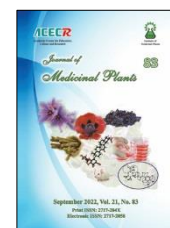




Institute of  
Medicinal Plants

## Journal of Medicinal Plants

Journal homepage: [www.jmp.ir](http://www.jmp.ir)



### Research Article

## Optimization of Essential oils production in *Mentha longifolia* L. using plant growth promoting cyanobacteria

Zeinab Shariatmadari<sup>1</sup>, Majid Ghorbani Nohooji<sup>2</sup>, Hossein Riahi<sup>1,\*</sup>, Fatemeh Heidary<sup>1</sup>

<sup>1</sup> Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

<sup>2</sup> Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

### ARTICLE INFO

#### Keywords:

Bioelicitor

Cyanobacteria

Essential oil

Menthol

*Mentha longifolia* L.

### ABSTRACT

**Background:** The cyanobacteria isolated and purified from soil can increase plant growth and productivity as bioelicitors. **Objective:** This study aimed was to investigate the effect of cyanobacteria as a bioelicitor on the production efficiency and essential oil composition of *Mentha longifolia* L. **Methods:** Two species of heterocystous cyanobacteria, *Anabaena vaginicola* ISB42 and *Nostoc spongiaeforme* ISB65, were used as bioelicitors to optimize the growth and productivity of *Mentha longifolia* L. Five pots were considered for each treatment and five others for the control plants. Treatment lasted for 100 days, and the treated plants were irrigated with 200 ml of cyanobacterial suspensions (0.2 %) every 21 days intervals. The experiment was performed in a randomized complete block design in an experimental greenhouse condition. After inoculation of treated plants with cyanobacterial bioelicitors, the vegetative factors, including shoot and root length, leaf area, as well as fresh and dry weight of plants were evaluated. In addition to growth factors, analyses of essential oils in studied plants were performed. **Results:** The results showed significant improvement in vegetative growth indices of the treated plants. Also, some economic and medicinal metabolites such as menthol, eucalyptol and phytol were increased in the essential oil of treated plants. The results also showed a significant increase in sesquiterpene hydrocarbons and oxygenated diterpenes in plants treated with cyanobacteria compared to controls. **Conclusion:** So, the use of cyanobacterial bioelicitors can be suitable to increase the yield as well as the economic and medicinal value of this medicinal plant.

### 1. Introduction

The genus *Mentha* commonly known as mint is a member of Lamiaceae family and has

economic and medicinal value. There are several species of *Mentha* were reported from Iran while some species were cultivated due to their

**Abbreviations:** MH, Monoterpene Hydrocarbons; OM, Oxygenated Monoterpenes; SH, Sesquiterpene Hydrocarbons; OS, Oxygenated Sesquiterpenes; OD, Oxygenated Diterpenes

\*Corresponding author: [h-riahi@sbu.ac.ir](mailto:h-riahi@sbu.ac.ir)

doi: [10.52547/jmp.21.83.47](https://doi.org/10.52547/jmp.21.83.47)

Received 6 April 2022; Received in revised form 27 August 2022; Accepted 28 August 2022

© 2020. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>)

economic importance [1-3]. *Mentha longifolia* L. is one of the species of this genus with natural growth in Iran which has been known for its pharmacological activities such as antimicrobial, anti-viral, anti-inflammatory, antioxidant and antimutagenic effects [4, 5]. The anti-parasitic and anti-insect properties are other activities, which have been reported from this aromatic plant [6, 7].

The unique content of essential oil in different types of mint and their valuable metabolites such as menthol are the main reasons for their commercial cultivation [8-10]. Due to the economic and industrial value of this genus and specific metabolites that can be extracted from mint species, attempts have been made to optimize plant production efficiency per unit area of cultivation. One of the new methods to raise the plant secondary metabolites content is the use of elicitors. The direct and indirect effects of elicitors on plant metabolic pathways can amplify the production of some secondary metabolites and could serve as a defense mechanism for the plant [11]. For example, some studies suggest the use of biotic elicitors such as *Rhizopus stolonifer* Vuillemin, *Trametes versicolor* (L.) Lloyd and *Mucor* sp. significantly increases the amount of  $\alpha$ -tocopherol in the cell culture of *Carthamus tinctorius* L. [12]. Another study indicated that inoculation of the *Mentha piperita* L. with arbuscular mycorrhizal fungi have positive effects on plant secondary metabolites, and can be improved the quantity and quality of active substances [13].

Today, various bioelicitors are used to optimize plant production. Many microorganisms in the plant rhizosphere such as cyanobacteria can act as bioelicitor and affect

plant metabolic pathways. Cyanobacteria are the oldest group of algae and morphologically are known as a diverse group of prokaryotes [14]. These microorganisms are useful in agricultural industries because of their capacity to produce plant growth stimulants including hormonal and non-hormonal substances [15-17]. Cyanobacteria also can affect the Physico-chemical properties of the soil, and the accumulation of their biomass in surface soils reduces soil erosion, improves soil texture and regulates soil acidity [18, 19].

In the last decade, the use of cyanobacteria as a biofertilizer to improve the quantity and quality of medicinal plants product has been considered [20-24]. The results of these studies showed a relative improvement in growth indices and the essential oil percentage in plants treated with cyanobacteria compared to controls. For example, a 1.5-fold increase in phenolic and flavonoid content of *Plantago* leaves, as well as 26.9 % increase in the chamazulene content of the *Matricaria* essential oil in plants treated with cyanobacteria were reported in previous studies [21, 22].

Considering the great economic value of different species of *Mentha*, it is essential to develop efficient and safe methods to optimize the production of these plants. Therefore, this study was conducted to investigate the effect of native cyanobacteria as a bioelicitors on the production efficiency of *M. longifolia* L. as common and economic medicinal plant in Iran. Additionally, the quantitative and qualitative changes in the essential oil compounds of this plant were analyzed under treatment conditions.

