Industrial Crops & Products 107 (2017) 97–104

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Research paper

Plant growth promoting rhizobacteria in an ecological cropping system: A study on basil (*Ocimum basilicum* L.) essential oil production

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ARTICLE INFO

Keywords:
Ecological inputs
Beneficial soil microorganisms
Medicinal plant
Nitroxin®
Azotobacter

ABSTRACT

The use of biofertilizers containing beneficial soil microorganisms as alternative for chemical fertilizers is an effective way to improve plant growth, environment health and soil productivity. According to the importance of the production of medicinal plants in sustainable agricultural systems, an experiment was conducted during growing seasons of 2013–2014 and 2014–2015 at the Agricultural Research Farm of Ferdowsi University of Mashhad, Iran. Trial was done in a randomized complete block design (RCBD) with three replications and four treatments. The treatments were seed inoculation with biofertilizers including: 1- Nitroxin® biofertilizer (containing *Azotobacter* sp. and *Azospirillum* sp. bacteria), 2- Biophosphorus® biofertilizer (containing *Bacillus* sp. and *Pseudomonas* sp. bacteria), 3- Mixture of Nitroxin® & Biophosphorus® and 4- The Control (no inoculation). The results showed the most amounts of plant height, number of branches, plant dry matter and leaf area index were obtained from the plants treated with Nitroxin®. The maximum number of branches (11.2) was obtained from the plants treated with mixture of Nitroxin® and Biophosphorus®. Seed production was at the most in plants under Biophosphorus® treatment. Biological fertilizers increased dry shoot yield compared with the control.The highest percentage of essential oil (0.83%) was observed in control. The traits associated with the yield were higher in the second cut compared with the first and the third cuts. There was a significant positive correlation ($r = 0.60^{**}$) between the plant height and the leaf yield. In general, the results suggest that biofertilizers especially Nitroxin® could improve growth characteristics and the yield of basil promising possibility of ecological production of this valuable medicinal plant indicating biofertilizers may be feasible in places with few resources.

1. Introduction

Nowadays ensuring the sustainable production of healthy food products regarded to environmental safety and social and economic issues, is not only an important topic in different sciences such as agriculture, ecology and the environment but considered by farmers, researchers, and policy makers (Neeson, 2004). Moreover, the physiological response of the crops to chemical fertilizers grew diminishing, and further studies show that, excessive use of chemical fertilizers reduces both environmental pollution and the crop yield ecological damages. Decline of biological activity, physical and chemical properties of soil and lack of micronutrients in chemical fertilizers are main reasons of crop yield reduction (Adediran et al., 2004).

Asia is the largest consumer of chemical fertilizers across the world (Pimentel et al., 2005). One option for increasing the sustainability of agricultural production and reduction in usage of chemical fertilizers is the use of soil beneficial microorganisms (Vessey, 2003; Gosling et al., 2006) known as Plant Growth Promoting Rhizobacteria (PGPRs). These microorganisms both directly and indirectly affect the plant growth (Vessey, 2003; Barea et al., 2005). The improvement of plant growth is achieved mainly through the release of metabolites from PGPRs. These bacteria increase the plant growth through the mechanisms which haven't still been entirely identified, but the most important ones are: a) production of the plant hormones such as Auxin, Gibberellin, Cytokinin and vitamins such as group B vitamins, b) fixing atmospheric N, c) solubilizing of inorganic phosphate and mineralizing organic phosphate and other nutrients, and d) dealing with phytopathogenic microorganisms by production of siderophores, antibiotics, enzymes and competing with detrimental microorganisms (Barea et al., 2005; Esitken et al., 2010).

Jahan et al. (2012a) reported that the application of biofertilizers significantly increased most traits of basil, including fresh and dry shoot yield, dry leaf yield and leaf area index. It has been reported that dual inoculation with mixture of *Azotobacter* sp., *Azospirillum* sp., application

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http://dx.doi.org/10.1016/j.indcrop.2017.05.020
Received 28 December 2016; Received in revised form 11 May 2017; Accepted 12 May 2017

of phosphate rock, inorganic nitrogen and inoculation with mycorrhizae, had improved the growth of *Datura stramonium* and *Ammi visnaga* Fam. Umbelliferae (Mahfouz and Sharaf-Eldin, 2007). The production of medicinal plants such as; fennel (Kapoor et al., 2004), black cumin (Shaalan, 2005), sena (Lakshmanan et al., 2005), oregano, caraway (Vestberg et al., 2005), majoran (*Origanum majorana* L.), thyme (*Thymus vulgaris* L.) and caraway (*Carum carvi* L.), (Richter et al., 2005) and mugwort (Kapoor et al., 2007) were improved by appling of biofertilizers by better qualitative and quantitative characteristics.

