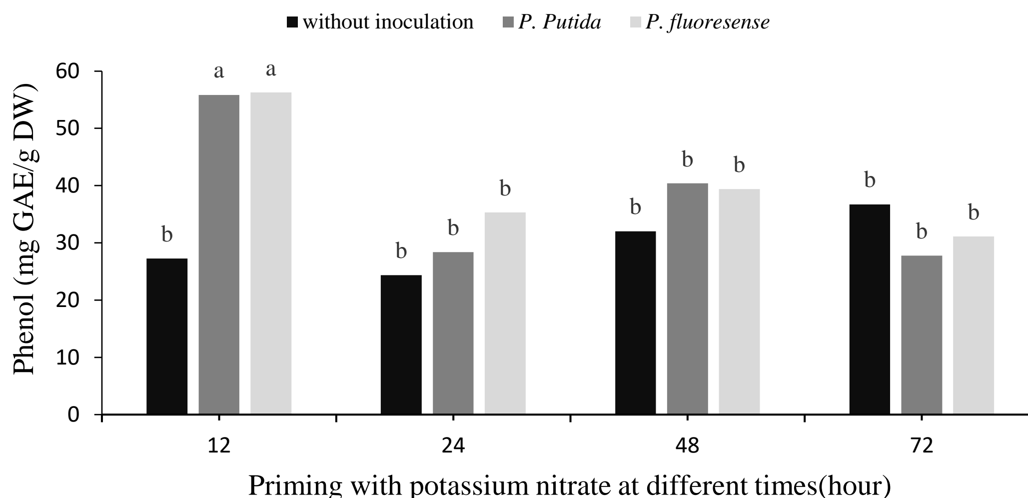

Fig. 1. Interaction of priming with potassium nitrate at different times with inoculation of plant growth-promoting rhizobacteria on the phenol content of lemon balm. Non-identical letters indicate a significant difference of 1% between the values based on Duncan's test.

Figure 2 illustrates the interaction of three priming treatments with potassium nitrate at varying concentrations and durations, combined with inoculation of plant growth-promoting rhizobacteria, on flavonoid content. The control (no bacterial inoculation and no potassium nitrate) during 48 hours of priming yielded the highest flavonoid content (4.24 mg quercetin g-1 DW). The lowest flavonoid content was observed with *Pseudomonas putida* after 12 hours of priming in the control (no potassium nitrate). Additionally, the control (no inoculation and no potassium nitrate) with *Pseudomonas fluorescens* did not show a significant difference with potassium nitrate concentration during 12 hours of priming.

The maximum antioxidant activity was observed with inoculation by *P. fluorescence* bacteria (50.05 µg mL^{-1}) as compared to *P. Putida* inoculation and the control (Fig. 3).

According to the interaction between priming with different concentrations of potassium nitrate and the duration of priming with inoculation of plant growth-promoting rhizobacteria on the percentage of essential oil (Fig. 4), the maximum essential oil percentage (0.2%) was observed in the control group (no bacterial inoculation and no potassium nitrate) after 72 hours of priming. The lowest percentage (0.04%) was recorded at a 1% concentration of potassium nitrate after 24 hours of priming with inoculation of *Pseudomonas putida* bacteria. Priming with 1% potassium nitrate for 12 hours with *P. putida* bacteria inoculum, as well as the control (without potassium nitrate) for 12 hours, did not yield statistically significant results. Overall, enhanced percentages of essential oils were obtained in treatments involving plant growth-promoting rhizobacteria inoculum compared to treatments with only potassium nitrate. However, when comparing the efficacy of the two rhizobacteria inoculums, *Pseudomonas fluorescens* showed better results than *P. putida* and the control group.

The analysis of essential oil in lemon balm was done using GC/MS. A total of 15 essential oil compounds were isolated, and all essential oil samples mainly contained oxygenated monoterpenes, including neral (citral), geranium, thymol, and then geranyl acetate and trans caryophyllene. These compounds accounted for approximately 84.4 – 94% of the total essential oil (Table 9). According to the data presented in Table 9, as a result of *P. putida* inoculation, the amount of carvacrol, thymol, geranyl acetate,

trans-caryophyllene and caryophyllene oxide in essential oil compounds increased, and *P. fluorescens* inoculation increased the amount of linalool, thymol, carvacrol, and geranyl acetate. All treatments caused the appearance of E-isocitral in the essential oil components, and as a result of the inoculation treatment with *P. fluorescence* and priming by nitrate, acetate1,8-cineol was added to the essential oil compounds. Also, the amounts of neral and geranial decreased in all treatment groups compared to the control.

