



## Effects of plant growth promoter bacteria on biomass and yield of basil (*Ocimum basilicum* L.)

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### ABSTRACT

The main objective of this study was to determine the effects of plant growth promoter bacteria on biomass and yield of basil. The experiment was carried out as randomized complete blocks design with eight treatments and three replications at research field of Agriculture Company of Ran in Firouzkuh of Iran in 2012. The treatments were [1] *Azotobacter chroococcum* (A), [2] *Azospirillum lipoferum* (B), [3] *Bacillus circulans* (C), [4] A + B, [5] A + C, [6] B + C, [7] A + B + C and [8] control (without fertilizer application). The present results have shown that the highest dry weight of plant, herb fresh yield, herb dry yield and essential oil yield were obtained after applying each three bacteria (A + B + C). The maximum plant height was obtained by using two bacteria (A + B). Generally, the maximum herb dry yield and essential oil yield were obtained with the integrated application of each three bacteria.

**Key Words:** Basil, *Azotobacter*, *Azospirillum*, *Bacillus*, Yield.

### INTRODUCTION

Applying plant growth promoter bacteria such as nitrogen fixing bacteria and phosphate solubilizing microorganisms in a sustainable agrosystem has led to an increase in quality agricultural products especially medicinal plants (Sharma, 2002). Free-living nitrogen fixing bacteria such as; *Azotobacter chroococcum* and *Azospirillum lipoferum*, were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (El Ghabban et al., 2006; Mahfouz and Sharaf Eldin, 2007). Phosphate solubilizing microorganisms such as; bacteria and fungi, are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization (Chen et al., 2006). Also, By using biofertilizers, quantity and quality of active substances of medicinal plants can be improved (Rashmi et al., 2008; Azzaz et al., 2009). Basil plant (*Ocimum basilicum*) is one of the most important aromatic plants which used to flavor foods and in traditional medicines. It is an annual and herbaceous plant, belonging to the Lamiaceae family. Its essential oils are synthesized and stored in glandular hairs and are used as flavorings in foods and beverages, as fragrances, as toiletry products such

as mouth washes and dental creams, as fungicides, or insecticides in pharmaceutical and industrial products (; Mondello et al. 2002; Khalid et al., 2006; Ziaei et al., 2012). Some studies have reported that plant growth promoter bacteria such as *Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus* sp and *Pseudomonas* sp could cause increased growth and yield of medicinal plants such as rosemary (Abdel-Aziz et al., 2007), fennel (Mahfouz and Sharaf Eldin, 2007; Azzaz et al., 2009; Moradi et al., 2011), turmeric (Velmurugan et al., 2008), mint (Abdel-Hadi Nadia et al., 2009), hyssop (Koocheki et al., 2009), geranium (Leithy et al., 2009), marjoram (Gharib et al., 2008; El-Ghandour et al., 2009; Al-Fraihat et al., 2011), davana (Kumar et al., 2009), dragonhead (Rahimzadeh et al., 2011) black cumin (Valadabadi and Farahani (2011), dill (Darzi et al., 2012), thyme (Yadegari et al., 2012) and basil (Makkizadeh et al., 2011; Jahan et al., 2013). Therefore, the main objective of the present experiment was to investigate the effects of plant growth promoter bacteria on biomass and yield of basil (*Ocimum basilicum* L.).

## MATERIALS AND METHODS

### Field Experiment

A field experiment, arranged in a randomized complete blocks design with three replications, was conducted in the Experimental field of the Agriculture Company of Ran, Firouzkuh, Iran during the growing season of 2012. The geographical location of the experimental station was 35° 45' N and 52° 44' E with the altitude of 1930 m. The eight treatments of experiment contain: [1] *Azotobacter chroococcum* (A), [2] *Azospirillum lipoferum* (B), [3] *Bacillus circulans* (C), [4] A + B, [5] A + C, [6] B + C, [7] A + B + C and [8] control (without fertilizer application). Inoculation was carried out by dipping the basil seeds in the cells suspension of  $10^8$  CFU/ml for 15 min. Several Soil samples (0–30 cm depth) were taken for the nutrient and trace element analysis prior to land preparation. Chemical and physical properties of the experimental soil is presented in Table 1. Each experimental plot was 3 m long and 2.4 m wide with the spacing of 10 cm between the plants and 40 cm between the rows. There was a space of one meter between the plots and 2 meters between replications. Basil seeds were directly sown by hand. There was no incidence of pest or disease on basil during the experiment. Weeding was done manually and the plots were irrigated weekly (as trickle irrigation system). All necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation. In this study, traits of plant height, dry weight of plant, herb fresh yield, herb dry yield and essential oil yield were evaluated. At the beginning of flowering, the plant height, from plant base to the tip of plant, was measured for each plot using a ruler ( $\pm 0.1$  cm) (Darzi et al., 2007; Azizi et al., 2008). Fresh weight of plant was calculated using a digital balance (Sartorius B310S;  $\pm 0.01$  g). For evaluating the dry weight of plant at the harvest time (full blooming), plants were put in the oven at 75° C for 48 h and dry weight was calculated using a digital balance (Sartorius B310S;  $\pm 0.01$  g) (Migahed et al., 2004; Badran and Safwat, 2004). Fifteen plants also were used at the harvest time to determination of herb fresh yield and herb dry yield.

