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Research Article

Effects of Plant Growth Promoting Bacteria on Growth and Essential Oil Production of Peppermint

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Abstract: Peppermint is abundant with organic compounds having therapeutic, coloring, preservative, and other uses for humans. Moreover, peppermint is used in food and perfume industry and as medicine in every corner over the world. Considering the economic and medicinal value of peppermint, there is a significant gap between supply and demand. Therefore, an analysis of the impact of bacteria that promote plant development was carried out on peppermints (*Pseudomonas putida* and *Curtobacterium* sp. *strain* LUW) essential oil production. Under the study, the plant growth parameters, essential oil, chlorophyll and carotenoids, and free proline of leaves were measured. The outcomes demonstrated that the use of both *Pseudomonas* and *Curtobacterium* (Plant Growth Promoting Bacteria) in the soil significantly increased root diameter, leaf area, leaf number, plant height, quantity of stems, root length, root volume, dry weight of leaf, and peppermint plants' relative water content by 34.29, 23.34, 36.57, 21.08, 87.5, 12.28, 20.37, 42.62, and 6.46 compared to the control conditions respectively. Furthermore, the application of bacteria in the soil that promotes plant growth raised the total quantity of essential oil, proline, and chlorophyll concentration in the leaf by 50.90, 20.90, and 33.35%, respectively, compared to the control conditions. Moreover, essential oil and proline content increased with *Curtobacterium* sp. *strain* application compared to *Pseudomonas putida.*

Keywords: *Curtobacterium* sp. *strain*, Essential Oil, Growth, Peppermint, *Pseudomonas putida*.

1. INTRODUCTION

It is well known that medicinal plants are abundant with chemicals having therapeutic, coloring, preservative, and other uses for human beings [1]. Peppermint (*Mentha piperita* L.) a perennial herbaceous plant, related to the family of Lamiaceae, genera *Mentha*, used as a medicine and immune-boosting food all over the world [2]. Water mint (*M. aqutica*) and spearmint (*M. spicata*) naturally crossed to produce peppermint [3]. Peppermint has a rhizome and short root, and the height of plant reaches about 30 to 90 cm. Peppermint has a straight, ascending, and branched stem, which is completely square in the upper part and it is reddish-purple or purple in color, and has two opposite leaves in each of the nodes. This plant has ovate leaves elongated to lance-shaped, petiolate, pointed toothed, slightly covered with hair, and opposite, 4 – 9 cm in length and 1.5 – 4 cm in width, whose upper surface is dark green. Peppermint has irregular flowers, mostly bisexual or hermaphrodite, having red, purple, or white flowers depending on the type of peppermint, and appear in the months of August and September. Its fruits are oval and hazelnut-shaped [4]. Approximately, all essential oils are synthesized and stored in the secretive hairs located on the surface of leaves [5]. Essential oil of peppermint has different contents such as menthyl acetate and menthone (monoterpenea), menthol (cyclic monoterpene alcohol), and a small amount of cineole and other terpenes.

Plant growth-promoting bacteria (PGPBs) include *Azospirillum*, *Azotobacter*, *Rhodococcus, Bacillus*, and *Pseudomonas* produce hormone-like substances, reduce ethylene levels, prepare and absorb plant-required elements such as

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phosphorus, iron, nitrogen, and potassium, and produce antimicrobial compounds which increase the host plants' ability to withstand a variety of environmental stresses including pests, diseases, salinity and drought [6]. *Azotobacter* is very important in the biological fixation of nitrogen and *Bacillus* and *Pseudomonas* in the transformation of phosphorus from its insoluble forms into soluble forms that plants can absorb. In addition, by generating siderophores for example, these bacteria promote plant growth in several ways, synthesis of antibiotics, and production of plant hormones [7]. Benchio *et al*. [8] reported that the application of *Bacillus* growth-promoting bacteria to basil plants resulted in a twofold increase in the amount of plant essential oil as well as an increase in biomass.

Considering the economic importance of peppermint plant in various pharmaceutical and food industries, this study aimed to examine the impact of *Pseudomonas putida* and *Curtobacterium* sp. *strain* LUW, two plant growth-promoting bacteria, on the development and yield of peppermint essential oil.

