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The Impact of Rhizospheric *Pseudomonas aeruginosa* on the Growth of *Melissa officinalis*

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Abstract

Microbial communities occupy a significant position in the functioning and productivity of agricultural ecosystems. The taxonomic genus *Pseudomonas* encompasses a group of bacterial species that can establish mutually beneficial partnerships with different plants. The objective of this study was to ascertain and isolate *Pseudomonas* strains that were obtained from the rhizosphere soil of *M. officinalis*. Furthermore, an examination was conducted on the morphological characteristics of *M. officinalis* that had been treated with standard and rhizospheric *Pseudomonas*. The experimental treatments consisted of 3 different groups: a rhizosphere *Pseudomonas* inoculation with a concentration of 106 and 109 CFU /ml, standard *Pseudomonas* inoculation with a concentration of 106 and 109 CFU /ml, and a control group that did not contain any bacteria. To effectively separate bacteria, specialized culture medium, such as the King B medium, were employed. The biochemical and molecular result revealed that the isolated strain was *Pseudomonas aeruginosa*. The standard *Pseudomonas* treatment with a concentration of 109 CFU/ml exhibited the greatest root length of 24.1 cm, while the treatment lacking the presence of *Pseudomonas* displayed the lowest root length of 12.9. Treatments with rhizospheric *P. aeruginosa* showed the greatest values for stem length, as well as root and stem weight. Meanwhile, the control treatment resulted in the lowest measurements for these mentioned characteristics. The absence of bacteria (microorganisms) is observed in this context. The present study's results demonstrated that the impact of rhizospheric *P. aeruginosa* on the growth and quality of *M. officinalis* was substantial, resulting in enhanced quantitative and qualitative growth of the plant. The highest magnitude of root length and weight was achieved through the inoculation of *P. aeruginosa*

Key words: *Melissa officinalis*, Rhizospheric, Rhizobacteria, *Pseudomonas aeruginosa*

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1. Introduction

Plant growth promoting bacteria (PGPR) exhibit various mechanisms including the biological fixation of atmospheric nitrogen, enhancement of nutrient availability in the rhizosphere, elevation of plant growth parameters, production of siderophores that chelate iron, synthesis of plant hormones, manufacture of antibiotics and fungicidal compounds, establishment of symbiotic interactions with plants, and a synergistic combination of these approaches for the enhancement of overall plant growth (Backer et al., 2018; Hatami et al. 2021; Vocciante et al. 2022; Fahde et al. 2023). Several species of bacteria possess the capacity to coexist harmoniously with plants, belonging to the taxonomic classifications of *Pseudomonas*, *Azotobacter, Azospirillium*, and *Bacillus* (Amin Deldar et al. 2014; Abdelaal et al. 2021; Minut et al. 2023). All strains of *Pseudomonas* possess the capability to produce auxin (Bakaeva et al. 2020). Various species within the *Pseudomonas* genus have demonstrated efficacy in managing pathogenic fungi through a multitude of mechanisms. These include the facilitation of siderophore production, antibiotic synthesis, and plant hormone production, enhancement of phosphorus absorption, nitrogen fixation, and enzymatic synthesis, which thereby regulates ethylene levels in plants and stimulates plant growth (Zboralskiet al. 2022). Previous studies have demonstrated the beneficial impact of employing *Pseudomonas* on the development of both aboveground and belowground anatomical structures. The primary factor behind these beneficial effects can be ascribed to the diverse properties of growth stimulants exhibited by these strains (Qessaoui et al. 2019). There are multiple reports discussing the positive effects of these bacteria on the different yields. The evidence from these accounts supports the notion that these bacteria increase nitrogen fixation and improve the absorption of important substances like phosphorus, potassium, and iron, hydration levels in plants and the production of plant hormones (Preston 2004). The observed increment is attributed to diverse mechanisms, including the provision of nitrogen for plant growth via N2 fixation, the synthesis of growth-promoting substances or phytohormones such as auxin, cytokinin, and gibberellin, as well as the application of biological agents to control soil-borne pathogens (Kavin et al., 2010; Shokati and Poudineh 2017). The botanical species *Melissa officinalis*, commonly referred to as lemon balm, belongs to the dicotyledonous plants within the order of flowers and the Lamiaceae family (Rasmussen et al. 2011; Miraj et al. 2017). The plant under consideration traces its origins to the Mediterranean and West Asia regions. Lemon balm essential oil is obtained from the flowers, as well as fresh or dried branches, through the processes of steam distillation or chemical extraction, often employing bergamot (Petrisor et al. 2022). In contemporary times, the field of agriculture and environment has encountered a multitude of issues that have given rise to concerns pertaining to pollution, particularly within water and soil resources. The implementation of biological fertilizers in conjunction with the utilization of appropriate organic substances is suggested as the most holistic and preferred approach to maintain the vital functionality of the soil system (Kurniawat et al. 2023). Currently, there is a significant undertaking aimed at discovering viable approaches to enhance soil quality, agricultural yields, and mitigate the presence of contaminants (Abraham et al., 2007; Sharma, 2004). This study aimed to investigate various morphological parameters of the *M. officinalis* plant after treatment with standard and rhizospheric *Pseudomonas aeruginosa*. This methodology is completely devoid of any potential chemical hazards or adverse effects.

