Agriculture & Food Security

RESEARCH Open Access

Salinity stress and PGPR effects on essential oil changes in *Rosmarinus officinalis* L.

Reza Dehghani Bidgoli[1*], Nazanin Azarnezhad[1], Maryam Akhbari[2] and Majid Ghorbani[3]

Abstract

Background: Medicinal plant species have been used by the ancestors around the world since ancient times. *Rosmarinus officinalis* is one of the most used medicinal plants, which belongs to the family Lamiaceae. To investigate the effects of different levels of salinity stress along with the induction of bacterial growth stimulation on the amount of essential oil composition in *R. officinalis,* an experiment was conducted in a randomized complete block design with 12 treatments and five replications. Salinity treatments included 0 (control), 2.5 (T1), 5 (T2), 7.5 (T3), 10 (T4) and 12.5 (T5) NaCl g/L, and the bacterium was pseudomonas fluorescence.

Results: The percentage of essential oils showed a significant relationship with increasing salinity either alone or in composition with plant growth-promoting rhizobacteria (PGPR) inoculation treatments and it increased with increasing salinity levels to treatment 4 (T4, 10 g/L NaCl) but decreased with further increases in salinity levels in treatments without using PGPR and it was constant in treatment with using PGPR. Phellandrene, one of the main compounds of essential oils, showed a trend like the whole amount of essential oils in both group of treatments.

Conclusion: Abiotic and biotic factors may influence the different mechanisms and limit the interactions between plant and beneficial bacteria, resulting in less-than-acceptable performance in plant growth promotion and management of diseases. In this context, the results revealed that the application of PGPRs can help improve the essential oil yield in *R. officinalis* even in salinity conditions.

Keywords: Bacteria, Essential oils, Rosemary, Medicinal plant, Salt stress, GC/MS

Introduction

Medicinal plants species and aromatic plants have been used by the ancestors around the world since ancient times [32]. *Rosmarinus officinalis* L. (rosemary) belongs to the family Labiatae or Lamiaceae and occurs as a shrub, under the shrub or herbaceous [3]. It is a dense aromatic plant with dark green lavender-like leaves and is a native of the Mediterranean region. The flowering tops and the rosemary leaves mainly contain flavonoids, phenolic acids, especially rosmarinic acid (choleretic activities), and an essential oil (containing pinene, camphene, cineole, borneol and camphor) to which it must have stimulatory effects [34]. Rosemary oils have been widely used for centuries as an ingredient in cosmetics, soaps, perfumes, deodorants, both for flavoring and for preservation of food products [2], and they have also many therapeutics and help the distribution of drugs and antiseptics [35]. Rosemary is used for treating different diseases in traditional medicine, including depression, insomniac and arthritic pains [28, 47].

According to Beattie [6], bacteria that reduce the incidence or severity of plant diseases are often referred to as biocontrol agents, whereas those that exhibit antagonistic activity toward a pathogen are defined as antagonists. The idea of using bacteria to sustain land productive for future generations is not new, and the utilization of bacteria to stimulate plant growth in agriculture has been practiced for millennia. There is increasing evidence that beneficial

*Correspondence: dehghanir@kashanu.ac.ir
[1] Department of Rangeland Management, College of Natural Resources and Earth Sciences, University of Kashan, Isfahan, Iran
Full list of author information is available at the end of the article

microbes can enhance plants' tolerance to adverse environmental stresses such as salinity stress [18], drought stress [46], weed infestation [4], nutrient deficiency and heavy metal contaminations [42]. PGPR, as biocontrol agents, can act through various mechanisms, regardless of their role in direct growth promotion, such as by the known production of auxin phytohormone [38], a decrease in plant ethylene levels [21] or nitrogen-fixing associated with roots [13]. Studies on the effect of salinity and PGPR on a plant have been neglected. Therefore, the present study aims to determine the changes in amount and performance of essential oils under the salinity stress either alone or in combination with PGPR bacteria in *R. officinalis*.