2. MATERIALS AND METHODS

This study was conducted at the research farm of Lorestan University, Iran, in 2022 (in greenhouse) located 33.38^{o} north and 48.35^{o} east, 1147 m above sea level, and an average temperature of 25±5 °C during the growing season. Rhizomes (*M. piperita* L.) were acquired from Research and Technology Complex of Medicinal Plants, Lorestan Province. Iran, and bacterial strains of *Curtobacterum* sp. *strain* LUW and *Pseudomonas putida* from the bacteriological collection from the Department of Plant Protection at the Agriculture Faculty, Lorestan University, Lorestan, Iran. Before planting the rhizomes, *Curtobacterium* and *Pseudomonas* bacteria were placed in separate pots between the pots filled with water, later peppermint rhizomes were placed between the pots containing the bacteria to inoculate the roots for a period of time. It was left in the pan for an hour. The strains were cultured in Vitro. Three treatments, i.e., control, *Pseudomonas putida and Curtobacterum* sp. *Strain* each with 8 replications were used. The rhizomes of peppermint were subjected to inoculation with two species of PGPBs. For this purpose, utilizing a fully randomized design, a factorial experiment was performed. Thereafter, the peppermint roots were inoculated with plant growth-stimulating bacteria, the inoculated rhizomes were grown in pots containing two kilograms of sterilized agricultural soil, manure, and cocopeat in a 2:1:1 ratio. The morphological traits including plant height, plant crown, number of leaves, branches and internode, length, volume and dry weight of root, leaf dry weight, length of internode, fresh and dry weight of stem were measured. The chlorophyll and carotenoids content were measured using Lichtenthaler method [9], free proline in leaves was measured using Bates method [10], and essential oil from dried leaves and petioles was measured using the distillation method [11]. The data were analyzed using Fisher LSD method and 95% confidence.

3. RESULTS AND DISCUSSION

3.1. Morphological Features

Variance analysis of data showed that *Pseudomonas putida* and *Curtobacterium* sp. *strain* LUW plant growth-promoting bacteria increased plant growth parameters such as plant height, leaves, leaf area, crown diameter, number of stems, roots' diameter, length, and volume, internode number, length of internode, weight of fresh and dry leaf, weight of fresh and dry stem, weight of fresh and dry root, and relative water content by 21.08, 36.57, 23.35, 31.13, 87.5, 34.29, 12.28, 20.37, 13.79, 18.54, 28.18, 42.62, 39.11, 52.20, 49.08, 40.06, and 6.460% respectively, in comparison to control. The results indicated that the application of PGPBs increased morphological characteristics such as plant height, quantity, and leaf surface, crown diameter, quantity of stems, diameter, volume, and length of the roots, number and length of internodes significantly. Which shows the positive effects of plant growth promoting bacteria about peppermint's development and growth (Table 1).

According to Ferreira *et al*. [11], the increase in the growth is because of nitrogen fixation, dissolution of mineral phosphates, iron absorption with siderophore, adjusting the level of plant hormones such as auxin or ethylene (through ACC (1-Aminocyclopropane-1-Carboxylate) deaminase activity), and combating pathogens by releasing hydrogen cyanide. Other studies' findings demonstrated that the use of PGPBs increases plant height, diameter of the stem, leaf count, length and quantity of branches, and dry and fresh weight of aerial parts [12]. Studies on black seed [13], sage

Table 1: Effect of PGPBs application on the morphological traits of peppermint.

Plant growth stimulating bacteria	Plant height (cm)	Leaves (number)	Shoots (number)	Root volume (cm^3)	Internode (number)	Dry weight of leaf (g)	Dry weight of stem (g)	Dry weight of root (g)
Control	22.50 b	45.58 b	5.41 b	10.75 b	6.58 b	1.15 b	0.83 b	1.43 c
Pseudomonas putida	28.33 a**	67.33 a**	6.75 a**	12.95 a**	7.33 ab**	1.39 a**	1.20 a**	1.91 b**
Curtobacterium strain	27.91 a**	65.41 a**	6.25 ab**	12.16 a**	7.58 a**	1.39 a**	1.20 a**	2.16 a**

** and * are significant at the level of 1 and 5% probability.

plant [14], and pumpkin [15], also figured out that the application of PGPBs increases the growth of plants. Numerous researches indicated that the increase in plant growth is due to PGPBs induction of growth-promoting hormones such as auxins and gibberellins, which increase the number and length of plant cells. By changing the structure of the root system, PGPBs enhance nutrient absorption, allocation of carbohydrates to the root, reduction of root peroxidase activity, synthesis of new proteins, and ultimately cause a rise in the growth of plant roots [16].