1.Materials and Methods

1.1.Sampling

Initially, specimens of M. *officinalis* were harvested from the rhizosphere soil located in the western region of Chadegan, characterized by a substantial concentration of flora. The forthcoming investigation is slated to take place in April 2016 within the esteemed research center and laboratory of Islamic Azad University, specifically at its Falavarjan branch. The present study employed *Pseudomonas aeruginosa* bacteria strain PTCC1573, which is a standard strain, in

conjunction with *Pseudomonas* bacteria isolated from the rhizosphere region.

1.2.Investigating the macroscopic and microscopic characteristics of rhizospheric *Pseudomonas*

In the initial phase, a microscopic evaluation was conducted to assess the phenotypic characteristics of the bacterial colonies on various culture media, namely King B, MacConkey agar, Muller Hinton Agar, and Blood agar (Merck, Germany). The gram staining procedure was conducted using the colonies obtained (Javaheri et al. 2022; Tabatabaei Ahmadrezaei et al. 2021; Yazdani et al. 2023).

1.3.Isolation of *Pseudomonas* bacteria from rhizosphere soil

A soil sample was collected from the *M. officinalis* to aid in the extraction of *Pseudomonas* bacteria present in the microbiome. In order to facilitate the extraction of *Pseudomonas* bacteria from the microbiome of basil plant root soil, a soil sample was collected and transported to the research laboratory of Falavarjan Azad University. Next, a total of 10 grams of highly fibrous soil, combined with the minute roots of the said plant, were carefully transferred into an Erlenmeyer flask that contained 90 ml of sterile distilled water. The process of dilution was extended until a magnitude of 109, subsequent to which, the resultant dilutions were subjected to cultivation in the form of grass on a solid culture medium known as King B (Merck, Germany). The petri dishes were subjected to incubation at a temperature of 28 °C for a time period of 24 hours. Subsequently, the colonies deemed suitable were chosen and subjected to linearization using King B's specialized culture medium. Samples were derived from suitable individual colonies and subsequently prepared for the purpose of identification. The KB Agar culture medium, also known as *Pseudomonas* Agar F or Flo Agar, is utilized in the enhancement of fluorescein production within the *Pseudomonas* group. This medium comprises pancreatic digest of casein, proteose peptone, dipotassium phosphate, magnesium sulfate, and agar.

1.4.Investigating the biochemical and molecular characteristics of rhizospheric *Pseudomonas*

The application of catalase, TSI, and oxidase tests was employed to initially discern and identify *Pseudomonas*. One can ascertain the existence of a particular gene fragment in bacteria through the implementation of polymerase chain reaction (PCR) methodology. In this protocol, a segment of every colony was solubilized in 10 microliters of sterile water by means of a sterile loop, and subsequently employed as a template for the reaction in this experimental approach. A fraction of every colony sample was suspended in a solution of denatured water, utilizing a sterile loop, to yield a final volume of 10 microliters. This diluted suspension served as the template for the polymerase chain reaction (PCR) amplification. Following the completion of the PCR, the resultant product was subsequently placed onto a 1% agarose gel.