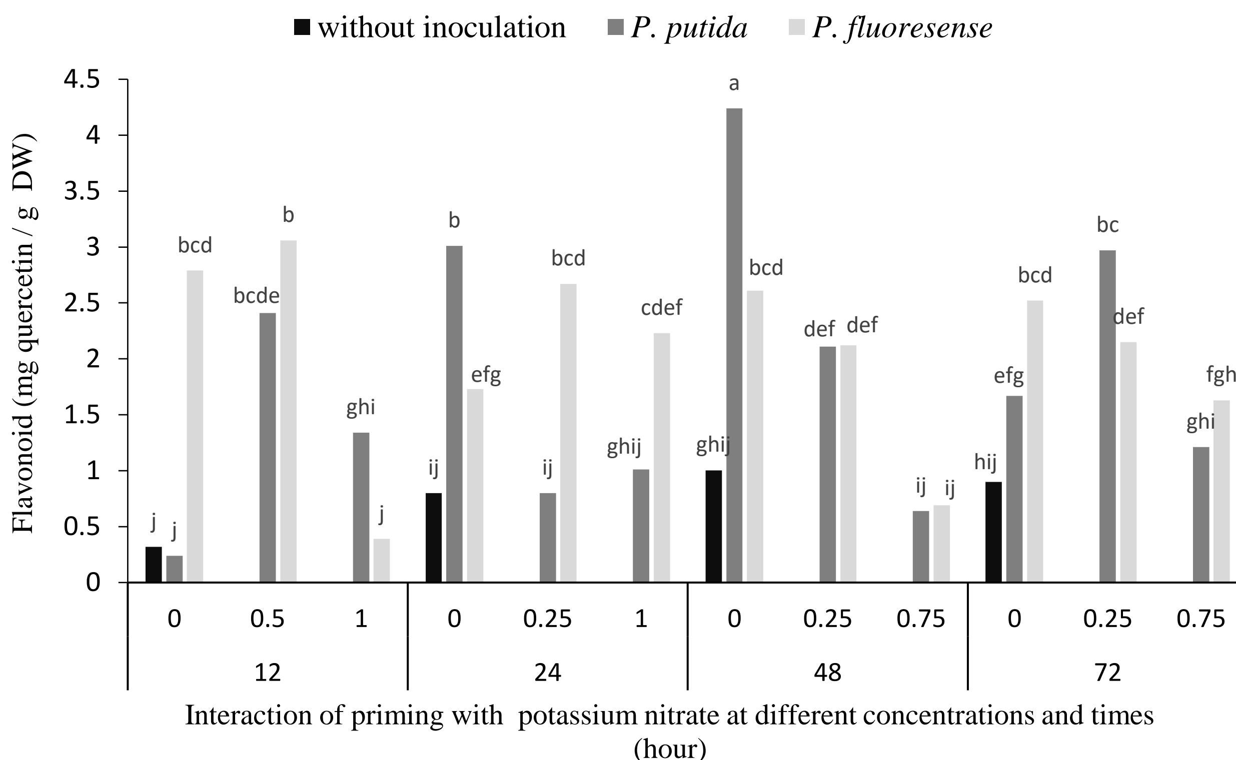

Fig. 2. Interaction of priming with potassium nitrate at different concentrations and time with inoculation of plant growth-promoting rhizobacteria on flavonoids in lemon balm. Non-identical letters indicate a significant difference of 1% among the values based on Duncan's test.

