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ORIGINAL ARTICLE

Investigation of PGPR on Antioxidant Activity of Essential Oil and Microelement Contents of Sweet Basil

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ABSTRACT

Ocimum basilicum L. (sweet basil) is an important medicinal plant, annual herb which grows in several regions all over the world. In this study, antioxidant activity of essential oil and microelements of *Ocimum basilicum* investigated. The control treatment was noninoculated, and the PGPR treatments were inoculated with *Pseudomonas putida* strain 41, *Azotobacter chroococcum* strain 5, and *zosprillum lipoferum* strain OF. In comparison to the control treatment, microelement contents and antioxidant activity of essential oil were increased by PGPR treatments. The maximum antioxidant activity of essential oil and Fe, Mn and Cu contents obtained in *Pseudomonas putida* strain 41+ *Azotobacter chroococcum* strain + *zosprillum lipoferum* strain OF treatment and maximum of Zn content found in *Azotobacter chroococcum* strain + *zosprillum lipoferum* strain OF treatment. It showed a synergistic effect between apply of *Pseudomonas* + *Azotobacter* + *Azosprillum* and *Azotobacter* + *Azosprillum* PGPR and an antagonism effect between usage of *Pseudomonas* + *Azotobacter* and *Pseudomonas* + *Azosprillum* PGPR on microelement contents and antioxidant activity of essential oil at treatments.

Key words: Ocimum basilicum, Pseudomonas, Azotobacter, Azosprillum, antioxidant activity, microelement

Introduction

Plant growth promoting rhizobacteria (PGPR) are root associated bacteria representing many different genera and species that colonize the rhizosphere, rhizoplane and improve plant growth when artificially introduced onto seeds, seed pieces, roots, or into soil. PGPR (plant-growth promoting rhizobacteria) or PGPF (plant-growth promoting fungi) enhance plant growth through numerous mechanisms including the protection of roots against infection by minor and major pathogens [1], enhancing the availability of nutrients to the host plant, lowering of the ethylene level within the plant or by the enhanced production of stimulatory compounds, such as plant growth regulators [2]. Different PGPR including associative bacteria such as *Azospirillum*, *Bacillus*, *Pseudomonas and Enterobacter* have been used for their beneficial effects on plant growth [3,4]. Many arketable biofertilizers are mainly based on plant growth promoting rhizobacteria (PGPR) that exert beneficial effects on plant development often related to the increment of nutrient availability to host plant [5].

Basil belongs to the genus Ocimum, derived from the Greek ozo which means to smell, in reference to the strong odors of the species within the genus [6]. Basil has been used as a folk remedy for an enormous number of ailments, including boredom, cancer, convulsion, deafness, diarrhea, epilepsy, gout, hiccup, impotency, insanity, nausea, sore throat, toothaches, and whooping cough. Basil has been reported in herbal publications as an insect repellent. [7] [recently the potential uses of O. basilicum essential oil, particularly as antimicrobial

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and antioxidant agents have also been investigated [8,9,10,11,12]. The O. basilicum essential oils exhibited a wide and varying array of chemical compounds, depending on variations in chemotypes, leaf and flower colors, aroma and origin of the plants [13,14]. It was reported that the leafy parts of basil had tonic, antiseptic [15] and insecticidal properties [16]. It is also known the leaves of basil are suitable for the treatment of pain and cough [17].] The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues [18]. Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E, and carotenoids [19]. Many herb spices, especially those belonging to the Lamiaceae family, such as sage, oregano, and thyme, show strong antioxidant activity [18]. The genus Ocimum, a member of the Lamiaceae family, contains between 50 and 150 species of herbs and shrubs [20]. A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts [21].

The purpose of this study was to evaluate the effect of inoculating *Ocimum basilicum* L. root with PGPR (*Pseudomonas putida* strain 41, *Azotobacter chroococcum* and *Azosprillum lipoferum*) on *Ocimum basilicum* L. antioxidant activity and microelements content. The results can be beneficial to farmers and people who focus on nutritional values of *Ocimumbasilicum* L.