Materials and methods

To investigate the effects of different levels of salinity stress along with the induction of bacterial growth stimulation on the amount of essential oils composition of *R. officinalis*, an experiment was conducted in a completely randomized block design with 12 treatments and five replications in the greenhouse of the Natural Resources Faculty at the University of Kashan. Salinity treatments in this experiment included 0 (control), 2.5 (T1), 5 (T2), 7.5 (T3), 10 (T4) and 12.5 (T5) gram NaCl per liter. A fresh culture of bacteria was used to prepare the suspension or inoculum. The bacterium in this experiment was *Pseudomonas fluorescens*, a strain of CHAO; nutrient broth medium was required to prepare the CHAO suspension. In the Soil Laboratory of the University of Tehran, the pure bacteria of CHAO were cultured in a solid nutrient agar medium and were located at a laboratory normal temperature for 36–48 h. Then, several lobes were removed from the new bacteria and cultivated in Nb (nutrient broth) fluid medium for 48 h on a shaker at 150–250 rpm and then were centrifuged at 4500 *g* for 10 min. The white cells of bacteria accumulated at the bottom of tubes were removed from the nutrient environment in this way, and they were mixed with distilled water. Some of the bacterial suspensions were placed into the spectrophotometer (Model 2100-UV) at 600 nm with the absorption of one (OD600 = 1) resulted in a concentration of 109 cfu/ml [44]. The cuttings of *R. officinalis* were transferred to plastic pots after 6 months when they were deployed and rooted. Soil characteristics were examined before starting the treatments presented in Table 1. Then, all plants (in the PGPR and salinity treatments) were inoculated with PGPR growth-stimulating bacteria and after that salinity stress was applied to the plants. The time of stress lasted 4 months in salinity stress either alone or in combination with PGPR. For all treatments, all planted seedlings were harvested and aerial parts of seedlings were dried and powdered as standard. The essential oils were separately extracted by the Clevenger device. Isolation and identification of rosemary essential oil compounds were performed by gas chromatography–mass spectrometry (GC/MS) machine in the laboratory. Statistical analyses were performed with mean comparison of Duncan's multiple range method using SPSS software version 24.0.

Results and discussion

Based on the analysis of variance, the percentage of essential oils in *R. officinalis* showed a significant relationship ($p < 0.01$) with increasing salinity either alone or in composition with PGPR inoculation treatments (Table 2). The results showed that the amount of essential oils increases with increasing salinity levels to treatment 4 (T4, 10 g/L NaCl) but decreased with further increases in salinity levels (Table 3, Figs. 1 and 2). These results were confirmed by Ghorbani et al. [20] studying *Nitraria schoberi* and Panahi et al. [36] studying *Salsola orientalis*, explaining that the moderate salinity levels can improve the growth parameters and the plant will be injured by increasing salinity levels.

In the other side, the results of treatments along with PGPR inoculation showed that the amount of essential oils increases with increasing salinity levels to treatment 4 (T4, 10 g/L NaCl) and it is constant with further increases in salinity levels (Table 3, Figs. 1 and 2). The highest amount of essential oils in *R. officinalis* is 0.882 and 0.784 in treatment 4 (T4) without using PGPR and in treatment 4 and 5 (T4, T5) with using PGPR, respectively (Table 3). The synergistic effects of combined inoculation of PGPRs have also been reported in various medicinal

Table 2 Analysis of variance for the impact of salinity either alone or in composition with PGPR on percentage of essential oils in *Rosmarinus officinalis*

S.O.V.	*df*	M.S	
		Treatment without PGPR	Treatment with PGPR
Salinity	5	0.219**	0.285**
Error	24	1.833×10^{-6}	3.433×10^{-6}

** Significant in $p < 0.01$

Table 1 Characteristics of soil used in the study

pH	EC (dS/m)	O.C (%)	SP (%)	Clay (%)	Silt (%)	Sand (%)	Texture class	N (%)	P (mg/kg)	K (mg/kg)
8.1	2.73	0.27	25.3	11	13	76	S.L.	0.025	39.5	192

Table 3 Average percentage of essential oils of *Rosmarinus officinalis* in different treatments of salinity alone and in composition with PGPR

Treatment	Control	T1	T2	T3	T4	T5
With PGPR	0.294	0.294	0.392	0.686	0.784	0.784
Without PGPR	0.294	0.392	0.392	0.588	0.882	0.490

