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THE EFFECT OF <u>HUMIC</u> ACID, PLANT GROWTH PROMOTING RHIZOBACTERIA AND SEAWEED ON GROWTH PARAMETERS, <u>ESSENTIAL OIL</u> AND CHLOROPHYLL CONTENT IN <u>SWEET</u> BASIL (OCIMUM BASILICUM L.)

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Abstract

Effect of Humic acid, plant growth promoting rhizobacteria and seaweed extract on growth parameters, essential oil and chlorophyll content in sweet basil (Ocimum basilicum L.) has been evaluated. The experiment was conducted in the form of factorial in randomized complete block design (RCBD) in the Research field of Russian State Agrarian University - Moscow Timiryazev Agricultural Academy in 2018. The treatments were: A) Humic acid at three levels (a_1 = nonuse, a_2 = foliar application [3 lit/ha], a₃= soil application [6 lit/ha], B) plant growth promoting rhizobacteria with two levels (b_1 = nonuse, b_2 = use [6lit/ha], C) seaweed extract (c_1 = nonuse, c_2 = soluble in irrigation water [1.5 lit/ha]). The results illustrated that the application of Humic acid causes increasing of all the traits studied. The highest amount of essential oil content (1.1%), fresh weight (0.9 kg/m^2) , dry weight (254.06 gr/m²), chlorophyll spad (10.98), height of the plants (48.62 cm), their root length (22.09 cm), shoot diameter (4.64 mm) and leaf width (3.08 cm) were observed at 6 lit/ha Humic acid. The results obtained also showed that application of seaweed extract results in increasing the fresh weight (0.89 kg/m²), chlorophyll spad (11.54), height (47.87 cm), root length (22.34_cm), shoot diameter (4.44mm), and leaf width (3.02_cm) of the plants. Plant growth promoting rhizobacteria had no significant effect on evaluated traits except essential oil content. Comparison of interaction effects between Humic acid levels, plant growth promoting rhizobacteria and seaweed extract showed that among the treatments, $a_3b_2c_2$ (soil application of Humic acid 6 lit/ha + plant growth promoting rhizobacteria and seaweed) caused the maximum expression of all the traits evaluated. Overall results showed that the effect of Humic acid on the traits studied was much more than that of plant growth promoting rhizobacteria and seaweed extract.

Keywords: sweet basil, Humic acid, plant growth promoting rhizobacteria, seaweed extract, growth parameters

Introduction

<u>Sweet basil</u> has been used for many years as a medicinal plant in traditional medicine to treat headaches, cough, parasitic diseases, and disorders of the kidney. The external uses of the plant can refer to <u>its</u> use as a tourniquet in place of stings and applying <u>the</u> oil directly on the skin to treat acne disease. <u>Mainly due to its</u> active ingredients (essential oils), <u>insect repelling properties and anti-parasitic and antibacterial activities, this plant is widely used in a number of industries, including the food, cosmetics, and pharmaceutical industries. <u>Sweet basil</u> is an annual herb which is economically <u>the</u> most important species among other species of basil. Nowadays it is cultivated and utilized in almost all warm temperate regions as a herb, spice and fresh vegetable plant [1].</u>

Humic <u>acid</u> is one of the most abundant organic constituents, which <u>occurs</u> in soils and other environments such as streams, oceans and lakes. <u>It</u> is a principal component of humic substances which are formed in the process of decomposition of organic matter in the soil. The effect of Humic <u>acid</u> on soils is undoubtedly improvement of <u>their</u> physical, chemical and biological properties, according to other studies [2,_3,_4]. Humic <u>acid</u> plays an extremely beneficial role in plant growth as a complementary agent in <u>both</u> organic <u>and</u> chemical fertilizers. It has been shown that humic substances (HS) act as a positive regulator of plant growth and enhance root, leaf and shoot growth as well as fruit yield [5]. Furthermore, an enhancement in yield of various plant species such as vegetables, root crops, flowers and cereals by humic substances have been reported [6].