Materials and methods

Microorganisms

Applied PGPR contained *Pseudomonas putida* strain 41, *Azotobacter chroococcum* strain 5 *and A. lipoferum* strain OF. To provide microbial inoculants, PGPR were inoculated in nutrient broth medium. Each bacterium was then removed at the end of logarithmic growth phase, and was asepticcally transferred to plastic containers containing sterile perlite and was then mixed well. PGPR concentration was adjusted to 1×108 (CFU/gr) in all inoculants.

Growth condition and plant materials

This study was conducted in experimental glasshouse of Islamic Azad University, Firoozabad Branch (28°35' N, 52°40' E; 1327 m above sea level). *Ocimum basilicum* L. seeds were inoculated and sown in a soil field, mixed with waterworn sand and peat (1/3, v/v each of them), in pots containing 7 kg of the mentioned (mixed) soil. Seven PGPR treatments were considered (*Pseudomonas putida, Azotobacter chroococcum, A. lipoferum, P. putida + A. chroococcum,P. putida + A. lipoferum, A. chroococcum + A. lipoferum and P. putida + A. chroococcum + A. lipoferum).* A non-inoculated treatment was set as control and N fertilizer was added to all the treatments according to the soil test. At full bloom, the plant herbage was cut 10 cm above the soil surface. Microelement contents were determined according to the lab manual of The Soil and Water Institute of Iran. The physicochemical properties of the soil were also determined (Table 1).

Isolation of Essential Oils

50 g sample of dried shoots was subjected to four-hours of hydro distillation using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate

Determination of Antioxidant Activity of Essential Oils with the 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

The antioxidant activity of essential oils was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (22).50 mL of methanolic solution of the essential oil (concentrations 20 g/L) was put into a cuvette, and 2 mL of 6×10^{-5} mol L^{-1} methanolic solution of DPPH was added. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined with spectrophotometer after 1 h for all samples. Methanol was used to zero the spectrophotometer. Percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen & Duh [23].

$$\% \text{ inhibition} = ((A_{C(o)} - A_{A(t)}) / A_{C(o)}) \times 100$$

where $A_{C(o)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t = 1 h.

Statistical analyses

Experiment was conducted based on a randomized complete block design (RCBD) with four replications. Means were compared with Duncan's new multiple range test (DNMRT) in SAS software for windows.

Results and discussion

The results showed that the PGPR have the capacity to increase *Ocimum basilicum* microelement contents. Data explained significantly differences between used PGPR treatments on antioxidant activity of essential oil, Fe, Zn, Mn and Cu contents (Table 2). Maximum antioxidant activity of essential oil was observed in the *Pseudomonas + Azotobacter + Azosprillum* treatment (78%) which

Table 1: Physicochemical properties of soil used

Parameter	value	Parameter	Value
Electrical conductivity (dsm^{-1})	0.83	Phosphorous(ppm)	7
PH of Saturated soil solution	7.43	Potassium(ppm)	292
Clay (%)	14	Iron(ppm)	4.11
Silt (%)	32	Zinc(ppm)	1.02
Sand (%)	54		
Nitrogen (%)	0.037		
Organic carbon (%)	0.41		

Table 2: Variance analysis of shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, shoot height, N,P,K content and essential oil yield in sweet basil.

SOV	Degree of Freedom(df)	Antioxidant activity (%))	Fe (ppm)	Mn (ppm)	Zn	Cu
Block	3	0.625ns	2470.30ns	1.07ns	5.67ns	0.12ns
Treatments	7	159.02**	97616.93**	949.85**	739.47**	65.31**
C.V%		1.38	7.22	2.39	3.67	3.69

Ns,** are levels of significance (not significant, p<0.01 respectively)
Sov: sources of varition

was significantly different when compared to other treatments. Minimum antioxidant activity was obtained in control treatment (58.15%). *Pseudomonas* + *Azosprillum* treatment (65.60%) had the lower antioxidant activity of essential oil compared to the other PGPR treatments (Fig. 1). It was reported that the usage of PGPR increased antioxidant activity of tomato fruit [24].