## 2. Materials and Methods

### 2.1. Isolation, purification and identification of cyanobacteria

Soil samples for isolation of cyanobacteria were collected from several medicinal plant fields (Iran, Mazandaran Province). The purification of the cyanobacteria was done through repeated sub culturing the colonies on the nitrate free BG11 solid medium [25]. The cyanobacteria were cultured under controlled laboratory conditions or artificial light illumination ( $74 \mu\text{mol photons/m}^2\cdot\text{s}$ ), with a 16/8-hour light-dark cycle, and  $25 \pm 2^\circ\text{C}$  temperature. Finally, purified taxa were identified by optical microscope (Olympus, Model BH-2), and based on valid identification key books [26]. For molecular determination of the isolates, 16S rRNA gene sequencing was performed.

### 2.2. Cyanobacterial suspension preparation

Algal suspensions of two isolated and purified species, *Anabaena vaginicola* ISB42 and *Nostoc spongiaeforme* ISB65, were prepared through homogenizing 0.2 g of cyanobacterial biomass after four weeks of culturing, in 100 ml of sterilized distilled water.

### 2.3. Pot culture and growing condition

Rhizomes of *M. longifolia* were obtained from Institute of Medicinal Plants (ACECR). The plants were previously collected and identified by Dr. Ghorbani Nohooji as expert botanist (Voucher specimen: 7021-IMPH). Similar and healthy rhizomes (15 for each treatment) were grown in three-liter pots containing 60 % peat, 25 % sand, and 15 % normal soil, for 100 days during April-July. The experiment was performed in a

randomized complete block design in an experimental greenhouse condition. For experimental treatment, 200 ml of cyanobacterial suspensions (0.2 g biomass in 100 ml distilled water) were added to each treated pot on the first day of planting and every 21 days thereafter. The control plants irrigated only with water.

### 2.4. Measurement of growth parameters

After 100 days of pot culture and inoculation of plants with cyanobacteria, treated and controlled plants were harvested and the root and aerial parts of them were separated. The parameters employed to assay for plant growth rates are as follows: root and shoot height (cm), fresh and dry weight of root and aerial parts of plants (g), number of leaves, leaf area ( $\text{cm}^2$ ), and stem ramification. The vegetative dimensions of the leaves were determined using imageJ software (version 1.44P; US National Institutes of Health, Bethesda, Maryland, USA). The wet and dry weight of roots and aerial parts of plant was also measured with a 0.001 g accuracy digital scale (Sartorius, Germany). Dry weight of plants was measured after placing roots and aerial parts in the oven at  $70^\circ\text{C}$  for 24 hours.

### 2.5. Essential oil extraction and GC-MS analysis

The mass of plant was transferred to a dark room away from moisture and completely dried by exposure to ambient air. Then they were turned into a fine powder by the mill and used for the next steps. According to the European Pharmacopoeia method, 100 g of dried biomass of plants (aerial parts at flowering stage) were hydro-distilled for 3 h using a clevenger-type apparatus to extract the essential oils (EOs) of each treatment [27, 28]. Essential oil yields were

calculated using the following equation:  $ROu = (M/B_m) \times 100$ , where (M) is the mass of the extracted oil (g) and ( $B_m$ ) is the initial plant biomass (g) [29]. The extracted EOs were dried using anhydrous sodium sulfate and stored in sealed vials at 4°C before gas chromatography (GC) and gas chromatography-mass spectrometric (GC-MS) analysis.

The GC-MS analyses were carried out in medicinal plants analysis and processing service center in Institute of Medicinal Plants (ACECR) to determine the different components of the volatile oils. The essential oils were analyzed by a gas chromatograph coupled with a mass spectrophotometer (GC-MS) (Agilent technologies 6890A), equipped with 5973 C mass selective detector and a BPX5 column of 30 m  $\times$  0.25 mm i.d., 0.25  $\mu$ m film, using Helium gas as the carrier gas with a flow rate of 1 ml/min and split ratio of 1:50. Oven temperature was programmed to remain at 50° C for 5 min, followed by an increase at a rate of 3 °C/min until 240 °C was reached, and after that programmed at 15 °C/min until reaching 300 °C, maintaining this temperature for 3 min; The injector and detector temperature were maintained at 250 °C and 220 °C, respectively. Compounds were identified by comparing retention indices (RI) with those reported in the literature and their mass spectrum with Wiley library [30, 31].

## 2.6. Statistical analysis

One-way ANOVA statistical analysis was performed employing SPSS software version 16 (Package for the Social Sciences, SPSS Inc., Chicago IL, USA). Means were separated using the Tukey HSD test at  $P < 0.05$ . Microsoft Office Excel 2007 was also used to draw the graphs.

## 3. Results

### 3.1. Plant growth parameters and cyanobacteria inoculation

In this study, two heterocystous cyanobacteria from two genera were isolated from medicinal plants bed soil and applied as bioelicitors. According to the results, there was a significant growth difference between treated and the control plants. In other words, two strains of cyanobacteria including *Nostoc spongiaeforme* ISB65 and *Anabaena vaginicola* ISB42, enhanced some growth parameters in roots and aerial parts of plants as compared with control (Table 1 and Fig. 1).

This increase was significant in growth parameters including the length of roots and shoots, as well as number of leaves. The fresh and dry weight of treated plants were also significantly higher than plants irrigated with water alone ( $P < 0.05$ ; Fig. 1, Table 1). Leaf area of plants treated with *Anabaena vaginicola* ISB42 was also significantly increased as compared with controls.

### 3.2. Essential oil composition

The GC/MS analysis of essential oil composition showed a quantitative and qualitative variation of metabolites in EOs of cyanobacterial treatments and control plants (Table 2, Fig. 2). The results show that the quantity of some metabolites such as menthol increased in response to both cyanobacterial suspensions, especially in plants treated with *Anabaena vaginicola* ISB42. In addition to a significant increase in menthol, a 2.7-fold increase in eucalyptol, and a 13-fold increase in phytol was reported in *Anabaena vaginicola* treated plants. Also, an 8-fold increase in phytol, and 1.6-fold increase in eucalyptol in *Nostoc spongiaeforme* treated plants were reported in our study.