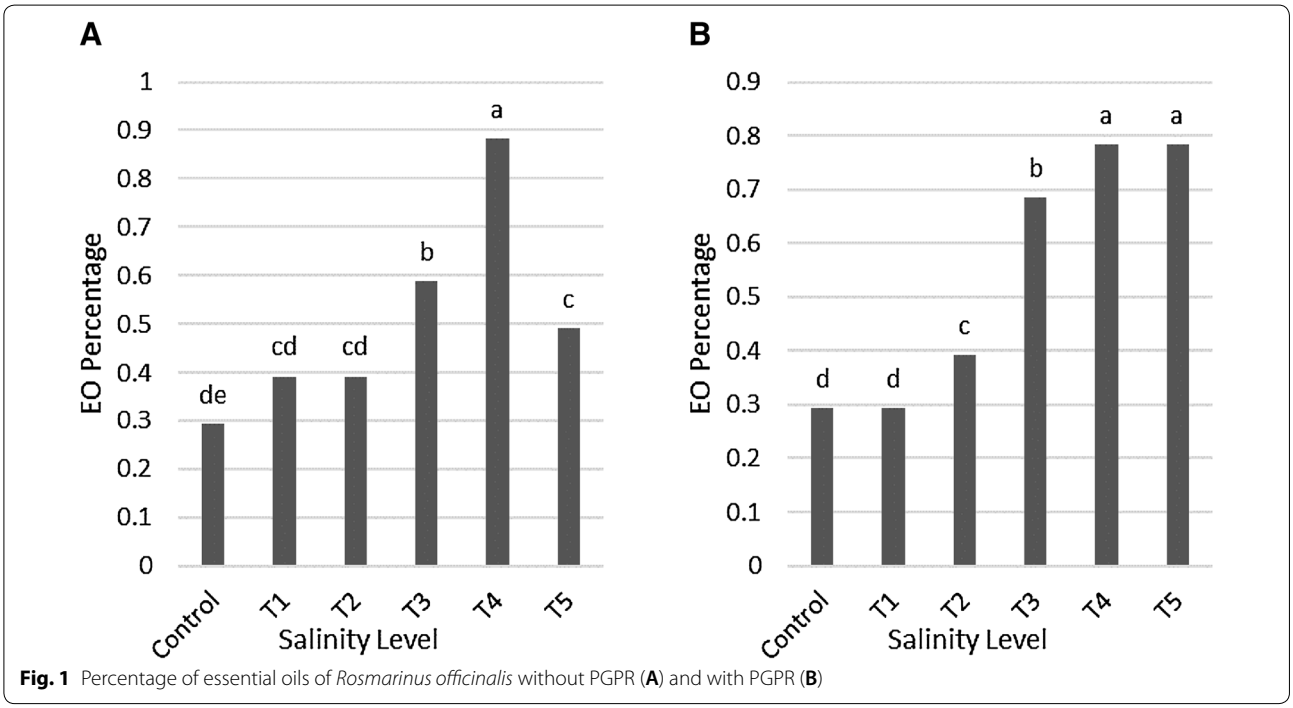

Fig. 1 Percentage of essential oils of *Rosmarinus officinalis* without PGPR (**A**) and with PGPR (**B**)

and aromatic plants (MAPs), for example in *Azadirachta indica* [43], Saracaasoca [25], *Phyllanthus amarus* [14], *Alpinia galanga* and *Coleus amboinicus* [31], *Ocimum basilicum* [23], *Calendula officinalis* [24] and *Silybum marianum* [15]. Beneficial rhizosphere bacteria are of two general types, those forming a symbiotic relationship with the plant and those that are free-living in the soil and root [5, 7, 27]. On the other hand, various PGPR strains have been also proven to be able to increase nutrient availability in the rhizosphere [8].

Based on the analysis of variance, the percentage of main compounds of essential oils in *R. officinalis* showed a significant relationship ($p < 0.01$) with increasing salinity either alone or in composition with PGPR inoculation treatments (Tables 4 and 5). The results showed that the amount of phellandrene content increases with increasing salinity levels to treatment 4 (T4, 10 g/L NaCl) but decreased with further increases in salinity levels (Fig. 3a) in treatments without PGPR inoculation. But in another group (treatments with both salinity and PGPR inoculation), the results showed an increasing trend in phellandrene content with increasing salinity levels (Fig. 3b). The highest amount of phellandrene is 57.48 in treatment 5 (T5, 12.5 g NaCl per liter) with using PGPR inoculation. Trends of other compounds are shown in Fig. 3 and Tables 4 and 5. Nevertheless, the application of PGPR has specifically shown a significant positive effect on essential oil production in *R. officinalis*.