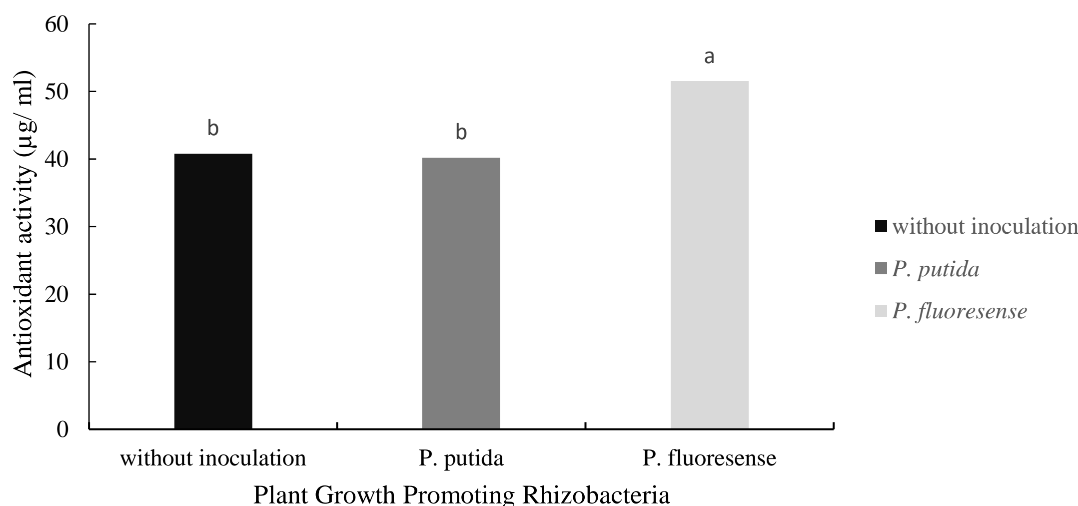

Fig. 3. Effect of plant growth-promoting rhizobacteria on antioxidant activity in lemon balm. Non-identical letters indicate a significant difference of 1% among the values based on Duncan's test.

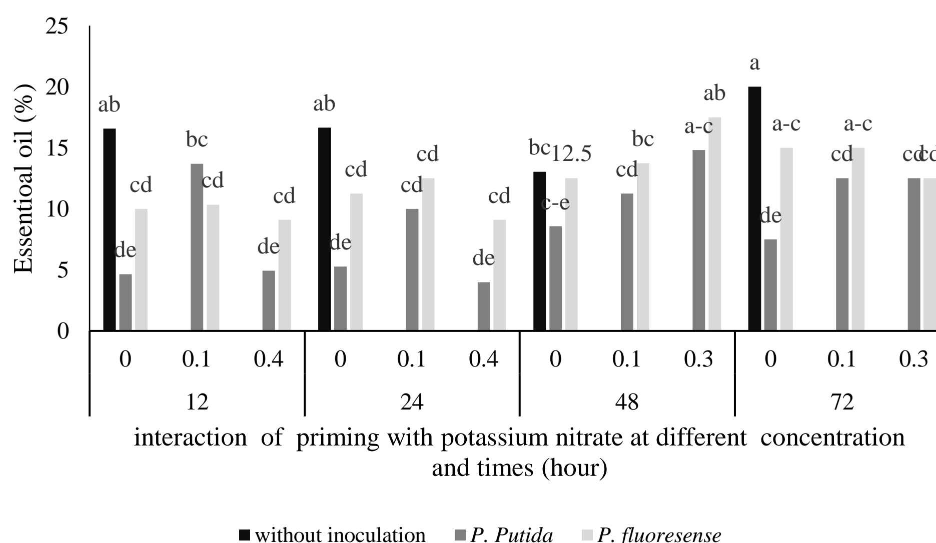

Fig. 4. Interaction of priming with potassium nitrate in different concentrations with inoculation of plant-growth promoting rhizobacteria of essential oil (%) in lemon balm.

Table 9. Compounds of essential oil after priming with potassium nitrate in different concentrations with inoculation of plant-growth promoting rhizobacteria.

No	RI	Rt GC 8	Constituents	A	B	C	D	E
1	989	7.23	Hepten-2-one<6-methyl-5->	0.8	0.5	0.5	0.5	0.7
2	1033	8.34	1,8-Cineol	-	-	-	-	0.5
3	1103	10.15	Linalool	0.2	0.2	0.3	0.2	0.2
4	1154	11.51	Citronella	1.5	0.9	1.4	0.8	1.2
5	1156	11.81	Isomenthone	0.8	0.3	0.5	0.2	4.6
6	1177	12.31	Isomenthol	1.5	0.4	0.9	0.2	1.4
7	1185	12.6	E-Isocitral	-	0.2	0.1	0.8	5.1
8	1245	14.07	Neral	34.9	32.5	29.1	26.8	30.6
9	1274	14.86	Geranial	46.4	44.9	39.2	36.9	42.1
10	1303	16	Thymol	3.9	7.4	17.6	22	3.5
11	1314	16.24	Carvacrol	0.3	1.2	1.2	2.1	0.5
12	1384	17.37	Geranyl acetate	6.4	6.8	6.8	6.5	6.3
13	1422	18.47	trans-Caryophyllene	1.9	2.4	1.3	1.4	1.9
14	1588	21.92	Caryophyllene oxide	0.4	0.7	0.1	0.5	0.1
15	1599	22.71	Hexadecane	0.8	1.4	0.7	0.8	08

A = control (no bacterial inoculation and no application of potassium nitrate). B = inoculation with *Putida*. C = inoculum with *fluorescens*. D = Interaction of potassium nitrate priming (0.75%) and 48 h priming with inoculation of *Putida* bacteria. E = Interactive effect of priming with potassium nitrate (0.75%) during 48 h of priming with inoculation of *P. fluorescens* bacteria.