During the last centuries, seaweeds have widely been used as a fertilizer in farming lands, both in agriculture and horticulture, particularly in Britain, France, Spain, Italy, Japan, and China. The results of many studies conducted recently have shown widespread use of marine macroalgae as a fertilizer in modern agriculture. It is reported that seaweeds contain all the required trace elements and plant growth hormones and seaweed manure is rich in potassium and poor in nitrogen and phosphorus. A positive effect of several species of algae (Ascophyllum spp., Laminaria spp., Ecklonia sp.) on the growth, development and, consequently, yields of field crops have been proved so far. Seaweeds constitute a source of many substances, valuable from the point of view of plant physiology, which particularly help plants adapt to stressful conditions. Biologically active Algonac acids, polyphenols, free amino acids, and particularly natural plant phytohormones: auxins, cytokinins, intensify plant growth, increase the root weight, and induce photosynthetic processes and as a result improve plant development [7, 8, 9, 10, 11]. In some findings, the authors particularly stress the importance of cytokines contained in seaweeds in creating plant resistance to diseases and nematodes [12, 13, 14]. Plant growth promoting rhizobacteria (PGPR) are groups of bacteria that actively colonize plant roots and are so useful for plant growth. The mechanism by which PGPR promote plant growth is still unknown, but it includes the ability to produce

phytohormones, a symbiotic <u>nitrogen</u> fixation, synthesis of antibiotics, enzymes and fungicidal compounds. It has been stated that application of PGPR enhanced the growth and yield of several major crops [15].

Material and methods

The experiment was designed as factorial in randomized complete block design (RCBD) with four replications per treatment. Prior to testing, the soil in the surface 0 - 30 cm layer was randomly sampled and sent to the laboratory in order to determine the soil physical and chemical properties (Table 1).

Table 1- Physical and chemical characteristics of the soil

zinc	Potash	Phosphate	Nitrogen	pH	EC_(ds/m)	Clay (%)	Silt (%)	Sand (%)
	(ppm))						
1	260.4	11.2	17	8	3.65	5	35	60

Experimental treatments were Humic <u>acid</u> in three levels including {A₁: nonuse (control), A₂: Foliar application at 3 liters per hectare and A₃: soil application at 6 liters per hectare}, PGPR treatment in two levels of {B₁: nonuse and B₂: consumption of 6 liters per hectare that PGPRs were including Azotobacter, Azospirillum and pseudomonas}. In this experiment seaweed was used in two different levels {C₁: nonuse, C₂: the use of 1.5 liter per hectare in irrigation water}, the Humic acid was used in this experiment was 80% and named Humax 95-WGS (Table 2). All three bacteria used in this study were native of Iran and they were separated and purred in Water and Soil Institute in Iran. The cultivation was in the form of ditch and hill. In this research, the distance between rows was 20 cm and <u>each plot had two rows of plants</u>.

Table 2- Characteristics of Humic acid used in the experiment

Trade name	Fulvic acid	K2O	Humic acid
Humax 95-WGS	15%	5%	80%

The seeds were mixed by PGPRs and then <u>planted</u> in <u>each</u> plot by the distance of 5_cm in a row and buried in the soil in depth of 0.5 to 1 cm. The <u>plantings</u> were irrigated immediately after <u>sowing</u> and <u>every four days thereafter</u>. Humic acid and seaweed <u>were applied</u> every 15 days. Four plants

in <u>each</u> row <u>were randomly chosen</u> and tagged for data recording purpose <u>[16]In</u> measure dry weight, samples were placed in oven for 48 hours <u>at</u> 70° C. Moreover, Minolta SPAD-520 chlorophyll meter was <u>used</u> to <u>determine the content of chlorophyll in the leaves</u>. <u>Selection</u> of the plants for measurements was randomized. This experiment was repeated at least for 3 random plants in each plot.

Statistical analysis

One-way ANOVA was applied to analyze the obtained data using SAS software (Version 9.1) [17]. For each source of treatment, the means of each trait were separated statistically using Duncan's multiple range test, with significance defined as $P \le 0.05$.

Essential oil

Table (3) shows that Humic acid and plant growth promoting rhizobacteria had significant effect on essential oil (P \leq 0.05). <u>Its content</u> ranged from 0.96 to 1.10%<u>increasing gradually with each increase in the level of Humic acid. Application of plant growth promoting rhizobacteria also had a positive effect on the essential oil content in the plants and increased it to 1.96%. As indicated in Table (3), there were no significant differences among seaweed extract treatments regarding the essential oil <u>content</u>. The effect of interaction between Humic acid, <u>plant</u> growth promoting rhizobacteria and seaweed extract on essential oil <u>content</u> is shown in Table (4). High Humic acid level (6 lit/ha) <u>in combination with</u> plant growth promoting rhizobacteria and seaweed extract had the highest essential oil of dill plants by 1.43%. Under organic conditions essential oil of <u>sweet</u> basil was two times as <u>high as in the case of mineral fertilizers application [18]</u>. It has been reported that of biological fertilizers <u>show a positive effect on</u> essential oil <u>content in plants [19, 20]</u>. Moreover, <u>some other studies have shown</u> that organic fertilizers can raise <u>the</u> amount of essential oils in medicinal plants [21, 22].</u>