On the other hand, the amount of some phytochemical constituents such as pulegone, piperitenone and piperitenone oxide decreased in treated plants. For example, piperitenone amount decreased from 14 % in control plants to 3.5 % and 3.77 % in *Anabaena* and *Nostoc* treated

plants respectively. The reduction of piperitenone oxide content from 51.87 % in control plants to 33.89 % and 10.61 % in *Anabaena* and *Nostoc* treated plants was reported in this study. The amount of pulegone in the treated plants also decreased significantly.

**Table 1.** Effect of cyanobacterial bioelicitors (*Anabaena vaginicola* ISB42, *Nostoc spongiaeforme* ISB65) on growth of *Mentha longifolia* after 100 days of planting (Mean  $\pm$  SE)

Parameters	Treatment		
	Control	<i>Anabaena vaginicola</i>	<i>Nostoc spongiaeforme</i>
Root length (cm)	20.33 $\pm$ 3.75	35.66 $\pm$ 2.96*	35.00 $\pm$ 2.88*
Shoot length (cm)	15.66 $\pm$ 2.33	27.66 $\pm$ 0.88*	27.33 $\pm$ 1.45*
Leaf number	18.66 $\pm$ 1.76	34.00 $\pm$ 2.30*	50.00 $\pm$ 1.76*
Fresh weight of stem and leaf (g)	1.31 $\pm$ 0.06	2.40 $\pm$ 0.36*	2.23 $\pm$ 0.24*
Dry weight of stem and leaf (g)	0.42 $\pm$ 0.01	0.80 $\pm$ 0.02*	0.76 $\pm$ 0.07*
Fresh weight of root (g)	1.35 $\pm$ 0.13	3.65 $\pm$ 0.47*	3.70 $\pm$ 0.35*
Dry weight of root (g)	0.50 $\pm$ 0.01	1.05 $\pm$ 0.04*	0.99 $\pm$ 0.06*
Leaf area (cm <sup>2</sup> )	1.01 $\pm$ 0.26	2.16 $\pm$ 0.16*	1.45 $\pm$ 0.05
Ramification (no.)	3.00 $\pm$ 0.00	4.66 $\pm$ 0.66	5.00 $\pm$ 0.57

\* Significant at the 0.05 level

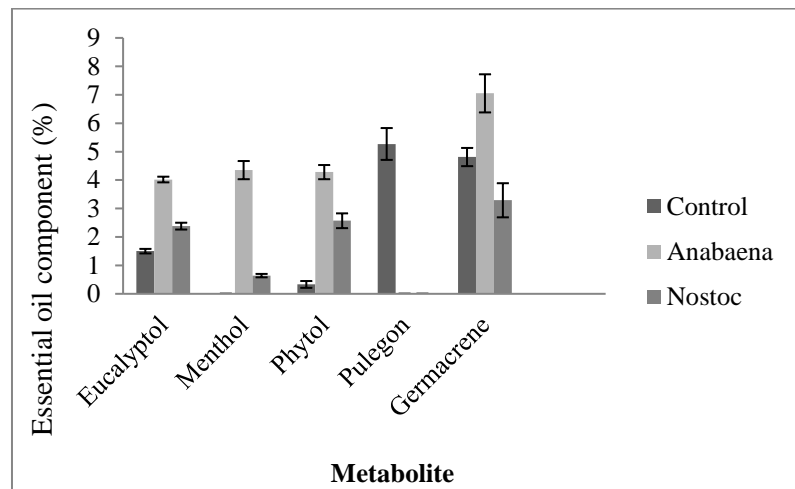


**Fig. 1.** Comparison between control and treated plants in growth parameters. Treatment conditions: 0.2 % algal suspensions were sprayed on soil of treated pots, 0. Plant irrigated by distilled water (Control plant), 1. Plant treated by *Anabaena vaginicola* ISB42 suspension, 2. Plant treated by *Nostoc spongiaeforme* ISB65 suspension (bar = 5 cm).

**Table 2.** Effects of cyanobacterial bioelicitors on essential oil components (%) in *Mentha longifolia* (Mean  $\pm$  SE).

No	Components	Compound Type	RI (Retention index)	Treatment		
				Control	<i>Anabaena vaginicola</i>	<i>Nostoc spongiaeforme</i>
1	Myrcene	MH	993	0.49	-	-
2	Limonene	MH	1033	0.38	-	-
3	Eucalyptol	OM	1038	1.50	4.02	2.38
4	<i>cis</i> -Sabinene hydrate	OM	1077	0.28	-	-
5	Linalool	OM	1108	0.28	-	-
6	Menthone	OM	1167	0.27	-	-
7	<i>neo</i> -Menthol	OM	1180	0.49	-	-
8	Menthol	OM	1189	-	4.35	0.64
9	$\alpha$ -Terpineol	OM	1208	0.63	-	-
10	Pulegone	OM	1251	5.27	-	-
11	<i>cis</i> -Piperitenone epoxide	OM	1267	-	-	1.76
12	<i>E</i> -Anethole	Other	1301	0.49	-	-
13	Thymol	OM	1308	0.54	-	0.53
14	Piperitenone	OM	1357	14.00	3.50	3.77
15	Eugenol	Other	1369	0.78	-	-
16	Piperitenone oxide	OM	1380	51.87	33.89	10.61
17	<i>Z</i> -Jasmone	Other	1407	0.56	-	-
18	<i>E</i> -Caryophyllene	SH	1427	2.51	11.03	4.42
19	<i>cis</i> -Muuro-la-3,5-diene	SH	1454	0.41	-	-
20	( <i>Z</i> )- $\beta$ -Faresene	SH	1457	-	-	0.60
21	Lavandulyl butyrate	OM	1461	0.26	-	-
22	<i>cis</i> -Muuro-la-4(14),5-diene	SH	1470	0.81	-	-
23	Germacrene D	SH	1489	4.81	7.05	3.29
24	Bicyclogermacrene	SH	1505	0.34	-	-
25	2,4-Di-tert-Butylphenol	Other	1519	-	-	2.69
26	<i>trans</i> -Calamenene	SH	1531	0.48	-	-
27	<i>cis</i> -Muuro-l-5-en-4- $\beta$ -ol	OS	1569	0.39	-	-
28	Caryophyllene oxide	OS	1596	0.39	-	0.57
29	Diethyl Phthalate	Other	1604	0.45	31.89	1.70
30	1,10- <i>di</i> -Epicubenol	OS	1630	1.64	-	-
31	$\alpha$ -Cadinol	OS	1673	1.72	-	0.47
32	Nonascosane	Other	1901	-	-	0.54
33	Dibutyl phthalate	Other	1971	0.29	-	-
34	<i>n</i> -Hexadecanoicacid	Other	1979	-	-	26.87
35	Chlorpyrifos	Other	1991	-	-	1.65
36	Phytol	OD	2058	0.33	4.27	2.57
37	Linolenic alcoh	Other	2077	-	-	10.47
38	Tetracosane	Other	2401	0.24	-	-
Total Identified				92.9	100	75.53

