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Effects of bio-stimulators and bio-fertilizers on morphological traits of basil (*Ocimum bacilicum* L.)

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ABSTRACT

To investigate the effects of bio-stimulators and bio-fertilizers on morphological traits of basil (Ocimum basilicum L.), a factorial experiment was conducted on the basis of randomized complete blocks design in three replicates at Institute of Medicinal Plants, ACECR. Bio-stimulators in four levels (control or no bio-stimulators and commercial formulations of aminolforte, kadostim and fosnutren at concentration of 1.5 L.ha^{-1}) and bio-fertilizers in four levels (control or no bio-fertilizes and commercial formulations of nitroxin, supernitro-plus and barvar II at concentration of 0.5 L.ha^{-1}) were the two studied factors. The results showed that the interaction effect of bio-stimulators and bio-fertilizers was significant (P<0.01) on all of parameters except for chlorophyll content (SPAD value). The maximum plant height (87 cm) in treatment of kadostim and nitroxin, secondary stem number (25.33) in treatment of kadostim and supernitro-plus, leaves number (206.33) and total dry weight (35.52 g) in treatment of aminolforte and nitroxin, stem diameter (7.16 mm) in treatment of fosnutren and nitroxin, and also flower dry weight (18.83 g) in treatment of fosnutren and bio-phosphur were obtained. In general, bio-stimulators and bio-fertilizers significantly increased the growth parameters of basil.

Keywords: *Ocimim basilicum* L., Bio-fertilizers, Bio-stimiumlators, Morphological traits

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INTRODUCTION

Basil (*Ocimum basilicum* L.) from Lamiaceae family is a medicinal and aromatic plant .The genus *Ocimum* consists of about 50–150 species [1] with a large number of varieties containing both terpene and non-terpene constituents in their essential oils [2]. Basil has a long history as culinary herb, thanks to its foliage which adds a distinctive flavor to many foods. Essential oil extracted from *Ocimum basilicum* L. has antioxidant and antimicrobial activity [3]. It is also considered as a source of aroma compounds, and it possesses a range of biological activities such as insect repellent, nematocidal, antifungal agents and antioxidants activities [1, 4, 5, 6].

The amino acids are essential constituents in all cells. In addition to their role in protein synthesis, they participate in both primary and secondary metabolic processes associated with plant development and in responses to stress. For example, glutamine, glutamate, aspartate, and asparagines serve as pools and transport forms of nitrogen, as well as

in balancing the carbon/nitrogen ratio. Other amino acids such as tryptophan, methionine, proline and arginine contribute to the tolerance of plants against biotic and abiotic stresses either directly or indirectly by serving as precursors to secondary compounds and hormones [7]. Manival et al. (1994) reported that application of bio-stimulators on tea plant increased photosynthetic CO_2 uptake [8]. Also, stomata control and photosynthetic CO_2 uptake are directly related to each other [9]. Mandal et al. (2007) stated that the most common growth sources include amino acid, a mixture of nutrients, hydrolysis proteins, triacotanol, humic acids, extracts of seaweed, and brassinolids [10]. The positive effect of bio-stimulators on production, quality and growth of vegetables, *Camellia* species and forage crops was reported [11, 12].

Bio-fertilizers are fertilizing compounds that are composed of one or more species of useful soil-living organisms and are presented on preservative substances. Bio-fertilizers are introduced as microbial inoculation stock as a compound with effective microbial strains and with high yield of supplying one or more nutritional elements. Bio-fertilizers are micro-organisms that are able to change nutritional elements from useless form to effective and useful compounds and this change is conducted in a biological process. Production expenses of bio-fertilizers are low and it does not cause pollution in the environment [13]. In an experiment on squash plants, effect of *Bacillus* bio-fertilizers as dissolving phosphate increased vegetative growth (plant height, stem diameter, leaves number and total dry weight), elements absorption (N, P, K, Mg and B), total amount of plant protein and amount of plant pigments (chlorophyll a, b and carotenoids) [14].

The aim of this study was to investigate the effects of bio-stimulators and bio-fertilizers on morphological traits of basil (*Ocimum bacilicum* L.).

MATERIALS AND METHODS

This experiment was carried out in 2011-2012 at Iranian Academic Centre for Education, Culture & Research (ACECR), Institute of Medicinal Plants (56° 35´ N and 50° 58´ E; 1500 m elevation). The soil s loam-silt with 0.071% N, 48.9 $mg.kg^{-1}$ Phosphorous, 33.6 $mg.kg^{-1}$ Potassium, EC 2.71 $ds.m^{-1}$, and pH 8.3.