The genus *Ocimum* belongs to Labiaceae family, which its Ecotypes have diversed morphological varieties, as some researchers have reported up to 150 species of this genus, while some others reported 65 species of this genus and the rest called as a synonyms (Labra et al., 2004; Telci et al., 2006). However, the genus includes at least, 60 species and numerous cultivars (Khalid et al., 2006). The most important ones are: *O. bacilicum, O. gratissimum*, *O. canum*, *O. americanum*, and *O. cranthum*. Among the species, regular basil (*O. bacilicum*) is the most important and used one and is cultivated all over the temperate climates. Basil is known as a medicinal and industrial plant and ranks second after spearmint. The basil leaves and essential oil have been being used for centuries in remediation of diseases such as headache, cold, digestive system disorders and kidney failure. Active ingredients of the vegetative part of basil are consumed as appetizers, which are used to treat bloating, improve digestion and reduce general body fatigue. This plant can be used to treat both some heart problems and enlarged spleens. In traditional medication, basil is used as an antiflatulence, antimalarial, a reducer of feversand a stimulant medication (Marrotti et al., 1996; Khalid et al., 2006; Telci et al., 2006; Chalchat and Ozcan, 2008).

The essential oil production does not depend only on plant genetics or developmental stage. The environment and its changes can influence in a significant way biochemical pathways and physiological processes that alter plant metabolism and, therefore, the essential oil biosynthesis (Sangwan et al., 2001) so essential oil ingredients are also varying according to ecological conditions. The oil is extracted from the vegetative parts (leaves, branches and fresh or dried flowers) through the hydrodistillation method by Clevenger apparatus and because it is lighter than water, separating the mixture of water-oil is comfortably possible. The dry matter yield of basil has been reported roughly as; 1.2–2 Mg ha^{-1}, the fresh shoot yield as 8–10 Mg ha^{-1} that sometimes exceed to 12 Mg ha^{-1} (Prakash, 1990; Jahan et al., 2012a).

To reduce the effects of agrochemicals use, it is necessary to pay attention to alternative approaches such as the use of biofertilizers. The long-term effects of the inoculation of basil seeds with PGPRs on plant growth is little known. Therefore, this study was designed, conducted and aimed to investigate the growth characteristics, the yield and the basil essential oil during two years of inoculation by biological fertilizers in a low input cropping system.

2. Material and methods

2.1. General information and experimental design

This study was conducted during the growing season of 2013–2014 and 2014–2015 at the Agricultural Research Farm of Ferdowsi University of Mashhad, Iran; which is located at 10 km southern east of the Mashhad city (longitude “28° 59 ‘E and latitude” 15° 36′ N and 985 m above the sea level). Average temperature and precipitation rate of the Research Farm for two years are shown in Fig. 1. The experiment conducted as a randomized complete block design (RCBD) with 3 replications and 4 treatments. The treatments were inoculation the basil seeds with: 1- Nitroxin® biofertilizer (containing *Azotobacter* sp. and *Azospirillum* sp. bacteria), 2-Biophosphorus® biofertilizer (containing *Bacillus* sp. and *Pseudomonas* sp. bacteria), 3- Mixture of Biophosphorus® and Nitroxin®, and 4- The control (no inoculation). Biofertilizers were produced by MehrAsia® Co. Ltd. Tehran, Iran, in liquid with 10^7 CFU/ml. Just before planting, soil samples were taken from 0 to 30 cm soil depth and the amount of the organic matter (Walkley Black’s method), nitrogen (Kjeldahl’s method), phosphorus (Olsen’s method), potassium (Flame Photometry method), pH and EC were determined according to FAO guide (Motsara and Roy, 2008) (Table 1).

2.2. Planting and management

The plots size was 4 × 3 m, containing 6 rows with 50 cm spacing. In both years of the experiment the seeds inoculation was performed with biofertilizers based on the standard method (Kennedy et al., 2004) and manufacturer's recommendations (with emphasis on avoiding direct sunlight and acceleration of planting after inoculation) (Jahan et al., 2013). Basil seeds which were selected from the local population with 98% vigor, were planted manually in a depth of 1–2 cm and within 6 cm distance on the rows, in the late May 2014 and 2015. Each plot was irrigated first immediately after planting and then in weekly intervals. The seeds emerged 7–14 days after sowing. In order to achieve the desired density, the plants had to be thinned in a six-leaf stage. Weeding was done 3 times in the periods of 21, 40 and 65 days after planting.

2.3. Measurements and calculations

During the growing season in two years, three cuts of vegetative growth were harvested when the plants reached to 5–10 percent of flowering stage (57, 79 and 118 days after planting, respectively). Before each cut, three plants in the area of 0.1 m^2 were randomly selected and the plant height, the number of secondary stems (branches number), the leaf/stem ratio, the leaf area index (LAI), fresh and dry shoot yield, dry leaf yield and the total dry matter per plant were measured. The leaf area was determined by Area Measurement System: Delta T, Ltd., UK. To determine the final yield of each plot, concerning the marginal effect of plots, plants were harvested and fresh yield and dry yield of the plants shoot and leaves were determined. At the end of the growing season; when the seeds were physiologically matured, the plants were harvested and the dry matter yield, the seed yield and the harvest index were determined. Before the harvest, three plants from each plot were randomly selected and 1000 seeds weight and seed weight per plant were determined.

