



Effects of mycorrhiza (*Glomus intraradices*), azotobacter (*Azotobacter chroococcum*) and vermicompost on yield and essential oil of basil (*Ocimum Basilicum* L.) in organic agriculture

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Abstract

Basil is an edible, medicinal and spice plants that contains rich source of essential oil. Basil fresh leaves are consumed as vegetable or use as flavour in foods and drinks. In present study, Biological fertilizers were used to increase yield of basil. Indeed, using biological fertilizers as alternative to chemical fertilizers in organic agriculture is an effective way to protect environment and human society. This study was carried out in an organic farm at research field of Islamic Azad University, Karaj branch in 2011-2012 growing season. Experiment was conducted in a Factorial arrangement based on randomized complete block design with four replications. Three factors include: mycorrhizae (*Glomus intraradices*), nitrogen-fixing bacteria (*Azotobacter chroococcum*) and vermicompost were randomized. Each factors had two levels useage and not use. Analysis variance results indicated that three-way interaction of mycorrhiza, azotobacter and vermicompost led to increase plant traits such as plant height ($p < 0.05$), shoot fresh weight ($p < 0.01$) and dry weight ($p < 0.05$). The treatments also increased essential oil significantly. The highest plant height, fresh and dry weight was associated with that three-way interaction of traetments. Thus, combined use of mycorrhiza, azotobacter and vermicompost have synergistic effect on basil yield and its essential oil..

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Introduction

Basil (*Osmium basilicum* L.) is one of annual herbaceous plant that belongs to family Lamiaceae (Labiatae). Basil is widely cultivated in Mediterranean climate with ecological demands such as air temperature between 7 to 27 °C, soil pH 4.3-8.2 and annual evaporation 0.6 to 4.2 m. Different environmental conditions, managements, planting and harvesting dates have caused to different growth and yield (Akbari nia *et al.* 2008). Fresh basil is used in foods as vegetables and salads. However, like other species of Lamiaceae, basil is known for its essential oil. Containing anti-fungal and anti-bacterial compounds caused that basil used in pest control and food industries. Khalid *et al.* (2006) reported that oil essential amount and compounds of oil essential can change from 0.5 to 1 percent in order to environmental conditions.

Applying biological fertilizers in organic agriculture is one of the efficient way to provide mineral nutrients. Biological fertilizers contain large variety of microorganisms which can exude plant growth hormones, have positive effects on plant growth. These characteristics have caused better development of root that followed by better minerals absorption and finally more crop yield (Daei *et al.* 2009).

By a research review, Omidbaigi (1997) declared that basil can produce 1.2 tone/ha dry matter. Root inoculation by mycorrhiza led to more plant height and yield in mint (Gupta *et al.* 2002). Kapoor *et al.* (2002) showed that shoot of Ajowan and Dill have significantly increased in order to applying mycorrhizal inoculation. In another study Kapoor *et al.* (2001) stated that mycorrhiza can increase Coriander yield. Applying vermicompost resulted in a remarkable increase height and yield of garlic (Arguello *et al.* 2006). Biological fertilizers caused plant nutrient uptake and growth improvement (Balakumbahan and Rajamani, 2010). Black cumin seed inoculation by biological fertilizers caused significant height increase and more crop growth rate (Khorramdel *et al.* 2008). In this context, Shalan

(2005) showed that biological fertilizers increased Black cumin growth characteristics.

It is not allowed to apply chemical fertilizers to provide plant nutrients in organic agriculture. Biological and organic fertilizers are the most important source to provide plant nutrients. In this regard, it is a necessity to study the effects of biological and organic fertilizers on crop growth. On the other hand a few researches have been done to investigate the effects of organic fertilizers on basil. Therefore the aim of present study was to investigate effects of vermicompost, azotobacter and mycorrhiza on Basil growth traits and its essential oil in organic agriculture condition.

