RESEARCH ARTICLE

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Effects of Biofertilizers on the Growth, Physiological Parameters, and Essential Oil Content of Basil (*Ocimum basilicum* L.)

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Article info:
Received: 2019
Accepted: 2019

ABSTRACT
Basil (*Ocimum basilicum* L.) is one of the widely cultivated medicinal crops that is used as kitchen herb, vegetables, spice, and especially its essential oil and extracts as therapeutics. One of the promising methods to improve basil growth, yield, and essential oil is use of biofertilizers. Biofertilizers are environmentally-friendly substances that enrich soil fertility and promote crop yield. In this study, we compared three treatments (methionine, humic acid, and nitroxin) arranged as 2*2*3 factorial based on completely randomized design with three replicates. All treatments significantly increased basil growth parameters, physiological traits, and content of essential oil ($P < 0.05$). Based on our results, the best and highest amount of parameters (plant height, number of leaves, leaf length, number of branch, dry weight, shoot diameter, root length, plant biomass, chlorophyll b, total chlorophyll, SPAD index, and essential oil) was observed in plants treated with 1 l/ha of methionine*5 l/ha of humic acid*3 l/ha of nitroxin ($P \leq 0.05$). In terms of root dry weight and chlorophyll a, the best mean was observed in plants treated with the mixture of 1 l/ha of methionine*5 l/ha of humic acid and the mixture of 5 l/ha of humic acid*3 l/ha of nitroxin ($P \leq 0.05$). Especially for content of essential oil (shoots and leaves), plants treated with 1 l/ha of methionine*5 l/ha of humic acid*3 l/ha of nitroxin showed +2.5-fold increase compared to control plants. In conclusion, results of this research suggest that the combination of methionine*humic acid*nitroxin could be an efficient and promising biofertilizers to improve the growth of basil, especially its essential oil.

Key words: Crop yield, Essential oil, Humic acid, Nitroxin, Physiology

Introduction

Basil (*Ocimum basilicum* L.; Lamiaceae family) is widely cultivated in many countries (Chalchat & Özcan, 2008). It is kitchen herb that comes from Iran and India, and has 20-60 cm length and white-purple flowers (Moghaddam et al., 2011). It is one of the important plants that is commonly used as vegetables, salad pizza, and medicinal plant. It has been showed that leaves of basil have beneficial effects on diuretic properties, mental fatigue, colds, spasms, and rhinitis (Chalchat & Özcan, 2008; Özcan & Chalchat, 2002). Gülçin et al. (2007) , determination of antioxidant and radical scavenging activity of basil, showed the water and ethanol extracts from basil tissues efficiently have DPPH radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, reducing power and metal chelating activities, protecting peroxidation of linoleic acid emulsion. In another study, Politeo et al. (2007) tested essential oil and antioxidant capacity of free volatile aglycones from basil. They showed that volatile aglycones like essential oil of basil has a good antioxidant activities. In one of the important studies on medical properties of basil, Kathirvel & Ravi (2012) with studying the essential oil from basil and its cytotoxicity against HeLa and HEp-2 human cancer cell lines and NIH 3T3 mouse embryonic fibroblasts, revealed that basil oil has potent cytotoxicity. Moreover, other studies have showed basil antimicrobial activities. Hossain et al. (2010) and Rattanachaikunsopon and Phumkhachorn (2010) reported that essential oil extracted from different parts of basil have antimicrobial activities on *Salmonella enteritidis, Bacillius cereus, B. subtilis, B. megaterium, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Shigella boydii, S. dysenteriae, Vibrio parahaemolyticus, V. mimicus, and Salmonella typhi*. It also has been indicated that basil seeds with 22% oil are a potential source for producing biodiesel fuel (Amini et al., 2017).