Fresh and dry weight

Results in Table (3) indicate that Humic acid, plant growth promoting rhizobacteria and seaweed extract had significant effect on fresh weight (P \leq 0.01). Fresh weight increase at 6_lit/ha with 0.9 (kg/m²) as compared to <u>the</u> controls. The plants grown in plots <u>with application of</u> seaweed extract and plant growth promoting rhizobacteria had significantly more fresh weight with 0.89 and 0.87 (kg/m²) in compared to <u>the</u> control, respectively. As <u>it</u> is seen in table (3), humic acid led to increase in dry weight of <u>sweet</u> basil (P \leq 0.01). Seaweed extract and plant growth promoting rhizobacteria had significant effect on dry weight. The highest amount of dry weight was found

at 6 lit/ha Humic acid with 254.06 (gr/m^2). According to Table (4), the high Humic acid level (6 lit/ha) in combination with plant growth promoting rhizobacteria and seaweed extract had the highest fresh and dry weight of dill plants by 0.99 and 273.8_(gr/m^2), respectively. There are several reports regarding the action mechanisms of humic substances which are divided into two groups: 1) direct effect on the plants and 2) indirect mechanisms through influencing the microorganisms in the soil, uptake of nutrients from the soil as well as physical properties of the soil.

Moreover, c <u>Humic</u> acid can act like a hormone and may exert its beneficial effects on the plant. Nevertheless, due to its instability in the <u>soil</u> it is assumed that another growth regulator like IAA, might <u>be</u> absorbed by humus_and as a result <u>promote</u> the growth of the plant. These results are consistent with the results of other researchers [23, 24, 25]. Nelson (2004) showed that PGPR has positive effects on plant growth. It has been also reported that PGPR promotes the growth and development of <u>sweet</u> basil through <u>stimulation of IAA synthesis</u>, <u>dissolution</u> of phosphorus <u>in the</u> <u>soil</u> and <u>increasing the</u> resistance <u>of plants</u> to pathogens and pests [26].

Spad

As indicate in Table (3), Humic acid and seaweed extract had significant effect on chlorophyll spad ($P \le 0.01$). Application of Humic acid at 6_lit/ha __caused essentially increasing of chlorophyll spad, i.e. by 10.98. Also, the plants grown in plots which received seaweed extract had significantly more chlorophyll spad with 11.54 compared to the control (Table 3). According to the table (4)₁ high Humic acid level (6 lit/ha) in combination with plant growth promoting rhizobacteria and seaweed extract had the highest chlorophyll spad of sweet basil plants, by 14.91Humic acid seems to stimulate chlorophyll synthesis or to arrest the degradation of the chlorophyll in sweet basil plant and thereby to affect the chlorophyll content [2]. In addition to this, seaweed extracts possess remarkable amount of hormones such as auxins, cytokines and betaines which can positively influence the synthesis of chlorophyll in the leaves [27]. Our findings are in accordance with previous findings of Whap ham et al. (1993), who observed that application of seaweed (*Ascophyllum nodosum*) increased the chlorophyll content in cucumber seedlings and tomato plants [28]. Also, seaweed extract spray enhanced the leaf chlorophyll in plants [7]. Recently Thirumaran et al. (2009) on *Cyamopsis tetragonoloba* indicated that seaweed extract increased photosynthetic pigments such as chlorophyll a, b and total chlorophyll and carotenoids [29].

Plant height

Results of the measurements of plant height are given in Table 3. Plant height was significantly affected by Humic acid and seaweed extract (P≤0.01). Plant height increased at 6 lit/ha with 48.62 (cm). Applications of seaweed extract led to rise in plant height with 47.87 (cm). Plant growth promoting rhizobacteria had no significant effect on plant height (Table 3). The effect of interaction between Humic acid, plant growth promoting rhizobacteria and seaweed extract on plant height is shown in Table (4). Application of high Humic acid level (6 lit/ha) in combination with plant growth promoting rhizobacteria and seaweed extract resulted in the highest plants, by 54.13 (cm). The increase of the height of plants can be explained by the fact that Humic acid makes nutrients in the soil readily available for plants as well as due to its growth and development promoting bioactivity. [30]. Moreover, humic acid enhances the soil porosity and significantly affects the development of the root which in turn can induce the shoot growth [31]. It has been also noted that humic substance has a remarkable impact on physical and chemical properties of the soil and consequently ameliorate the mineral nutrients uptake and aerial soil properties [32]. Meanwhile, an observed enhance in plant height in our study might be due to presence of the auxins in seaweed extract which can induce cell division and elongation and as a result enhance the shoot growth, leaf size and fresh and dry weigh [33]. Our findings are in agreement with the results of other researchers [34, 35].