Dehydration, salinity, low- and high-temperature stresses and other abiotic stresses lead to metabolic toxicity, generation of ROS, membrane disorganization, prevention of photosynthesis, reduced nutrient acquisition and altered hormones levels [9]. Accumulation of osmoprotectants, production of superoxide radical scavenging mechanisms, exclusion or compartmentation of ions by the efficient transporter and symporter systems and production of specific enzymes involved in the regulation of plant hormones are among the mechanisms that plants have evolved for adaptation to abiotic stresses [12, 29, 37, 40, 41]. Similar to these, findings of PGPR have been reported by some other workers [19, 26].

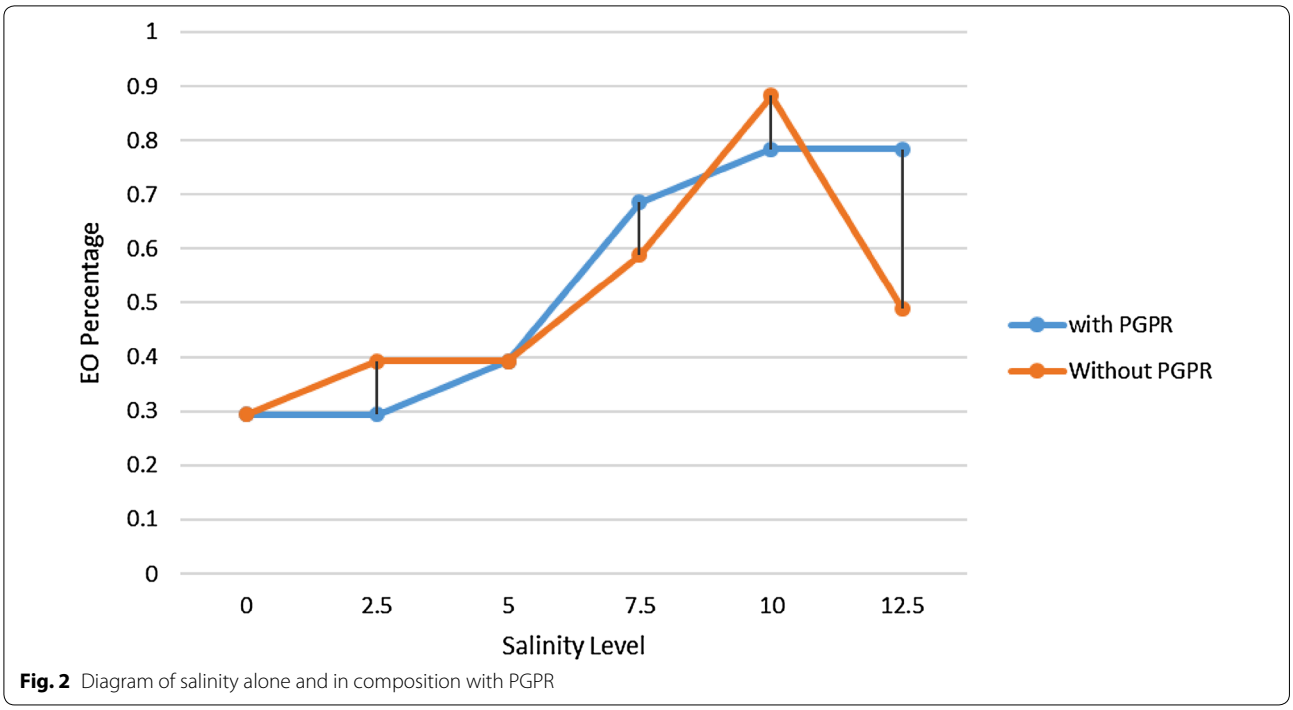

Fig. 2 Diagram of salinity alone and in composition with PGPR

Table 4 Results of analysis of variance for the impact of salinity without PGPR on the rate of main compounds of essential oils in *Rosmarinus officinalis*

S.O.V.	*df*	M.S.						
		Phellandrene	Limonene	Dill ether	Dihydrocarvone	Thymol	Myristicin	Dillapiole
Salinity	5	345.444**	35.975**	9.921**	2.441**	0.136**	0.159**	13.312**
Error	24	0.000	7.667×10^{-5}	8.000×10^{-5}	0.000	0.010	7.667×10^{-5}	0.000

** Significant in $p < 0.01$

Table 5 Results of analysis of variance for the impact of salinity with PGPR on percentage of main compounds of essential oils in *Rosmarinus officinalis*

