Advances in Environmental Biology, 7(1): 113-115, 2013
ISSN 1995-0756

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Impact of *Azotobacter* on Growth and Total Phenolic Content of Garden Thyme

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ABSTRACT

Garden thyme, member of Lamiaceae family, is one of the herbaceous perennial plants. The green part of thyme plant contains the most popular herbal medicine and spice, used in all developing countries. The plant growth promoting rhizobacteria (PGPR) can influence plant growth and metabolism. *Azotobacter* is one of the PGPR. In this study we applied different strains of *Azotobacter* in order to determine their effects on growth and total phenolic content of garden thyme. The treatments were different strains of *Azotobacter* (strains 5, 9, 12, 30) and they compared to control (without using bacteria). Experiment was carried out using a completely randomized design (CRD) with three replications. The highest value of shoot height (16.11 cm) was obtained by using strain 30. The maximum shoot fresh and dry weights were achieved on strain 30. Root fresh and dry weights were the maximum when strain 30 used. Total phenolic content was maximum (6.40 mg GAE/g dw) by application of strain 5.

Key words: phenolics, *Thymus vulgaris*, PGPR, biofertilizer, medicinal plants.

Introduction

Garden thyme (*Thymus vulgaris* L.), member of Lamiaceae (Labiatae) family, is one of the herbaceous perennial plants. Garden thyme is native to the western Mediterranean region and Southern Italy [7]. The green part of thyme plant contains the most popular herbal medicine and spice, used in all developing countries. Thyme has a very important role in phytotherapy [11]. Polyphenolic compounds are commonly found in medicinal plants and they have been reported to have multiple biological effects, including antioxidant activity [18].

Agronomical factors and geographical environment can alter composition and quantity of active substances from a particular species of thyme plant [8,9].

Biofertilizers are microbial inoculants contain living cells of micro-organism such as bacteria, algae and fungi which may help plant growth. Biological activities are markedly enhanced by microbial interactions in the rhizosphere of plants [16]. The plant growth promoting rhizobacteria (PGPR) can influence plant growth directly through the production of phytohormones and indirectly through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens [4].

Application of biofertilizers as substitute for inorganic fertilizers should not be considered as a simple objective and short term benefits, but as a mean to improve environmental conditions and human health.

Azotobacter is one of the PGPR. In this study we applied different strains of *Azotobacter* in order to determine their effects on growth and total phenolic content of garden thyme.

Materials and Methods

Plant materials and experimental conditions:

This study was conducted in experimental glasshouse of Islamic Azad University, Firoozabad Branch, Iran (28°35' N, 52°40' E; 1327 m above sea level). Thyme seeds were sown in the pots containing 2/3 soil and 1/3 sand (v/v) and thinned at 4-6 leaves stage to one plant per each pot. The pot mixture were tested before applying treatments and the texture was clay loam with PH=7.12, organic C=0.03%, total N=0.05%, available P=3.8 mg/kg, available K=560 mg/kg and EC=0.58 dS/m. Plants kept at 23±3/15±3°C day/night temperatures. The treatments were different strains of *Azotobacter* (strains 5, 9, 12, 30) and comparing them to control (without using bacteria). The strains were prepared from Soil and Water Research Institute, Karaj, Iran. Inoculation was conducted via soil injection. Experiment was carried out using a completely randomized design (CRD) with three replications. Each replicate contained 5 pots. Plants were harvested at vegetative

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stage. Shoot height and fresh weights of shoot and root were measured. Shoots were dried at room temperature and roots were dried at 60°C for 72 hours.

Determination of total phenolic content:

250 mg of ground dried shoots was measured into a microtube. The sample was extracted with methanol 85% (with acetic acid) in ultrasonic bath for 15 minutes. Then the samples were centrifuged for 20 minutes at 10000 rpm then adding n-hexane to supernatant and were centrifuged for 10 minutes at 10000 rpm again. Bottom phase contain polyphenols. Total phenolic content was determined by Folin-Ciocalteu method. 200 µl of shoot extracts was added to 1 ml Folin- Ciocalteu's reagent and 800 µl of 75 g/L sodium carbonate was added to the solution in the microplate. The plate was incubated in a dark condition for 90 minutes in room temperature. The absorbance was measured at 765 nm using a Microplate Reader. Total phenolic content of shoot extracts were expressed as mg gallic acid equivalent per gram of dried shoots (mg GAE/g dw).

Statistical analysis:

The data were subjected to analysis of variance (ANOVA) using SPSS computer software and means compared with DNMRT at 5% level of probability.

Results and Discussion

Azotobacter strains altered growth characteristics and total phenolic content of garden thyme (Table 1). The highest value of shoot height (16.11 cm) was obtained by using strain 30 which was significantly different when compared to control. The maximum shoot fresh and dry weights (7.60 g/plant and 3.55 g/plant, respectively) were achieved on strain 30 which were not significantly different when compared to other strains. Root fresh and dry weights were the maximum when strain 30 used. Total phenolic content was maximum (6.40 mg GAE/g dw) by application of strain 5.

PGPR help plants via different mechanisms. One is production of secondary metabolites like antibiotics, cyanide, and hormonelike substances. Another mechanism is antagonism to soilborne root pathogens and phosphate solubilization. Improvement of phosphorus nutrition is one of the factors involved in plant growth promotion by PGPR. These bacteria may improve plant P acquisition by solubilizing organic and inorganic phosphate sources through phosphatase synthesis or by lowering the pH of the soil [3,5,15]. Omidbaigi and Arjmandi [10], indicated that nitrogen and phosphorous are necessary for producing the highest yield of thyme. Udagawa [17], showed increasing in leaves fresh and dry weights of thyme by increasing in nutrients concentration in aquatic growing medium. According Sharafzadeh [14], nutrients are able to change growth and total phenolic content of garden thyme.