2.4. Essential oil extraction

To investigate the qualitative traits, 50 g of the dried leaves per plot were randomly selected and the essential oil was measured by a Clevenger apparatus. At first, each sample was grinded and poured into a one-liter flask and then 600 ml of distilled water was added. Then samples were placed in the Clevenger apparatus for 4 h and the percentage and the yield of the essential oil of each sample were determined (Richter and Schellenberg, 2007).

2.5. Statistical method and analysis

The statistical analysis (ANOVA) was performed by SAS Ver. 9.1 software. Using a combined analysis, data were analyzed for vegetative growth; so that, the treatments were considered as main plot, the cuts as sub-plot and the year as the time factor. The data which were related to the reproductive growth were analyzed as a split plot in time design, and for this purpose, the experimental treatments were considered as the main plot factor and the year as the sub plot factor. The curves fitting and graphs drawing, were done by using the Slide Write Ver. 2 and the MS-EXCEL Ver. 14 Software. Mean comparisons were done by Duncańs multiple range test at the 5% probability level.

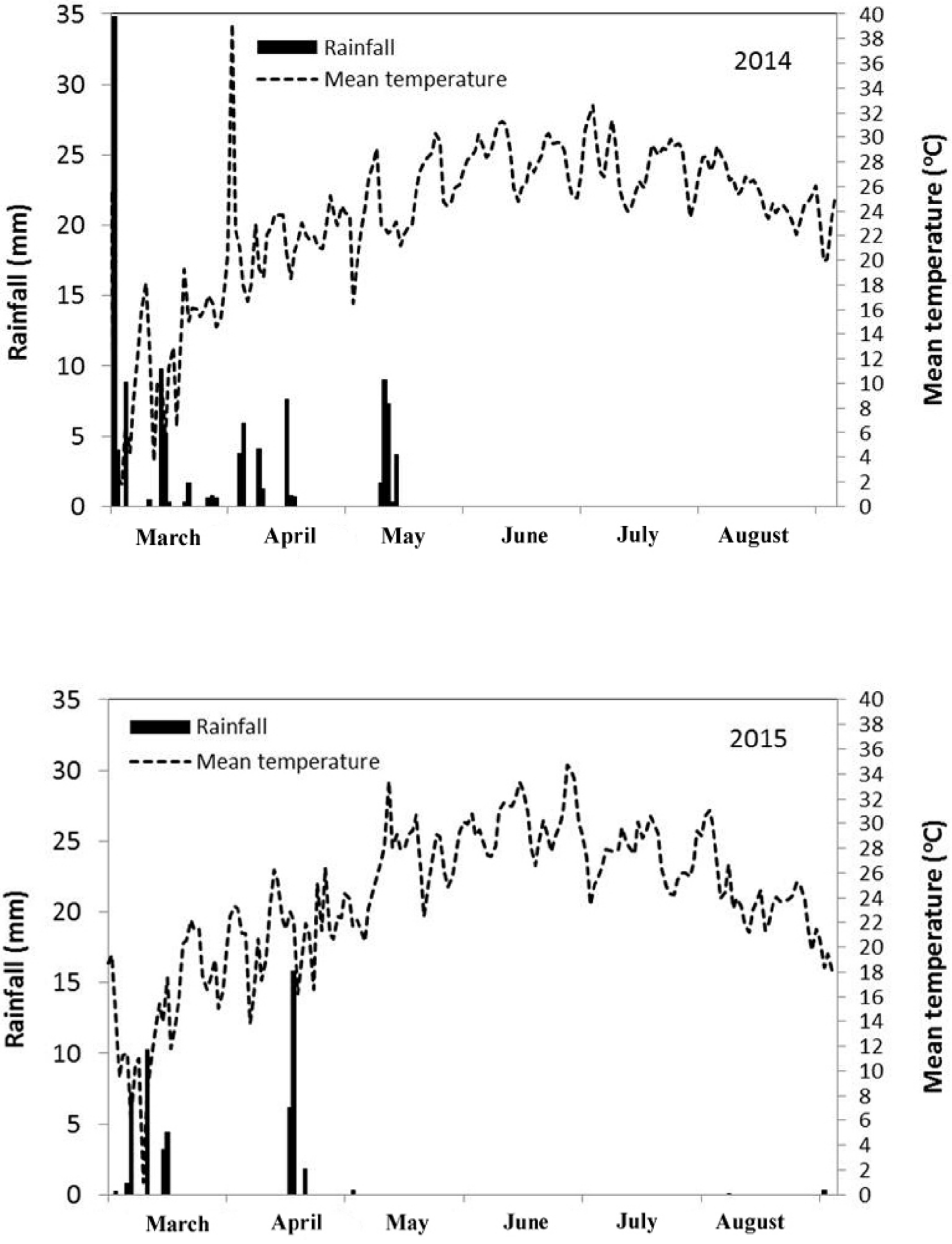

Fig. 1. Trend of average temperature (°C) and precipitation rate (mm) for two years of experiment in Mashhad, Iran.

Table 1
Soil properties of the experimental field (mean of two years).