S.O.V.	*df*	M.S.						
		Phellandrene	Limonene	Dill ether	Dihydrocarvone	Thymol	Myristicin	Dillapiole
Salinity	5	442.204**	37.009**	11.647**	2.057**	0.024**	0.348**	10.338**
Error	24	0.000	0.000	667×10^{-5}	7.667×10^{-5}	7.667×10^{-5}	7.000×10^{-5}	5.000×10^{-5}

** Significant in $p < 0.01$

Essential oil yield can be increased by plant in association with mycorrhiza and humic substances, which benefit root ramification, improving water absorption and phosphorus uptake. Furthermore, they can also influence the chemical composition of EOs [10, 22]. Adesemoye and Kloepper [1] compiled the benefits derivable from plant–PGPR interactions to include the following: improvements in seed germination rate, root development, yield, leaf area, shoot and root weights, chlorophyll content, protein content, hydraulic activity and nutrient uptake (including phosphorus and nitrogen). The bacteria, with their physiological adaptation and genetic potential for increased tolerance to drought, increasing salt concentration and high temperatures, could improve plant production in degraded sites [30, 45] (Tables 6 and 7).

Conclusion

The plant growth-promoting microorganisms were found to have a great potential for use as bioinoculants to increase production of medicinal and aromatic plants [11]. The literature clearly demonstrates that PGPR induces plant growth and development through their numerous direct and indirect mechanisms of action [33]. In this work, synthesis of herbal organs for essential oils of *R. officinalis* was

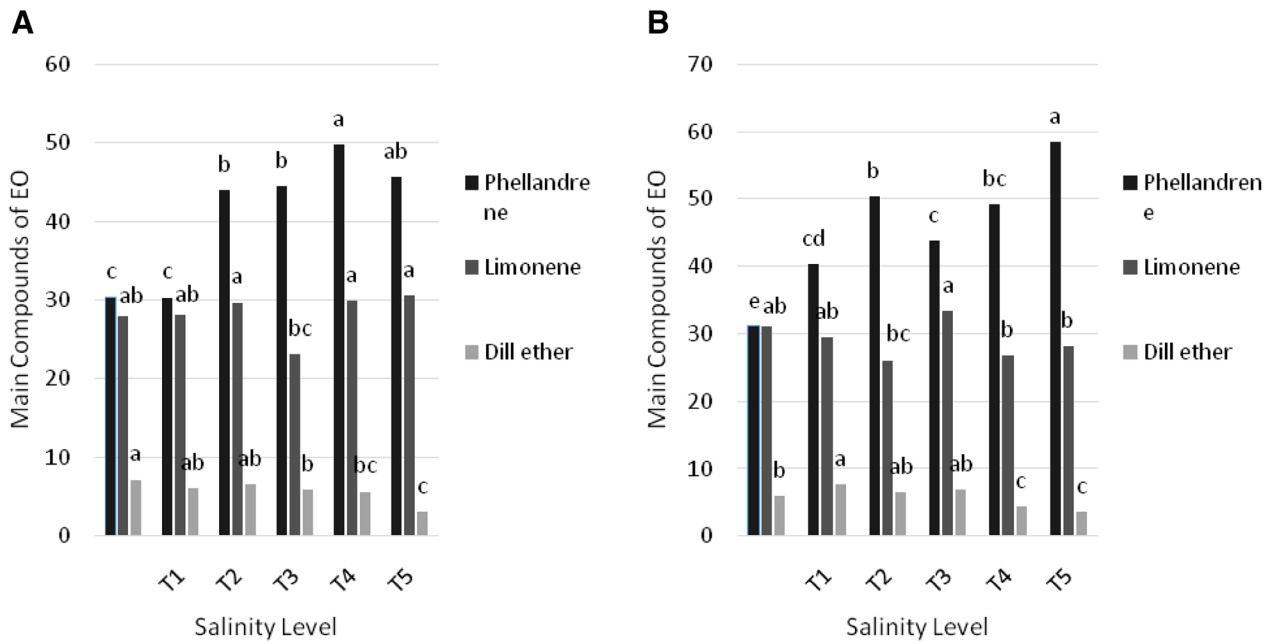

Fig. 3 Main compounds of essential oils *Rosmarinus officinalis* without PGPR (**A**) and with PGPR (**B**)