This study has been conducted on the base of factorial experiment in randomized complete block design with 16 treatments and three replications. The treatments were foliar application of bio-stimulators in four levels (control or no bio-stimulators and commercial formulations of aminolforte, kadostim and fosnutren at concentration of 1.5 $L.ha^{-1}$) and seed inoculation with bio-fertilizers in four levels (control or no bio-fertilizes and commercial formulations of nitroxin, supernitro plus and barvar II). Twenty seeds were sown at each pot and after thinning, 5 plants were remained in each pot.

Three commercial formulations of bio-stimulators including aminolforte, kadostim and fosnutren were supplied by Inagrosa Industries Agro Biologicals, Madrid, Spain. The details of the formulations are mentioned in Table 1. All of the treatments were sprayed in four growth stages including: 3, 4 and 5 weeks after seedling emerging and in plant flowering stage.

Bio-fertilizers used in this experiment are nitroxin, supernitro-plus and barvar II (bio-phosphor) which were applied each of them 500 ml in 5 Kg seed. The most effective method for application of bio-fertilizers was seed inoculation with these compounds in sowing time. Bio-fertilizer of supernitro-plus was composed of different species of N stabilizing bacteria, controllers of soil-living pathogens and growth stimulating bacteria (PGPR) like *Basillus subtilis*, *Azospirillum* spp., and *Pseudomonas* spp. Bio-fertilizer of nitroxin was composed of *Pseudomonas* genus. Concentration of nitrogen stabilizing bacteria and growth stimulators in bio-fertilizers of supernitro-plus and nitroxin was 10^8 living cells (CFU). Bio-fertilizer of barvar II was composed of phosphate solvent bacteria including different genera of *Pseudomonas*/*Bacillus*. Number of living cells (CFU) was 10^7 living cells from each of bacteria genera per ml of bio-fertilizer of barvar II. These bacteria had the ability of production of mineral and organic acids and phosphatise enzyme secretion and in this way it will change the sources of mineral and organic phosphorous in soil to useful form for plant.

All operations were done regularly during the growing season. The studied parameters were: plant height (cm), secondary stem number, leaves number, total dry weight (g), basal stem diameter (mm), and flower dry weight (g). In order to measure total dry matter, the five plants from each pot were harvested and then were placed in the electric oven of 75° C until the constant weight was gained. For measurement of leaf chlorophyll content (SPAD

value), 5 leaves of each plant were selected and mean of leaf chlorophyll content (SPAD value) was measured by device of SPAD (Minolta, 50 II). Analysis of variance of the results was done using the SPSS software (ver.17), and means in the results were compared using the Fisher's protected Least Significant Differences (LSD) Test.

Table 1. Formulation of bio-stimulators used in the experimental treatments

Biostimulators*	Formulation of compounds **
Aminolforte	Free amino acids 3750 mg.L^{-1}, organic components 2% and total N 1.1% (urea N 0.8% and organic N 0.3%)
Kadostim	Free amino acids 3750 mg.L^{-1}, organic components 2% and total N 4.2% (amonia N 0.8%, nitric N 3.1% and organic N 0.3%) and potassium (K_2O) 6%
Fosnutren	Free amino acids 3750 mg.L^{-1}, organic components 2% and total N 3.8% (amonia N 2.1%, nitric N 1.4% and organic N 0.3%) And phosphorous (P_2O_5) 6%

**Biostimulators supplied by Inagrosa Industries Agro Biologicals are compatible to the climate of Iran.*
*** Quantity and kind of free amino acids applied in the formulation of bio-stimulators in this experiment based on the percent of total amino acids are as follows: Glysin 11.2% , Valine 5.1%, Proline 8.3%, Alanin 13.2%, Aspartic acid 4.4%, Arginine 8.3%, Glutamic acid 0.9%, Lysine 5.1%, Lucine 16.4%, Isolucine 4.4%, Phenylalanin 5.1%, Methionine 4.2%, Serin 3.9%,Treonine 0.3%, Histidine 0.3%, Tyrosine 1.5%, Glutamine 0.9%, Systein 0.3%, Aspargine 0.4%, Tryptophan 0.4%.*