Humic substances are the major components of soil and their beneficial effects have been revealed on plant growth and development as well as plant-soil system (Vaccaro et al., 2015). Several studies have showed that application of humic substances have a positive effects on crop yield, shoot and root development of plants, chlorophyll content, nutrient uptake, and many biological pathways (Canellas & Olivares, 2014; Thiele-Bruhn, 2010; Vaccaro et al., 2015). Befrozfar et al. (2013) reported that application of humic acid (seed inoculation and foliar) and its combination with vermicompost and plant growth promoting bacteria (PGPRs) significantly improves plant height, leaf area, shoot dry/fresh weight, and chlorophyll content. In another study, Jamali et al. (2015) , assessing effects of humic acid, compost and phosphorus on growth traits and some micronutrient uptake in basil medicinal plant, showed plants treated with humic acid compared to control have significantly more height, number of lateral branches and shoot dry weight.

Moreover, Khaled & Fawy (2011) reported that different levels of humic acid increase basil growth and nutrient uptake under salinity stress, and ameliorate effects of the stress on basil.

Biofertilizers have been found environmental-friendly substances that can improve both morphological characteristics and physiological/biochemical properties of crops (Bhardwaj et al., 2014). In addition, it has well been indicated that biofertilizer application enhances crop tolerance to a variety of abiotic/biotic stresses such as drought, salinity, and diseases (Bhardwaj et al., 2014). Nowadays biofertilizers are considered as one of the promising options to replace and reduce the usage of chemical fertilizers (Tahami et al., 2017). The way by which biofertilizers improve plant yield is not identified completely, but it is believed that these types of fertilizers act as supplementary materials, associate with root to absorb nutrition, stimulate production of phytohormones, and enrich soil around the root (Tahami et al., 2017). Nitroxin as one of important and common biofertilizers, especially containing nitroxin-fixing microorganisms, has remarkable effects on soil fertility and on improving plant yield. Studies on nitroxin effects and its combinations with other biofertilizers such as biological phosphorus on basil, have showed increased growth parameters in this plant. Weisany et al. (2012) showed that application of nitroxin and its mixture with biological phosphorous increases the dry weight of total shoots, plant height, number of leaves, leaf dry weight, photosynthesis, transpiration, essential oil content and yield, root dry weight, number of flowering branches and chlorophyll content in basil. Jahan et al. (2012) reported that plants treated with nitroxin (containing *Azotobacter* spp. and *Azospirillum* spp.), biophosphorous (containing *Bacillus* sp. and *Pseudomonas* sp.) and nitroxin + biophosphorous have significantly increased growth parameters compared to the control group (without treatment). Recently, to use the highest potentials of biofertilizers, different mixture of them in different levels are combined and applied on plants. Studies have been found that in most cases, plants treated with mixture of biofertilizer in appropriate levels have more and better growth parameters compared to plants treated by one type of biofertilizers (Azhgan, 2016; Barghamadi, 2013; Barghamadi & Najafi, 2016; Befrozfar et al., 2013).

To the best our knowledge, the use of mixture of methionine, humic acid, and nitroxin on basil is still poorly studied, despite their important contribution to the sustainability of crop production and increasingly importance of basil in many countries. The aim of this research was to assess the effects of three biofertilizers (methionine, humic acid, and nitroxin) in different levels and their interaction effects on the growth and physiological parameters as well as essential oil of basil. Findings of the study could be used to improve basil yield and production.

Materials and methods

Experimental Conditions and Treatments

The experiment was carried out in a greenhouse with temperature 25±2 ºC, relative humidity 50% and 60% during day and night respectively, and 14h photoperiod throughout the experiment. Seeds of Iranian basil (*O. basilicum*) were sown in pots with a depth of 25 cm and a diameter of 20 cm filled with clay loam (pH of 7.5, 10% organic material, 1.30 dS/m, 50% field capacity). Seeds were tested for their vigor and sown in a depth of 1 cm. In each pot, four healthy plants were grown.