Materials and method

Field experiment

The present study took place in an organic farm at research field of Islamic Azad University, Karaj branch, Iran. A one year experiment was conducted in a Factorial arrangement based on randomized complete block design with four replications in 2011-2012 growing season. Three factors include: mycorrhizae (*Glomus intraradices*), nitrogen-fixing bacteria (*Azotobacter chroococcum*) and vermicompost were randomized. Each factor had two levels useage and not use. Azotobacter were inoculated with seeds before planting. Mycorrhiza and vermicompost were added into soil just before planting. After adjustment of temperature for Basil germination and growth, on May 20th seeds were planted in 50 cm space rows and space between plants was 3 cm. Each plot was 9 m² with 6 rows. In order to adjust plant space in row (3 cm), plants were thinned in 4 leaves stage. Purple nutsedge, Purslane, Common lambsquarters and pigweed were dominant weeds in plot. Weeds were controlled by hand weeding using a hoe and/or a rototiller whenever necessary. It should be notice that no chemical herbicide was used during the experiment. The first irrigation was carried out immediately after planting and subsequent irrigations were carried out to the end of growing season once a week. Plots were irrigated to 70% of field capacity. Samples consisted

of 4 rows of the center rows of each plot after leaving two rows in the border areas to avoid border effects. Ten plants were selected randomly and their traits such as Plant height, shoot fresh weight, shoot dry weight were measured. In order to measure dry weight, samples were dried for 48 hours in 70 °C oven.

Extraction of essential oil

The aerial parts were transferred to the lab to determine the essential oil content. Using Clevenger-type apparatus, the essential oil of samples was extracted by hydro-distillation of 100 gr dried weight Basil. This method for the extraction of oils is recommended by the European Pharmacopoeia (European Pharmacopoeia, 1983).

Statistical analysis

Analysis of variance (ANOVA) and mean separations were performed using the general linear model (GLM) procedure of SAS Institute Inc. (2002). The assumptions of variance analysis were tested by

ensuring that the residuals were random, homogenous, and with a normal distribution about a mean of zero. The least significant difference procedure (LSD) at a probability level of 0.05 was used to determine statistically significant differences among treatment means.

Results and discussion

Analysis variance results (Table 1) showed that main effects of azotobacter and vermicompost and three-way interactions of mycorrhiza, azotobacter and vermicompost had significant effect on plant height ($p < 0.05$). Respect to the means comparison (Table 6), three-way interactions of mycorrhiza, azotobacter and vermicompost result in maximum height (81.563 cm) and minimum height was shown in control (68.358 cm). There was a significant difference between maximum and minimum height. Providing plant nutrition is one of the main factors determining plant height. Biological fertilizers provided nutrition in root zone and hereby caused to increase height.

Table 1. Analysis of variance of measured traits in Basil.

Source variations	of DF	Mean of squares			
		Plant height	Shoot fresh weight	Shoot dry weight	Essential oil
Repeat	3	19.4783917	124808.833	4102.2813	0.03886146 ^{ns}
Mycorrhiza (M)	1	5.6616125 ^{ns}	596778.125 ^{ns}	289370.2813 ^{**}	0.16387813 ^{**}
Azotobacter (B)	1	117.4278125 [*]	1458632 ^{**}	243427.5313 ^{**}	0.30615313 ^{**}
Vermicompost (V)	1	155.2322 [*]	5040312.5 ^{**}	178652.5313 ^{**}	0.80327813 ^{**}
M*B	1	28.05005 ^{ns}	2476425.125 ^{**}	10046.5313 ^{ns}	0.00262812 ^{ns}
M*V	1	6.7896125 ^{ns}	137550.125 ^{ns}	12760.0313 ^{ns}	0.11640312 [*]
B*V	1	4.7895125 ^{ns}	12640.5 ^{ns}	1696.5313 ^{ns}	0.05362813 ^{ns}
M*B*V	1	136.29005 [*]	2550411.125 ^{**}	39691.5313 [*]	0.08715313 [*]
Error (E)	21	22.725625	162689.02	9046.7336	0.01913527
C.V		6.367117	17.5607	12.70998	18.14911

Table 2. Mean comparison of main effects on some basil traits.