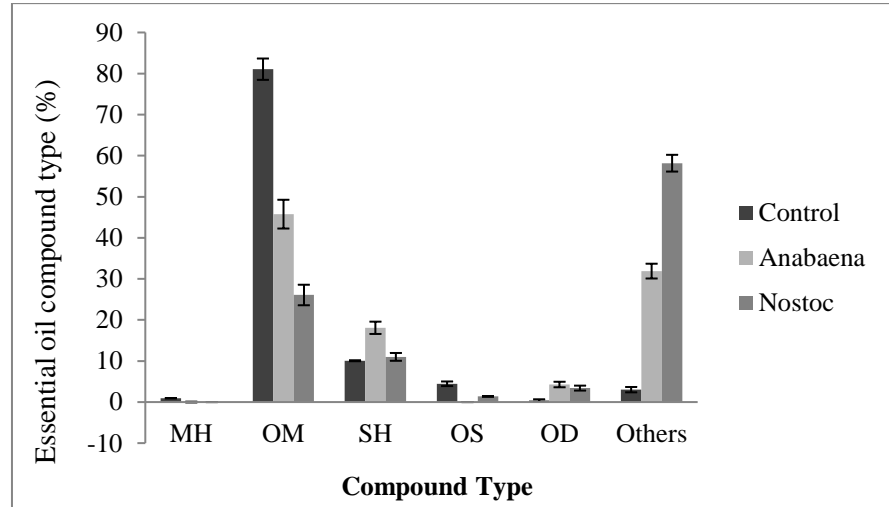
OM: Oxygenated Monoterpenes, SH: Sesquiterpene Hydrocarbons, OS: Oxygenated Sesquiterpenes, OD: Oxygenated Diterpenes



**Fig. 2.** Comparison between control and treated plants in some commercial metabolites in essential oil component (%)

The results also showed a significant increase in Sesquiterpene Hydrocarbons (SH) and Oxygenated Diterpenes (OD) in plants treated with cyanobacteria compared to controls. A 1.8-fold increase in SH and 12.2-fold increase in OD was reported in *Anabaena vaginicola* treated

plants. In *Nostoc spongiaeforme* treated plants 9.7-fold increase in OD were also reported. While in control plant Oxygenated Monoterpenes (OM) were dominant components (Fig. 3).



**Fig. 3.** Comparison between control and treated plants in essential oil compound types (%)

Anabaena: *Anabaena vaginicola* ISB42, Nostoc: *Nostoc spongiaeforme* ISB65, MH: Monoterpene Hydrocarbons, OM: Oxygenated Monoterpenes, SH: Sesquiterpene Hydrocarbons, OS: Oxygenated Sesquiterpenes, OD: Oxygenated Diterpenes

#### 4. Discussion

According to previous studies, medicinal plant's biomass and essential oil yield of them are influenced by soil condition and its

microflora [21, 32]. Similar to previous studies, the results of our study revealed significant changes in most growth factors, especially in the root and the aerial parts of the treated plants. The

[Downloaded from jmp.ir on 2025-07-13]  
[DOR: 20.1001.1.2717204.2022.21.83.5.5]  
[DOI: 10.52547/jmp.21.83.47]

root - as an absorbing organ of plants and the source of some plant metabolites such as phytohormones - is an important part of plants. The positive effect of plant growth-promoting rhizobacteria (PGPR) on root growth indices such as root length and dry weight of roots, as well as density of the hairy roots have been reported in previous studies [33]. It is evident that with improved root growth, as water and mineral absorbing organ, the growth of plants shoot and their productivity also improved [34].

The results of the present study also showed an increase in shoot length and the number of leaves in plants treated with cyanobacteria. Based on our findings, it can be argued that the biomass of the aerial part of treated plants was increased significantly due to the influence of cyanobacterial elicitors (Table 1). Previous studies have noted an increase in vegetative indices such as the number of leaves and shoot length of some medicinal plants caused by the usage of cyanobacterial biofertilizers. These reports attribute the significant increase in the biomass of the aerial parts of medicinal plants such as *Satureja hortensis* L., *Mentha piperita* L., *Plantago major* L., and *Matricaria chamomilla* L. to the use of cyanobacterial elicitors in greenhouse conditions [21-24]. Therefore, cyanobacteria can be useful elicitors for achieving robust plants and leading to beneficial influences on soil nutrient availability and plant yield [20, 35].