The experiment was arranged as 3*2*2 factorial with three replicates based on completely randomized design (CRD). Totally, 36 unites (pots) were produced and three treatments were compared: (a) methionine (0 and 1 liter/hectare); (b) humic acid (0 and 5 liter/hectare); and (c) nitroxin (0, 3, and 6 liter/hectare). Spraying with biofertilizers (methionine and humic acid) was performed after 15 and 45 days of cultivation, and nitroxin was used to treatment of seeds before cultivation. Three healthy and uniform plants were selected at last stage of vegetative growth after 75 days of cultivation and used to analysis their growth and physiological parameters.

Growth Parameters

The morphological parameters of basil measured in this experiment were plant height (cm), leaves (n/plant), leaf length (cm), branch (n/plant), dry weight (g/plant), shoot diameter (mm), root length (cm), root dry weight (g/plant), and plant biomass (%). Plant height was measured from the crown to the tip of the stem. Fresh root was carefully washed with tap water after harvest and measured from the crown to the tip of the main root. To measure dry weight, plants were dried in oven with 72°C temperature for 72 hours.

Chlorophyll Content (a, b, and total chlorophyll)

0.2 g of fresh leaves were grinded within 10 mL of 99% methanol, then centrifuged at 3000 rpm for 5 min. The extract was used to measure light absorption at 653, 470, and 666 nm (Lutts et al., 1996). The following equations were used for calculating chlorophyll content:

CHLa= 15.65 A666-7.34 A653

CHLb= 27.05 A653-11.21 A666

Cx+c= 1000 A470 -2.860 CHLa- 129.2CHLb

CHLt= CHLa+CHLb+Cx+c

where CHLa; chlorophyll a, CHLb; chlorophyll b, Cx+c; carotenoid, and CHLt; total chlorophyll.

Relative Water Content (RWC)

The Sanchez described method (Sánchez et al., 1998) was followed to evaluate plant water state. First, the fresh weight (FW) of leaves measured and then turgor weight (TW) of samples was measured after soaking within distilled water for 12 h at room temperature. Subsequently, samples located at 75°C and their dry weight (DW) was measured. The following equation was used for calculating RWC:

RWC%= [(FW-DW)/(TW-DW)] ×100

SPAD index

The SPAD index of leaves were measured by SPAD-502 in three times (Konica, Minolta, Tokyo). The chlorophyll meter (SPAD 502) is a non-destructive method to determine chlorophyll content. This method estimates the relative quantity of chlorophyll by measuring the transmittance of the leaf at a wavelength of 650 nm and 940 nm (Yamamoto et al., 2002). Because of presence of some differences in regression equations for chlorophyll content and SPAD index, this measure is used as complementary to total chlorophyll content (Fanizza et al., 1991).

Essential Oil Content

To extract essential oil of basil, we used water distillation in which 25 g of leaves and stem tissues dried in oven, were homogenized and boiled in 600 mL of distilled water in Clevenger for 3 hours. Then, water was gently removed from the tank and the amount of extracted essential oil was measured.

Statistical Analysis

The data were analyzed by three-way ANOVA using JMP8—Statistics Software. Treatment means were separated with Duncan's multiple range test (P ≤ 0.05).

Results

Morphological Traits

Interaction effect of three treatments (methionine*humic acid*nitroxin) was significant (P < 0.01) on plant height, number of leaves, leaf length, number of branches, shoot dry weight, shoot diameter, root length, and plant biomass (Table 1). Interaction effect of methionine*nitroxin and humic acid*nitroxin were significant (P < 0.05) on root dry weight (Figure 1).