Maximum Zn content was showed in *Azotobacter* + *Azosprillum* treatment (110 ppm) which had significantly difference than the other treatments. Data explained it was no significantly difference between apply *Azosprillum* treatment (84.40 ppm) and *Pseudomonas* + *Azotobacter* treatment (82.26 ppm). Between PGPR treatments, *Pseudomonas* + *Azosprillum* treatment (81.18 ppm) had minimum of Zn content (Fig. 2).

Maximum Fe, Mn and Cu contents were obtained in *Pseudomonas* + *Azotobacter* + *Azosprillum* treatment (respectly 1466.73ppm, 141.52ppm and 30.67ppm) which had significantly difference than the other treatments. At all treatment, minimum of Fe, Mn and Cu contents were found in control treatment (respectly 905.55ppm, 90.50ppm and 17.60ppm). Data showed *Pseudomonas* + *Azosprillum* treatment and *Pseudomonas* + *Azotobacter* treatment had minimum Fe and Cu content compared to the other PGPR treatment.(Fig. 3, Fig. 4, Fig. 5)

Based on the results, it was showed a synergistic effect between apply of *Pseudomonas* + *Azotobacter* + *Azosprillum* and *Azotobacter* + *Azosprillum* PGPR and an antagonism effect between usage of *Pseudomonas* + *Azotobacter* and *Pseudomonas* + *Azosprillum* PGPR on microelement contents and antioxidant activity of essential oil at treatments.

Many studies showed that bacterial inoculation significantly increased nutrient contents of plants.

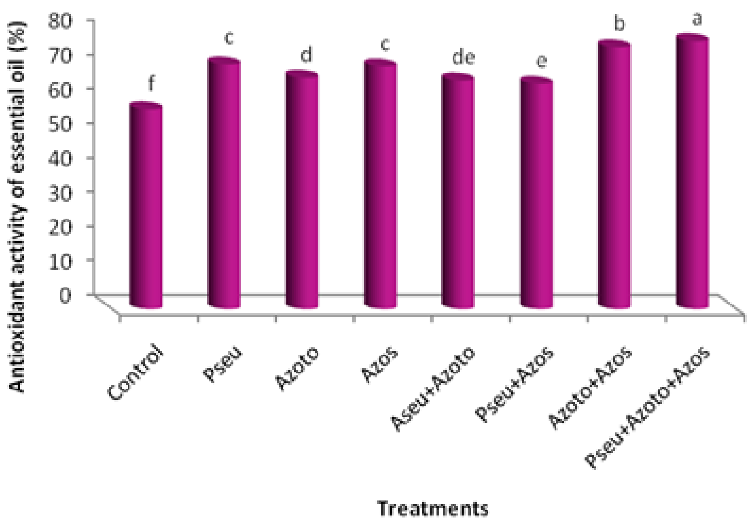

Fig. 1: Antioxidant activity of essential oil (%) in respond to treatments in *Ocimum basilicum* L.

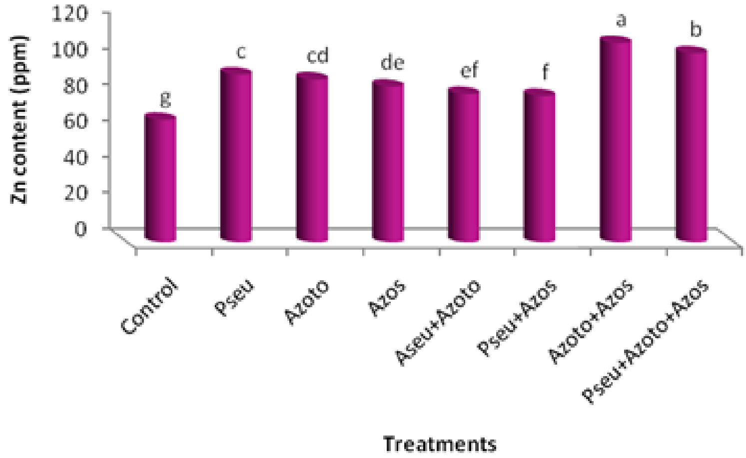

Fig. 2: Zn content (ppm) in respond to treatments in *Ocimum basilicum* L.