Root length, shoot diameter and leaf width

The data presented in Table-3 illustrated that the effect of <u>Humic_acid application and seaweed</u> extract on <u>root_length</u>, <u>shoot_diameter</u> and leaf width was significant ($P \le 0.05$). Plant growth promoting rhizobacteria had no significant effect on <u>the above_mentioned</u> traits (Table 3). Consumption of Humic acid at 6_lit/ha <u>led</u> to increase in <u>root_length</u>, <u>shoot_diameter</u> and leaf width with 22.9 (cm), 4.64 (mm) and 3.08 (cm), respectively. The plants grown in plots received seaweed extract had significantly more root length, shoot diameter and leaf width 22.34 (cm), 4.44 (mm) and 3.02(cm), respectively. The effect of interaction between humic acid, plant growth promoting rhizobacteria and seaweed extract on root length, shoot diameter and leaf width is shown in Table (4). High Humic acid level (6 lit/ha) and consumption of plant growth promoting rhizobacteria and seaweed extract had the highest root length, shoot diameter and leaf width of dill plants by 24.62 (cm), 5.21 (mm) and 3.15 (cm), respectively. Application of the humic acid and seaweed remarkably enhanced the above traits. An increase in growth parameters of <u>sweet_basil</u> by application of humic acid can be partially ascribed to the better absorption of nutrients by the plant. It has been stated that <u>application_of</u> humic acid enhanced the nitrogen uptake by the roots of olive plants [36]. According to the previous published reports, favorable effects of humic acid on plant

growth and development is attributed to its hormone-like mode of action which implicate in several aspects of plant metabolisms including cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and various enzymatic reactions [37, 38, 39]. It is assumed that the mechanism of the action of humic acid on plant growth is identical to that of plant hormones, but it is still not clear that humic acid possess hormone-like components [39] although it has been shown that humic acid might have these components [40]. Apart from humic acid, seaweed extracts also have cytokines and can influence various physiological activities such as chlorophyll synthesis which leads to an increase in photosynthesis and as a result induces the growth of the plant [41]. The presence of macronutrients such as nitrogen, potassium and phosphorous in seaweed extractcan also enhance the growth parameters of the plant [42].

Conclusion

This experiment <u>has</u> uncovered the physiological mechanisms by which <u>Humic</u> acid and seaweed extract can influence plant growth and <u>their</u> essential oil <u>content</u> positively and produce significant increases in measured traits. <u>Humic</u> acid and seaweed <u>were shown to be</u> more effective than plant growth promoting rhizobacteria <u>as to</u> studied traits in <u>sweet</u> basil plant. According to the above findings, the use of <u>Humic</u> acid and seaweed extract <u>due to their</u> less harmful and <u>more</u> positive effects on plant growth <u>are to be considered as effective means for replacing expensive</u> chemical fertilizers <u>with</u> sustainable cultivation of <u>sweet</u> basil in Iran and other developing countries.

Tuble 5 Effect of fluine acta levels, plant growth promoting finzoodeteria and seaweed extract on studied thats of sweet basin plant

	Essential oil	Fresh weight	Dry weight	Chlorophyll	Plant height	Root length	Shoot diameter	Leaf width
Source of variation	(%)	(kg/m ²)	(kg/m ²)	spad	(cm)	(cm)	(mm)	(cm)
Humic acid								
0	0.962 ^b	0.77 ^b	239.063 ^c	8.326 ^c	44.344 ^b	17.001 ^c	4.187 ^b	2.875 ^b
3_lit/ha	0.979 ^b	0.78^{b}	247.813 ^b	9.954 ^b	47.922 ^a	20.244 ^b	4.274 ^b	3.073 ^a
6_lit/ha	1.108^{a}	0.90 ^a	254.063 ^a	10.988 ^a	48.625 ^a	22.909 ^a	4.644 ^a	3.088 ^a
PGPR								
0	0.971 ^b	0.77 ^b	246.66 ^a	7.9652 ^b	46.823 ^a	21.986 ^a	4.244 ^a	3.011 ^a
6_lit/ha	1.961 ^a	0.87 ^a	247.292 ^a	8.5483 ^a	48.104 ^a	22.316 ^a	4.492 ^a	3.013 ^a
Seaweed extract								
0	0.973 ^a	0.70 ^b	246.66 ^a	7.3958 ^b	45.052 ^b	19.762 ^b	3.291 ^b	2.096 ^b
1.5_lit/ha	1.060 ^a	0.89 ^a	247.29 ^a	11.547 ^a	47.875 ^a	22.340 ^a	4.445 ^a	3.028 ^a
Mean of square								
H×PGPR	$0.025^{n.s}$	0.73 ^{n.s}	525**	46.681 [*]	83.816 ^{n.s}	4.979 ^{n.s}	$0.777^{n.s}$	$0.009^{n.s}$
H×seaweed	0.157**	0.368 ^{n.s}	131.25 ^{n.s}	28.039 ^{n.s}	61.759*	7.642 ^{n.s}	$0.495^{n.s}$	0.4^{*}
PGPR×seaweed	$0.003^{n.s}$	3.956*	379.688 ^{n.s}	44.382^{*}	20.345 ^{n.s}	0.154 ^{n.s}	$0.144^{n.s}$	0.103 ^{n.s}
H×PGPR×seaweed	0.188^{*}	1.293*	1918.75**	51.29*	31.103*	24.779 [*]	0.462^{*}	0.416^{*}
Error	0.028	0.558	94.46	10.6	15.708	5.574	0.262	0.051

