Journal of Crop Nutrition Science
ISSN: 2423-7353 (Print) 2538-2470 (Online)
Vol. 7, No. 3, 2021
http://JCNS.iauahvaz.ac.ir **OPEN ACCESS**

Growth and Biochemical Properties of Green Basil (*Ocimum basilicum* L.) Affected by Foliar Application of Biostimulants

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RESEARCH ARTICLE

ARTICLE INFO.
Received Date: *4 Jul. 2021*
Received in revised form: *7 Aug. 2021*
Accepted Date: *8 Sep. 2021*
Available online: *30 Sep. 2021*

To Cite This Article:
Mozhgan Alibakhshi, Hossein Ali Asadi-Gharneh. Growth and Biochemical Properties of Green Basil (*Ocimum basilicum* L.) Affected by Foliar Application of Biostimulants. ***J. Crop. Nutr. Sci.,*** 7(3): 34-45, 2021.

ABSTRACT

BACKGROUND: Green basil is a nutritional and medicinal plant due to its valuable properties. Improving qualitative and quantitative traits of plants with appropriate methods in sustainable agricultural were emphases. Biostimulants derived from organic materials and stimulate natural process in plants and improve crop performance.

OBJECTIVES: In this research the effects of some biostimulants (amino acids, humic acid and seaweed extract) on morpho-physiological properties of green basil were evaluated.

METHODS: This research was carried out under greenhouse condition. The experiment was conducted based on Completely Randomized Design with ten treatments and three replications. The treatments included control (distilled water), seaweed extract (0.5, 1 and 2 $gr.l^{-1}$), amino acid (1, 2 and 3 $gr.l^{-1}$) and humic acid (1, 2 and 3 $ml.l^{-1}$).

RESULT: The results showed that application of different biostimulants positive effects on vegetative parameters and biochemical properties of green basil plants. The highest leaf numbers (21.67) was measured in amino acid application at 1 $g.l^{-1}$ concentration. The effect of humic acid was more noticeable on increased plant height (37.00- 47.67 cm) in compare to other biostimulants. The plants that received biostimulants had higher plant fresh weight than control treatment. Seaweed extract and humic acids increased significantly carotenoid content of plants. The highest ascorbic acid content was measured in treated plants at levels of 2 and 3 $gr.l^{-1}$ amino acid. Application of all biostimulants were used in this study, had positive effects on concentration of total phenolic content of basil plants.

CONCLUSION: According to the results of the present study, it can be concluded that, application of biostimulants at proper concentration due to positive effect on quality and quantity of growth parameters and biochemical properties of green basil plants are recommended.

KEYWORDS: *Leaf area, Plant height, Carotenoid, Ascorbic acid, Total phenolic content.*

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1. BACKGROUND

Basil is one of the most important plants belongs to the Lamiaceae family. Basil is annual herbaceous plant with originated from the warm tropical regions and cultivated in different parts of the world (Putievsky and Galambosi, 1999). This plant has 30-60 cm height and can be harvested 2-3 times during the seasons and has been used as a medicinal and aromatic plants for centuries (El-Sayed *et al*., 2015). Basil considered as one of the most important culinary herbs grown for flavoring and confectionery of foodstuffs and condiments due to its valuable properties (Chanwitheesuk *et al*., 2005; Maach *et al*., 2020). Basil has different implications in nutrition and medicine. For example, it is an important source of different phenolic compounds, flavonoids, carbohydrates, proteins, fibers and vitamins and can be used for treating different diseases such as migraine, sore throat and asthma (Huang *et al*., 2009). In recent years, excessive application of chemical fertilizers, not only effects on production efficiency, but also caused to environmental pollutions and therefor, reduce use of chemical fertilizers and alternative was to improve yield quality and quantity were emphases (Barker and Pilbeam, 2007). One such approach can be obtained with use of biostimulants in agricultural systems (Mafakheri and Asaghari, 2018). The terms of biostimulants are refer to substances that derived from organic materials containing bioactive substance that have the capacity to stimulate several natural processes that benefit for plan growth and metabolism and improve crop performance (Du Jardin, 2015). They are supplied at very low concentrations in order to induce beneficial effects and aid to plants to overcome in stress conditions and increase crop yield and quality (Nardi *et al*., 2021; Mzibrz *et al*., 2021). Humic acid and humic substances are an organically charged biostimulants that significantly increases plant physiological activities and impact plant growth, nutrient uptake by plants and improving soil health (Hatice and Gusler, 2005). El-Sayed *et al*. (2015) found that humic acid increased all growth parameters (plant height, number of branches, leaf area, herb fresh and air-dry weights) of basil. Seaweed extract contain biochemical compounds such as polysaccharides, proteins, polyunsaturated fatty acids, pigments, minerals and plant hormones and can act as biostimulants to enhanced physiological activities related to enhanced crop yield and quality (González *et al*., 2013) and resistance to environmental stress (Thambiraj *et al*., 2012). In recent years, use of seaweed extract has been explored in modern agriculture (Erulan *et al*., 2009). Seaweed extracts can improved plant growing by supplying macro and micro elements in order to physiological activities of higher plant (Hernandez *et al*., 2014). Amino acids are biomolecules and affecting different plant enzymatic and metabolic activities. These compounds with high amount of nitrogen increasing plant chlorophyll content and improve plant tolerance to stress conditioning and enhancing the physiological activities of growth (Bashir *et al*.,