Soil texture	Total Nitrogen (ppm)	Available Phosphorus (ppm)	Available Potassium (ppm)	Organic matter (%)	pH	EC (dS m^{-1})
Silty loam	800	13	119	0.94	7.4	1.2

3. Results and discussion

3.1. Vegetative growth

3.1.1. Plant height

The results showed that the highest (49.58 cm) and the lowest (37.78 cm) plant height were observed in plants treated with Nitroxin® and the control, respectively (Table 2). The plant height was significantly different among the cuts and increased from the first to the third cut gradually (Table 3). According to Table 4, basil plants in the second year of experiment had higher height.

Timely supply of plants required nutrients is one of the main factors affecting the plant height. Increasing of plant height in plants treated with biofertilizer, could be assigned to soil biological activity and more availability of micro and macro elements for plants. Therefore, this could preserve the plants of facing the loss of nutrients and reduction of growth and height. In an experiment, different levels of nitrogen positively affected the height of the basil plants, although there was no significant difference between nitrogen levels (Sifola and Barbieri, 2006).

The less plant height in the first cut and its increase during the next cuts, may be due to the slow release of nutrients into the soil solution and their gradual facilitating effect on promoting plant growth by biological fertilizers during the growing seasons facilitated. Ending the peak at warm temperature (late June and early July) and confronting gradually to the favorable environmental conditions for the plant growth during the second and the third cuts may have been other factors that can delay the onset of flowering stage and increase the vegetative growth and the plant height.

Table 2
Mean comparisons of some basil growth characteristics as affected by biofertilizers (Each value was averaged of two years).

	Plant height (cm)	Number of branches per plant	Leaf weight/stem weight	Dry matter (g plant^{-1})	Shoot fresh yield (kg ha^{-1})	Essential oil (%)	Yield of essential oil (L ha^{-1})
Nitroxin®	49.58*a*	9.88*b*	1.64*a*	11.77*a*	15774*ab*	0.59*b*	10.28*a*
Biophosphorus®	44.45*b*	8.63*c*	1.29*a*	8.59*b*	14677.7*bc*	0.55*b*	8.53*a*
Nitroxin® + Biophosphorus®	43.49*b*	11.25*a*	1.44*a*	7.73*b*	17656.1*a*	0.52*b*	8.47*a*
Control	37.87*c*	8.38*c*	1.37*a*	5.39*c*	12569.4*c*	0.83*a*	8.23*a*

In each column, means which share the same letter(s) are not significantly different *($p \le 0.05$)*.

3.1.2. Branches number per plant

The results showed that the most number of branches were obtained from plants inoculated by the mixture of Biophosphorus® plus Nitroxin®, and Nitroxin® solely inoculation (Table 2). Plants in the third and the first cut had the most branch number (11.2 and 10.5, respectively) and the plants in the second cut had the lowest (6.87) branch number (Table 3). Branch number in the first year, was about 70% more than the second year (Table 4). Empowerment of the plant-rhizosphere system and the increase of the vegetative growth, particularly before flowering stage, can lead to the emergence of more branches. Application of 30 Mg ha^{-1} of manure increased the *Matricaria chamomilla* L. branches (Jahan and Jahan, 2010). However, the result of a two-year study carried out by Mahfouz and Sharaf-Eldin (2007) on the fennel, showed that the applying of different types of biological and chemical fertilizers in the first year had no significant effect on the number of branches.

3.1.3. The leaf to stem ratio

The effect of different treatments on the leaf to stem ratio was not significant (Table 2), but for the cuts and the years, the plants of the first cut and the first year had a highest ratio ($p \le 0.05$) (Tables 3 and 4). According to the results, although the biological fertilizers increased the leaf and stem growth simultaneously, they did not change the allocation patterns of assimilates. Hence, the percentage of leaf, the percentage of stem and finally leaf to stem ratio did not vary amongst the treatments. Since basil leaves have the most essential oil and therefore the most food & medicinal industries consumption compared with the other parts of the plant, it could be concluded that a higher leaf to stem ratio is more appropriate for the essential oil extraction.

3.1.4. Leaf Area Index (LAI)

The results demonstrate the effectiveness of the inoculation of the basil seed by biological fertilizers. The plants treated with Nitroxin® had the highest LAI (about 4), and after that the mixture of Biophosphorus® + Nitroxin® and Biophosphorus® had the second and third level amounts which significantly differed from the control (Table 2). During the growing season, the plants were treated with Nitroxin® had the highest LAI in 105 days after planting, whereas in other treatments, the highest LAI was observed in 90 days after planting and then it declined (Fig. 2).

In a similar experiment on sesame, the linear growth of LAI was started in 40th day after emergence; however, the growth rate was higher in the plants inoculated with biofertilizers compared with the control. Therefore, sesame LAI reached to the maximum in 60th day after emergence as a result of biofertilizer inoculation, whereas it did so, 15 days after the control treatment, i.e. almost in 75th day after emergence. At this stage; On the contrary, the sesame LAI as a result of Biosulfur® inoculation was more than LAI in the control (Jahan et al., 2013). Biological fertilizers, through influence on availability of the nutrients, promote vegetative growth of basil by increasing the number of leaves, which in turn caused more LAI (Sifola and Barbieri, 2006). Results of an experiment on basil showed that, the plants inoculated with Nitroxin® and phosphate biofertilizers produced more leaves than plants which treated by chemical fertilizers (Weisany et al., 2012).