Discussion

Effect of priming with potassium nitrate on germination indices

The results obtained indicate that the interaction between priming, varying concentrations of potassium nitrate, and the duration of exposure significantly influenced all germination traits and seed parameters (Tables 2 and 3). Priming with 0.25% potassium nitrate for 12 hours yielded the highest germination percentage. Potassium nitrate directly affects the seed's respiratory system by enhancing oxygen absorption, which acts as a crucial factor in conjunction with phytochromes (Hilhorst et al., 1997). Many seeds that require light for germination are sensitive to potassium nitrate, and the observed increase in germination under its influence can be attributed to this requirement (Gonzalez Benito et al., 1995). However, as shown by the mean square results in Table 3, high concentrations of potassium nitrate

reduced the average germination percentage, likely due to ionic toxicity and the creation of severe osmotic conditions that inhibit germination (McDonald et al., 1997). In the present study, it was noted that the rate of germination under the interaction of priming with potassium nitrate concentrations and time duration reached its peak in the control group during 72 hours of priming. This increase in germination rate in the control may be linked to elevated metabolic activity (McDonald, 2000).

The findings of Shim et al. (2008) align with our results, as they investigated the effect of potassium nitrate on the germination of *Paspalum dilatatum*. They observed a significant increase in germination percentage when seeds were treated with potassium nitrate at concentrations of 0.2% and 0.5% under a temperature of 25°C for 48 to 72 hours. Similarly, Lara et al. (2014) studied the impact of potassium nitrate on tomatoes and found improved germination characteristics, such as mean germination time and germination speed, attributed to the activity of nitrite reductase enzymes, which facilitate the production of nitrite and nitric oxide, leading to enhanced sprouting.
Further, Bahmani et al. (2013) reported accelerated germination of *Capparis spinosa* var. *Parviflora* seeds treated with 200 mL of potassium nitrate and a 48-hour priming period. Kartoolinejad et al. (2015) also highlighted that potassium nitrate priming significantly contributes to seedbed breakdown, increased germination percentage, and improved germination characteristics in the medicinal plant *Capparis cartilaginea*. They recommended 200 mL of potassium nitrate and 72 hours of priming as the optimal treatment for enhancing the germination traits of this medicinal plant.

Similar results were observed in a study on *Glycine max*, where potassium nitrate improved germination traits such as germination percentage, bud emergence, stem and root length, and seedling dry weight (Ahmadvand et al., 2012). Khan et al. (2022) further emphasized that KNO3 priming is an essential seed priming practice for improving maize growth and biomass production under alkaline stress. Likewise, Baluchi et al. (2015) studied the effects of potassium nitrate on the medicinal plant *Carthamus tinctorius* (L) and found that it significantly increased the dry weight of radicles and plumules, seed index, and uniformity in germination, consistent with the findings of the present study.

Morphological traits

The results demonstrated that priming with potassium nitrate at varying concentrations and durations, combined with the inoculation of plant growth-promoting rhizobacteria, had a positive effect on most morphological traits. These findings are consistent with those of Mahfouz et al. (2007), who reported that the application of biofertilizers such as *Azotobacter chroococcum*, *Azospirillum liboferum*, and *Bacillus megaterium* significantly enhanced vegetative growth in Foeniculum vulgare, including increases in plant height, number of branches, and both fresh and dry biomass per plant. Additionally, another study by Pierzad et al. (2010) investigated the effect of Azotobacter on plant height and found a significant increase in this trait.
Similarly, Gorbanpour et al. (2014) attributed the observed increase in root dry weight to the secretion of hormones by these bacteria, particularly those influenced by *Pseudomonas putida*. These hormones promote the development of branched and thinner roots, ultimately enhancing root growth by improving contact with water and nutrients. These findings align with the data from the current study.
In the present research, the application of potassium nitrate significantly improved morphological traits such as plant height, the number of primary and secondary branches, fresh and dry root weight, and dry stem weight. These results are in agreement with the study by Jabeen et al. (2011), which concluded that potassium nitrate application had a substantial effect on the morphological traits of sunflower flowering, including increased leaf area and fresh and dry weight. Furthermore, another study by Hegazi et al. (2011) showed that potassium nitrate enhanced vegetative growth and yield in olive plants. Similarly, Kazemi (2013) reported that potassium nitrate application significantly improved plant height, dry weight, yield, vegetative growth, and fertility in cucumber plants.