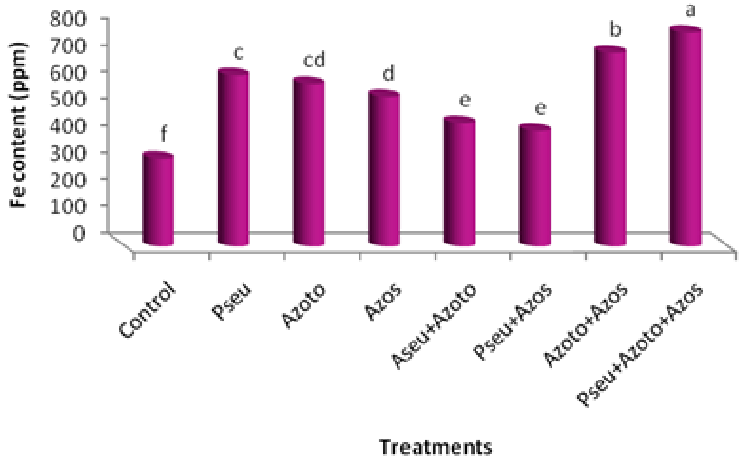

Fig. 3: Fe content (ppm) in respond to treatments in *Ocimum basilicum* L.

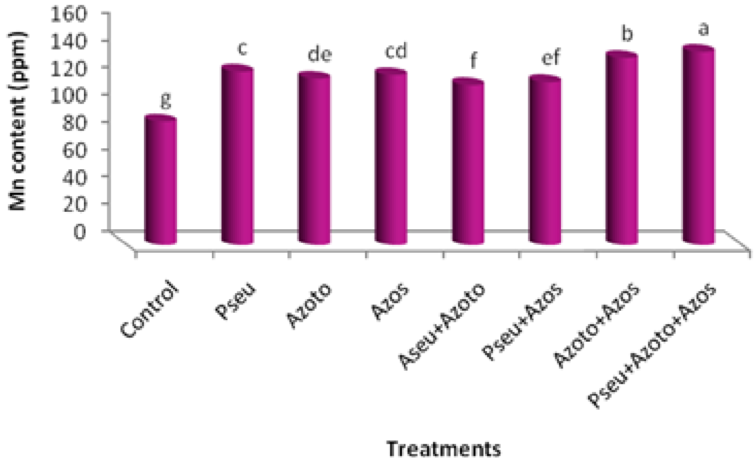

Fig. 4: Mn content (ppm) in respond to treatments in *Ocimum basilicum* L.

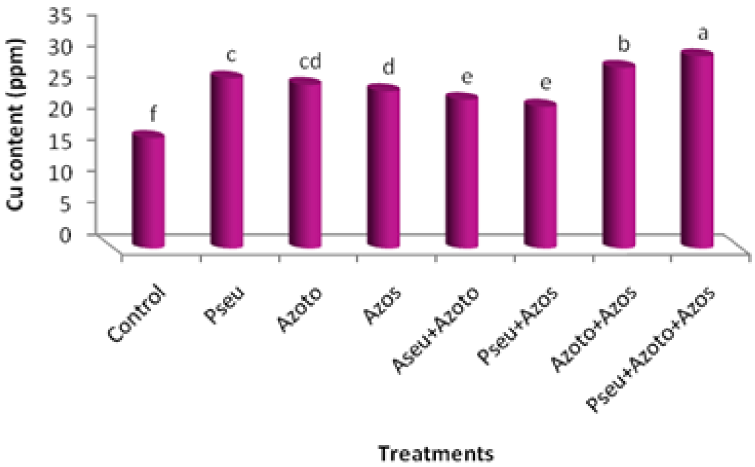

Fig. 5: Cu content (ppm) in respond to treatments in *Ocimum basilicum* L.