As it is shown in Fig. 2, the plants which were inoculated with three biological fertilizers had more LAI during the growing season compared with the control plants. In an experiment on *Celosia argentea*, it was found that the increase in leaf area and chlorophyll content of the plants inoculated with nitrogen-fixing free living bacteria was due to the increase in nitrogen absorption (Rawia et al., 2006).

3.1.5. Fresh and dry shoot yield, dry leaf yield

The results showed that inoculation with biological fertilizers increased the yield of basil compared with the control. The most fresh shoot yield (17656.1 kg ha^{-1}) was obtained from the plants treated with mixture of Nitroxin® and Biophosphorus® and then from the plants inoculated with Nitroxin® (15774 kg ha^{-1}) and finally the ones which were inoculated with Biophosphorus® (14667.7 kg ha^{-1} with no significant difference compared with control) (Table 2). Biofertilizers increased significantly the dry shoot yield and dry leaf yield compared with the control (Table. 2). Basil produced more shoot and leaf yield in the second year than in the first year (Table 4).

Facilitated absorption of the nutrients, vitamins secretion, fixing atmospheric nitrogen, solubilizing the minerals like phosphorus, production of siderophore and chelates, synthesis of the enzymes and phytohormons including Auxin, Cytokinin and Gibberellin that affect the different stages of the plant growth and development, are among the direct effects of the plants growth, promoting bacteria through which they can enhance the crops yield (Glick et al., 1995; Lucy et al., 2004; Gray and Smith, 2005). Fatma et al. (2008) reported that the biological fertilizers containing *Azotobacter* sp., *Azospirillum* sp. and phosphate solubilizing bacteria, significantly affected the growth indices and the amount of essential oil in Majorana (*Majorana hortensis*). They also reported that characteristics of essential oil of Majorana on Gram positive and Gram negative bacteria, fungi and yeasts were affected by inoculation.

Table 3
Mean comparisons of some basil growth characteristics as affected by biofertilizers between three cuts (Each value was averaged of two years).

	Plant height (cm)	Number of branches per plant	Leaf weight/ stem weight	Dry matter (g plant^{-1})	Shoot fresh yield (kg ha^{-1})	Shoot dry yield (kg ha^{-1})	Leaf dry yield (kg ha^{-1})	Essential oil (%)	Yield of essential oil (L ha^{-1})
First cut	37.7*c*	10.5*a*	1.86*a*	6.28*c*	11967.9*c*	1903.3*b*	1342.4*b*	0.52*b*	5.52*c*
Second cut	44*b*	6.87*b*	1.29*b*	10.31*a*	17660.9*a*	3217.2*a*	1761.5*a*	0.59*b*	10.26*a*
Third Cut	49.5*a*	11.25*a*	1.12*b*	8.19*b*	15879*b*	3211*a*	1356.4*b*	0.75*a*	10.75*a*

In each column, means which share the same letter(s) are not significantly different *($p \le 0.05$)*.

Table 4
Mean comparisons of some basil growth characteristics as affected by biofertilizers between two years.

	Plant height (cm)	Number of branches per plant	Leaf weight/ stem weight	Dry matter (g plant^{-1})	Shoot fresh yield (kg ha^{-1})	Shoot dry yield (kg ha^{-1})	Leaf dry yield (kg ha^{-1})	Essential oil (%)	Yield of essential oil (L ha^{-1})
First year	34.08*b*	12.02*a*	1.82*a*	5.66*b*	8260*b*	1531.1*b*	855*b*	0.64*a*	5.16*b*
Second year	52.5*a*	7.05*b*	1.09*b*	10.51*a*	22079*a*	4023.3*a*	2040.6*a*	0.60*a*	12.08*a*

In each column, means which share the same letter(s) are not significantly different ($p \le 0.05$).

3.1.6. Total dry matter per plant

The results indicated that biological fertilizers had so positive effects on total dry weight per plant as the highest dry matter (11.77 g plant^{-1}) and lowest (5.39 g plant^{-1}) were obtained from plants treated with Nitroxin® and the control, respectively (Table 2). Amongst the cuts, the highest total dry matter per plant (10.31 g) was obtained from the second cut. The total dry matter per plant in the second year was more than what was in the first year, for about 85 percent (Tables 3 and 4). Organic and biological fertilizers cause more yield and plants growth by increasing availability of macro and micro nutrients required by plants, improvement of physiochemical and biological soil characteristics, raising water-holding capacity of the soil. Biofertilizers particularly affect root density, developing patterns and architecture by improvement of soil structure and producing plant hormones and promoting the uptake and transporting of minerals (Khalid et al., 2006; Fatma et al., 2008). Biological fertilizers significantly increased the plant weight in the second and third cuts compared with the first cut due to the slow release of essential elements for the plant growth and synchrony of this release with plant uptake (Table 3). These results are in agreement with the findings of Barea et al., 2005.

