EFFECT OF BIOLOGICAL, ORGANIC AND CHEMICAL FERTILIZERS ON SOME ANTIOXIDANT ACTIVITIES AND YIELD OF BASIL (*Ocimum basilicum* L.)

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Abstract

To investigate the effect of combined application of organic and bio-chemical fertilizers on the quantitative and qualitative characteristics of basil (*Ocimum basilicum* L.), an experiment was conducted in a randomized complete block design with six treatments and three replications at Urmia University Research Field. Treatments including: control (no fertilizer), Azotobacter biofertilizer, Azotobacter + livestock manure, Azotobacter + vermicompost, Azotobacter + phosphorus biofertilizer, Azotobacter + chemical fertilizers were considered. The studied traits included essential oil content, phenolic and flavonoid content, DPPH radical scavenging, superoxide radical scavenging, and nitric acid radical scavenging activity. The results showed that the application of organic and biofertilizers had the greatest effect on increasing the studied traits including essential oil content, total phenol content. The results of this study suggest that the use of organic and bio-fertilizers and the reduction of the use of chemical fertilizers are recommended because of the remarkable results in the experiment.

Keywords: Basil, essential oil, vermicompost, azotobacter, phenolic content

1. INTRODUCTION

Throughout human history, natural products derived from plants have been widely used for nutrition, health, or disease control (Pateraki et al., 2015). The importance, position and role of medicinal and industrial plants in increasing sustainable management, especially in the macro-economic, environmental, health, employment, food security and genetic resources of the national and global arena, to a great extent. The power of rehabilitation today and their role, especially in the provision of medicine, is considered as one of the indicators of development in the country (Pouryousef, 2015). With the advent of science and the world's attention to the detrimental effect of the use of chemicals and synthetic materials, the world has again resorted to the use of herbal products, which is said to be the 21st century, the century of medicinal plants (Amanzadeh et al., 2011). Cultivation of medicinal plants in agronomic ecosystems plays an important role in creating diversity and stability in these systems. Maintaining the quality and sustainability of production relative to the quantity of the product in the field of medicinal plants further reveals the importance of sustainable management systems for agricultural systems (Koocheki et al., 2012).

Basil (*Ocimum basilicum* L.) is one of the most important plants of the Lamiaceae (Makari and Kintzios, 2008). Different species of basil have been used for medicinal, spice, and vegetable purposes for many years (Zheljazkov et al., 2008). Medicinal properties and the presence of aromatic compounds make basil one of the most famous herbs in the world. Basil essential oil and its extracts are widely used in food, pharmacy and perfume industries (Simon et al., 1999).

It seems that the presence of micro-organisms due to the use of biofertilizers in the root environment and the production and secretion of plant growth stimulating compounds by these organisms have a positive effect on plant growth and lead to increased percent essential oils and essential oil yields (Bastami and Majidian, 2016). Many studies have already been done to compensate for nitrogen deficiency by methods other than the application of chemical fertilizers, such as inoculating seeds with microorganisms such as Azotobacter (Bahamin et al., 2019). The results of the study on fennel (*Foeniculum vulgare* L.) and anise (*Pimpinella anisum* L.) also showed that the combined use of organic and biofertilizers compared to the control, increased essential oil yield (Behzadi and Salehi, 2017). A study on *Zingiber officinale* Rosc. showed that under conditions of improved soil properties and as a result of increased photosynthesis, flavonoid and phenol content in this plant increased, which led to increased antioxidant activity of the plant. (Ghasemzadeh and Jaafar, 2011). An experiment on sour tea (*Hibiscus sabdariffa* L.) found that inoculation with biofertilizers increased plant diameter and the highest diameter was related to nitroxin inoculated seeds treatment, which was about 2% higher than control (Nemati and Dahmardeh, 2015). In the study of biofertilizers and biosulfur on morphological and phytochemical traits of the puppet pup (*Physalis alkekengi*) reported that the highest number of sub-branches was obtained by the combination of four liters of nitroxin plus four kg of biosulfur. A study on *Rosmarinus officinalis* also reported that combined vermicompost and nitroxin increased plant dry weight and essential oil percentage (Noorbakhsh et al., 2016).

The purpose of this research was to study the single and combined effects of organic and bio-fertilizers on the essential oil of basil and to determine the best treatment for achieving the highest antioxidant activities.