1.5.The effect of rhizospheric bacteria on the morphological characteristics of the Melissa *officinalis*

In this experimental design, a total of 60 plant seeds were individually positioned at uniform intervals within each Petri dish. The Petri dishes were then sealed with Parafilm and placed in a growth chamber under controlled conditions. In the subsequent experiment, 2 concentrations (106 and 109 CFU/ml) of standard *P. aeruginosa* inoculum (PTCC1573) and 2 concentrations (106 and 109 CFU/ml) of *Pseudomonas* inoculum extracted from the rhizosphere were employed. The tested plant's contamination was subjected to the germinator (model JTGL200) set at a temperature of 25 °C and a photoperiod consisting of 16 hours of light and 8 hours of darkness. Simultaneously, the control sample (representing the environment devoid of bacteria) was also placed in the same setting. Regular monitoring of seed germination was conducted on a daily basis over a span of 10 consecutive days at a predetermined time. Subsequently, the enumeration process ceased, and the percentage of germinated seeds was ascertained by utilizing the prescribed equation. Once the plant attained the three-leaf stage, the dimension of both the root and stem were measured employing a ruler possessing a precision of 1 mm.

1.6. Statistical analysis

This study was conducted using a factorial

experiment with a completely randomized design that included three replications. The data underwent analysis of variance using SPSS19 software, and the comparison of means was conducted utilizing Duncan's method ($P<0.05$) at the examined level. Graphs were generated using Excel software.

2.Results

2.1. Macroscopic identification of isolate

The *Pseudomonas* colonies that were gathered were subjected to microscopic examination using differential and specific medium. Table 1 illustrated the findings generated from macroscopic examinations. The microscopic result indicated Gram-Negative and rod-shaped bacterium.

2.2.Biochemical identification of rhizobacteria *pseudomonas* isolate

According to the findings presented in Table 2, the biochemical identification of the bacteria revealed that it was capable of thriving in MHA, MC, and KB media. In addition, the catalase and oxidase tests exhibited positive results for this bacterial strain.

2.3.Molecular identification of rhizobacteria *pseudomonas* isolate

Following the assessment of the PCR volume obtained from the amplification process of the targeted 16S rRNA bacterial genes, the resultant PCR product was subsequently loaded onto a 1% agarose gel for further examination of its quality. In reference to Figure 1, it is evident that the product has generated a band size measuring 350 bp (base pairs).

2.4.The effect of standard and rhizospheric *Pseudomonas aeruginosa* on the germination

The germination characteristics of *Melissa officinalis* were observed to be influenced by the presence of *Pseudomonas* rhizospheric bacteria and standard bacteria, resulting in statistically significant effects at a significance level of 5%. The rhizosphere bacteria treatment exhibited a noticeably superior germination rate of 98% when subjected to a concentration of 109 CFU/ml. The germination rate of *M. officinalis* was observed to be 60%, which was found to be the lowest when the treatment lacked the presence of *Pseudomonas* bacteria.

2.5.The effect of standard and rhizospheric *Pseudomonas aeruginosa* on the Shoot length

The examination of variance within the experimental data indicated that the application of rhizobacteria *Pseudomonas* isolate has a significant impact on the shoot length of *M. officinalis* at a confidence level of 95%. The analysis of the average rice stem lengths revealed that the treatment containing rhizospheric *Pseudomonas* isolate with a concentration of 109 CFU/ml exhibited the highest mean stem length at 11.9 cm, whereas the control treatment had the lowest mean stem length recorded at 6.2 cm.

2.6.The effect of standard and rhizospheric *Pseudomonas aeruginosa* on the Shoot weight

The treatment of rhizobacteria *Pseudomonas* isolate affected the shoot weight characteristic of *Melissa officinalis* plant at a statistical level of 5%. Comparison of the average of different treatments revealed that the highest and lowest stem weight is 2.5 and 1.4 grams are present in standard *Pseudomonas* bacteria treatments with a concentration of 106 and 109 CFU/ml the control treatment, respectively

Table 1. The results of microscopic tests of rhizobacteria *pseudomonas* isolate on various culture media

Medium	Macroscopic characterization
King's B Medium (KB)	Round and smooth colonies, large size with smooth margins.
MacConkey agar (MC)	Medium-sized and pink colonies
Muller Hinton Agar (MHA)	Blue-green colonies
Blood Agar (BA)	Dull, gray opaque, relatively large and usually beta hemolytic colonies

Table 2. The results of a number of biochemical tests to detect rhizobacteria *pseudomonas* isolate