Table 6 Amount of the different compounds of essential oils in salinity treatment without PGPR in *Rosmarinus officinalis*

Compounds	Retention time	Control	T1	T2	T3	T4	T5
Phellandrene	15.41	30.31	30.31	43.98	44.40	49.75	45.54
Limonene	16.60	27.86	28.14	29.66	23.17	29.85	30.59
Dill ether	24.12	7.13	6.04	6.62	5.95	5.67	3.08
Dihydrocarvone	24.91	1.71	1.85	1.26	0.52	1.35	0.06
Thymol	29.41	0.42	0.05	0.03	0.00	0.37	0.25
Myristicin	38.87	0.35	0.36	0.45	0.00	0.20	0.07
Dillapiole	43.28	4.43	1.44	1.74	0.00	0.41	0.38

Table 7 Amount of the different compounds of essential oils in salinity treatment with PGPR in *Rosmarinus officinalis*

Compounds	Retention time	Control	T1	T2	T3	T4	T5
Phellandrene	15.41	31.09	40.34	50.24	43.69	49.22	58.48
Limonene	16.60	31.04	29.42	26.11	33.33	26.79	28.11
Dill ether	24.12	5.95	7.63	6.48	6.88	4.44	3.61
Dihydrocarvone	24.91	1.51	1.59	1.18	1.46	0.02	0.50
Thymol	29.41	0.29	0.16	0.25	0.29	0.12	0.25
Myristicin	38.87	0.30	0.47	0.66	0.10	0	0.57
Dillapiole	43.28	3.35	3.50	1.52	0.52	0	1.45

described. Optical properties were established as strongly dependent on the application of PGPR. In this context, our results revealed that the application of PGPRs can help to improve the essential oil yield in *R. officinalis* even in the salinity conditions. The essential oils represent an important part of the folk medicine for their medicinal properties such as the antioxidant activity [39]. Notably, abiotic and biotic factors may influence the different mechanisms and limit the interactions between plant and beneficial bacteria, resulting in less-than-acceptable performance in plant growth promotion and management of diseases [16, 17]. Finally, it can be stated that PGPR can help to improve the essential oil yield in normal and salinity conditions, but further investigations are needed to evaluate its performance in different conditions and under multi-stress situations.

Abbreviations
PGPR: plant growth-promoting rhizobacteria; Nb: nutrient broth; GC/MS: gas chromatography–mass spectrometry; g/L: gram per liter.

Authors' contributions
Contribution of authors is defined as the priority of authorship. All authors read and approved the final manuscript.

Author details
[1] Department of Rangeland Management, College of Natural Resources and Earth Sciences, University of Kashan, Isfahan, Iran. [2] Natural Essential Oils Institute, University of Kashan, Isfahan, Iran. [3] Department of Desertification, College of Natural Resources and Earth Sciences, University of Kashan, Isfahan, Iran.

Acknowledgements
The authors thank the University of Kashan for funding this project.

Competing interests
The authors declare that they have no competive interests.

Availability of data and materials
Data and information about this project were provided by supervisor (corresponding author).

Consent for publication
All authors agree to publish this article in this journal.

Ethics approval and consent to participate
This research was approved in University of Kashan with No: 1975/305 and has not been published elsewhere before.

Funding
The funding for this research has been provided by University of Kashan.

Publisher's Note

Received: 18 March 2018 Accepted: 21 December 2018
Published online: 03 January 2019