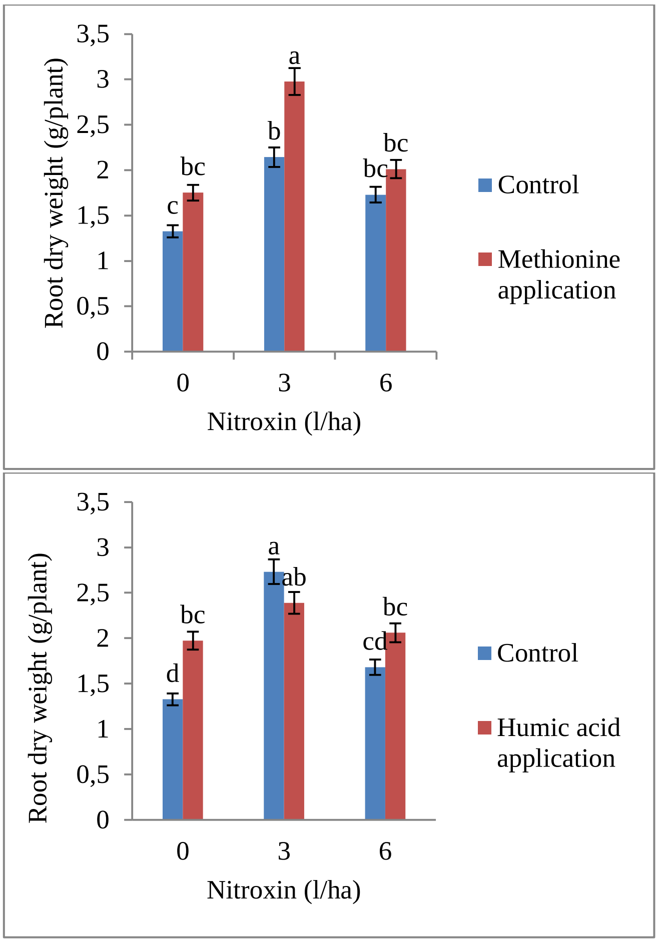

Figure 1. *Interaction effect of methionine*nitroxin and humic acid*nitroxin on the root dry weight of basil (Ocimum basilicum L.). Different letters above each bar indicate significant differences according to Duncan's multiple-range test (P ≤ 0.05).*

The basil height was significantly increased by the combination of 1 l/ha (liter per hectare) of methionine*5 l/ha of humic acid*3 l/ha of nitroxin compared to control plants (+189.19%) and other treatments (~ +102.70%). The highest increase in the combination of 1 l/ha of methionine*5 l/ha of humic acid*3 l/ha of nitroxin treatment was also observed for leaf length (+154%), shoot dry weight (189.36%), shoot diameter (+260%), and root length (+181%). The highest increase for number of leaves and number of branches was observed in the combination of 5 l/ha of humic acid*6 l/ha of nitroxin treatment.

Table 1. *Interaction effects of biofertilizer application (methionine, humic acid, and nitroxin) on the growth parameters of basil (Ocimum basilicum L.).*

Methionine	Humic acid	Nitroxin	Plant height (cm)	Leaves (n/plant)	Leaf length (cm)	Branch (n/plant)	Dry weight (g/plant)	Shoot diameter (mm)	Root length (cm)	Plant biomass (%)
Treatment (liter/hectare)			**Growth parameters**							
0	**0**	**0**	49.33f	38.33f	4ef	9f	5.55d	1.60f	27h	30.08e
		3	73.33bc	61.66a	7.50a	9.66ef	5.50d	2.83cd	39cde	48ab
		6	56ef	54.66bc	5.33bcde	14.33ab	11a	2.90bcd	34.33fg	38.66d
	5	**0**	60de	50cd	5.50bcd	13bc	7.66bc	2.12ef	36def	43.08c
		3	68cd	51cd	6.66ab	15a	9.53ab	3.33bc	40bcd	38.84d
		6	59.33de	57.66ab	6bc	15a	6.72cd	3.50b	34fg	45.16bc
1	**0**	**0**	56ef	50cd	4.75cdef	11de	6.61cd	2.95bcd	31gh	43.33c
		3	61.33de	51.33c	7.48a	13.66abc	7cd	2.78cd	42.33bc	43.33c
		6	59.33de	45.33de	3.66f	12.33cd	6.34cd	2.83cd	33.76fg	44c
	5	**0**	63.33de	42ef	4.50def	13.33abc	8.02bc	2.32de	35.66ef	51.33a
		3	93.33a	45.33de	6.16abc	12.33cd	10.51a	4.16a	49a	51.51a
		6	80b	53.33bc	7.58a	14abc	8.99b	2.83cd	44.07b	45.33ab
Significance										
N			**	**	**	**	*	**	**	**
H			**	ns	*	**	**	**	**	**
M			**	**	ns	ns	ns	ns	**	**
N*H			ns	**	**	ns	**	*	ns	**
N*M			ns	**	ns	**	ns	**	ns	**
H*M			**	ns	ns	**	ns	ns	*	ns
N*H*M			**	**	**	**	**	*	**	**