In the present study, the use of PGPBs increased the growth coefficient in peppermint plants. Thus, the increase and improvement in growth were perhaps because of auxin and gibberellin production and absorption of nutrients like nitrogen, phosphorus, potassium, magnesium, and boron. Several other researches also reported the positive influence of growth-stimulating bacteria on the height of various plants [17]. Similar results having positive effect of PGPBs on the increase of the total number of leaves was reported by Fasihi *et al*. [18], and the total number of branches by Yasri *et al*. [19].

3.2. Essential Oil

Application of *Pseudomonas putida* and *Curtobacterium* sp. *strain* LUW (PGPBs) increased the percentage of plant essential oil by 34.61 and 50.90% respectively, compared to the control. The amount of peppermint essential oil significantly increased when PGPBs were used. Leithy *et al*. [20] reported that the use of *Azotobacter*, *Azospirillium,* and phosphate-dissolving bacteria increased essential oil production in *Mazorana hortensis*. According to Singh *et al*. [21] essential oil is increased in leaves by using Nitrogen (*Mentha arvensis* and *Mentha piperita*). Benchio *et al*. [8] also stated that the utilization of Bacillus plant growth stimulating bacteria on the basil plant raised biomass and the amount of basil essential oil by two times. Rati *et al*. [22], concluded that the application of phosphate-dissolving bacteria together with insoluble inorganic phosphate called tricalcium phosphate caused a significant improvement in the concentration of phosphorus in the stem and a notable rise in essential oil percentage compared to control.

3.3. Chlorophyll and Carotenoids

Inoculation of peppermint rhizomes and soil with *Curtobacterium strain* and *Pseudomonas putida* plant growth-promoting bacteria, increased the amount of chlorophyll in the whole leaf by 33.35% (chlorophyll-a 37.91% and chlorophyll-b by 26.10%) and 25.15% (chlorophyll-a 33.56% and chlorophyll-b 13.72%) in contrast to the treatments under control. The content of carotenoids in the plant leafs increased by 49.98% and 56.88% in contrast to the control with the application of *Pseudomonas putida* and *Curtobacterium strain* plant growth-promoting bacteria, respectively. However, the differences between *Pseudomonas* and *Curtobacterium* bacteria were not statistically significant (Table 2).

The rise in the amount of chlorophyll with growth-promoting bacteria may result from the remarkable superiority in producing siderophores in these above-mentioned bacteria. Additionally, auxin is important in raising the amount of chlorophyll, so in an experiment, the treatment of wheat leaves with 100 mg/kg indole-3-acetic acid caused a 31% increase in the chlorophyll content in contrast to the treatment under control [17]. The research conducted by Iftikhar *et al*. [23] suggested

Table 2. Effect of PGPBs application on the production of essential oil, chlorophyll carotenoid, and leaf proline.

Plant growth-promoting bacteria	Essential oil %	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid	Leaf proline
Control	0.39 b	6.26 c	4.60 b	10.87 c	1.37 b	0.43 a
Pseudomonas putida	0.53 a**	8.36 b**	5.23 a**	13.60 b**	2.20 a**	0.33 b**
Curtobacterium strain	0.60 a**	9.45 a**	5.82 a**	15.28 a**	2.15 a**	0.39 a**

** and * are significant at the level of 1 and 5% probability.

that PGPBs with the ability to dissolve phosphate by producing acidic compounds not only can increase the phosphorus's solubility, but additionally boost the uptake of magnesium and iron, which are essential elements for the production of chlorophyll.

3.4. Proline

Inoculation of peppermint rhizomes and soil with *Pseudomonas putida* and *Curtobacterium sp.* PGPBs enhanced the concentration of plant proline by 20.90% compared to the control treatments. Applying PGPBs effectively increased the proline content of leaves (Table 2). Proline is produced by the nitrogen metabolism of plants, where nitrate is first transformed into nitrite and then ammonia, following which glutamine and glutamate are used to convert it to amino acids. Therefore, PGPBs contribute to the above process by fixing nitrogen and increasing the plant's proline. The results of the study show that PGPBs can fix nitrogen. Consequently, the increase in proline in peppermint can be linked to the peppermint plant's bacteria producing more nitrogen [18].

4. CONCLUSIONS

According to the Findings of the study, application of both *Pseudomonas* and *Curtobacterium* (PGPBs) in the soil significantly enhanced plant height, leaf area, number of leaves, root diameter, number of stems, root length, root volume, dry leaf weight, and relative water content of peppermint plants in comparison to the control circumstances. Furthermore, applying of plant growth stimulating bacteria in the soil increased the total amount of essential oil, Carotenoid, and chlorophyll content in the leaf.

5. CONFLICT OF INTEREST

The authors declare that there is conflict of interest.

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