References

1. Adesemoye AO, Kloepper JW. Plant-microbes interactions in enhanced fertilizer use efficiency. J Appl Microbiol Biotechnol. 2009;85:1–12.
2. Arnold N, Valentini G, Bellomaria B, Hocine L. Comparative study of the essential oils from *Rosmarinus eriocalyx* Jordan & Fourr. From Algeria and *Rosmarinus officinalis* L. from other countries. J Essent Oil Res. 1997;9(2):167–75.
3. Atikbekkara F, Bousmaha L, Talebbendiab SA, Boti JB, Casanova J. Chemical composition of essential oil of *Rosmarinus officinalis* L. grown in the Tlemcen region. J Biol Health. 2007;7:6–11.
4. Babalola OO. Beneficial bacteria of agricultural importance. Biotechnol Lett. 2010;32:1559–70.
5. Barriuso J, Pereyra MT, Lucas García JA, Megías M, Gutierrez Mañero FJ, Ramos B. Screening for putative PGPR to improve establishment of the symbiosis *Lactarius deliciosus–Pinus* sp. J Microbial Ecol. 2005;50(1):82–9.
6. Beattie GA. Plant-associated bacteria: survey, molecularphylogeny, genomics and recent advances. In: Gnanamanickam SS, editor. Plant-associated bacteria. Dordrecht: Springer; 2006. p. 1–56.
7. Bianciotto V, Andreotti S, Balestrini R, Bonfante P, Perotto S. Extracellular polysaccharides are involved in the attachment of *Azospirillum brasilense* and *Rhizobium leguminosarum* to arbuscularmycorrhizal structures. Eur J Histochem. 2001;45(1):39–49.
8. Cakmakci R, Donmez D, Aydýn A, Sahin F. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. J Soil Biol Biochem. 2005;38:1482–7.
9. Carmen B, Roberto D. Soil bacteria support and protect plants against abiotic stresses. Institute of Genetics and Biophysics Adriano BuzzatiTraverso. Chapter 7. Italy 2011.
10. Copetta A, Lingua G, Berta G. Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. J Mycorrhiza. 2006;16:485–94.
11. Da Silva JAT, Egamberdieve D. Plant-growth promoting rhizobacteria and medicinal plants. RPMP. Vol. 38- Essential Oils III and Phytopharmacology. 2014; 25–42.
12. Des Marais DL, Juenger TE. Pleiotropy, plasticity, and the evolution of plant abiotic stress tolerance. Ann N Y Acad Sci. 2010;1206:56–79.
13. Döbereiner J. History and new perspectives of diazotrophs in association with non-leguminous plants. J Symbiosis. 1992;13:1–13.
14. Earanna N, Bagyaraj DJ. Influence of AM fungi and growth promoting rhizomicroorganisms on growth and herbage yield of *Phyllanthusamarus* Schum. and Thom. J Geobios. 2004;31:117–20.
15. Egamberdieva D, Jabborova D, Mamadalieva N. Salt-tolerant *Pseudomonas extremorientalis* able to stimulate growth of *Silybum marianum* under salt stress condition. J Med Aromat Plant Sci Biotechnol. 2013;7:65–75.
16. Egamberdiyeva D, Hoflich G. Root colonization and growth promotion of winter wheat and pea by *Cellulomonas* spp. at different temperatures. J Plant Growth Regul. 2002;38:219–24.
17. Egamberdiyeva D, Hoflich G. Influence of growth promoting bacteria on the growth of wheat at different soils and temperatures. J Soil Biol Biochem. 2003;35:973–8.
18. Egamberdiyeva D. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl J Soil Ecol. 2008;36:184–9.
19. Gabriele B, Christin Z, Jana L, Monika G, Rodrigo C, Kornelia S. Impact of plant species and site on rhizosphere-associated fungi antagonistic to *Verticillium dahliaekleb*. J Appl Environ Microbiol. 2005;71(8):4203–13.
20. Ghorbani M, Ranjbar Fardoyi A, Panahi F, Attarha J, Marzbani N, Moases M. Salinity and *Nitraria schoberi*: growth parameters, chlorophyll content and ion accumulation. Int J Agric Crop Sci. 2014;7–11:853–62.
21. Glick BR, Cheng Z, Czarny J, Duan J. Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol. 2007;119:329–39.
22. Hazzoumi Z, Moustakime Y, Elharchili EH, Joutei KA, et al. Effect of arbuscular mycorrhizal fungi (AMF) and water stress on growth, phenolic compounds, glandular hairs, and yield of essential oil in basil (*Ocimumgratissimum* L.). J Chem Biol Technol Agric. 2015;2:10–21.
23. Hemavathi VN, Sivakumr BS, Suresh CK, Earanna N. Effect of *Glomus fasciculatum* and plant growth promoting rhizobacteria on growth and yield of *Ocimum basilicum*. Karnataka J Agric Sci. 2006;19:17–20.
24. Hosseinzadah F, Satei A, Ramezanpour M. Effects of mycorrhiza and plant growth promoting rhizobacteria on growth, nutrient uptake and physiological characteristics in *Calendula officinalis* L. Middle East. J Sci Res. 2011;8(5):947–53.