Increased nutrient uptake by plants inoculated with plant growth promoting bacteria has been attributed to the production of plant growth regulators at the root interface, which stimulated root development and resulted in better absorption of water and nutrients from the soil [25,26,4]. Different PGPR including associative bacteria such as *Azospirillum, Bacillus, Pseudomonas, Entero bacter* have been used for their beneficial effects on plant growth [27; 28]. Abbass and Okon, [29] explained that *Azotobacter* has positive effect on plant growth. Synergistic effects of com-bined inoculation of PGPRs have also been reported in various crops, for examples tomatoes [30], potatoes 31], rice [32], sugar beet and barley [33].

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References

1. Whipps, J.M., 2001. Microbiol interactions and biocontrol in the rhizosphere. J., Expe. Bot., 52: 487-511.
2. Antoun, H. and D. Pre´vost, 2005. Ecology of plant growth promoting rhizobacteria. In: Siddiqui, Z.A. (Ed.), PGPR: Biocontrol and Biofertilization. Springer, Dordrecht, pp: 1-38.
3. Kloepper, J.W. and C.J. Beauchamp, 1992. A review of issues related to measuring of plant roots by bacteria. Can. J. Microbiol., 38: 1219-1232.
4. Ho¨flich, G., G. Ku¨hn, 1996. Fo¨rderung das Wachstums und der Na¨hrstoffaufnahme bei kurziferen O¨ l- und Zwischenfruhten durch inokulierte Rhizospherenmikroorganismen. Zeischrift fu¨ r Pflanzenerna ¨hrung und Bodenkunde, 159: 575-578.
5. Vessey, K.J., 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255: 571-586.
6. McIntosh, Charles, 1853. The Book of the Garden. Edinburgh and London: W. Blackwood, 237.
7. Duke, James A. Culinary Herbs: A Potpourri. Buffalo, NY: Bonch Magazine, Limited, 1985.
8. Lee, S.J., K. Umano, T. Shibamoto and K.G. Lee, 2005. Identification of volatile components in basil (Ocimum basilicum L) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chemistry, 91: 131-137.
9. Politeo, O., M. Jukic and M. Milos, 2007. Chemical composition and antioxidant capacity of free volatile aglycones from basil (Ocimum basilicum L.) compared with its essential oil. Food Chemistry, 101: 379-385.
10. Sartoratotto, A., A.L.M. Machado, C. Delarmelina, G.M. Figueira, M.C.T. Duarte and V.L.G. Rehder, 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Brazilian Journal of Microbiology, 35: 275-280.
11. Suppakul, P., J. Miltz, K. Sonneveld and S.W. Bigger, 2003. Antimicrobial properties of basil and its possible application in food packing. Journal of Agriculture and Food Chemistry, 51: 3197-3207.
12. Wannissorn, B., S. Jarikasem, T. Siriwangchai, and S. Thubthimthed, 2005. Antibacterial properties of essential oils from Thai medicinal plants. Fitoterapia, 76: 233-236.