Physiological traits

Potassium is a highly mobile element in plant tissues and plays a crucial role in plant metabolism and enzyme activity, particularly in carbohydrate metabolism. It is also essential for transporting water and minerals throughout the xylem. The application of potassium nitrate has been shown to enhance plant growth by improving physiological processes, as potassium is integral to stomatal conduction, osmotic pressure regulation, protein synthesis in plants (Ashraf et al., 2005), and nitrate metabolism

(Jeschke et al., 1985).
The findings of the present study indicate an increase in the chlorophyll index and photosynthetic pigments under the influence of 0.25% potassium nitrate, although this treatment did not exhibit a statistically significant effect on ion leakage or relative water content. These results are consistent with those of Kazemi (2013), who reported that a concentration of 100 mg L^{-1} potassium nitrate increased chlorophyll index and soluble sugars in the grasses *Paspalum vaginatum* and *Cynodon dactylon*. The researchers concluded that a concentration of 10 mL potassium nitrate increased relative water content compared to the control. This effect is attributed to the accumulation of potassium nitrate ions in plant leaf cells, which is believed to help maintain cell integrity under biotic and abiotic stress conditions, thereby preventing damage (Asadi Wafa et al., 2015).

The results of this study also align with Elhindi et al. (2016), who found that potassium nitrate increased photosynthetic activity and chlorophyll content in *Coriandrum sativum*, while simultaneously reducing ion leakage. Relative water content is a measure of the moisture retained in leaves, and in this study, ion leakage and relative water content did not show statistically significant effects under the interaction studied.
Basetmia et al. (2010) reported that plant growth-promoting rhizobacteria (PGPR) increased chlorophyll content in bananas. Similarly, Bano et al. (2015) evaluated the effect of *Pseudomonas* bacteria on wheat and found that it increased chlorophyll content by 24-26%, while *Bacillus* bacteria increased it by up to 32%. These findings were further confirmed by Sheikhi-Ghahfarokhi et al. (2014), who reported an increase in chlorophyll a content in *Calendula officinalis* following the application of Bacillus, *P. putida*, *P. fluorescens*, and *Azotobacter*, as compared to control plants.

Metabolic traits
The present results demonstrated that a 0.5% concentration of potassium nitrate positively correlated with increased levels of total phenols and flavonoids. The concentration of essential oil and its bioactive compounds, which impact the nutraceutical and commercial value of medicinal plants, are significantly influenced by genetic, environmental, and agronomic factors (Yang et al., 2018). Potassium nitrate, in conjunction with bacterial inoculation, proved to be an effective elicitor for the synthesis of Phenylalanine Ammonia Lyase (PAL) and Chalcone Synthase (CHS) enzymes, leading to increased total phenol and antioxidant activity. Studies have shown that Plant Growth Promoting Rhizobacteria (PGPR) enhance phenol and flavonoid levels in various plants, such as *Solanum viarum* and *Begonia malabarica* (Hemashenpagam et al., 2011; Selvaraj et al., 2008).
Antioxidant activity typically correlates directly with the levels of total phenols and flavonoids, based on the quantity of free radical-trapping substances measured by the 2,2-Diphenyl-picrylhydrazyl (DPPH) assay. In this study, the antioxidant activity was notably enhanced by the Pseudomonas fluorescens inoculum in lemon balm, while Pseudomonas putida inoculation and the control (non-inoculation) did not show significant improvements. The enhanced antioxidant activity can be attributed to *P. fluorescens* bacteria triggering malonic acid pathways, significantly increasing its production. The highest antioxidant activity was observed in the lemon balm herbal extracts. Total phenols, which are crucial plant secondary metabolites, help counteract biotic and abiotic stresses. Due to their hydroxyl groups, phenols effectively neutralize free radicals (Fukumoto et al., 2000). In this study, PGPR led to a significant increase in total phenols compared to the control (treatments without rhizobacteria inoculum). These results align with Cappellari et al. (2013), who observed a substantial increase in phenols in Mexican marigold plants treated with *P. fluorescens* and *Azospirillum brasilense*. Similarly, Bencho et al. (2010) reported a significant increase in phenols in Mexican marigold plants treated with *Azospirillum brasilense* and *Pseudomonas fluorescens*.
This study aimed to evaluate the role of potassium application and the combination of rhizobacteria on secondary metabolites, antioxidant production, and essential oil synthesis in the medicinal herb lemon balm. Previous research has investigated potassium nitrate's role as a fertilizer in enhancing essential oil synthesis by activating related enzymes. Khalid (1996) reported that potassium application increased essential oil quantity in umbelliferous plants. El-Gendy et al. (2015) examined the effects of nitrogen and potassium fertilizers on essential oil production in *Anthriscus cerefolium* (L.) and found that these fertilizers improved plant yield and essential oil percentage. Mishra et al. (2010) tested the effects of *P. fluorescens* and *Bacillus subtilis* on essential oil yield in Pelargonium graveolens, noting that these bacteria increased the essential oil yield. Ghorbanpour et al. (2013) reported that *Pseudomonas putida* strains