Treatment	Plant height (cm)	Shoot fresh weight (kg/ha)	Shoot dry weight (kg/ha)	Essential oil (gr/100 gr dry matter)
Mycorrhiza				
Use	75.292 a	2433.4 a	843.44 a	0.83375 a
Not use	74.451 a	2160.3 a	653.25 b	0.69063 b
Azotobacter				
Use	76.787 a	2510.4 a	835.56 a	0.86000 a
Not use	72.956 b	2083.4 b	661.13 b	0.66438 b
Vermicompost				
Use	77.074 a	2693.8 a	823.06 a	0.92063 a
Not use	72.669 b	1900.0 b	673.63 b	0.60375 b

The result of analysis variance (Table 1) depicted that main effects of azotobacter and vermicompost and two-way interactions of mycorrhiza and azotobacter had a significant effect on shoot fresh weight ($p < 0.01$). highest shoot fresh weight was gained in three-way interaction ($3650 \text{ kg}\cdot\text{ha}^{-1}$). Control had lowest shoot fresh weight. Shoot dry matter was also affected by treatments (Table 1). Shoot dry matter was affected by main effects ($p < 0.01$) and three-way interaction ($p < 0.05$). the highest dry matter was observed in three-way interaction ($1071 \text{ kg}\cdot\text{ha}^{-1}$). Indeed, all experimental factors had effect on height and shoot fresh weight and in this way resulted in increasing dry matter. Mycorrhiza can increase plant growth and development. According to Podila and Douds (2010) mycorrhizal plant can uptake plant nutrients especially phosphorus, more than control. Plant phosphorus content affects physiological processes such as photosynthesis, and in this way it

leads to improve crop yield. In the other hand, azotobacter through producing biological nitrogen fixation and plant growth promoters such as Vitamin B, nicotinic acid, pantothenic acid, cytokinin and gibberellin acid, eventually caused plant growth and yield (Kader, 2002). Other studies indicated that biological fertilizers increase plant height via growth promoting hormones production. Indeed, azotobacter caused to produce Indole-3-acetic acid (IAA) and affect plant growth. Vermicompost as an enrichment organic manure, provide mineral nutrient. Vermicompost also contains enzymes and growth hormones (Rigi, 2003). Kumar *et al.* (2001) stated that vermicompost was able to increase phosphorus solubility and hyphae of mycorrhiza absorb it. Thus vermicompost associated with mycorrhiza increase plant growth especially in shoot. It was also reported by Balakumbahan and Rajamani (2010), Khorramdel *et al.* (2008) and Shalan (2005).

Table 3. Mean comparison of two-way interaction of Mycorrhiza and Azotobacter on some Basil traits

Treatment		Plant height (cm)	Shoot fresh weight (kg/ha)	Shoot dry weight (kg/ha)	Essential oil (gr/100 gr dry matter)
Mycorrhiza	Azotobacter				
use	use	78.144 a	2925.1 a	948.38 a	0.92250 a
	Not use	72.440 b	1941.8 b	738.50 b	0.74500 b
Not use	use	75.430 ab	2095.6 b	722.75 b	0.79750 ab
	Not use	73.471 ab	2225.0 b	583.75 c	0.58375 c

Table 4. Mean comparison of two-way interaction of Mycorrhiza and Vermicompost on some Basil traits

Treatment		Plant height (cm)	Shoot fresh weight (kg/ha)	Shoot dry weight (kg/ha)	Essential oil (gr/100 gr dry matter)
Mycorrhiza	Vermicompost				
use	use	77.034 a	2895.9 a	938.13 a	1.05250 a
	Not use	73.550 a	1971.0 b	748.75 b	0.61500 c
Not use	use	77.114 a	2491.6 a	708.00 b	0.78875 b
	Not use	71.788 a	1829.0 b	598.50 c	0.59250 c