The positive effect of cyanobacteria on plant growth and productivity has been attributed to various factors including the nitrogen fixation ability of these microorganisms, biosynthesis of plant growth-promoting hormonal substances (e.g., auxins), as well as biosynthesis of non-hormonal substances such as amino acids, sugars, and vitamins [17, 35, 36, 37]. According to previous studies, the heterocystous

cyanobacteria can fixate atmospheric nitrogen under natural conditions, and this capability can be one of the important reasons for their positive effect on plant growth [17, 34]. Also, the biosynthesis of some growth-promoting substances such as auxin compounds by these prokaryotic algae can be regarded as another factor in improving the growth of plants treated with cyanobacterial suspensions. Previously, the presence of plant growth-promoting phytohormones such as indole-3-butyric acid and indole-3-acetic acid has been reported from the biomass of the studied cyanobacteria [16, 34].

Auxins, especially indole-3-butyric acid, play an important role in stimulating plant rooting. Previous studies have noted an increase in the formation of lateral roots in the seedlings of various plants as a result of applying indole-3-butyric acid [38]. There is also evidence supporting the ability of cyanobacteria to produce auxins such as indole-3-butyric acid and indole-3-acetic acid [16, 21, 22]. In these studies, the production of auxins is considered as an important factor in improving the vegetative growth of plants using cyanobacterial fertilizers.

In another part of the study, we evaluated metabolic profile changes of plant under treatment conditions. The most important compounds in the metabolic profile of members of this species are menthol, menthone, pulegone, eucalyptol, piperitenone and piperitenone oxide [39, 40]. The results of our study showed that *Anabaena* and *Nostoc* inoculation increases some metabolites such as menthol, eucalyptol, phytol and germacrene (Fig. 2).

Menthol is one of the main components of the essential oil of *Mentha* whose level increased in the metabolic profile of the treated plants (Table 2). It is the most well-known monoterpene compound ( $C_{10}H_{20}O$ ) that is used in food, pharmaceutical, and cosmetic industries [41].



Eucalyptol (1,8-cineole) is another metabolite whose level increased in the metabolic profile of the plants treated with cyanobacteria (Table 2). This metabolite has various pharmacological effects and is used to treat some diseases such as sinusitis and asthma [42].

Phytol similar to menthol and eucalyptol is an industrial metabolite whose level increased in the metabolic profile of treated plants (Table 2). Phytol is used in the fragrance industry as well as in cosmetics, shampoos, soaps, household cleaners, and detergents [43]. Germacrenes was other metabolite with antimicrobial and insecticidal properties that showed an increase in the metabolic profile of treated plants in our study (Fig. 2).

In addition to our study and the report of significant changes in the essential oil of *M. longifolia* treated with cyanobacterial bioelicitors, there are other studies confirming similar changes in relation to other bioelicitors. In this regard, some researchers suggest that the plant growth and quality of secondary metabolites in medicinal plants are affected and improved by using mycorrhiza-based biofertilizers, green algae, growth-promoting bacteria, and some compound fertilizers [13, 44, 45]. In one of these studies, the effectiveness of mycorrhiza-based biofertilizers, algae, and a combination of these fertilizers was evaluated in relation to the general performance of *Ocimum basilicum* L., and the quantity of phenolic and flavonoid compounds of this plant [44, 45]. It was found that the levels of phenolic and flavonoid compounds and the antioxidant properties of *O. basilicum* increase significantly under treatment conditions. This increase was particularly remarkable in plants treated with mycorrhiza-algae biofertilizer. Previous studies have also shown that cyanobacterial bioelicitors cause quantitative changes in the flavonoid

content of *Plantago major* L. and so enhance its medicinal effects [22]. The significance increases of effective metabolites (e.g., chamazulene) in the essential oil of *Matricaria chamomilla* L. under the influence of cyanobacterial elicitors also reported in another study [21].

The positive effect of biotic elicitors on the secondary metabolites of *Calendula officinalis* L. has also been reported in previous studies [46]. According to results of this study, the level of secondary metabolites is dependent on various environmental factors such as chemical composition of soil [46]. Some scientists believe that metabolite change may be due to the activation of specific antioxidant enzymes triggered by treatment with biotic elicitors [44]. The relationship between primary and secondary metabolic pathways also reported in previous studies [47]. They explained that carbohydrates as a key metabolite in plants can act as a precursor to promote other specific metabolic pathways [47]. Therefore, any factor that enhances the general performance of a plant's production and its photosynthetic efficiency can also serve to increase the production efficiency of secondary metabolites. Phytohormones have also been introduced in some studies as an influencing factor on performance of a plant's production and the metabolic composition of them [48-50].

## 5. Conclusion

According to the results of the present study, heterocystous cyanobacteria have a substantial role in rising the quality and quantity of valuable plant metabolites, and they can be used to improve the production efficiency of medicinal plants. Therefore, we can recommend the use of cyanobacterial strains used in this study as a suitable and economical method for the safe and

cost benefit production of medicinal plants such as *M. longifolia*. Finally, considering the increased level of valuable metabolites such as menthol and eucalyptol in *Mentha longifolia* specimens treated with *Anabaena vaginicola* ISB42, we can expect the antibacterial properties of these plants to amplify compared to normal conditions.

### Author contributions

Z. Sh.: Wrote the paper; designed the experiments and analysis. M. Gh. N.: Performed the analysis; contributed in field and laboratory studies. H. R: Project management; Designed

the experiments and analysis. F. H.: Collected the data; Contributed in field and laboratory studies.

### Conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

### Acknowledgment

The authors wish to thank Iran National Science Foundation (INSF) for funding this project (Grant No: 95814237). This article is part of a research project supported by the INSF as supervisor.