Isolate	Catalase	Oxidase	OF	Growh on MHA	Growth on MC	Growth on King B	Gelatinase
P. aeruginosa	+	+	+	+	+	+	-

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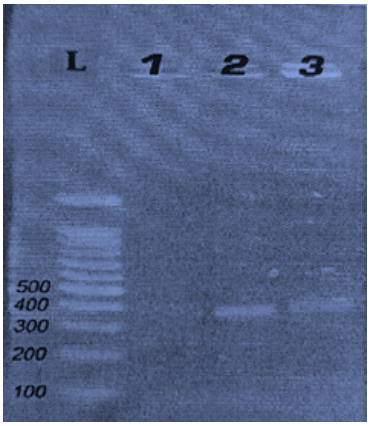

Fig. 1. The results of electrophoresis of PCR product, *Pseudomonas* strain on 1% agarose gel, PCR product was 350bp. Line L: Ladder, line 1: Negative control; line 2: rhizobacteria *Pseudomonas aeruginosa*; line 3: Positive control

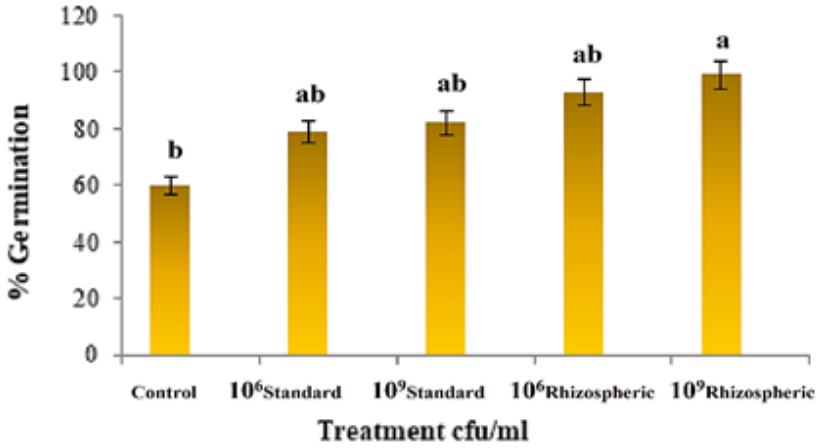

Fig. 2. A comparative study was conducted to assess the average impact of the *Pseudomonas* isolate of rhizobacteria on the germination characteristics of *M. officinalis*. The experimental treatments involved a control group without the presence of Pseudomonas bacteria, as well as groups treated with 106 and 109 CFU/ml of standard *Pseudomonas* bacteria and 106 and 109 CFU/ml of rhizosphere bacteria. Statistical analysis revealed that the letter (a) indicated bacteria that do not differ significantly, the letter (b, c) indicated bacteria that are significantly different; (a) P≤0.05, (b) P≤0.01, (c) P≤0.001.

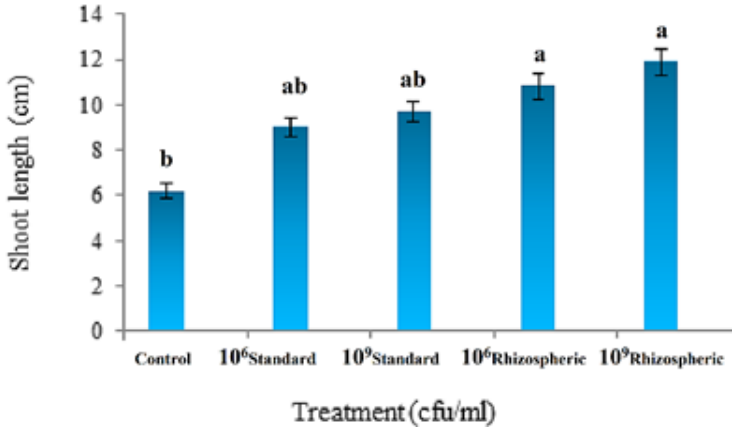

Fig .3. A comparative study was conducted to assess the average impact of the *Pseudomonas* isolate of rhizobacteria on the germination characteristics of *M. officinalis*. The experimental treatments involved a control group without the presence of Pseudomonas bacteria, as well as groups treated with 106 and 109 CFU/ml of standard *Pseudomonas* bacteria and 106 and 109 CFU/ml of rhizosphere bacteria. Statistical analysis revealed that the letter (a) indicated bacteria that do not differ significantly, the letter (b, c) indicated bacteria that are significantly different; (a) P≤0.05, (b) P≤0.01, (c) P≤0.001.