2. MATERIALS AND METHODS

An experiment was conducted at the experimental open field of Urmia University, Iran on 2017. The mean annual rainfall and temperature of studied area were 63.2 mm and 5.4˚C respectively. This study was conducted on a randomized complete block design with six treatments and three replications. Treatments included control (No fertilizer), Azotobacter biofertilizer, Azotobacter + livestock manure, Azotobacter + vermicompost, Azotobacter + phosphorus biofertilizer, Azotobacter + chemical fertilizers. Azotobacter species of the bacterial strain, which actively stabilizes the air nitrogen available to plants. Phosphorus (P) biofertilizer, phosphate fertilizer-2 biological fertilizers were used, which contains two types of phosphate solubilizing bacteria Pantua (P5 strain) and *Pseudomonas putida,* each package of this biofertilizer can be substituted make up 2 to 5 percent of the phosphate fertilizer needed by the plant. Chemical fertilizer was used as NPK (12-12-36 + 2 MgO). Prior to the experiment to determine the physical and chemical properties of the soil, samples were taken from 0 to 30 cm depth and analyzed in the soil laboratory (Table 1). The method of application of bio-fertilizers was sprayed to the roots of transplants during planting. Foliage was transplanted on the roots of the transplants, then planting and irrigation were performed. Subsequent irrigation was carried out once every 6 days if needed. Weed control was done by hand weeding. Sampling was done by removing the marginal rows and half of the plots from the beginning. No specific disease or pest was observed.

Table 1. Main physiochemical properties of soil sample

pH	EC (dS m^{-1})	OC (%)	Clay (%)	Silt (%)	Sand (%)	Olsen-P (mg kg^{-1})	K (mg/kg^{-1})
7.2	0.54	0.94	32	27	21	7.6	395

The shoots were sampled at 50% flowering stage and then were stored in shade at room temperature in dry paper bags (Safaei-Ghomi et al., 2009). The plants were harvested on September. Essential oil extraction was performed using Clevenger apparatus (distilled water). Then, 10 g of dried leaves were poured into a 1000 ml balloon, and about 100 ml of distilled water was added and extraction was performed. The extraction time was about 3 hours. During this time, the volatile compounds were extracted with water vapor and after cooling, a distinct layer on the surface of the water was visible in the graduated tube of the Clevenger apparatus.

To extract the extract, 2 g of basil was extracted in Chinese mortar and then extracted with 25 ml of methanol at room temperature on a magnetic stirrer for 3 h. 1 ml of Folin-Ciocalteu reagent was added to 50 µl of the plant extract. The resulting solution was then mixed with 1 ml of sodium carbonate (10%) and incubated for 60 minutes at room temperature and in the dark. Finally, the absorbance of the solution was measured using a spectrophotometer at 750 nm (Oki et al., 2002). Total phenol content was expressed as mg / kg of gallic acid in 100 g of extract using standard curve of gallic acid.

To determine the flavonoid content, 50 ml of the extract was mixed with 1 ml of distilled water, then 0.075 ml of sodium nitrite (5%) was added and after 5 minutes 0.15 ml of $AlCl_3$ solution (10%) was then added to 0.5 ml NaOH (1 M) and the absorbance intensity was measured by the appearance of pink at 510 nm by spectrophotometer (Jia et al., 1999). Total flavonoid content was expressed as mg of quercetin in 100 grams of extract using standard quercetin curve.

The degree of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging was determined by the modified Burits and Bucar method (Burits and Bucar, 2000). Approximately 40 µl of the extract was mixed with 2 ml of DPPH methanol solution (0.004%) and the absorbance was read at 305 minutes incubation at 517 nm. The DPPH radical was calculated using the percent ratio:

DPPH (%) = $(1-A_{sample} / A_{blank}) \times 100\%$ MoH radical DPPH

A_{sample} is the absorbance of the extract at t = 60 min and blank A is the control absorbance at t = 0 min.