2018). Application of amino acid increased stem diameter, whole stem weight, number of total leaves per plant, and fresh weights of total yield and marketable yield of Chinese cabbage (El-Afifi *et al*., 2014) and enhanced the vegetative traits and chemical compositions of basil leaves (Azza and Yousef, 2015).

2. OBJECTIVES

The goals of this research were to determine the effects of foliar application of humic acid, Seaweed extract and Amino acid on morpho-physiological properties of green basil.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out under natural light and temperature conditions in the Greenhouse Research Center of Islamic Azad University (IAU), Isfahan (Khorasgan) Branch, Iran in 2020. Green basil seeds were purchased of Pakan Bazr Company in Isfahan and were sown in the plastic pots (40 × 30 × 15 cm). The medium of pots was prepared by mixing field soil, sand and cow manure at 2, 1 and 1 ratio, respectively. The physiochemical analysis of pot soil was composed of pH=7.14, N (0.18%), P_2o_5 (98.34 mg $kg^{-1)}$, K_2O (1845 mg kg^{-1}), Cu (0.9 $mg.kg^{-1}$), Mn (7.66 $mg.kg^{-1}$), Fe (1.4 $mg.kg^{-1}$), Zn (2.76 $mg.kg^{-1}$), Na (311 mg kg^{-1}) and organic matter (1.79%). Also, the soil texture was loam. The treatments included of different biostimulants with three factors of commercial seaweed extract (0.5, 1 and 2 $gr.l^{-1}$), amino acid (1, 2 and 3 $gr.l^{-1}$) and humic acid (1, 2 and 3 $ml.l^{-1}$) in three replications. A control treatment (distilled water) was also used.

3.2. Greenhouse Management

Seaweed extract (Algline®) containing *Ascophyllum nodosum* extract is composed of organic matter (44.5% w/w), alginic acid (1%), gibberellic acid (1ppm) and K_2O (19.3%). Hubest® organic fertilizer, obtained from Hasel Novin Agriculture Company used as a source of humic acid which contains K_2O (8.0%), humic and fulvic acid (60%), sulfur (3.7%), iron (1.2%) and MgO (0.2%). Amino acid (Kimitec Bombardier), as a natural effective plant growth activator, purchased from Abroon company and contains free amino acids (15.4%), nitrogen (8.83%), total organic matter (59.43%), phosphorous (P_2O_5, 0.55%) and total fulvic acids (24.72%). Plants were irrigated every 3 days with top water to field capacity. After germination of seeds and in two-leaf stage, the seedlings were thinned and finally 10 uniform plants reaming per each pot. Foliar applications of treatments were done by a hand sprayer, between 6-8 A.M. at intervals of 15 days. During the grow period the daily and nightly temperatures were 27±2 and 20±2, respectively. No additional fertilizer was applied.

3.3. Measured Traits

Plant height (Cm) was measured by a ruler from the ground surface to extreme growing tip. Leaf area (mm^2) was measured by using an area meter (Area Meter AM, 200) and expressed as mm^2. Number of leaves and the lateral shoots

per plants were determined by counting themes at the end of experiment. Also, the diameter of stem was measured by a digital caliper with an accuracy of 0.01mm. In order to determination of fresh and dry weight, the plants were removed from above ground surface and the fresh weight of plant were recorded, then the plant dried in oven at 70°C overnight and dry weight of each sample recorded by a digital scale (Shehata and Nosir, 2019).