### References

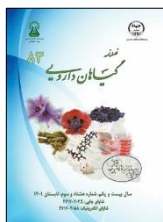
1. Mozaffarian V. Identification of Medicinal and Aromatic Plants of Iran. Tehran: Farhang Moaser Publishers; 2013: 514-523.
2. Yahyaabadi Y, Mahmoudi Otaghvari A and Nazifi E. Phytochemical and palynological study of several species of *Mentha* L. in north of Iran. *J. Plant Res.* 2020; 33(4): 843-853. doi: 20.1001.1.23832592.1399.33.4.8.4.
3. Mokhtarikhah G, Ebadi MT and Ayyari M. Agro-morphological and phytochemical studies of spearmint landraces (*Mentha spicata* L.) in Iran. *Ind Crops Prod* 2022; 176: 114367. doi: 10.1016/j.indcrop.2021.114367.
4. Anwar F, Abbas A, Mehmood T, Gilani AH and Rehman N. *Mentha*: a genus rich in vital nutra-pharmaceuticals- a review. *Phytother. Res* 2019; 33(10): 2548-2570. doi: 10.1002/ptr. 6423.
5. Farzaei MH, Bahramsoltani R, Ghobadi A, Farzaei F and Najafi F. Pharmacological activity of *Mentha longifolia* and its phytoconstituents. *J. Tradit. Chin. Med* 2017; 37 (5): 710-720. doi: 10.1016/S0254-6272(17)30327-8.
6. Kouhestani F, Hashemi SA and Sabzevari S. An overview of insecticidal properties of *Mentha longifolia* L. in Iran. *JNKUMS* 2020; 11 (4): 93-97. doi: 10.29252/nkjmd-110413.
7. Kumar P, Mishra S, Malik A and Satya S. Insecticidal properties of *Mentha* species: A review. *Ind. Crops Prod* 2011; 34(1): 802-817. doi: 10.1016/j.indcrop.2011.02.019.
8. Thakur S, Walia B and Chaudhary G. *Mentha arvensis* (Pudina): A review based upon its medicinal properties. *Res. J. Pharmacognosy. Phytochem.* 2021; 13 (3): 143-148. doi: 10.52711/0975-4385.2021.00024.
9. de Sousa Guedes JP, da Costa Medeiros JA, de Souza e Silva RS, de Sousa JMB, da Conceição ML and de Souza EL. The efficacy of *Mentha arvensis* L. and *Mentha piperita* L. essential oils in reducing pathogenic bacteria and maintaining quality characteristics in cashew, guava, mango, and pineapple juices. *Int. J. Food Microbiol.* 2016; 238: 183-192. doi: 10.1016/j.ijfoodmicro. 2016.09.005.
10. Hou T, Sans SS, Li H, Xing Y, Nanda A, Netala VR and Zhang Z. Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Biosci* 2022; 47: 101716. doi: 10.1016/ j.fbio.2022. 101716.

11. Kokate P, Purohit AP, Gokhale SB. Pharmacognosy, 20<sup>th</sup> Ed, Nirali Publication, India; 2002.
12. Chavan SP, Lokhande VH, Nitnaware KM and Nikam TD. Influence of growth regulators and elicitors on cell growth and  $\alpha$ -tocopherol and pigment productions in cell cultures of *Carthamus tinctorius* L. *Appl. Microbiol. Biotechnol* 2011; 89(6): 1701-1707. doi: 10.1007/s00253-010-3014-4.
13. Goudarzian A, Pirbalouti AG and Hossaynzadeh M. Menthol, balance of menthol/menthone, and essential oil compositions of *Mentha*  $\times$  *Piperita* L. under foliar-applied chitosan and inoculation of arbuscular mycorrhizal fungi. *J Essent Oil Bear Pl* 2020; 23(5): 1012-1021. doi: 10.1080/0972060X.2020.1828177.
14. Waterbury JB. The cyanobacteria-isolation, purification and identification. *Prokaryotes* 2006; 4: 1053-1073. doi: 10.1007/0-387-30744-3-38.
15. Singh JS, Kumar A, Rai AN and Singh DP. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Front. Microbiol* 2016; 7: 529. doi: 10.3389/fmicb.2016.00529.
16. Seyed Hashtroudi M, Ghassempour AR, Riahi H, Shariatmadari Z and Khanjir M. Endogenous auxins in plant growth promoting cyanobacteria – *Anabaena vaginicola* and *Nostoc calcicola*. *J. Appl. Phycol* 2013; 25: 379-386. doi: 10.1007/s10811-012-9872-7.
17. Santini G, Biondi N, Rodolfi L and Tredici MR. Plant biostimulants from cyanobacteria: an emerging strategy to improve yields and sustainability in agriculture. *Plants (Basel)*. 2021; 10(4): 643. doi: 10.3390/plants10040643.
18. Abinandan S, Subashchandrabose SR, Venkateswarlu K and Megharaj M. Soil microalgae and cyanobacteria: the biotechnological potential in the maintenance of soil fertility and health. *Crit. Rev. Biotechnol* 2019; 39(8): 981-998. doi: 10.1080/07388551.2019.1654972.
19. Lichtfouse E. Genetic engineering, biofertilisation, soil quality and organic farming. Springer, Dordrecht; 2010. doi: 10.1007/978-90-481-8741-6.
20. Bharti A, Prasanna R, Kumar G, Nain L, Rana A, Ramakrishnan B and Singh Shivay Y. Cyanobacterial amendment boosts plant growth and flower quality in Chrysanthemum through improved nutrient availability. *Appl Soil Ecol* 2021; 162: 103899. doi: 10.1016/j.apsoil.2021.103899.
21. Zarezadeh S, Riahi H, Shariatmadari Z and Sonboli A. Effects of cyanobacterial suspensions as bio-fertilizers on growth factors and the essential oil composition of chamomile, *Matricaria chamomilla* L. *J. Appl. Phycol*. 2020; 32 (2): 1231-1241. doi: 10.1007/s10811-019-02028-9.
22. Chookalaili H, Riahi H, Shariatmadari Z, Mazarei Z and Seyed Hashtroudi M. Enhancement of total flavonoid and phenolic contents in *Plantago major* L. with plant growth promoting cyanobacteria. *J. Agric. Sci. Technol* 2020; 22(2): 505-518. doi: 10.1007/s10811-019-02028-9.
23. Shariatmadari Z, Riahi H, Abdi M, Seyed Hashtroudi M and Ghassempour AR. Impact of cyanobacterial extracts on the growth and oil content of the medicinal plant *Mentha piperita* L. *J. Appl. Phycol* 2015; 27 (6): 2279-2287. doi: 10.1007/s10811-014-0512-2.
24. Riahi H, Shariatmadari Z, Khanjir M and Azizi A. Effect of *Anabaena vaginicola* inoculum on growth of pot plants. *Acta Hort* 2013; 1013:507-513. doi:10.17660/ActaHortic.2013.1013.63.