Legend: Different letters within each column indicate significant differences according to Duncan's multiple-range test (P ≤ 0.05). ns, non significant, *P < 0.05 and **0.01, indicate level of significance.

For some traits, the highest increase was observed in more than one treatment for example in the term of number of leaves and leaf length, the combination of 5 l/ha of humic acid*6 l/ha of nitroxin treatment and the main effect of 3 l/ha of nitroxin produced same result and no significant difference observed between their mean (Table 1). According to results shown in Table 1, six traits of nine traits showed the highest amount of increase compared to control plants when 1 l/ha of methionine*5 l/ha of humic acid*3 l/ha of nitroxin was applied.

Data analysis showed the highest dry weight of root compared to control plants (+209%) and other treatments (~ +150%) in the combination of 1 l/ha of methionine*3 l/ha of nitroxin and the combination of 5 l/ha of humic acid*3 l/ha of nitroxin treatments (Figure 1). The main effect of nitroxin at 5 l/ha level and the combination of methionine*nitroxin at 1 and 3 l/ha, respectively, produced same results and no significant difference (P ≤ 0.05) was observed between them.

Basil plants treated with 3 l/ha of nitroxin, 1 l/ha of methionine, methionine (1 l/ha)*humic acid (5 l/ha)*nitroxin (3 and 6 l/ha) had a highest amount of plant biomass (shoots, roots, and leaves) (Table 1). Under these treatments, plants showed about +171% increases compared to control group.

Physiological Traits

The effect of methionine (0 and 1 l/ha), humic acid (0 and 5 l/ha), nitroxin (0, 3, and 6 l/ha) and their combination on physiological characteristics are reported in Table 2 and Figure 2.

Interaction effect of methionine*humic acid*nitroxin was significant (P < 0.01) on chlorophyll b, total chlorophyll and SPAD index, and also was significant on relative water content at 0.05 level. In the term of chlorophyll a, the combinations of methionine*nitroxin and humic acid*methionine were significant (P < 0.01). The treatments including 3 l/ha of nitroxin, 1 l/ha of methionine*3 and 6 l/ha of nitroxin, and 1 l/ha of methionine*5 l/ha of humic acid*3 and 6 l/ha of nitroxin significantly increased chlorophyll b (~ +300%), total chlorophyll (~ +240%), relative water content (~ +169%) and SPAD index (~ +174%) compared to control plants. Chlorophyll a was increased about +197% and +166% as result of interaction effect of methionine*nitroxin and humic acid*nitroxin compared to control plants.

Table2. *Interaction effects of biofertilizer application (methionine, humic acid, and nitroxin) on the physiological parameters of basil (Ocimum basilicum L.).*