3.3.1. Chlorophyll and carotenoid contents

In order to determination of chlorophyll and carotenoid content of sample 0.5 g of fresh plant leaves were ground in a mortar and 50 ml acetone 80% (20 distilled water: 80 acetone) was added. The mixture was centrifuged at 5000 rpm. Eventually, the transparent acetone extract was separated to obtain 5 ml solution with pure acetone. Finally, chlorophyll a, b and carotenoid contents were measured using spectrophotometry at 633 nm, 645 nm and 470 nm, respectively. The following equations were used to determine chlorophyll a, b, and total and also carotenoids (Car.).

Equ.1. Chl.a ($mg.g^{-1}$) = [(12.7×Abs_{663}) - (2.6×Abs_{645})] × V/W × 1000

Equ.2. Chl.b ($mg.g^{-1}$) = [(22.9×Abs_{645}) - (4.68×Abs_{663})] × V/W × 1000

Equ.3. Chl.total ($mg.g^{-1}$) = Chl.a + Chl.b

Equ.4. Car = (1000 A_{470} - 1.82 Chl.a - 85.02 Chl.b)/198

Where Chl.a, Chl.b, Chl.total, and Car. is chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid, respectively. Chlorophyll and carotenoid concentrations were expressed in $mg.g^{-1}$ fresh weight and mg/100 g fresh tissue (Li *et al*., 2009).

3.3.2. Ascorbic acid

Ascorbic acid content in basil plant leaves were measured using indophenol method according to AOAC (1984). 10 g of fresh leaves were homogenized with 48 ml metaphosphoric acetic acid and 2 ml of sodium citrate solution. The samples were filtered using a Buchner funnel and suction pump and 10 milliliters of the filtrates were rapidly titrated with the standardized 2, 6 dichlorophenolindophenol solution till formation of a permanent pink color.

3.3.3. Total phenolic content

The total phenolic content of samples was measured using a spectrophotometer at the visible zone of 765 nm according to method described by Singleton and Rossi (Singleton and Rossi, 1965). Accordingly, 1 g of plant sample treated with liquid nitrogen using a crucible. Then, 10 cc pure methanol was added to extract phenolic compounds and the solution was passed through filter paper. In order to obtain the standard solution, gallic acid solution, (0.1 g gallic acid in 100 ml pure methanol), Folin (5 ml Folin in 50 ml distilled water), and sodium carbonate 7.5% (1.5 g sodium carbonate in 20 cc distilled water) were mixed.

3.4. Statistical Analysis

The experiment was conducted based on Completely Randomized Design with ten treatments and three replications. Data were analyzed using MSTATC Software and means were com-

pared by Duncan multiple range test method at 5% probability level.

4. RESULT

The effects of foliar application of biostimulants on growth parameters of the basil plant were shown in tables 1. As shown, application of different biostimulants significantly changed amount of vegetative growth parameters (leaf number, plant height, lateral stem number, stem diameter, leaf area) and plant fresh and dry weight. All applications of seaweed extract, amino acid and humic acid increased significantly leaf number of sweet basils. The highest and lowest leaf numbers were measured of 1 $g.l^{-1}$ amino acid rate (21.67) and control treatment (13.33), respectively. The obtained data reviled that the effects of seaweed extract and humic acid were increased with concentration of these treatments (Table 1). The highest plant height was observed with application of 1 $ml.l^{-1}$ humic acid (47.67 cm) and significantly different from control treatment (32.00 cm). The effect of humic acid was more noticeable on increased plant height (37.00- 47.67 cm) in compare to other biostimulants (Table 1). The data related to the effect of different biostimulants application rates on the number of lateral branches of sweet basil indicated that there are significant differences among the studied treatments. According to our data, it is a gradual increase in number of lateral branches with the concentration of amino acid from 1 to 3 $gr.l^{-1}$ (3.00-6.33). There was no difference between humic acid levels (Table 1). The results of mean comparison indicated that the stem diameter varied from 0.12 mm to 0.29 mm. The control grope and the concentration of 3 $gr.l^{-1}$ amino acid treatment had the lowest and the highest stem diameter. Furthermore, spraying the seaweed, humic and amino acid had a gradual increase on stem diameter of treated plants (Table 1). In terms of leaf area, the basil plants which received different levels of biostimulants were varied from 3225 mm^2 to 3665 mm^2 and had a significant enhancing leaf area. The highest leaf area was measured at the lowest amino acid (3965 mm^2) and humic acid (3944 mm^2) concentrations (Table 1). It was observed that at all rates tested the plants that received biostimulants had higher plant fresh weight than control treatment. The results of mean comparison showed that amount of plant fresh weight varied from 5.60 g to 14.76 g. The effect of the amino acid treatments (7.37-14.76 gr) was more evident on the fresh weight of the plant than the other treatments. The amount of highest fresh weight was three times higher than control treatment. There was no difference between different levels of humic acid treatments (Table 1). Dry weight of basil plant was significantly affected by application of different biostimulants. In this regard, the control grope and the highest concentration of amino acid had the lowest (1.27 gr) and the highest (7.85 gr) dry weight value, respectively (Table 1). The data related to the effects of different biostimulants rates on biochemical characteristics of basil plants are presented in Table 2. The obtained results indicated that the seaweed extract, humic acid and amino acid treatments sig-