In an experiment which was done on Marjoram (*Majorana hortensis*), using biofertilizers and compost whether combined or separately, increased the plant fresh and dry weight, compared with chemical fertilizers (Fatma et al., 2008). In a greenhouse experiment, inoculation by the mixture of phosphate biofertilizer and Nitroxin®, compared with separately inoculation by Nitroxin® and phosphate biofertilizers, had a greater impact on increasing the total dry matter of basil shoots (Weisany et al., 2012).

3.1.7. Essential oil percentage & yield

The results showed that a significant increase in the essential oil percentage of the plants which were under control compared with biofertilizers, and no significant differences were seen between biofertilizers (Table 2). Inoculation with biofertilizers had no significant effect on essential oil yield; however, the plants treated with Nitroxin® had the most essential oil yield (Table 2). The lower essential oil percentage in plants inoculated with biofertilizers may be related to reduced stress levels resulted from their inoculations (Vessey, 2003); in contrast, control treatment plants experienced more stress in absence of rhizobacteria.

Basil harvested in the third cut, produced more essential oil percentage than the one in the first and the second cut (Table 3), but there was no significant difference between two years of the experiment (Table 4). Growing period of basil ended to third cut was coincided with warm and drought of mid late of summer (Fig. 1), so it was expected essential oil percentage increases as a response to warm weather. Essential oil percentage is negatively correlates with essential oil yield in general (Sangwan et al., 2001; Tahami et al., 2011; Jahan et al., 2012b). The essential oil yield in the first cut got the lowest (Table 3). However, the essential oil yield in the second year was significantly more than the one in the first year (134% higher than the first year) (Table 4).

The high percentage of the essential oil in control plants and its low percentage in plants under biofertilizers treatments which had the most yield, may be due to the higher levels of the secondary metabolites under environmental stress conditions and nutrient deficiencies; because the biofertilizers have provided favorable conditions for the plants and secondary metabolites in these plants, would not increase as a result of providing appropriate soil nutrients for the plant growth. Tahami et al. (2011) reported that the basil essential oil increased as a result of biofertilizers inoculation under an organic cropping system. Jahan and Jahan (2004) by investigating the effect of organic fertilizers on German chamomile (*Matricaria chamomilla*), reported similar results.

The essential oil yield depends on the leaf yield and the essential oil percentage. Thus, an insignificant difference in the essential oil yield can be due to the decrease in the essential oil percentage in the plants inoculated with biofertilizers. In a research, basil grown under organic conditions came about to have more essential oil yield than when it was grown with common chemical fertilizers (Khalid et al., 2006). Similar reports (Qandeel et al., 2002), indicated an increase in the basil essential oil in a productive system which was based on the use of the combination of organic and inorganic nitrogen fertilizers. The highest zucchini squash seed oil and the protein percent were obtained through the application of chicken manure. However, there was no

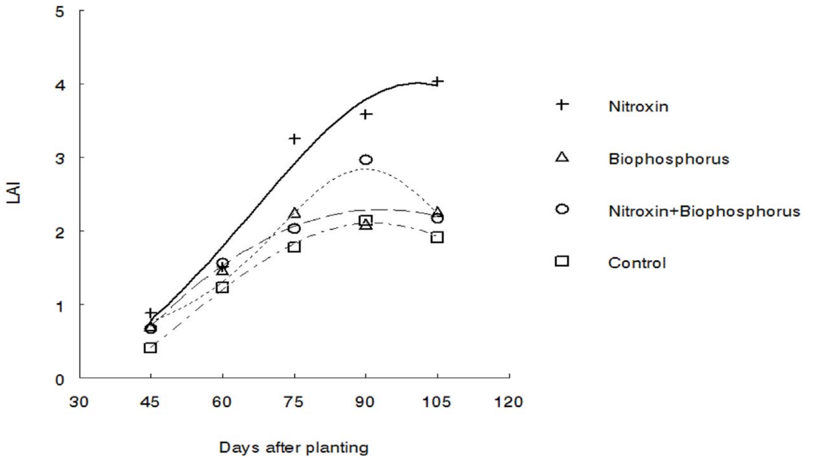

Fig. 2. Changes in leaf area index of basil as affected by biofertilizers during the growing season (values were averaged of two years).

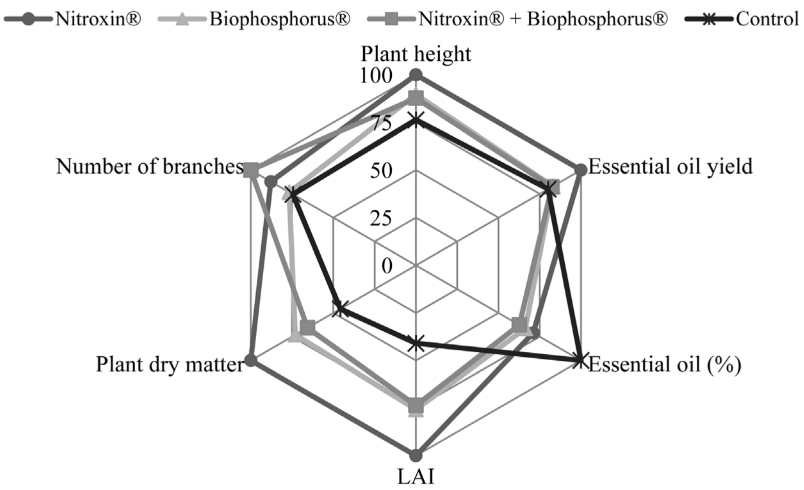

Fig. 3. The relative comparison of the effects of biofertilizers on some quantitative and qualitative characteristics of basil (All values were averaged of two years). The comparisons were done assuming Nitroxin® values as 100 percent and other fertilizers values were calculated regarding it.