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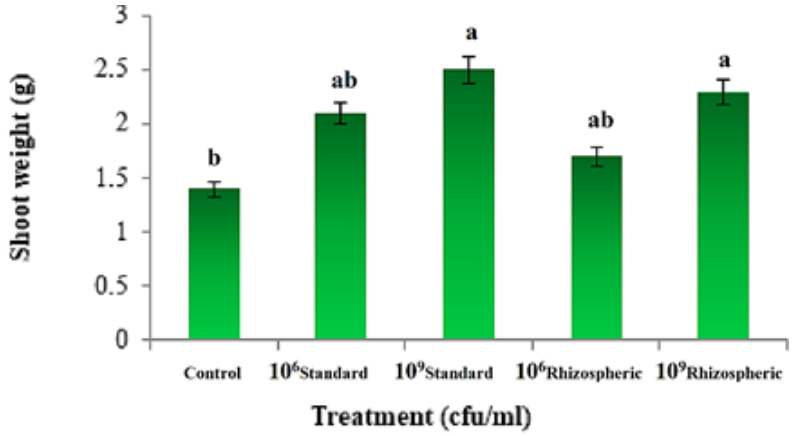

Fig. 4. A comparative study was conducted to assess the average impact of the *Pseudomonas* isolate of rhizobacteria on the germination characteristics of *M. officinalis*. The experimental treatments involved a control group without the presence of *Pseudomonas* bacteria, as well as groups treated with 106 and 109 CFU/ml of standard *Pseudomonas* bacteria and 106 and 109 CFU/ml of rhizosphere bacteria. Statistical analysis revealed that the letter (a) indicated bacteria that do not differ significantly, the letter (b, c) indicated bacteria that are significantly different; (a) P≤0.05, (b) P≤0.01, (c) P≤0.001.

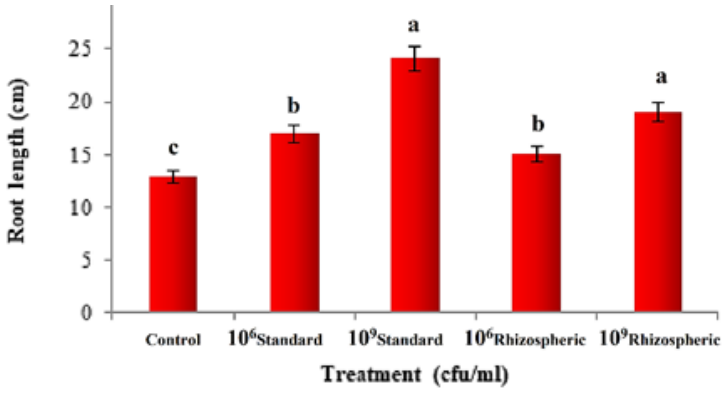

Fig.5. A comparative study was conducted to assess the average impact of the *Pseudomonas* isolate of rhizobacteria on the root length of *M. officinalis*. The experimental treatments involved a control group without the presence of *Pseudomonas* bacteria, as well as groups treated with 106 and 109 CFU/ml of standard *Pseudomonas* bacteria and 106 and 109 CFU/ml of rhizosphere bacteria. Statistical analysis revealed that the letter (a) indicated bacteria that do not differ significantly, the letter (b, c) indicated bacteria that are significantly different; (a) P≤0.05, (b) P≤0.01, (c) P≤0.001.

2.7.The effect of standard and rhizospheric

The treatment of rhizobacteria *Pseudomonas* isolate affected the root length characteristic of *M. officinalis* plant at a statistical level of 5%. Although the maximum root length of 24.1 cm belonged to the standard *Pseudomonas* bacteria treatment with concentration 109 CFU/ml, but the difference between this treatment and the rhizosphere *Pseudomonas* bacteria treatments with concentration 109 CFU/ml was not significant. The lowest plant root length of 12.9 cm was also observed in the treatment without the presence of *Pseudomonas* bacteria, which showed a decrease of about 45% compared to the superior treatment.