nificantly increased the chlorophyll a content of the plants, compared with the content control treatment (0.059 $mg.g^{-1}$). The highest concentration of chlorophyll b content (0.105 $mg.g^{-1}$) was measured by the application of 2 and 3 $gr.l^{-1}$ of seaweed extract and amino acid, respectively. There was a significant effect of seaweed, amino acid and humic acid treatments in high levels on the total chlorophyll content of the plant, compared with the control treatment (0.107 $mg.g^{-1}$).

Table 1. Effects of seaweed extract, amino acid and humic acid application on some growth parameters of basil

Treatment	Leaf number	Plant height (cm)	Lateral branches number	Stem diameter (mm)
Control	13.33^{f}	32.00^{e}	1.00^{d}	0.12^{f}
Seaweed 0.5 $gr.1^{-1}$	18.33^{cd}	41.33^{bc}	3.67^{bc}	0.16^{de}
Seaweed 1 $gr.1^{-1}$	18.67^{bc}	42.67^{b}	3.33^{c}	0.20^{c}
Seaweed 2 $gr.1^{-1}$	20.33^{ab}	40.33^{bc}	3.33^{c}	0.21^{c}
Amino acid 1 $gr.1^{-1}$	21.67^{a}	39.00^{cd}	3.00^{c}	0.16^{de}
Amino acid 2 $gr.1^{-1}$	16.67^{de}	37.00^{d}	5.00^{ab}	0.27^{b}
Amino acid 3 $gr.1^{-1}$	16.60^{de}	33.33^{e}	6.33^{a}	0.29^{a}
Humic acid 1 $ml.l^{-1}$	15.33^{e}	47.67^{a}	6.00^{a}	0.14^{ef}
Humic acid 2 $ml.l^{-1}$	15.67^{e}	37.00^{d}	6.00^{a}	0.17^{d}
Humic acid 3 $ml.l^{-1}$	18.65^{bc}	39.67^{cd}	5.00^{ab}	0.26^{b}

*Means with similar letters in each column are not significantly different by Duncan's test at 5% probability level.

Continue table 1.

Treatment	Leaf area (mm^2)	Plant fresh weight (gr)	Plant dry weight (gr)
Control	3450^{cd}	5.60^{f}	1.03^{f}
Seaweed 0.5 $gr.1^{-1}$	3527^{bcd}	9.15^{d}	1.33^{ef}
Seaweed 1 $gr.1^{-1}$	3524^{bcd}	12.65^{b}	2.65^{b}
Seaweed 2 $gr.1^{-1}$	3457^{cd}	6.86^{ef}	1.13^{ef}
Amino acid 1 $gr.1^{-1}$	3965^{a}	7.37^{e}	1.36^{ef}
Amino acid 2 $gr.1^{-1}$	3225^{d}	11.71^{bc}	1.99^{cd}
Amino acid 3 $gr.1^{-1}$	3773^{abc}	14.76^{a}	3.52^{a}
Humic acid 1 $ml.l^{-1}$	3944^{a}	10.59^{cd}	2.25^{bc}
Humic acid 2 $ml.l^{-1}$	3505^{bcd}	10.61^{cd}	1.57^{de}
Humic acid 3 $ml.l^{-1}$	3816^{ab}	10.02^{d}	1.40^{ef}

*Means with similar letters in each column are not significantly different by Duncan's test at 5% probability level.