Brown [2], revealed that *Azotobacter paspali* can release IAA in the medium. Reda et al. [12], reported that growth regulators increase total phenolic content in thyme.

PGPR can biologically fix nitrogen [4]. Facilitating plant nutrition could be the mechanism by which PGPR enhance plant growth, since the nutritional plants status is enhanced by increasing the availability of nutrients in the rhizosphere [1,13]. Nguyen and Niemeyer [9], illustrated that nutrient availability affects the production of polyphenolic compounds in three cultivars (Dark Opal, Genovese, and Sweet Thai) of basil.

The growth and phenolic content could be markedly affected by the geographical environment, places that plants is grown, physical and chemical characteristics of soil and the method used for determining phenolic content.

In each column, means with the same letters are not significantly different at 5% level of Duncan's new multiple range test.

Table 1: Effects of different strains of *Azotobacter* on growth and total phenolic content of garden thyme.

Azotobacter strains	Shoot height (cm)	Shoot FW (g/plant)	Shoot DW (g/plant)	Root FW (g/plant)	Root DW (g/plant)	Total phenolic content (mg GAE/g dw)
Control	13.90 b	3.49 b	1.30 b	0.29 d	0.05 c	5.39 a
Strain 5	14.78 ab	6.25 a	2.71 a	1.16 c	0.17 c	6.40 a
Strain 9	13.24 b	7.13 a	3.06 a	2.63 b	0.47 b	5.52 a
Strain 12	12.76 b	7.12 a	3.30 a	2.49 b	0.46 b	6.22 a
Strain 30	16.11 a	7.60 a	3.55 a	3.61 a	0.77 a	6.08 a

References

1. Bar-Ness, E., Y. Hadar, Y. Chen, V. Romheld, H. Marschner, 1992. Short term effects of rhizosphere microorganisms on Fe uptake from microbial siderophores by maize and oat. Plant Physiol., 100: 451-456.
2. Brown, M.E., 1974. Seed and root bacterization. Ann Rev Phytopathol., 12: 181-197.
3. Dart, P.J., 1986. Nitrogen fixation associated with non-legumes in agriculture. Plant Soil., 90: 303-334.
4. Glick, B.R., 2003. Plant growth promoting bacteria. In: Glick BR, Pasternak JJ (eds.),

Molecular Biology-Principles and Applications of Recombinant DNA, ASM Press, Washington DC, USA.
5. Goldstein, A.H., 1986. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Altern Agric. 1: 51-57.
6. Jordan, M.J., R.M. Martinez, K.L. Goodner, E.A. Baldwin, J.A. Sotomayor, 2006. Seasonal variation of *Thymus hyemalis* Lange and Spanish *Thymus vulgaris* L. essential oils composition. J Indust Crops Prod., 24: 253-263.
7. Matiljan, J., 2008. Foundation, the World's Healthiest Foods. Retrieved from: (www.whfoods.com/genpage.php?tname=foods pice&dbid=77).
8. Naghdi badi, H., D. Yazdani, S. Mohammad Ali, F. Nazari, 2004. Effects of spacing and harvesting time on herbage yield and quality/quantity of oil in thyme, *Thymus vulgaris* L. J Indus Crops Prod., 19: 31-236.
9. Nguyen, P.M., E.D. Niemeyer, 2008. Effect of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). Retrieved from (http://www.southwestern.edu/academics/bwp/vol8/niemeyer-vol8.pdf).
10. Omidbaigi, R., A. Arjmandi, 2002. Effects of NP supply on growth, development, yields and active substances of garden thyme (*Thymus vulgaris* L.). Acta Hort., 576: 263-265.
11. Razic, S., A. Onjia, B. Potkonjak, 2003. Trace elements analysis of *Echinacea purpurea*-Herbal medicinal. J Pharm Biomed Anal., 33: 845-850.
12. Reda, F., Abdel E.A. Rahim, G.S.A. El Baroty, H.S. Ayad, 2005. Response of essential oils, phenolic components and polyphenol oxidase activity of thyme (*Thymus vulgaris* L.) to some bioregulators and vitamins. J Agr Biol., 7: 735-739.
13. Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust J Plant Physiol., 28: 897-906.
14. Sharafzadeh, Sh., 2011. Effect of nitrogen, phosphorus and potassium on growth, essential oil and total phenolic content of garden thyme (*Thymus vulgaris* L.). Adv Environ boil., 5: 699-703.
15. Sharafzadeh, Sh., 2012. Effects of PGPR on growth and nutrients uptake of tomato. Int J Adv Eng Tech., 2: 27-31.
16. Tilak, K.V.B.R., B.S. Reddy, 2006. *B. cereus* and *B. circulans* novel inoculants for crops. Curr Sci., 5: 642-644.
17. Udagawa, Y., 1995. Some responses of dill (*Anethum graveolens*) and thyme (*Thymus vulgaris*) grown in hydroponic, to the concentration of nutrient solution. Acta Hort., 396: 203-210.
18. Wojdylo, A., J. Oszmianski, R. Czemerys, 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem., 105: 940-949.