### Extraction of Essential oil

In order to determine the essential oil content (%), a sample of 100 g of basil dried herb from the each plot were mixed with 500 ml distilled water and then were subjected to hydro-distillation for 3 h using a Clevenger-type apparatus (Sajjadi, 2006; Darzi et al., 2012). Essential oil yield also was calculated with by using essential oil content and herb dry yield.

**Table 1.** Some Traits of Physical and Chemical of soil in experiment site

| Cu<br>(mg/kg) | Fe<br>(mg/kg) | K<br>(mg/kg) | P<br>(mg/kg) | N<br>(%) | O.C<br>(%) | EC<br>(ds/m) | pH  | Texture    |
|---------------|---------------|--------------|--------------|----------|------------|--------------|-----|------------|
| 1.2           | 8             | 720          | 48           | 0.127    | 1.86       | 1.55         | 7.6 | Clay-Loamy |

### Statistical Analysis

All the data were subjected to statistical analysis (one-way ANOVA) using SAS software (SAS Institute, version 8, 2001). Differences between the treatments were performed by Duncan's Multiple Range Test (DMRT) at 5% confidence interval. Transformations were applied to the data to assure that the residuals had normal distribution (Zar, 1996).

## RESULTS AND DISCUSSION

### Plant height

The present results have indicated that plant height was significantly affected by the application of different treatments of plant growth promoter bacteria (Table 2). The highest plant height (46.3 cm) was obtained by applying two bacteria (azotobacter + azospirillum) and the lowest plant height (22.6 cm) was indicated by control treatment (Figure 1). According to the present analysis, Nitrogen fixing bacteria have increased plant height by enhancing the nitrogen content and the rate of photosynthesis (Migahed et al., 2004). The present result were derived from the improvement of nitrogen fixing bacteria' activities in soil, which is in agreement with the previous studies carried out on the rosemary, fennel, turmeric, marjoram, hyssop, geranium (Abdel-Aziz et al., 2007; Mahfouz and Sharaf Eldin, 2007; Velmurugan et al., 2008; Gharib et al., 2008; Koocheki et al., 2009; Leithy et al., 2009; Al-Fraihat et al., 2011).

### Dry weight of plant

The results presented in Table 2 have demonstrated that dry weight of plant was influenced by the application of different treatments of growth promoter bacteria, significantly. Among various treatments, treatment of the application of azotobacter plus azospirillum plus bacillus (A + B + C)(31.55 g) have indicated maximum increase in dry weight of plant (Figure 2). Integrated application of nitrogen fixing bacteria and phosphate solubilizing bacteria, through the increased nitrogen and phosphorus uptake (Rashmi et al., 2008; Azzaz et al., 2009), caused higher biomass production contain plant height and fresh weight of plant which leads to improvement of dry weight of plant. The result of present work are in agreement with the reports of Mahfouz and Sharaf Eldin (2007) on *Foeniculum vulgare*, Abdel-Aziz et al. (2007) on *Rosmarinus officinalis*, Kumar et al. (2009) on *Artemisia pallens*, Valadabadi and Farahani (2011) on *Nigella sativa*, Al-Fraihat et al. (2011) on *Majorana hortensis* and Yadegari et al. (2012) on *Thymus vulgaris*.