Treatment (liter/hectare)			**Physiological parameters**			
Methionine	**Humic acid**	**Nitroxin**	**Chlorophyll b (mg/gFW)**	**Total chlorophyll (mg/gFW)**	**Relative water content (%)**	**SPAD index**
0	**0**	**0**	1.28e	5.35d	48.04e	31.66e
		3	6.57a	12.87a	77.96ab	37.06d
		6	3.04cd	10.65bc	66.32cd	42.23bc
	5	**0**	3.21cd	9.37c	66.12cd	42.86bc
		3	3.25de	10.22bc	74.07bc	39.90cd
		6	4.12bc	12.91a	68.13cd	44.56b
1	**0**	**0**	4.32bc	10.24bc	62.08d	39.33cd
		3	3.37cd	12.25ab	73.49bc	42.60bc
		6	3.55cd	11.54ab	83.28a	39.20cd
	5	**0**	5.38ab	11.19abc	72.90bc	44.46b
		3	3.91bcd	13.27a	81.14ab	54a
		6	3.63bcd	10.34bc	73.84bc	42.26bc
Significance						
N			ns	**	**	**
H			ns	ns	*	**
M			ns	**	**	**
N*H			**	*	**	*
N*M			**	**	ns	**
H*M			ns	ns	ns	ns
N*H*M			**	**	*	**

Legend: Different letters within each column indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$). ns, non significant, *$P < 0.05$ and **0.01, indicate level of significance.

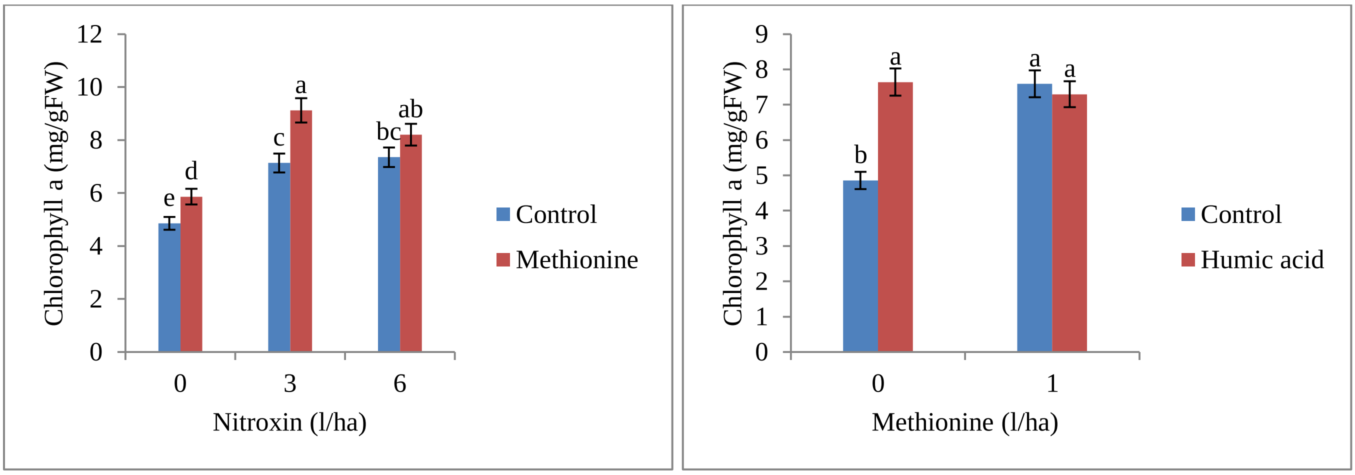

Figure 2. *Interaction effect of methionine*nitroxin and humic acid*methionine on the chlorophyll a content of basil (Ocimum basilicum L.). Different letters above each bar indicate significant differences according to Duncan's multiple-range test ($P \leq 0.05$).*

Essential Oil Content

Interaction effect of biofertilizer application (Methionine, humic acid, and nitroxin) had a significant effect on essential oil of bail (P < 0.05). As shown in Figure 3, the combination of 1 l/ha of methionine*5 l/ha of humic acid*3 l/ha of nitroxin produced the highest content of essential oil (leaves and shoots) compared to other treatments. The content of essential oil was increased about 269% (~ +2.5 fold) compared to control plants.