RESULTS AND DISCUSSION

Effect of bio-stimulators on plant height was statistically significant (P<0.01), while regarding bio-fertilizers insignificant (Table 2). The interaction effect of bio-stimulators and bio-fertilizers on plant height was statistically significant (P<0.01) (Table 2). The results indicated that the maximum plant height (87.00 cm) was obtained by kadostim and nitroxin (Table 5). The least plant height (57.66 cm) was observed by control treatment (Table 5). Moreover, these results are according to Karima & Abdel Wahed (2005). They reported that use of amino acids significantly increased height of German chamomile. Abou Dahab & Abd El-Aziz (2006) suggested that use of amino acids (especially tryptophan) were significantly effective on height of philodendron so that in 1st and 2nd year, the height of samples increased from 25.60 cm to 46.30 cm and from 27.30 cm to 47.30 cm, respectively.

According to variance analysis, main effects of bio-stimulators and bio-fertilizers were significant (P<0.01) on total dry weight (Table 2). Also, the interaction effect of bio-stimulators and bio-fertilizers on total dry weight was statistically significant (P<0.01) (Table 2). The results indicated that the maximum total dry weight (48.52 g) was obtained by fosnutren and nitroxin (Table 5). The minimum total dry weight (4.09 g) was observed by application of barvar II and control treatment (Table 3). These results are according to Dinno et al. (2009) study on application of perfectoz bio-stimulator on pepper. Also, Rafiee et al. (2011) reported that application of bio-stimulators increased total dry weight of Pot marigold seedlings. It is reported that bio-fertilizers of nitrogen (nitroxin) had positive effect on growth of aerial parts of the plant [19].

Bio-stimulators and bio-fertilizers had significant (P<0.01) effect on total fresh weight (Table 2). Also, the interaction effect of bio-stimulators and bio-fertilizers on total fresh weight was statistically significant (P<0.01) (Table 2). The maximum total fresh weight (87.33 g) was obtained by fosnutren and nitroxin (Table 5). The minimum total fresh weight (28.32 g) was observed by application of barvar II (bio-phosphor) and fosnutren treatment (Table 3). In an experiment, Sanchez et al. (2005) reported that application of biological fertilizers could increase yield of *Matricaria recutita*.

The main and interaction effects of bio-stimulators and bio-fertilizers on chlorophyll content (SPAD value) were statistically insignificant (Table 2). The main effects and interaction of bio-stimulators and bio-fertilizers were significant (P<0.01) on secondary stem number of basil (Table 2). The results indicated that the most secondary stem number (25.33) was obtained by kadostim and supernitro-plus (Table 4). These results are in line with Nahed et al (2009) study on use of amino acids tirozin, thiamin and tryptophan on *Thuja Orientalis* L. They concluded that all growth parameters improved with increase in concentration of amino acids. Positive effect of amino acids on plant performance might be due to stimulating effect of amino acids on plant cells growth. However, amino acids were introduced by Goss (1973) as a source of energy during lack of carbohydrates. These results are in according to Christopher et al. (2007) on *Basella rubra* L. They reported that application of bio-fertilizers increased stem diameter, secondary stem number, leaves number and flower number.

The main effect of bio-stimulators and bio-fertilizers and its interaction on leaf number was significant (P<0.01) (Table 2) and maximum number of leaves (25.33) was obtained by kadostim and barvar II (bio-phosphur) (Table 5).

Our results are not in according to the results of Abdel-Mawgoud et al. (2011) study. They were reported that with increasing concentration of mani-plex and amino-green, the number of leaves of green bean was decreased. However, the results are in line with that of Ayman et al. (2009) experiment on *Vicia faba* L. concerning interaction effect of humic acid and amino acid in isolation and in presence of chelated micro nutrients and the results by Shekari et al. (2012) on *Plantago psyllium* L. with bio-stimulator spray. Increase in yield and growth parameters is proved to be feasible using amino acids. Therefore, supply of nutritious sources to form protein tissue is essential [27]. Bacteria of *Pseudomonas* and *Basillus* change insoluble phosphorous to soluble form and in other words they are phosphate dissolving bacteria [28]. Spices of *Pseudomonas fluorescens* with different mechanisms like synthesis of anti-biotic, growth regulators and enzymes regulating ethylene synthesis in plant improve plant growth [29].