25. Andersen RA. Algal culturing techniques. Elsevier academic Press; 2005.
26. Komárek J. Süßwasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota 3. Teil / 3rd part: Heterocytous Genera. Springer Spektrum; 2013.
27. Amini Sh, Ghorbani Nohooji M, Khani M, Labbafi MR and KhalighiSigaroodi F. Biological activity of some essential oil constituents in four *Nepeta* L. species against *Sitophilus oryzae* L. *Biodiversitas* 2019; 20(2): 338-343.
28. Anonymous. European Pharmacopoeia, 5th edition. Strasbourg- version 5.1. EDQM; 2005.
29. da Costa OB, Del Menezzi CHS, Benedito LEC, Resck IS, Vieira RF and Bizzo HR. Essential oil constituents and yields from leaves of *Blepharocalyx salicifolius* (Kunt) O. Berg and *Myracrodruon urundeuva* (Allemão) collected during daytime. *Int. J. For. Res.* 2014; 982576, 6 p. doi:10.1155/2014/982576.
30. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation: Carol Stream, Texas; 2007.
31. McLafferty FW and Stauffer DB. The Wiley / NBS registry of mass spectral data. Wiley, New York; 1989.
32. Salehi B, Stojanović-Radić Z, Matejić J, Sharopov F, Antolak H, Kręgiel D, Sen S, Sharifi-Rad M, Acharya K, Sharifi-Rad R, Martorell M, Sureda A, Martins N and Sharifi-Rad J. Plants of genus *Mentha*: from farm to food factory. *Plants (Basel)* 2018; 7(3): 70. doi: 10.3390/plants7030070.
33. Pan B, Bai YM, Leibovitch S and Smith DL. Plant growth promoting rhizobacteria and kinetin as ways to promote corn growth and yield in a short growing season area. *Eur. J. Agron* 1999; 11: 179-186. doi: 10.1016/S1161-0301(99)00029-5.
34. Shariatmadari Z, Riahi H, Seyed Hashtroudi M, Ghassempour AR and Aghashariatmadary Z. Plant growth promoting cyanobacteria and their distribution in terrestrial habitats of Iran. *Soil. Sci. Plant Nutr* 2013; 59:535–547. doi: 10.1080/00380768.2013.782253.
35. Santini G, Rodolfi L, Biondi N and et al. Effects of cyanobacterial-based biostimulants on plant growth and development: a case study on basil (*Ocimum basilicum* L.). *J. Appl. Phycol.* 2022; 34:2063–2073. doi: 10.1007/s10811-022-02781-4.
36. Gonçalves AL. The use of microalgae and cyanobacteria in the improvement of agricultural practices: a review on their biofertilising, biostimulating and biopesticide roles. *Appl. Sci.* 2021; 11: 871. doi: 10.3390/app11020871.
37. Tan CY, Dodd IC, Chen JE, Phang SM, Chin CF, Yow YY and Ratnayake S. Regulation of algal and cyanobacterial auxin production, physiology, and application in agriculture: an overview. *J. Appl. Phycol.* 2021; 33:2995-3023 doi: 10.1007/s10811-021-02475-3.
38. Mobli M and Baninasab B. Effect of Indole butyric acid on root regeneration and seedling survival after transplanting of three *Pistacia* species. *J. Fruit Ornament. Plant. Res.* 2009; 17(1): 5-13.
39. Hajlaoui H, Trabelsi N, Noumi E, Snoussi M, Fallah H, Ksouri R and Bakhrouf A. Biological activities of the essential oils and methanol extract of two cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. *World J. Microbiol. Biotechnol* 2009; 25: 2227–2238. doi: 10.1007/s11274-009-0130-3.
40. Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A and Ozkan H. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*.

- Food Chem* 2007; 103: 1449-1456. doi: 10.1016/j.foodchem.2006.10.061.
- 41.** Croteau RB, Davis ME, Ringer KL and Wildung MR. Menthol biosynthesis and molecular genetics. *Naturwissenschaften* 2005; 92: 562-77. doi: 10.1007/s00114-005-0055-0.
- 42.** Yin C, Liu B, Wang P, Li X, Li Y, Zheng X, Tai T, Wang C and Liu B. Eucalyptol alleviates inflammation and pain responses in a mouse model of gout arthritis. *Br J. Pharmacol.* 2020; 177(9): 2042-2057. doi: 10.1111/bph.14967.
- 43.** Alamgir ANM. Therapeutic Use of Medicinal Plants and their Extracts: Volume 2: Phytochemistry and Bioactive Compounds (Progress in Drug Research Book 74). Springer International Publishing AG, Bangladesh; 2018.
- 44.** Hristozkova M, Gigova L, Geneva M, Stancheva I, Vasileva I, Sichanova M and Mincheva J. Mycorrhizal fungi and microalgae modulate antioxidant capacity of basil plants. *J. Plant Prot. Res* 2017; 57(4): 417-426. doi: 10.1515/jppr-2017-0057.
- 45.** Hristozkova M, Gigova L, Geneva M, Stancheva I, Velikova V and Marinova G. Influence of mycorrhizal fungi and microalgae dual inoculation on basil plants performance. *Gesunde Pflanz* 2018; 70(2): 99-107. doi: 10.1007/s10343-018-0420-5.
- 46.** Hormozinejad E, Zolfaghari M, Mahmoodi Sourestani M and Enayati Zamir N. Effects of plant growth promoting rhizobacteria and chemical fertilizer on growth, yield, flowering, physiological properties, and total phenolic content of *Calendula officinalis* L. *Iranian Journal of Medicinal and Aromatic Plants Research* 2018; 34(4): 684-696. doi: 10.22092/ijmapr.2018.109899.1985.
- 47.** Dewick PM. Medicinal natural products: a biosynthetic approach, 3rd Edition. John Wiley & Sons, Ltd, Publication, United Kingdom; 2009.
- 48.** Ahmadi S, Mehrab Y and Behzad H. Foliar application effects of salicylic acid and indole acetic acid on the essential oil composition of *Mentha piperita* L. and *Melissa officinalis* L. *Journal of Plant Process and Function* 2018; 7(26): 251-262.
- 49.** Del Roario Cappellari L, Santoro M, Schmidt A, Gershenzon J and Banchio E. Induction of essential oil production in *Mentha × piperita* by plant growth promoting bacteria was correlated with an increase in jasmonate and salicylate levels and a higher density of glandular trichomes. *Plant. Physiol. Biochem.* 2019; 141: 142-153. doi: 10.1016/j.plaphy. 2019.05.030.
- 50.** Riahi L, Cherif H, Miladi S, Neifar M, Bejaoui B, Chouchane H, Masmoudi AS and Cherif A. Use of plant growth promoting bacteria as an efficient biotechnological tool to enhance the biomass and secondary metabolites production of the industrial crop *Pelargonium graveolens* L'Hér. under semi-controlled conditions. *Ind Crops Prod* 2020; 154: 112721. doi: 10.1016/j.indcrop.2020.112721.