significantly increased the essential oil percentage in *Salvia officinalis*. Similarly, Cappellari et al. (2013) observed a 70% increase in essential oil yield in plants treated with *Pseudomonas fluorescens* and *Azospirillum brasilense* without altering the oil composition compared to control plants. Banchio et al. (2010) reported a 2.5% increase in essential oil yield in inoculated *Origanum majorana* plants compared to controls. Azizi et al. (2014) found that potassium nitrate application significantly affected essential oil content and yield in *Artemisia dracunculus*, with a maximum essential oil content of 0.57% at a concentration of 6 g L^{-1} potassium nitrate. In contrast, Ezzeld et al. (2010) reported reduced essential oil content and fruit yield in *Carum carvi* (L.) with increased potassium levels. Other studies by Zaghloul et al. (2009) and Said-al Ahl et al. (2009) also observed that potassium humate increased essential oil content in tomatoes and citrus fruits. Additionally, inoculation of *Hyoscyamus niger* with *Pseudomonas putida* (pp-168) and Pseudomonas fluorescens (pf-187) significantly increased both hyoscyamine and scopolamine alkaloids (Gorbanpour et al., 2013). Banchio et al. (2009) observed a notable increase in the two main components, α-terpineol and eugenol, in *Ocimum basilicum*, with increases of up to 2 and 10 times, respectively.

Conclusion

The germination experiment on Lemon balm (*Melissa officinalis*) demonstrated that priming with different concentrations of potassium nitrate and durations led to improved uniformity and enhanced germination traits. A significant increase in germination percentage and vigor index was observed with a 0.25% potassium nitrate solution and a 12-hour priming period. Similarly, the greenhouse results highlighted notable improvements in morphological traits.

Interactions between potassium nitrate priming at varying concentrations and durations, combined with the inoculation of two bacterial species, *Pseudomonas putida* and *Pseudomonas fluorescens*, revealed that the control (without potassium nitrate) showed enhanced root fresh and dry weight, as well as stem fresh and dry weight when inoculated with *P. putida*. The 0.25% potassium nitrate concentration was particularly effective in combination with this bacterium during 72 hours of priming. Additionally, the application of *P. fluorescens* improved traits such as the number of primary and secondary branches.

Significant improvements were also observed in physiological traits, including photosynthetic pigments. The optimal results were achieved with a 0.25% potassium nitrate solution during 72 hours of priming combined with *P. putida* inoculation. The highest chlorophyll index, measured four months after culture, was recorded in the control (without potassium nitrate) during 72 hours of priming with *P. putida*. The methanolic extracts of lemon balm showed increased antioxidant properties with the interaction of potassium nitrate priming at various concentrations and durations, along with *P. putida* and *P. fluorescens* inoculation. Specifically, flavonoid content reached 4.24 mg of quercetin per mL of extract. Both phenols and antioxidant activity were elevated under the influence of these bacterial inoculants. The best results for phenols and antioxidant activity were obtained with a 0.5% potassium nitrate solution during 12 hours of priming with *P. fluorescens*.

Essential oil percentage was also measured, with the control (no bacterial inoculation and no potassium nitrate) showing the best performance during 72 hours of priming. Although some essential oil components, such as geranial, citronella, and neral, showed a slight decrease compared to the control, other compounds, including carvacrol, thymol, and isomenthone, demonstrated significant increases.

Given the positive effects of *P. putida* and P. *fluorescens* on lemon balm, plant growth-promoting rhizobacteria could be recommended as alternatives to chemical fertilizers, potentially reducing chemical fertilizer use and promoting the production of chemical-free medicinal plants.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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