Means with the same letter in a column are not significantly different according to DMRT at 1% level of probability.

H: Humic acid, PGPR: Plant growth promoting rhizobacteria

ns, * and ** are not significant and significant at 5 and 1% level of probability, respectively

Table 4- Interaction Effect between humic acid levels, plant growth promoting rhizobacteria and seaweed extract on studied traits of basil.

	Mean							
	Essential oil	Fresh weight	Dry weight	Chlorophyll	Plant height	Root length	Shoot diameter	Leaf width
Treatment	(%)	(kg/m^2)	(kg/m^2)	spad	(cm)	(cm)	(mm)	(cm)
a1b1c1	0.908 ^b	0.531 ^c	230 ^c	5.761 ^e	43.56 ^{bc}	19.08 ^c	4.118 ^{ab}	2.577 ^b
a1b1c2	0.931 ^b	0.583 ^{bc}	232 ^c	7.39 ^b	47.75 ^{abc}	21.73 ^{abc}	4.39 ^{ab}	3.123 ^a
a1b2c1	1.103 ^b	0.818 ^{ab}	251.3 ^{ab}	10.97 ^d	43.38 ^{bc}	21.32 ^{abc}	3.877 ^b	2.777 ^{ab}
a1b2c2	0.907 ^b	0.804 ^{ab}	243.8 ^b	9.19 ^c	42.69 ^c	21.26 ^{abc}	4.71 ^{ab}	3.023 ^{ab}
a2b1c1	0.932 ^b	0.810 ^{ab}	245.3 ^b	9.879°	46.94 ^{abc}	22.69 ^{abc}	4.025 ^b	3.135 ^a
a2b1c2	1.085 ^b	0.873 ^a	246.3 ^{ab}	7.693 ^d	43.88 ^{bc}	19.69 ^{bc}	4.398 ^{ab}	3.055 ^{ab}
a2b2c1	0.981 ^b	0.878 ^a	261.3 ^a	11.26 ^b	51.5 ^{abc}	23.42 ^{ab}	4.322 ^{ab}	3.01 ^{ab}
a2b2c2	1.034 ^b	0.839 ^{ab}	260 ^a	11.48 ^b	52.19 ^{ab}	23.79 ^a	4.003 ^b	2.91 ^{ab}
a3b1c1	0.938 ^b	0.854^{ab}	242.5 ^{ab}	7.609 ^d	47.75 ^{abc}	22.60 ^{abc}	4.193 ^{ab}	3.14 ^a
a3b1c2	0.974 ^b	$0.957^{\rm a}$	242.5 ^{ab}	9.46 ^c	45.06 ^{bc}	22.11 ^{abc}	4.34 ^{ab}	3.048 ^{ab}
a3b2c1	0.973 ^b	0.912 ^a	246.3 ^{ab}	11.59 ^b	44.75 ^{bc}	22.31 ^a	4.83 ^{ab}	3.115 ^a
a3b2c2	1.434 ^a	0.990^{a}	273.8 ^a	14.91 ^a	54.13 ^a	24.62 ^a	5.21 ^a	3.152 ^a

Means with the same letter in a column are not significantly different according to DMRT at 1% level of probability.

a: Humic acid{ a1= control, a2= foliar application 3lit/ha, a3= soil application 6lit/ha}

b: Plant growth promoting rhizobacteria{b1= nonuse, b2= 6lit/ha}

c: Seaweed{c1= nonuse, c2= 1.5lit/ha}



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