Table 5
The correlation coefficients between some basil growth characteristics as affected by biofertilizers (All values averaged of 2 years).

	Plant height	Number of branches per plant	Leaf to stem ratio	Dry matter yield	LAI	Shoot fresh yield	Shoot dry yield	Leaf dry yield	Essential oil%
Number of the branches	−0.41**								
Leaf to stem ratio	−0.53**	0.15 *ns*							
Dry weight	0.54**	−0.53**	−0.20 *ns*						
LAI	−0.13 *ns*	0.00 *ns*	0.29*	0.32**					
Shoot fresh yield	0.76**	−0.54**	−0.44**	0.40**	−0.25*				
Shoot dry yield	0.74**	−0.45**	−0.51**	0.32**	−0.29*	0.86**			
Leaf dry yield	0.60**	−0.55**	−0.31**	0.39**	−0.18 ns	0.86**	0.80**		
Essential oil%	−0.02 *ns*	0.16 *ns*	−0.09 *ns*	−0.09 *ns*	−0.25*	−0.12 *ns*	0.07 *ns*	−0.18 *ns*	
Essential oilyield	0.53**	−0.21 *ns*	−0.35**	0.33**	−0.21 *ns*	0.54**	0.67**	0.54**	0.62**

*, ** and *ns*, shows a significant difference at 5% and 1% probability level, not significant, respectively.

Table 6
Mean comparisons of the yield and the yield components of basil as affected by biofertilizers.

	seed yield per plant (g)	1000 seed weight (g)	dry matter yield (kg ha^{-1})	seed yield (kg ha^{-1})	harvest index (%)
Nitroxin®	5.01*c*	2.13*a*	22575*a*	1537.4*b*	8.93*d*
Biophosphorus®	8.92*a*	1.80*b*	23799.5*a*	4118.9*a*	16.47*a*
Nitroxin® + Biophosphorus®	6.43*bc*	2.07*ab*	20200*b*	1971.1*b*	11.71*c*
Control	6.96*b*	1.90*ab*	23330*a*	3555*a*	14.06*b*

In each column, means followed by the same letter(s) are not significantly different ($p \leq 0.05$).

Table 7
Mean comparisons of the reproductive traits of basil as affected by biofertilizers between 2 years of experiment.

	seed yield per plant (g)	1000 seeds weight (g)	dry matter yield (kg ha^{-1})	seed yield (kg ha^{-1})	harvest index (%)
The first year	6.39*a*	1.94*a*	11255*b*	1575.3*b*	13.95*a*
The second year	7.27*a*	2.01*a*	31646*a*	3639*a*	11.42*b*

In each column, means followed by the same letter(s) are not significantly different ($p \leq 0.05$).

significant difference between inorganic and chemical fertilizers concerned to seed oil percent (Jahan et al., 2012b). Sifola and Barbieri (2006) reported that different levels of nitrogen application increased basil essential oil yield. They concluded that it was due to the effect of nitrogen on the dry matter accumulation and the essential oil percentage.

3.1.8. A relative comparison of the effects of biofertilizers on some quantitative and qualitative characteristics of basil

Fig. 3 shows the effect of biofertilizers inoculation on some growth characteristics of basil. The most plant dry matter, plant height and essential oil yield were observed in the plants inoculated with Nitroxin®. The Plants inoculated with the mixture of Biophosphorus® and Nitroxin® had maximum number of branches and the control plants had the most essential oil percentage. Overall, Nitroxin® superiority over other treatments can be related to positive effects of rhizobacteria (*Azotobacter* sp. and *Azospirillum* sp.). These bacteria; in addition to providing the appropriate ratio of nitrogen, enhance growth and development of the plants, through the synthesis and the secretion of growth stimulators (Tilak et al., 2005). Nitrogen and phosphorus play an important role in the essential oil structure and composition, like Isopentenyl-pyrophosphate (IPP) and dimethyl-allyl pyrophosphate (DMAPP) (Sangwan et al., 2001).