13. Da-Silva, F., R.H.S. Santos, E.R. Diniz, L.C.A. Barbosa, V.W.D. Casali and R.R. De-Lima, 2003. Content and composition of basil essential oil at two different hours in the day and two seasons. Revista Brasileira de Plantas Medicinais, 6(1): 33-38.
14. Sajjadi, S.E., 2006. Analysis of the essential oils of two cultivated basil (Ocimum basilicum L.) from Iran. Daru, 14(3): 128-130.
15. Kosekia, P.M., A.L. Villavicencioa and M.S. Britoa, 2002. Effects of irradiation in medicinal and eatable herbs. Radiat Phys Chem., 63: 681-684.
16. Umerie, S.C., H.U. Anaso and L.J.C. Anyasoro, 1998. Insecticidal potentials of Ocimum basilicum leaf-extract. Bioresourch Technol., 64: 237-239.
17. Basilico, M.Z. and J.C. Basilico, 1999. Inhibitory effects of some spice essential oils on Aspergillus ochraceus NRRL 3174 growth and ochratoxin. A production. Lett Appl Microbiol., 29: 238-241.
18. Hirasa, K. and M. Takemasa, 1998. Spice science and technology. Marcel Dekker: New York.
19. Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural Food & Chemistry, 46P: 4113-4117.
20. Simon, J.E., M.R. Morales, W.B. Phippen, R.F. Vieira and Z. Hao, 1999. Basil: a source of aroma compounds and a popular culinary and ornamental herb. In J. Janick (Ed.), Perspectives on new crops and new uses, pp: 499-505. Alexandria, VA: ASHS Press.
21. Nakatani, N., 1997. Antioxidants from spices and herbs. In F. Shahidi (Ed.), Natural antioxidants: chemistry, health effects, and applications, pp: 64-75. Champaign, IL: AOCS Press. M.S. Blois, Nature, 181(1958): 1199-1200.
22. Politeo, O., M. Jukic and M. Milos, 2006. Chemical Composition and Antioxidant Activity of Essential Oils of Twelve Spice Plants. CCACAA, 79(4): 545-552.
23. Yen, G.C. and P.D. Duh, 1994. J. Agric. Food Chem., 42: 629-632.
24. Ordookhani, K., K. Khavazi, A. Moezzi and F. Rejali, 2010. Influence of PGPR and AMF on antioxidant activity, lycopene and potassium contents in tomato. African Journal of Agricultural Research, 5(10): 1108-1116.
25. Kloepper, J.W., R.M. Zablowicz, B. Tipping, R. Lifshitz, 1991. Plant growth mediated by bacterial rhizosphere colonizers. In: Keister, D.L., Gregan, B. (Eds.), the rhizosphere and plant growth, 14. BARC Symposium, pp: 315-326.
26. Zimmer, W., K. Kloos, B. Hundeshagen, E. Neiderau and H. Bothe, 1995. Auxin biosynthesis and denitrification in plant growth promotion bacteria. In: Fendrik J, De Gallo Vandeleyden J, De Zamoroczy D. (Eds.), Azospirillum VI and related microorganisms, Series G: Ecological, 37: 120-141.
27. Kloepper, J.W. and C.J. Beauchamp, 1992. A review of issues related to measuring of plant roots by bacteria. Canadian Journal of Microbiology, 38: 1219-1232.
28. Ho¨flich, G., W. Wiehe, G. Kuhn, 1994. Plant growth stimulation with symbiotic and associative rhizosphere microorganisms. Experientia, 50: 897-905.
29. Abbass, Z. and Y. Okon, 1993. Plant growth-promotion by Azotobacter paspali in the rhizosphere. Soil Biol. Biochem., 25: 1075-1083.
30. Gamalero, E., M.G. Martinnoti, A. Trotta, P. Lemanceau and G. Berta, 2004. Morphogenetic modifications induced by Pseudomonas fluorescens A6RI and Glomus mosseae BEG12 in the root system of tomato differ according to plant growth conditions. New Phytol., 155: 293-300.
31. Kundu, B.S. and A.C. Gaur, 1980. Effect of phosphobacteria on the yield and phosphate uptake of potato crop. Curr. Sci., 49: 159.
32. Tiwari, V.N., L.K. Lehri, A.N. Pathak, 1989. Effect of inoculation crops with phospho-microbes. Exp. Agr., 25: 47-50.
33. Çakmakçi, R., F. Kantar and Ö.F. Algur, 1999. Sugar beet and barley yields in relation to Bacillus polymyxa and Bacillus megaterium var. phosphaticum inoculation. J. Plant Nutr. Soil Sci., 162: 437-442.