### Herb fresh yield

The results presented in Table 2 have revealed that studied various treatments had significant effects on the herb fresh yield. The maximum herb fresh yield (8675 kg/ha) was obtained by using integrated application of each three bacteria and the minimum herb fresh yield (3813 kg/ha) was indicated in control

treatment (Figure 3). Improved Biomass and eventually herb fresh yield by bacteria has been attributed both to production of plant hormones, especially growth promoters and by supplying nitrogen phosphorus. The present result is in agreement with previous studies on medicinal plants such as Abdel-Aziz *et al.* (2007) on rosemary, Leithy *et al.* (2009) on geranium, Abdel-Hadi Nadia *et al.* (2009) on mint, Gharib *et al.* (2008), El-Ghandour *et al.* (2009) and Al-Fraihat *et al.* (2011) on marjoram.

### Herb dry yield

The results presented in Table 2 have showed that plant growth promoter bacteria had significant effects on the herb dry yield. The highest herb dry yield (694.2 kg/ha) was obtained by integrated applying of bacteria (azotobacter + azospirillum + bacillus) and the minimum herb dry yield (524.9 kg/ha) was showed in control treatment (Figure 4). Increased herb dry yield in mentioned treatment can be owing to the improvement of yield components such as; plant height, fresh weight and dry weight of plant. The present result is in agreement with the report of Abdel-Hadi Nadia *et al.* (2009) on *Mentha piperita*, El-Ghandour *et al.* (2009) and Al-Fraihat *et al.* (2011) on *Majorana hortensis*, Makkizadeh *et al.* (2011) and Jahan *et al.* (2013) on *Ocimum basilicum* and Rahimzadeh *et al.* (2011) on *Dracocephalum moldavica*.

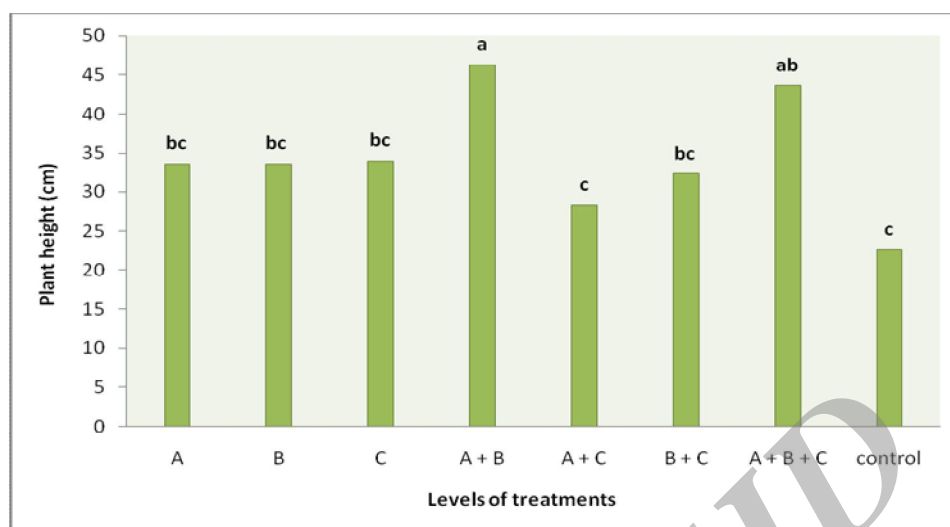
### Essential oil yield

The present results have indicated that essential oil yield was significantly affected by the application of different treatments of growth promoter bacteria (Table 2). The maximum essential oil yield (1573.3 g) was obtained by using integration of three bacteria of *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Bacillus circulans* and the minimum essential oil yield (537.0 g) was showed in control treatment (Figure 5). Integrated application of Plant growth promoter bacteria contain nitrogen fixing bacteria and phosphate solubilizing bacteria through the improvement of essential oil yield components such as essential oil content and herb dry yield caused more essential oil yield. This finding is in accordance with the observations of Abdel-Aziz *et al.* (2007) on *Rosmarinus officinalis*, Gharib *et al.* (2008), El-Ghandour *et al.* (2009) and Al-Fraihat *et al.* (2011) on *Majorana hortensis*, Koocheki *et al.* (2009) on *Hyssopus officinalis*, Makkizadeh *et al.* (2011) on *Ocimum basilicum*, Yadegari *et al.* (2012) on *Thymus vulgaris* and Darzi *et al.* (2012) on *Anethum graveolens*.