To measure superoxide radical inhibition, superoxide anion radicals were generated by a pyrogallol autoxidation system (Ling and Zhao, 1995). The test tube was incubated in Ben-Marie (25 ° C) for 9 minutes in Tris buffered saline (pH = 8.2, 50 mmol L^{-1}). Then 40 µl of incubated pyrogallol solution was injected into the top of the test tube and mixed. The mixed sample was incubated for 25 minutes at 25 ° C and then immediately dripped into one drop of ascorbic acid (0.035%) to complete the reaction. The adsorption of the mixture at 420 nm was recorded as A_o (pyrogallol autoxidation rate) after 5 min. The A1 auto oxidation rate was increased by the same method. Only a certain amount of extract (10 µL) was added to Tris buffer. The percentage of radical accumulation was calculated using:

Radical scavenging activity = $((A_0-A_1) / A_0) * 100\%$ superoxide radical scavenging

Nitric oxide radical inhibition was calculated using the Illosvoy Griess reaction. Approximately 3 ml of the reaction solution was incubated with 2 ml of sodium nitroprusside (10 mM), 0.5 ml of saline phosphate buffer, and 40 ml of the plant extract for 25 minutes at 25 ° C. Then, 0.5 ml of the resulting solution was mixed with 1 ml of sulfanilic acid (0.33% in 10% glacial acetic acid) and allowed to stand for 5 min to complete diazotization. Then 1 ml of naphthylethylenediamine dihydrochloride was added to the mixture and allowed to stand for 30 minutes at 25 °C. The absorbance of the solution (light pink) was read at 540 nm with the control. The percentage of nitric oxide radicals was calculated using the following formula:

Nitric oxide radical accumulation percentage = $(A_0 – A1) / A_0 * 100$

Analysis of variance was performed by ANOVA, averages were performed with SAS software version 9.1 at the probability levels of $p<0.05$ and $p<0.01$.

3. RESULTS AND DISCUSSION

3.1. Antioxidant activity

3.1.1. Total phenol, Total flavonoid, DPPH radical scavenging activity

The analysis of variance and mean values of studied properties of basil were shown in Table 2. According to the results, significant differences were observed in various phytochemical properties as affected by fertilizer treatments. Fertilizer phosphorus biofertilizer contains two types of phosphorus solubilizing bacteria from *Bacillus lentus* and *Pseudomonas putida* species that decompose insoluble and thus absorbable phosphorus compounds using two mechanisms of secretion of organic acids and acid phosphorus, respectively (Malbubi, 2007). The results of comparison of mean data (Table 2) show that the highest total phenolic content of basil in fertilizer treatments was obtained in Azotobacter + phosphorus biofertilizer (43.08 mg GAE g^{-1} DW) , as well as Azotobacter + vermicompost (41.73 mg GAE g^{-1} DW) and the lowest total phenolic content was observed in control (23.86 mg GAE g^{-1} DW) treatment.

Table 2. Mean values of studied properties according to fertilizer treatments

Treatment	Total phenol (mg GAE g^{-1} DW)	Total flavonoid (mg QE g^{-1} DW)	DPPH radical (%)	Super oxide radical (%)	Nitric oxide radical (%)	Essential oil (%)
Control	23.86 c	0.59	71.33 c	23.71 c	16.24 d	2.80 c
Azotobacter biofertilizer	31.52 b	0.66	72.96 bc	26.77 bc	20.48 c	2.83 bc
Azotobacter+ livestock manure	38.78 b	0.68	73.98 ab	26.02 bc	21.77 bc	3.03 a
Azotobacter+ v.compost	41.73 a	0.72	74.70 ab	27.87 b	22.40 b	3.04 a
Azotobacter+phosphorus biofertilizer	43.08 a	0.71	75.28 a	33.57 a	27.20 a	3.06 a
Azotobacter+chemical fertilizer	38.14 b	0.60	73.58 ab	29.13 b	22.61 b	2.96 b
Fertilizer treatment	**	ns	*	**	**	**
CV (%)	3.78	10.73	1.20	6.27	3.34	2.47

*There were no significant differences between the mean values shown with the same letters at a 5% probability level, CV: Coefficient of variation, ns: Not significant, *: $p<0.05$, **: $p<0.01$*

In terms of total flavonoid content, there was no statistically difference between fertilizer treatments. Total flavonoid content varied between 0.59-0.72 mg QE g^{-1} DW. The scavenging activity of DPPH radical of basil, statistical significant difference was found between fertilizer treatment. Although the highest DPPH activity was obtained from the treatment of Azotobacter + phosphorus (75.28 %), there was no statistically difference between the other fertilizer treatments in which Azotobacter was included. Researchers have stated that high concentrations of phenol in florets are related to the role of organic fertilizers in the production of substances that induce the chemical pathway of acetic acid and thus the production of more flavonoids and phenolic substances (Naguib et al., 2012). Research on ginger showed that under conditions of improved soil properties and as a result of increased photosynthesis, flavonoid and phenol content in this plant increased, which led to increased antioxidant activity of the plant (Ghasemzadeh and Jaafar, 2011). The use of organic fertilizers due to the high amounts of humic compounds results in the synthesis of phenolic compounds such as flavonoids and anthocyanins in plants (Theunissen et al., 2010).