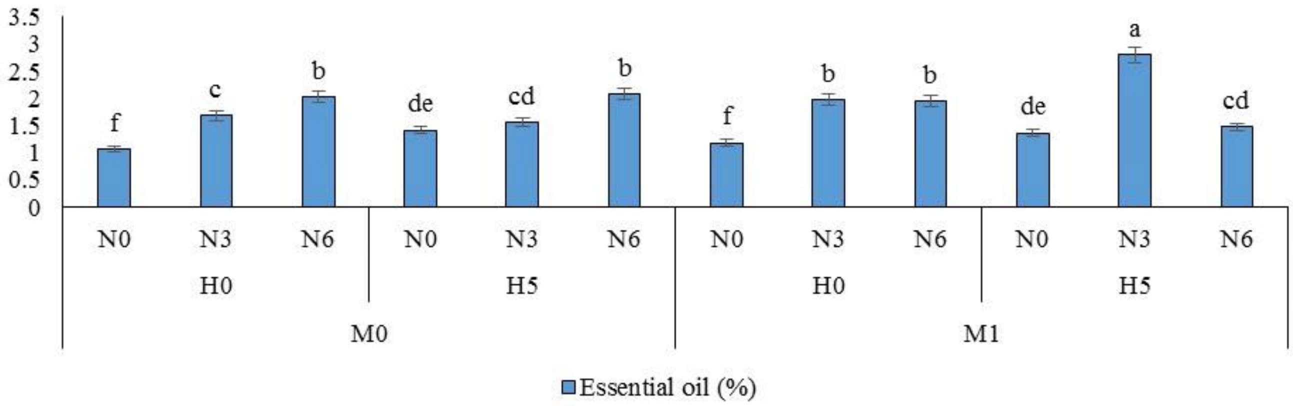

Figure 3. *Interaction effect of biofertilizer application (Methionine, humic acid, and nitroxin) on the essential oil content of basil (Ocimum basilicum L.). Different letters above each bar indicate significant differences according to Duncan's multiple-range test (P ≤ 0.05).* ***Legend:*** N: nitroxin, H; humic acid, M; Methionine, N0, 3, and 6; concentrations of nitroxin in 0, 3, and 6 liter per hectare, H0 and 5; humic acid in 0 and 5 liter per hectare, M0 and 1; Methionine in 0 and 1 liter per hectare.

Discussion

Basil (*O. basilicum*), as one of the most important medicinal plants, is commonly cultivated as commercial plants in many countries (Hussain et al., 2008). Essential oils and herb extracts of basil have natural antioxidant potentials and biologically active compounds (Hussain et al., 2008; Lee et al., 2005). Moreover, because of a specific aroma and flavor, this plant is used in food industries as a spice (Lee et al., 2005). These properties along with many others potentials have converted basil to one of plants that day by day its cultivation increases (Lee et al., 2005; Simon et al., 1990). Considering the importance of basil and need to increase the yield and performance of this plant as well as to the best of our knowledge, effects of using biofertilizers (methionine, humic acid, and nitroxin) and their combination in different concentrations were investigated.

There are not reports studying the effects of methionine, humic acid and nitroxin on basil's growth parameters, therefore discussion of our study findings is based on the results available from other plant species. Biofertilizers have been found to promote the morphological traits in many medicinal plants, in terms of promoted and increased plant height, number of leaves and branches, shoot and root dry weight, and root length (Atiyeh et al., 2002; Das et al., 2007; Gharib et al., 2011; Nurzyńska-Wierdak, 2013; Yadegari & Mosadeghzad, 2012). In this study, the application of biofertilizers (methionine, humic acid, and nitroxin) and their combination increased morphological characteristics of basil (Table 1 and Figure 1). There is no report that shows the effects of methionine on growth traits of plants, but Galili and Amir (2013) reviewed studies in which fortifying with methionine increases plant nutritional values. In agreement with our findings, several studies have found that humic acid increases the root growth of maize (*Zea mays*) (Canellas et al., 2002), above-ground parameters of durum wheat (Delfine et al., 2005), growth parameters (plant height, number of leaves and branches) of faba bean and tomato (El-Ghamry et al., 2009; Lazcano et al., 2009). Regarding effects of nitroxin on growth parameters of plants, Rahi (2013a) reported an increase in plant height, dry weight of shoot and root in *Amaranthus retroflexus* treated with nitroxin. In another study, Nejatzadeh-Barandozi and Gholami-Borujeni (2014) studied application of nitroxin in dill (*Anethum graveolens* L.) and its effects on morphological traits, and found that plant height, number of umbel per plant, and number of umbellet per umbel are increased by application of nitroxin.