Analysis variance results (Table 1) demonstrated that main effects of azotobacter, mycorrhiza and vermicompost at P level of 0.01, two-way interaction of mycorrhiza and vermicompost at P level of 0.05 and three-way interaction ($p < 0.05$) had significant effect on Basil essential oil. Respect to mean comparison (Table 6), three-way interaction was associated with highest essential oil ($1.1525 \text{ gr}/100 \text{ gr dry matter}$). Lowest amount of essential oil was observed in

control. In this regard, Ajimoddin *et al.* (2005), Vinutha (2005) and Banchio *et al.* (2009) reported that biological fertilizers increased Basil essential oil. Because nitrogen and phosphorus is essential for regeneration of ATP and NADPH that are needed to biosynthesize terpenoid compounds (manufacturers of essential oil), therefore, biological fertilizers can increase plant essential oil (Loomis and Corteau, 1972).

Table 5. Mean comparison of two-way interaction of Azotobacter and Vermicompost on some Basil traits

Treatment		Plant height	Shoot fresh weight	Shoot dry weight	Essential oil
Azotobacter	Vermicompost	(cm)	(kg/ha)	(kg/ha)	(gr/100 gr dry matter)
use	use	78.603 a	2887.4 a	903.00 a	0.97750 a
	Not use	74.971 ab	2133.4 b	768.13 b	0.74250 b
Not use	use	75.545 ab	2500.1 ab	743.13 b	0.86375 ab
	Not use	70.366 c	1666.6 c	579.13 c	0.46500 c

Table 6. Mean comparison of three-way interaction of treatments on some Basil traits.

Treatment			Plant height	Shoot fresh weight	Shoot dry weight	Essential oil
Mycorrhiza	Azotobacter	Vermicompost	(cm)	(kg/ha)	(kg/ha)	(gr/100 gr dry matter)
Use	Use	Use	81.563 a	3650.0 a	1071.00 a	1.15250 a
		Not use	74.725 abc	2200.3 c	825.75 b	0.69250 cd
	Not use	Use	72.505 bc	2141.8 c	805.25 b	0.95250 ab
		Not use	72.375 bc	1741.8 c	671.75 b	0.53750 de
Not use	Use	Use	75.643 abc	2124.8 c	735.00 b	0.80250 bc
		Not use	75.218 abc	2066.5 c	710.50 b	0.79250 bc
	Not use	Use	78.585 ab	2858.5 b	681.00 b	0.77500 bc
		Not use	68.358 c	1591.5 c	486.50 c	0.39250 e

Conclusion

In summary, our results demonstrated that the combined use of mycorrhiza, azotobacter and vermicompost significantly improved shoot fresh weight, shoot dry weight and essential oil in compared with control. In fact, there is an increasing relationship between mycorrhiza, azotobacter and vermicompost that shows they are Supplementary for each other. Therefore combined use of them leads to increase plant height, dry matter and essential oil. In addition to effect on Basil growth and its essential oil, biological fertilizers can play effective role to approach to organic agriculture.

References

Ajimoddin I, Vasundhara M, Radhakrishna D, Biradar SL, Rao GGE. 2005. Integrated nutrient management studies in sweet basil (*Ocimum basilicum* L.). *Indian Perfume* **49**, 95-101.

Akbarinia A, Jalilee Vand H, Kazemi F. 2008. *Cultivation of Medicinal Plants* **2**, Qazvin, Press Sayehgostar.

Arguello JA, Ledesma A, Nunez SB, Rodriguez CH, Goldfarb MD. 2006. Vermicompost effects on bulbing dynamics, nonstructural carbohydrate content, yield, and quality of Rosado paraguayo garlic bulbs. *Horticulture Science* **41(3)**, 589-592.

Balakumbahan R, rajamani K. 2010. Effect of biostimulant on growth and yield of senna (*cassia angustifolia* var KKM.1) *journal of horticultural science & ornamental plants*, IDOSI publication **2(1)**, 16-18.