The photochemical compounds in plants are considered to be antioxidants that have similar antioxidant capacity to synthetic antioxidants without side effects (Salmanian et al., 2013). Antioxidants exist in both natural and synthetic forms. In recent years, the use of synthetic antioxidants such as TBHQ, BHT and BHA like other chemical additives has been limited due to their potential toxicity and carcinogenicity. Nowadays most of the research is done on using new antioxidants without risk from plant sources, animal, microbial and food are concentrated (Mohammadi et al., 2014). Research on ginger showed that under conditions of improved soil properties and as a result of increased photosynthesis, flavonoid and phenol content in this plant increased, which led to increased antioxidant activity of the plant (Ghasemzadeh and Jaafar, 2011). The results of asparagus (*Asparagus racemosus* Willd.) and medicinal plant (*Andrographis paniculata* L.) native to East Asia indicate a significant effect of vermicompost on antioxidant activity of these plants (Saikia and Upadhyaya, 2011). The antioxidant capacity of the plant increases with increasing levels of phenolics and flavonoids during the use of organic fertilizers (Aminifard et al., 2012).

3.1.2. Superoxide and nitric oxide radical inhibition

Highest superoxide radical inhibition (33.57 %) and nitric oxid radical inhibition (27.20 %) were recorded in Azotobacter + phosphorus biofertilizer treatment and the lowest of them were observed in control (23.71 % and 16.24 % respectively).

Types of reactive oxygen include free oxygen radicals such as superoxide (O^{2-}) and hydroxyl radical (OH−) radicals as well as hydrogen peroxide (Lima et al., 2002) by oxidizing photosynthetic pigments, lipids. Membranes, proteins, and nucleic acids cause oxidative damage (Sairam and Srivastava, 2002). Nitric oxide is actually a chemical intermediate that is produced by endothelial cells, macrophages, neurons, etc., and is involved in the regulation of many physiological processes. Nitrite oxide has antioxidant properties and its role in inhibiting carcinogenesis and tumor growth is well known (Lata and Ahuja, 2003). In a study on walnut leaf extract, it was found that they showed high purification ability of nitric oxide radicals in these leaves (Almeida et al., 2008).

3.2. Essential oil content

The effect of organic and biofertilizer treatments on essential oil content was significant (Table 2). The highest essential oil content was Azotobacter + phosphorus biofertilizer (3.06 %), Azotobacter + vermicompost (3.04 %) and Azotobacter + livestock manure (3.03 %). The lowest essential oil was control (2.80 %) treatment. Findings from a study on fennel and anise plants revealed that the combined use of organic fertilizers increased the essential oil yield (Behzadi and Salehi al., 2017). In another experiment on lemon balm, it was observed that the combined application of 2 tons of organic fertilizer (bovine) and nitroxin biofertilizer increased the percentage and yield of essential oil (Razipour et al., 2016). A study on Acacia Mountain also reported that the combination of vermicompost and nitroxin increased plant dry weight and essential oil content (Noorbakhsh et al., 2016). Also in a research on savory, it was found that the use of nitrogen fixative bacteria (Azospirillum) had a positive effect on the percentage of basil in the essential oil (Bashirifar et al., 2016). Various studies have shown the effect of vermicompost on increasing phosphorus uptake; for example, an increase in phosphorus accumulation in anise seed (*Pimpinella anisum* L.) has been reported (Khalesro et al., 2012).

4. CONCLUSION

The results of experiment showed that organic and biofertilizers had superiority to chemical fertilizer and positive response of basil to fertilizer application. Non-use of chemical inputs in the production of medicinal plants and their products is their main condition of being healthy and natural, generally by using appropriate amounts of organic and biological fertilizers in a sustainable agricultural system while producing healthy crops and improved quantitative and qualitative yields and the removal of fertilizers reduce the harmful environmental hazards in agriculture.

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