The stem diameter main effects of bio-stimulators ($P<0.01$) and bio-fertilizers ($P<0.05$) and interaction effect ($P<0.01$) were significant. The most stem diameter (7.16mm) was obtained by fosnutren and nitroxin (Table 5). The results of this study are according to Mazher et al. (2011) on *Codiaeum variegatum* L., Farooqi et al. (1996) on *Artemisia annua* L., and Kim et al. on *Dendranthama grandiflorum* L. The reports showed that amino acids are transferred fast in phloem exudates and xylem exudates [33]. It is reported that application of *Pseudomonas fluorescens* on *Catharanthus roseus* increased yield of the plant [32].

Table 2. Analysis of variance for measured traits in sweet basil (*Ocimum basilicum* L)

S.O.V	DF	Mean Squares							
		Plant height	Secondary stem number	Leaf number	Stem diameter	Flower dry weight	Total dry weight	Total fresh weight	chlorophyll content (SPAD value)
Rep.(Block)	2	148.550	9.438	451.937	1.335	0.893	10.944	215.082	12.608
bio-stimulator(S)	3	412.876 **	177.389**	6193.833**	5.233**	59.394**	87.717**	878.987*	37.482^{ns}
bio-fertilizer(F)	3	78.371 ^{ns}	41.722**	1745.833**	2.552*	20.096**	29.499**	292.953*	43.363^{ns}
S×F	9	3184.940**	104.407**	7068.704**	4.068**	45.798**	260.441**	1239.17**	20.898^{ns}
Error	30	52.617	5.971	216.893	0.812	1.608	4.341	99.380	15.011
CV (%)		10.03	19.16	14.87	16.07	13.88	15.61	16.61	10.370

,, ns shows significant in 5%, 1%, and insignificant ,respectively*

Table 3. Mean comparison of bio-stimulators on morphological traits of basil (*Ocimum bacilicum* L.)

Treatment	Plant height (cm)	Secondary stem num. per plant	Leaf num. per plant	Stem diameter (mm)	Flower dry weight(g)	Total dry weight (g)	Total fresh weight (g)	Chlorophyll content (SPAD value)
Control	66.89^{b}	8.75^{b}	73.75^{c}	5.02^{b}	6.90^{c}	10.86^{c}	49.53^{c}	34.9
Aminolforte	73.20^{b}	16.00^{a}	102.17^{b}	5.98^{a}	10.14^{b}	16.97^{a}	63.25^{ab}	39.07
Kadostim	80.15^{a}	16.08^{a}	128.17^{a}	5.05^{b}	7.68^{c}	11.79^{c}	69.65^{a}	37.5
Fosnutren	68.92^{b}	10.16^{b}	91.91^{b}	6.33^{a}	11.73^{a}	13.75^{b}	57.47^{bc}	37.95

**Values in a column bearing different superscript are significantly different at 0.01 levels*

Table 4. Mean comparison of bio-fertilizers on Morphological traits of basil (*Ocimum bacilicum* L.)

Treatments	plant height (cm)	Secondary stem number Per plant	Leaf number per plant	Stem diameter (mm)	Flower dry weight(g)	Total dry weight(g)	Total fresh weight(g)	Chlorophyll content (SPAD value)
Control	69.87^{a}	10.75^{b}	84.66^{c}	4.94^{b}	7.73^{c}	11.84^{b}	55.35^{b}	35.94
Nitroxin	75.11^{a}	15.25^{a}	113.92^{a}	5.67^{ab}	8.64^{bc}	15.42^{a}	66.62^{a}	39.96
Supernitro plus	73.78^{a}	12.66^{b}	100.75^{b}	5.77^{a}	9.28^{b}	13.62^{b}	60.65^{ab}	37.57
Barvar II (Biophosphor)	70.40^{a}	12.33^{b}	96.66^{bc}	6.01^{a}	10.81^{a}	12.49^{b}	57.29^{b}	35.95

**Values in a column bearing different superscript are significantly different at 0.01 levels*

The main effect of bio-stimulators and bio-fertilizers and its interaction on flower dry weight was significant at 1% (Table 2). Considering interaction effect of bio-stimulators and bio-fertilizers application, the most flower dry weight (18.83g) was obtained by fosnutren and barvar II (bio-phosphur) (Table 3). The results here were according to that of Nahed et al. (2009) on *Salvia farinarcea* L., Wahba et al. (2002) on *Antholyza aethiopica* L. and El-Fawakhri (2003) on *Chrysanthemum* spp. The results showed that use of amino acids improved blossoming and develop of flowers arrangements with preferred quality. Amino acids affect synthesis of bio-materials such as

protein, amino, pyrimidine, alkaloids, enzymes, and trypenoids [22]. According to results of Dehghani Moshkani et al. (2010), bio-fertilizers increased yield of capitula dry weight of *Matricaria recutita* L.