25. Lakshmipathy R, Chandrika K, Gowda B, Balakrishna AN, Bagyaraj DJ. Response of *Saraca asoca* (Roxb.) de Wilde to inoculation with *Glomusm osseae*, *Bacillus coagulans* and *Trichoderma harzianum*. J Soil Biol Ecol. 2001;21:76–80.
26. Lugtenberg B, Chin-A-Woeng T, Antonie BG. Effects of salinity stress and plant growth promoting rhizobacteria on *Medicago sativa*. Van Leeuwenhoek. 2002;81:373–83.
27. Lugtenberg B, Kamilova F. Plant growth-promoting rhizobacteria. J Annu Rev Microbiol. 2009;63:541–56.
28. Lugtenberg BJJ, Dekkers L, Bloemberg GV. Molecular determinants of rhizosphere colonization by *Pseudomonas*. J Annu Rev Phytopathol. 2001;39:461–90.
29. Mahaian S, Tuteja N. Cold, salinity and drought stresses: an overview. J Arch Biochem Biophys. 2005;444:139–58.
30. Maheshwari DK, Dubey RC, Aeron A, Kumar B, Kumar S, Tewari S, Arora NK. Integrated approach for disease management and growth enhancement of *Sesamum indicum* L. utilizing *Azotobacter chroococcum* TRA2 and chemical fertilizer. World J Microbiol Biotechnol. 2012;28(10):3015–24.
31. Mani N. Phytochemical and antimicrobial studies on *Alpinia galangal* and *Coleus amboinicus* as influenced by native AM fungi. PhD thesis, BharathidasanUniviersity, India. 2004.
32. Morelli F, Ferarrese L, Munhoz CL, Alberton O. Antimicrobial activity of essential oil and growth of *Ocimum basilicum* L. inoculated with mycorrhiza and humic substances applied to soil. J Genet Mol Res. 2017;16(3):1–11.
33. Ngoma L, OlurantiBabalola O, Ahmad F. Ecophysiology of plant growth promoting bacteria. J Sci Res Essays. 2012;7(47):4003–13.
34. Oluwatuyi M, Kaatz GW, Gibbons S. Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. Phytochemistry. 2004;65(24):3249–54.
35. Palevitch PD, Yaniv Z. Medicinal plants of Holy land. Tel-Aviv, Tammuz Publisher Ltd. 1991;1:2–4.
36. Panahi F, Asareh MH, Jafari M, Givar A, Tavili A, Arzani H, Ghorbani M. The responses of *Salsola orientalis* to salt stress. Int J Adv Biol Biomed Res. 2015;3(2):163–71.
37. Parida AK, Das AB. Salt tolerance and salinity effects on plants: a review. J Ecotox Environ Saf. 2005;60:324–49.
38. Patten CL, Glick BR. Role of *Pseudomonas putida* in doleacetic acid in development of the host plant root system. Appl J Environ Microbiol. 2002;68:3795–801.
39. Rozza AL, Pellizzon CH. Essential oils from medicinal and aromatic plants: a review of the gastroprotective and ulcer-healing activities. Fundam Clin Pharmacol. 2013;27:51–63.
40. Santner A, Calderon-Villalobos LIA, Estelle M. Plant hormones are versatile chemical regulators of plant growth. J Nat Chem Biol. 2009;5:301–7.
41. Shao HB, Chu LY, Jaleel CA, Manivannan P, Panneerselvam R, Shao MA. Understanding water deficit stress-induced changes in the basic metabolism of higher plants-biotechnologically and sustainably improving agriculture and the ecoenvironment in arid regions of the globe. J Crit Rev Biotech. 2009;29:131–51.
42. Sheng XF. Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus. J Soil Biol Biochem. 2005;37:1918–22.
43. Sumana DA. Influence of VA mycorrhizal fungi and nitrogen fixing and mycorrhization helper bacteria on growth of neem (*Azadirachta indica* A. Juss). PhD. thesis, University of Agriculture Sciences, India 1998.
44. Thompson DC. Evaluation of bacterial antagonists for reduction of summer patch symptoms in Kentucky blue grass. J Plant Dis. 1996;80:850–62.
45. Yang J, Kloepper JW, Ryu C-M. Rhizosphere bacteria help plants tolerate abiotic stress. J Trends Plant Sci. 2009;14(1):1–4.
46. Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. J Microbiol Biotechnol. 2008;18:958–63.
47. Zargari A. Medical plants. 5th ed. Tehran: Tehran University Press; 1995.