How to cite this article: Shariatmadari Z, Ghorbani Nohooji M, Riahi H, Heidary F. Optimization of Essential oils production in *Mentha longifolia* L. using plant growth promoting cyanobacteria. *Journal of Medicinal Plants* 2022; 21(83): 47-59. doi: 10.52547/jmp.21.83.47



## فصلنامه گیاهان دارویی

Journal homepage: [www.jmp.ir](http://www.jmp.ir)

مقاله تحقیقاتی

## بهینه‌سازی تولید اسانس در گیاه *Mentha longifolia* L. با استفاده از سیانوباکتری‌های تحریک کننده رشد گیاه

زینب شریعتمداری<sup>۱</sup>، مجید قربانی نهوجی<sup>۲</sup>، حسین ریاحی<sup>۱\*</sup>، فاطمه حیدری<sup>۱</sup><sup>۱</sup>گروه علوم و زیست فناوری گیاهی، دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران<sup>۲</sup>مرکز تحقیقات گیاهان دارویی، پژوهشگاه گیاهان دارویی جهاد دانشگاهی، کرج، ایران

اطلاعات مقاله	چکیده
گل‌واژگان:	مقدمه: عوامل زیستی نظیر سیانوباکتری‌های خاکزی می‌توانند به‌عنوان عوامل تحریک‌کننده زیستی موجب افزایش رشد و محصول گیاهان دارویی شوند. هدف: هدف از این مطالعه، ارزیابی اثر سیانوباکتری‌ها بر تولید و محتوای اسانس گیاه دارویی <i>Mentha longifolia</i> L. است. روش بررسی: در این مطالعه دو گونه از سیانوباکتری‌های دارای هتروسیست شامل <i>Anabaena vaginicola</i> ISB42 و <i>Nostoc spongiaeforme</i> ISB65، به‌منظور بهینه‌سازی رشد، و نیز افزایش کارایی تولید اسانس گیاه دارویی <i>M. longifolia</i> L. انتخاب شدند. در مجموع پنج گلدان به ازای هر تیمار و نیز به‌عنوان شاهد در نظر گرفته شدند. گیاهان تیمار به مدت ۱۰۰ روز و در فواصل ۲۱ روزه با ۲۰۰ میلی‌لیتر سوسپانسیون ۰/۲ درصد سیانوباکتریایی آبیاری شدند. سپس ارزیابی و مقایسه صفات رویشی شامل طول ریشه و بخش هوایی، سطح برگ، و نیز وزن تر و خشک گیاهان تیمار و شاهد صورت گرفت. علاوه بر صفات رویشی، آنالیز اسانس و ارزیابی کمی و کیفی اسانس گیاهان نیز انجام شد. نتایج: نتایج حاصل از این مطالعه نشان‌دهنده افزایش معنی‌دار مقادیر مربوط به پارامترهای رویشی گیاهان تیمار در مقایسه با گیاهان شاهد بود. همچنین، برخی متابولیت‌های اقتصادی و دارویی این گیاه نظیر منتول، اوکالیتول و فیتول در اسانس گیاهان تیمار افزایش نشان داد. در این مطالعه همچنین افزایش معنی‌دار سزکونی‌ترین‌های هیدروکربنه و دی‌ترین‌های اکسیژنه در گیاهان تیمار شده با سیانوباکتری‌ها مشاهده شد. نتیجه گیری: بر اساس نتایج به‌دست آمده، به‌نظر می‌رسد استفاده از تیمارهای سیانوباکتریایی به‌عنوان عوامل تحریک‌کننده زیستی می‌تواند جهت افزایش محصول و نیز بهبود ارزش دارویی و اقتصادی این گیاه دارویی در نظر گرفته شود.

مخفف‌ها: MH، هیدروکربن‌های مونوترپن؛ OM، مونوترپن‌های اکسیژنه؛ SH، هیدروکربن‌های سزکونی‌ترین؛ OS، سزکونی‌ترین‌های اکسیژنه؛ OD، دی‌ترین‌های اکسیژنه

\* نویسنده مسؤول: [h-riahi@sbu.ac.ir](mailto:h-riahi@sbu.ac.ir)

تاریخ دریافت: ۱۷ فروردین ۱۴۰۱؛ تاریخ دریافت اصلاحات: ۵ شهریور ۱۴۰۱؛ تاریخ پذیرش: ۶ شهریور ۱۴۰۱

doi: [10.52547/jmp.21.83.47](https://doi.org/10.52547/jmp.21.83.47)© 2020. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>)