It is clear from table 2, that basil plants which were sprayed with seaweed extract and humic acids increased significantly in terms of carotenoid content. Carotenoid content increased with increasing seaweed extract and humic acid levels and highest amount of carotenoid was measured with spraying of seaweed extract at three levels of treatment. There was no difference in carotenoid content due to amino acid treatment to compare with control plants. According to the results, the highest ascorbic acid content was measured in treated plants at levels of 2 and 3 $gr.l^{-1}$ amino acid which is significantly higher

than the control treatment. In overall, the results revealed that the application of seaweed extract, amino acid and humic acid significantly increases ascorbic acid content in basil plants compared to control treatment (Fig. 1). In this study, application of biostimulants had outstandingly positive effects on concentration of total phenolic compounds. In this regard, foliar application of 1 and 2 $gr.l^{-1}$ seaweed extract, amino acid at rate of 1, 2 and 3 $gr.l^{-1}$ and usage of 3 $ml.l^{-1}$ humic acid, indicated significant higher phenolic compounds in comparison to other treatments. As shown in table 2, in treated basil plants, a gradual increase in total phenolic content was observed.

Table 2. Effects of seaweed extract, amino acid and humic acid application on biochemical properties of basil

Treatment	Chlorophyll a ($mg.g^{-1}$)	Chlorophyll b ($mg.g^{-1}$)	Total chlorophyll ($mg.g^{-1}$)
Control	0.05^{g}	0.04^{e}	0.09^{d}
Seaweed 0.5 $gr.1^{-1}$	$0.06f^{g}$	0.05^{e}	0.11^{d}
Seaweed 1 $gr.1^{-1}$	0.09^{e}	$0.06cd^{e}$	0.15^{c}
Seaweed 2 $gr.1^{-1}$	0.20^{b}	0.10^{a}	0.30^{a}
Amino acid 1 $gr.1^{-1}$	0.07^{f}	0.05^{de}	0.12^{cd}
Amino acid 2 $gr.1^{-1}$	0.12^{d}	0.10^{ab}	0.22^{b}
Amino acid 3 $gr.1^{-1}$	0.22^{a}	0.10^{a}	0.32^{a}
Humic acid 1 $ml.l^{-1}$	0.07^{f}	0.08^{abc}	0.15^{c}
Humic acid 2 $ml.l^{-1}$	0.15^{c}	0.08^{e}	0.23^{b}
Humic acid 3 $ml.l^{-1}$	850.22^{a}	0.07^{bcd}	0.29^{a}

*Means with similar letters in each column are not significantly different by Duncan's test at 5% probability level.

Continue table 2.

Treatment	Carotenoid ($mg.g^{-1}$)	Total phenolic ($mg.kg^{-1}$)
Control	0.08^{de}	457.02^{c}
Seaweed 0.5 $gr.1^{-1}$	0.142^{ab}	694.05^{b}
Seaweed 1 $gr.1^{-1}$	0.146^{a}	969.36^{a}
Seaweed 2 $gr.1^{-1}$	0.158^{a}	973.87^{a}
Amino acid 1 $gr.1^{-1}$	0.062^{e}	1034.05^{a}
Amino acid 2 $gr.1^{-1}$	0.081^{de}	1076.17^{a}
Amino acid 3 $gr.1^{-1}$	0.083^{d}	988.62^{a}
Humic acid 1 $ml.l^{-1}$	0.105^{c}	667.28^{b}
Humic acid 2 $ml.l^{-1}$	0.116^{c}	987.13^{b}
Humic acid 3 $ml.l^{-1}$	0.123^{bc}	1034.05^{a}

*Means with similar letters in each column are not significantly different by Duncan's test at 5% probability level.

5. DISCUSSION

The use of medicinal plants as a source of different metabolites for nutritional and pharmaceutical properties has more popular in the world. The global approach for these plants has been directed towards improving the quantity, quality and health of the active ingredient. Therefore, it is necessary to find methods which supported this idea. The excesses application of chemical fertilizers damaged the texture of crop soils