3.1.9. Correlation coefficients between some basil traits

As it is shown in Table 5, the leaf yield and the dry shoot yield are negatively correlated with the leaf to stem ratio ($r = -0.31^{**}$ and $r = -0.51^{**}$ respectively). There is a negative correlation between the number of branches and plant height ($r = -0.41^{**}$). The number of branches had a strong negative correlation with the plant dry matter

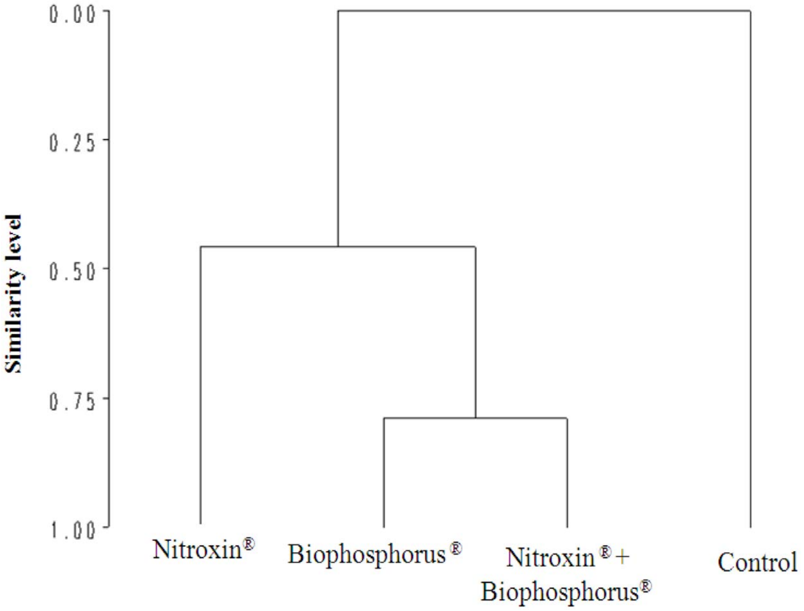

Fig. 4. The cluster analysis of the treatments based on the impact of biofertilizers on vegetative and reproductive characteristics of basil averaged of two years of experiment.

and the leaf yield ($r = -0.53^{**}$ and $r = -0.55^{**}$ respectively).

3.2. Reproductive growth

3.2.1. Yield components

The mean comparisons showed that the highest seed yield per plant (8.92 g) resulted from the plants inoculated with Biophosphorus®, but the lowest; contrary to the expectation, obtained from the plants which were inoculated with Nitroxin®. The highest and the lowest 1000 seeds weight (2.13 g and 1.8 g respectively), were observed in the plants inoculated with Nitroxin® and Biophosphorus®, respectively ($p \leq 0.05$) (Table 6). There was no significant difference considering seed yield per plant and 1000 seeds weight between two years of experiment (Table 7).

The highest seed yield (4118.9 kg ha^{-1} and 3555 kg ha^{-1}) was resulted from the plants inoculated with Biophosphorus® and the control plants, and the lowest seed yield was obtained from the plants under Nitroxin® and the mixture of Nitroxin® and Biophosphorus® treatments (Table 6). Dry matter and seed yield of basil in the second year were higher than the ones in the first year (Table 7).

3.2.2. Dry matter yield & harvest index

The results showed a significant reduction in dry matter yield of the plants inoculated with the mixture of Nitroxin® and Biophosphorus®. Other treatments were not differed in this aspect (Table 6). Although all biofertilizers treatments had no significant effects on harvest index, the highest and the lowest harvest index were observed respectively in Biophosphorus® (16.47%) and Nitroxin® (8.93%) treatments (Table 6). Basil harvest index in the first year was about 22 percent higher than the one in the second year (Table 7). This could be assigned to more development and activities of soil microorganisms in the second year.

3.2.3. Cluster analysis of treatments based on impact of biofertilizers on vegetative and reproductive traits of basil

As it is shown in Fig. 4, the results of cluster analysis on two years of experiment data at 75% similarity level indicates that the treatments were placed into three groups, so that Nitroxin® was placed in the first cluster, Biophosphorus® and the mixture of Nitroxin® and Biophosphorus® were placed in the second cluster and the control was placed in a separate cluster. Another point to be noted is similarities and difference between the three biofertilizers with the control treatment; nearly at 50% similarity level (Fig. 4).

4. Conclusions

The results indicated that the biological fertilizers particularly the Nitroxin® had a positive effect on the yield and the growth characteristics of basil. Plant heights, number of branches, dry matter and shoot fresh yield in plants inoculated by Nitroxin®, were the highest. The most amount of seed yield were resulted from plants inoculated with Biophosphorus®. Concerning the essential oil, biofertilizers were not significantly different. Considering the most traits associated with the yield, the second cut was better than the first and the third, which can be due to the depletion of the nutrients which were released during the growth season. Hence, it is recommended to check the more effective use of biofertilizers, and those should be used more than once during the growing season. Avoiding the use of chemical inputs in the production of the medicinal plants and their products is of the great importance in food and drugs industries. In general, it seems that the use of biofertilizers, due to the reduction of the chemicals use and the environmental degradation, is a healthy and sustainable production method for the medicinal and herbal plants.

Acknowledgements

Financial support of Ferdowsi University of Mashhad and Center of Excellence for Special Crops (CESC) facilities for conducting these experiments is acknowledged. Authors would like to thank Prof. M. Nassiri Mahallati for valuable advices and Dr. S. Bajouri for editing corrections.

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