Table 5. Mean comparisons for interaction effects of bio-stimulators and bio-fertilizers on measured parameters.*

Treatments		plant height (cm)	Secondary stem number Per plant	Leaf number per plant	Stem diameter (mm)	Flower dry weight (g)	Total dry weight (g)	Total fresh weight (g)	Chlorophyll content (SPAD value)
Control	Control	57.66^{f}	8.00^{de}	78^{d}	3.11^{h}	7.70^{efg}	10.38^{fgh}	64.91^{bcd}	31.53
	Nitroxin	78.40^{abcd}	10.00^{d}	78.66^{d}	4.75^{fg}	3.35^{i}	9.66^{gh}	43.12^{cfg}	37.10
	Super nitroplus	70.16^{bcdef}	8.33^{d}	98.66^{cd}	6.72^{abcd}	9.54^{cdef}	19.32^{b}	51.57^{def}	35.53
	Barvar II (Biophosphor)	61.33^{ef}	8.66^{d}	39.66^{f}	$5.52^{abcdefg}$	7.03^{gh}	4.09^{i}	38.52^{fg}	35.43
Aminol forte	Control	74.83^{abcde}	16.33^{c}	48.66^{ef}	5.00^{defg}	8.80^{defg}	2.84^{i}	37.75^{fg}	35.66
	Nitroxin	69.66^{bcdef}	16.33^{c}	206.33^{a}	4.89^{efg}	10.24^{cd}	35.52^{a}	76.92^{abc}	43.86
	Super nitroplus	72.66^{bcde}	9.66^{d}	72^{de}	7.04^{ab}	11.66^{bc}	12.18^{efg}	59.65^{cde}	38.63
	Barvar II (Biophosphor)	75.66^{abcd}	21.66^{d}	81.66^{d}	6.98^{abc}	9.86^{cde}	17.36^{bcd}	78.69^{ab}	38.13
Kadostim	Control	68.66^{cdef}	7.66^{de}	96^{cd}	5.06^{defg}	10.64^{cd}	19.43^{b}	75.09^{abc}	38.30
	Nitroxin	87.00^{a}	16.00^{c}	95.66^{cd}	5.90^{abcdef}	7.34^{fgh}	7.83^{h}	59.13^{cde}	41.23
	Super nitroplus	81.66^{abc}	25.33^{a}	138.67^{b}	4.00^{gh}	5.26^{hi}	9.17^{gh}	60.77^{bcde}	38.93
	Barvar II (Biophosphor)	83.30^{ab}	15.33^{c}	182.33^{a}	5.27^{cdefg}	7.50^{fgh}	10.73^{fgh}	83.63^{a}	31.53
Fosnutren	Control	78.33^{abcd}	11.00^{d}	116^{bc}	6.58^{abcde}	3.79^{i}	14.72^{cde}	43.66^{efg}	38.26
	Nitroxin	65.40^{def}	18.66^{bc}	75^{d}	7.16^{a}	13.65^{b}	6.68^{gh}	87.33^{a}	37.66
	Super nitroplus	70.63^{bcdef}	7.33^{de}	93.66^{cd}	5.32^{bcdef}	10.66^{cd}	13.80^{def}	70.60^{abc}	37.20
	Barvar II (Biophosphor)	61.33^{ef}	3.66^{e}	83^{d}	6.26^{abcdef}	18.83^{a}	17.80^{bc}	28.32^{g}	38.70

**Values in a column bearing different superscript are significantly different at 0.01 levels*

CONCLUSION

In this experiment, commercial formulation of bio-stimulators and bio-fertilizers had significantly positive effect on growth parameters of basil (*Ocimum bacilicum* L.). The effectiveness of bio-stimulators can be due to existence of amino acids that can promote metabolic activities. Bio-fertilizers as compounds with effective microbial strains and high yield of supplying one or more nutritional elements improved the vegetative parameters. In interaction effects bio-stimulators and bio-fertilizers improved vegetative and reproductive traits. In general, these compounds can be supply essential nutrients like nitrogen, phosphorous and potassium and affect on morphological growth of the plant.

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