**Table 2.** Analysis of variance of studied traits

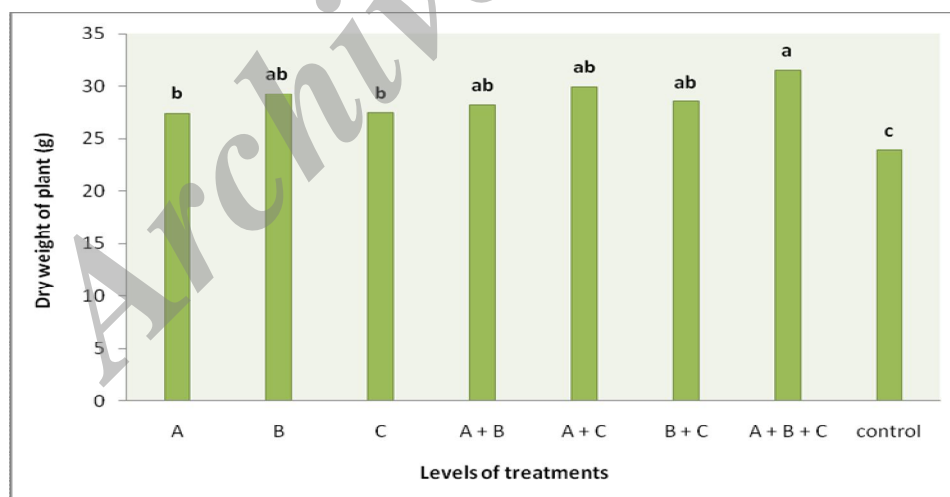
|             | S. O. V | df           |                       |                         |                       |                       | MS |  |
|-------------|---------|--------------|-----------------------|-------------------------|-----------------------|-----------------------|----|--|
|             |         | Plant height | Dry weight of plant   | Herb fresh yield        | Herb dry yield        | Essential oil yield   |    |  |
| Replication | 2       | 152.541*     | 10.0358 <sup>ns</sup> | 5034870.5 <sup>ns</sup> | 4850.84 <sup>ns</sup> | 7176.54 <sup>ns</sup> |    |  |
| Treatments  | 7       | 174.952**    | 15.1845**             | 6669831.1*              | 7360.97**             | 311524.2**            |    |  |
| Error       | 14      | 35.8273      | 3.16436               | 2057717.3               | 1533.10               | 50553.06              |    |  |

\* and \*\*: Significant at the 5 and 1% levels of probability, respectively. ns: Non-significant.