Banchio E, Xie X, Zhang H, Pare PW. 2009. Soil bacteria elevate essential oil accumulation and emissions in sweet basil. *Journal of Agricultural and Food Chemistry* **57**, 653-657.

<http://dx.doi.org/10.1021/jf8020305>.

Daei G, Ardakani MR, Rejali F, Teimuri M, Miransari S. 2009. Alleviation of salinity stress on wheat yield, yield components, and nutrient uptake using arbuscular mycorrhizal fungi under field conditions. *Journal of plant physiology* **166**, 612-625.

<http://dx.doi.org/10.1016/j.jplph.2008.09.013>

- Gupta ML, Prasad A, Ram M, kumar S.** 2002. Effect of the vesicular-arbuscular mycorrhiza(VAM)fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol mint (*menthe arvensis*)under field conditions. Bio resource Technology **81**, 77-79.
- Kader MA.** 2002. Effects of *Azotobacter* inoculant on the yield and nitrogen uptake by wheat .Journal of biological sciences **2**, 259-261.
<http://dx.doi.org/10.3923/jbs.2002.259.261>.
- Kapoor R, Giri B, Mukerji KG.** 2001. Mycorrhization of coriander (*Coriandrum sativum* L.) to enhance the concentration and quality of essential oil. Journal of the Science of Food and Agriculture **82(4)**, 339-342.
<http://dx.doi.org/10.1002/jsfa.1039>
- Kapoor R, Giri B, Mukerji KG.** 2002. *Glomus macrocarpum*: a potential bioinoculant to improve essential oil quality and concentration in Dill (*Anethum graveolens* L.) and carum (*Trachyspermum ammi Sprague*). World Journal of Microbiology and Biotechnology **18(5)**, 459-463.
<http://dx.doi.org/10.1023/A:1015522100497>.
- Khalid A, Hendawy SF, El-Gezawy E.** 2006. *Ocimum basilicum* L. Production under Organic Farming. Research Journal of Agriculture and Biological Sciences **2(1)**, 25-32.
- Khorramdel S, Koocheki A, Nassiri Mahallati M, Ghorbani R.** 2009. Application effects of biofertilizers on the growth indices of black cumin (*Nigella sativa*L.). Journal of Agricultural Research of Iran **6(2)**, 285-294.
- Kumar V, Singh KP.** 2001. Enriching vermicompost by nitrogen fixing and phosphate solubilizing bacteria. Bio resource Technology **76**, 173-175.
[http://dx.doi.org/10.1016/S0960-8524\(00\)00061-4](http://dx.doi.org/10.1016/S0960-8524(00)00061-4)
- Loomis WD, Croteau R.** 1972. Essential oil biosynthesis. Recently Advance Phytochem **6**, 147-185.
- Omidbaigi R.** 1997. Approaches to Production and Processing of Medicinal Plants.Vol.2. Tarrahan e Nashr Publication. Tehran.
- Ozcan M, Derya AM, Unver A.** 2005. Effect of drying methods on the mineral content of Basil (*Ocimum basilicum*) . Journal Food Engine **69**, 375-379.
<http://dx.doi.org/10.1016/j.jfoodeng.2004.08.030>
- Podila GK, Douds DD.** 2001. Current advances in Mycorrhizae Research. APS Press, St. Paul. 2001, p. 127 - 40.
- Rigi MR.** 2003. Study of greenhouse effect three types of vermicompost and nitrogen on yield and chemical composition of corn and rice. M.Sc. Thesis. University of Shiraz, 5-7 P.
- SAS Institute Inc.** 2002. The SAS System for Windows, Release 9.0. Statistical Analysis Systems Institute, Cary, NC, USA.
- Shalan MN.** 2005. Influence of biofertilizers and chicken manure on growth,yield and seeds quality of (*nigella sativa* L.) Plants. Egyptian journal of Agriculture Research **83**, 811-828.
- Vinutha T.** 2005. Biochemical studies on *Ocimum* sp. inoculated with microbial inoculants. M.Sc thesis, University of Agricultural Sciences, Bangalore, India.