2.8.The effect of standard and rhizospheric *Pseudomonas aeruginosa* on the root weight

The examination of the means pertaining to various treatments indicated that the treatment involving a concentration of 109 CFU/ml of *Pseudomonas* rhizospheric bacteria demonstrated the highest root weight of 3.2 grams. The observed treatment did not yield any statistically significant distinction compared to the standard treatment approach involving *Pseudomonas* bacteria at a concentration of 109 CFU/ml. The investigation revealed that the absence of *Pseudomonas* bacteria in the treatment resulted in the lowest recorded plant root weight of 2.1 grams, demonstrating a significant reduction of approximately 34% in comparison to the superior treatment.

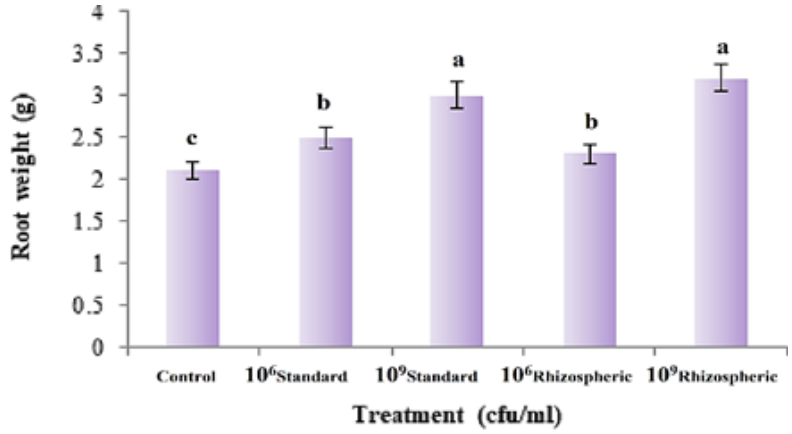

Fig.6. A comparative study was conducted to assess the average impact of the *Pseudomonas* isolate of rhizobacteria on the root weight of M. *officinalis*. The experimental treatments involved a control group without the presence of *Pseudomonas* bacteria, as well as groups treated with 106 and 109 CFU/ml of standard *Pseudomonas* bacteria and 106 and 109 CFU/ml of rhizosphere bacteria. Statistical analysis revealed that the letter (a) indicated bacteria that do not differ significantly, the letter (b, c) indicated bacteria that are significantly different; (a) P≤0.05, (b) P≤0.01, (c) P≤0.001.

3.Discussion

Enhancing plant growth has been observed to be positively influenced by specific soil microorganisms. These microorganisms, commonly known as isobacteria, can stimulate the growth of plants. *Pseudomonas* spp is a highly stimulating and efficacious bacterial genus within the field of biofertilizers, contributing significantly to the growth and development of various plant species (Vazques et al. 2000). The use of rhizobacteria, specifically called plant-growth promoting rhizobacteria (PGPR), has attracted considerable attention among scientists as a possible replacement for chemical pesticides. The rhizobacteria possess various mechanisms that facilitate plant growth, regulate plant pests, and induce resistance against diverse abiotic stresses. In this review, a thorough analysis is conducted on how rhizobacteria aid in plant growth promotion, pest control, and soil remediation. The study also places emphasis on investigating the impacts of PGPR inoculation on plant growth and survival in the presence of environmental stressors (Saeed et al. 2021). In other investigation, the impact of development advancing microscopic organisms (PGPB) on chemical-biological properties of soil, surrender and surrender components of two assortments of Khazar and Hashemi rice was examined. In this investigate, cultivar figure in 2 levels (Hashemi and Khazar rice) and seed inoculation with microscopic organisms in 8 levels (*P. fluorescens* strain 93, P. fluorescens strain 103, *P. fluorescens* strain 136, *P. fluorescens* strain 168, *P. fluorescens* strain 169, *P. fluorescens* strain 177, *P. fluorescens* strain 4, beside a control treatment (without microbes)) were considered. The characteristics examined were: soil nitrogen, soil phosphorus, soil potassium, soil causticity, soil electrical conductivity, the number of microorganisms within the soil, the number of seeds per plant, the number of seeds per cluster, the weight of 1000 seeds, organic abdicate and seed surrender. In this explore, the cultivar impact and bacterial impact on most of the examined characteristics were noteworthy. The comes about of this try appeared that seed vaccination with microbes moved forward the retention of supplements, surrender and surrender components of rice (AminDeldar et al, 2014). Several other researchers conducted investigations in line with our study, examining how seed hydropriming and the introduction of plant growth promoting rhizobacteria (namely, *Pseudomonas fluorescens* and *Pseudomonas putida*) as a biological stimulant influence various physiological characteristics of M. *officinalis* in the soil. These traits include photosynthetic action, pigments, phenol content, flavonoids, percentage yield, and essential oil production. A study conducted by researchers has demonstrated the demonstrated efficacy of rhizobacteria in promoting the growth of the *M. officinalis* plant. This finding suggests