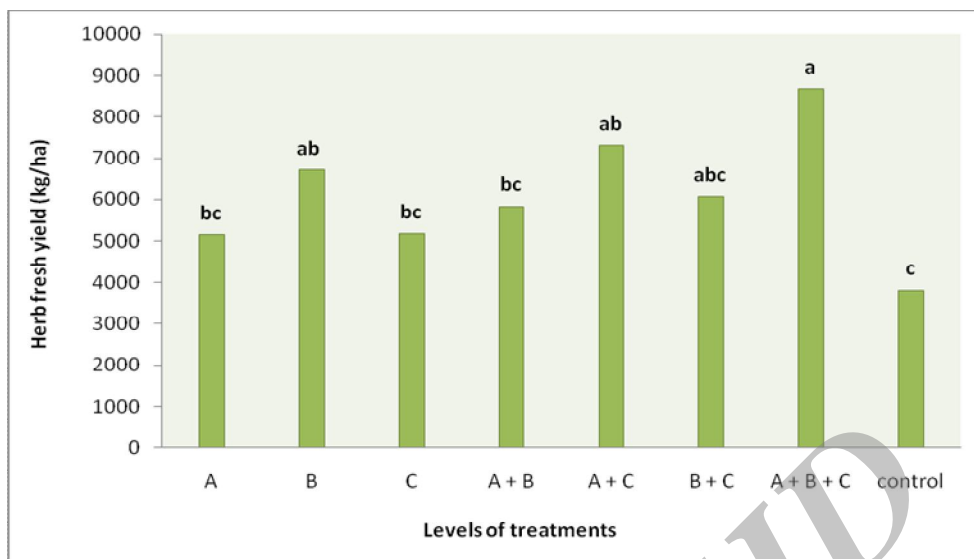


**Figure 1.** Mean comparison for plant height in different levels of plant growth promoter bacteria treatments

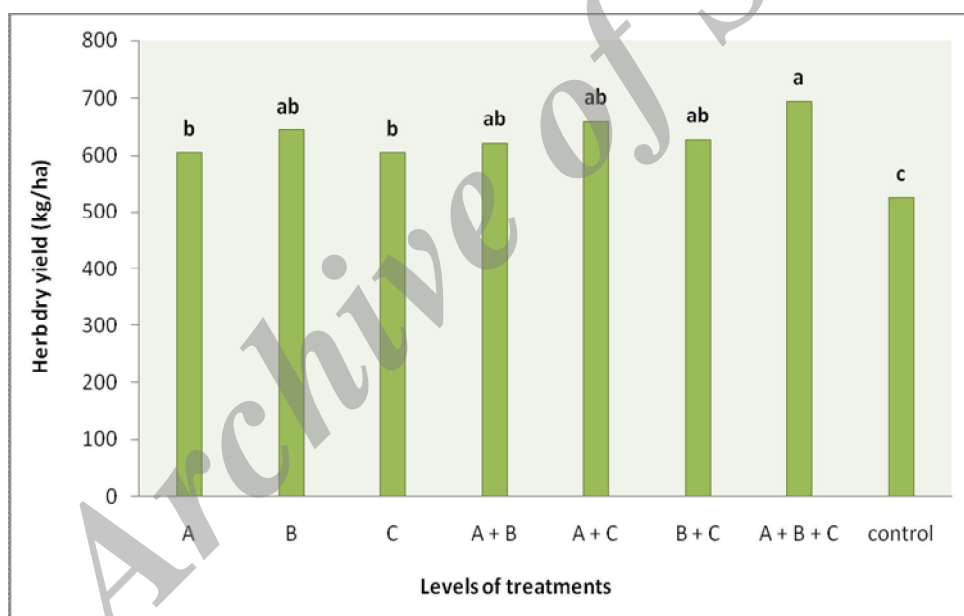
A, B, C, A + B, A + C, B + C, A + B + C and control represent azotobacter, azospirillum, bacillus, azotobacter + azospirillum, azotobacter + bacillus, azospirillum + bacillus, azotobacter + azospirillum + bacillus and without fertilizer application, respectively.



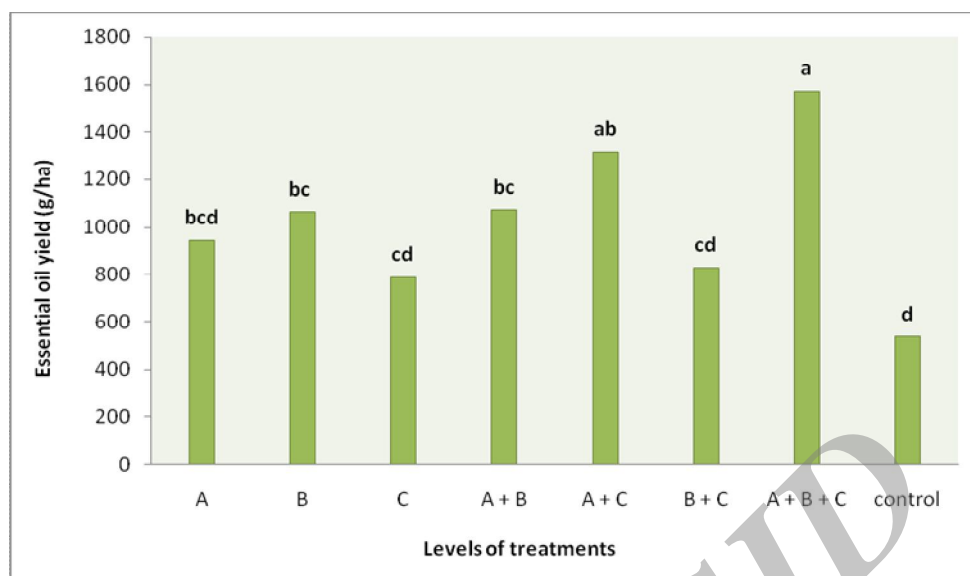
**Figure 2.** Mean comparison for dry weight of plant in different levels of plant growth promoter bacteria treatments



**Figure 3.** Mean comparison for herb fresh yield in different levels of plant growth promoter bacteria treatments



**Figure 4.** Mean comparison for herb dry yield in different levels of plant growth promoter bacteria treatments



**Figure 5.** Mean comparison for essential oil yield in different levels of plant growth promoter bacteria treatments

## CONCLUSION

It is clear from the present study that, Integrated application of plant growth promoter bacteria positively influenced on biomass and yield of basil, as the highest herb dry yield and essential oil yield were obtained by using each three bacteria (azotobacter + azospirillum + bacillus).

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