and has caused environmental pollution and effects on human health (Dixon, 2018). Due to these problems, application of safety materials can be improving quantity and qualities of crops without any side-effects have been more emphasis (Ji *et al*., 2019. In recent years, biostimulants are used to increase yield and improve crop quality (Maacha *et al*., 2020). In this research application of some biostimulants at different rates on growth parameters and biochemical properties of green basil were evaluated. The obtained results indicated that application of humic acid, seaweed extract and amino acid has significant effects on growth vegetative and biological traits of green basil plants. Control plants produced lower plant fresh weight, leaf number, stem diameter, lateral shoots and leaf area than plants treated with the amino acid, seaweed extract and humic acid. More yields were obtained when the biostimulants were applied in higher levels. Similar results reported by Hernandez *et al*. (2014) in evaluation of liquid seaweed extracts on growth of tomato (*Solanum lycopersicum* L.) plants. The positive effects of amino acid application on morphological traits can be related to providing of nitrogen and organic carbon for basil plants. Increases of vegetative characteristics by application of biostimulants confirmed by other researchers. The seaweed extract increased vegetative parameters such as plant length, number of leaves, number of shoot and root growth of watermelon (Abdel-Mawgoud *et al*., 2010) and fennel plant (Mahfouz and Sharaf-Eldin (2007).

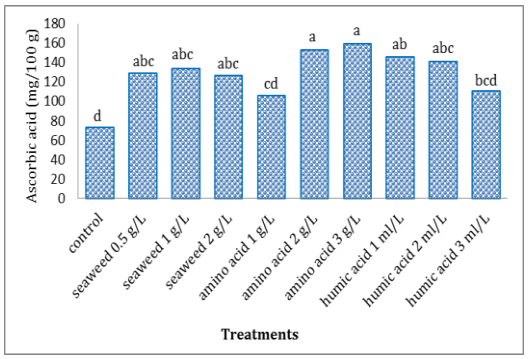

Fig. 1. The effect of different biostimulants on ascorbic acid content of basil plants. Means with similar letters in each column are not significantly different by Duncan's test at 5% probability level.

Also, according to Khan *et al*. (2009) the reason for increase of root growth of plants treated by biostimulants is improving of nutrient element uptake. In terms of biochemical properties, parallel to our results, other researchers reported that application of brown seaweed extract significantly enhanced the phenolic content of cabbage (Lola-luz *et al*., 2013) and cowpea (*Vinga unguiculata* L. Walp) by Vasantharaja *et al*. (2019). Biostimulants were used in this study contains nitrogen, potassium and boron which are essential for growth and production of plants (Maacha *et al*., 2020). Nitrogen is a constituent of important biomolecules such as auxin, cytokines and chlorophyll and facilitates rapid growth and helps improved leaf and forage crop production. Potassium is an activator of enzymes used in photosynthesis and respiration. It is helps in photosynthesis by the formation of a chlorophyll precursor (Bhatla and Lal, 2018). The increase of chlorophylls pigment and carotenoid content may be due to the stimulating effect on leaves and more production and transport of

photosynthetic materials (Porsakhi and Asadi-Gharneh, 2019). It seems that magnesium and irons which are ingredients in biostimulants, had positive effects on synthesis of chlorophyll and carotenoids content (Pise and Sabale, 2010). Magnesium is an important constituent of chlorophyll and is also an activator of enzymes contributed in dark reaction in photosynthesis. Iron is present as an enzyme cofactor in plants and is required for chlorophyll formation and synthesis (Bhatla and Lal, 2018). On the other hand, increased in total chlorophyll content in plants can be due to improvement of water status of plants and more absorption of minerals (Delshadi *et al*., 2017), which causes effected on growth parameters of plants. The results of this research revealed positive effects of biostimulants on ascorbic acid content of green basil. Ascorbic acid is one of the important antioxidants and displays a key role in the reduction of diseases. Plants are rich sources of phytochemical or bioactive compounds and are considered as beneficial sources of antioxidants. These compounds have the important roles in preventing or ameliorating various chronic human diseases. For this reason, plant with the high amounts of bioactive compound are generally associated with health promotion properties. Phenolic compounds are secondary metabolites that are synthesized in many plants. The amount of phenolic compounds can be increased in the plant tissue due to plant nutrition. It is clear that the increase of biochemical traits in plants contributed to improve the human health (Lin *et al*., 2016).

6. CONCLUSION

The factors affecting on biochemical properties of plants for medicinal and nutritional usage and health purpose are very important. Therefore, finding methods which help to this purpose are valuable. According to the results of the present study, it can be said that biostimulants as a suitable bio fertilizers affected on morphological and biochemical properties of green basil. Therefore the application of these bio fertilizers at proper concentration due to positive effect on growth vegetative and health properties of the plant are recommended.

ACKNOWLEDGMENT

The authors thank all colleagues and other participants, who took part in the study.

FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

FUNDING/SUPPORT: This study was done by support of Department of Horticulture, Islamic Azad University, Isfahan (Khorasgan) Branch.

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