Our results showed that the combination of biofertilizer improves the growth parameters of plants significantly better and more than treatments that only one biofertilizer applied (Table 1 and Figure 1). In agreement with this finding, Kholdi et al. (2015) reported that the combination of humic acid*nitroxin increases shoot height, shoot dry weight, root dry weight, and number of pods in faba bean compared to faba bean that only treated with humic acid or nitroxin. Same results have been reported for Ajowan (*Carum copticum* L.) (Barghamadi, 2013) and oilseed crops (Yadav et al., 2018).

The chlorophyll content is one of the important factors for maintaining photosynthetic capacity of plants and correlates with promoted growth of plants (Jiang and Huang, 2001; Sairam et al., 2002). SPAD index is a logarithmic scale of chlorophyll content which are directly associated with the efficiency and capacity of the photosynthetic apparatuses. Moreover, it determines the greenness and thickness of leaves and provides information about changes in pigmentation caused by an increase in chlorophyll content under a long-term stress factor (Richardson et al., 2002). Moreover, relative water content is one of the important indices that being used for assessing effect of factors on plants (Matin et al., 1989). In line with our results (Table 2 and Figure 2), other studies have indicated the positive effects of biofertilizers (humic acid and nitroxin) on the physiological parameters (Karakurt et al., 2009; Nikbakht et al., 2008; Rahi, 2013a). Karakurt et al. (2009) reported that humic acid application using two methods including foliar and soil fertilization positively influences chlorophyll content, especially chlorophyll b. A study on *Amaranthus retroflexus* showed that nitroxin improves chlorophyll a, b, and total chlorophyll (Rahi, 2013b). The positive effects of humic acid in combination with nitroxin on physiological parameters (chlorophyll content and relative water content) have been reported in faba bean (Kholdi et al., 2015), Ajowan (*Carum copticum* L.) (Barghamadi, 2013), and capsicum (Meena, 2015).

According to findings by Azhgan (2016) for sweet basil and by Barghamadi and Najafi (2016) for Ajowan (*Carum copticum* L.), our treatments using biofertilizers increased essential oil in basil (Figure 3). Rahimi Shokooh et al. (2013) reported that nitroxin and nitroxin in combination with other biofertilizers (super-nitro plus and barvar II) improve essential oil. In another study, Azhgan (2016) reported that application of humic acid at both 3 and 6 l/ha and in combination with nano fertilizer (Pharmks®) significantly improves biochemical characteristics of basil. In this study, we found that 5 l/ha of humic acid*6 l/ha of nitroxin remarkably increases essential oil compared to control plants (~+1.6 fold), and when 1 l/ha of methionine added to this treatment (1 l/ha of methionine*5 l/ha of humic acid*3 l/ha of nitroxin, Figure 3) the essential oil are increased by +2.5 fold compared to control plants.

Conclusion

Therapeutic potentials and its use in food industries have converted basil to a widely cultivated medicinal crop. Therefore, it is important that yield and performance of basil are increased. Based on our data, application of biofertilizers (methionine, humic acid, nitroxin) improved the growth and physiological as well as essential oil of basil. The combinations of these biofertilizers were more efficient, especially the combination of 1 l/ha of methionine*5 l/ha of humic acid*3 l/ha of nitroxin in which the highest morphological/physiological traits and essential oil were observed. Overall, it is suggested that the combination of methionine*humic acid*nitroxin could be an efficient and promising material to improve the growth of basil, especially its essential oil.

Conflict of Interest

Authors declare that there is no conflict of interest.

Authors' contributions

All authors contributed equally to this work.

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