that plant growth stimulating rhizobacteria hold promise as a viable substitute for chemical fertilizers in cultivation practices. Furthermore, such an approach ensures the cultivation of medicinal plants without the reliance on chemical agents (Hatami et al. 2021). Gholami et al examined the influence of plant growth-promoting rhizobacteria (PGPR) on the early stages of seed germination, subsequent growth of seedlings, and final yield of maize grown in a field environment. In their investigation, some bacterial strains, namely *Pseudomonas putida*, *Pseudomonas fluorescens*, Azospirillum lipoferum and *Azospirillum brasilense*, were employed in the conducted experiments. Seed inoculation significantly boosted both seed germination and seedling vigor of maize, as evidenced by the initial study results. In the subsequent experiment, the application of bacterial inoculation demonstrated a significant increase in the dry weight of both the leaves and shoots, as well as a noticeable expansion in leaf surface area, observed across both sterilized and non-sterilized soil samples. The findings from the study indicated that the application of bacterial treatments had a more pronounced impact on the growth and development of plants in nonsterile soil, as compared to sterile soil (Gholami et al. 2009). The research by Williams et al focused on investigating how the presence of *Pseudomonas* rhizospheric bacteria and arbuscular mycorrhizal fungus influences the growth and production of strawberries. The findings of their investigation showcased the ability of these microorganisms to exert a positive influence on the growth and development of strawberries (Williams et al. 1992). Kaur and her colleagues studied the potential use of a rhizosphere fungus and lemongrass extract's antimicrobial and proteolytic properties in treating human ailments. Microorganisms and the medicinal plant lemongrass are highly abundant in secondary metabolites. Fungi play a significant and essential role within the soil environment by serving as primary decomposers of plant remnants. In doing so, they facilitate the release of crucial nutrients and uphold the maintenance as well as enhancement of plant growth (Kaur et al. 2015). The findings obtained from the present study are in line with previous research by other scholars in demonstrating that treatment with rhizospheric bacteria leads to an augmentation in the concentration of vital compounds within both annual medicinal plants, such as basil (*Ocimum basilicum*), and perennial plants, such as marjoram (*Origanum majorana*) (Banchio et al. 2008; Maricel et al. 2011). *Pseudomonads putida* as well as Fluorescence as divergent growth stimulator characteristics were employed in Ghorbanpour's study. The findings demonstrated a significant increase in the dry weight of both the plant's root and shoot following the inoculation of the specified bacteria. The findings of their study indicated a notable influence exerted by rhizospheric bacteria of the *Pseudomonas* genus on the development of roots and leaves, as well as the enhancement of essential oil production and efficacy in sage plants. The application of rhizospheric bacteria treatment yielded a significant enhancement in select primary and crucial components of the essential oil, in contrast to the control group (Ghorbanpour et al. 2014). Ahemad and colleagues conducted an investigation into the effects of growth-promoting bacteria on the augmentation of growth in various medicinal plants, as well as their potential for biological control. This article presents a study wherein the researchers examined a comprehensive set of 112 bacterial cultures obtained from the rhizosphere of eleven medicinal plants. Eleven bacterial strains with selected vessel and morphological characteristics displayed discernible efficacy in promoting plant growth and exerting antifungal activity against two prevalent phytopathogenic fungi in liquid culture. The most robust isolates were identified using the 16S rRNA gene sequence, comprising of *Bacillus thuringiensis* and *Pseudomonas florens* (Ahemad et al. 2014).

4.Conclusion

The presence of *Pseudomonas* rhizospheric bacteria has been proven to have a noticeable and remarkable influence on various plant features, including germination, stem and root length, and stem and root weight. Also, the results of this study revealed that the presence of bacteria, both in the rhizosphere state and in the standard state, had a substantial impact on the growth and quality of the *M. officinalis* plant in various aspects.

Conflict of interest

The authors declare no conflict of interests.

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