

Effect of Seaweed Extract, Humic Acid and Chemical Fertilizers on Morphological, Physiological and Biochemical Characteristics of *Trigonella foenum-graecum* L.

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ABSTRACT

Modern agriculture is searching for new biotechnologies that would allow for a reduction in the use of chemical inputs without negatively affecting crop yield or farmers' income. Seaweed extract and humic acid are used as nutrient supplements, biostimulants, or biofertilizers in agriculture and horticulture to increase plant growth and yield. To investigate the effects of SeaWeed Extract (SWE), humic acid, and chemical fertilizers on the growth, physiological and biochemical characteristics of *Trigonella foenum-graecum* L., a greenhouse experiment was conducted in 2016. Results showed that foliar applications of seaweed extract enhanced growth parameters. Among the different treatments, the plants that received SWE showed maximum shoot lengths, fresh and dry weights, number of pods per plant, chlorophyll "a", chlorophyll "b", total chlorophyll, and carotenoids. Also, application of SWE increased the amount of total phenolic and flavonoid contents in fenugreek. All fertilizer treatments increased significantly 2,2-DiPhenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity of the plants in comparison to the control. Plants treated with SWE showed stronger activity against α -glucosidase with IC_{50} value of $171.37 \mu\text{g mL}^{-1}$ in comparison with acarbose ($IC_{50}= 19.7 \mu\text{g mL}^{-1}$) as the reference α -glucosidase inhibitor. The data generated by this study revealed that SWE could be used as foliar spray to maximize the quality and quantity of fenugreek.

Keywords: Antioxidant activity, Biofertilizers, Fenugreek, Phenolic compound

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual crop belonging to the Fabaceae family. This crop is native to an area extending from Iran to Northern India, but is now widely cultivated in China, North and East Africa, Ukraine and Greece (Petroponlas, 2002). Its seeds are a good source of protein, vitamins, alkaloid *trigonellin*, and essential oil, with immense medicinal value, particularly against digestive disorders (Rizvi *et al.*, 2013). The leaves of fenugreek plant are edible and often used as a vegetable dish in Iran. It is

also widely used in traditional medicine as tonics, as a remedy against stomach disorders, diabetes, fever, anaemia, constipation and as a galactagogue as well as for stimulation of appetite. It also has been used in alleviating high cholesterol plasma levels, diabetes and oxidative damage (Acharya *et al.*, 2007).

Any improvement in agricultural system that results in higher production should reduce the negative environmental impact of agriculture and enhance the sustainability of the system. One such approach is the use of bio stimulants, which can enhance the effectiveness of conventional mineral fertilizers. Some substances affect plant

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growth and its physiological activities, including Humic Acid (HA) and SeaWeed Extract (SWE). HA and its derivatives are complex compounds that exist in soils with high levels of organic matters and quinine functional groups, which are formed by microbial decay of plant tissues (Zhang *et al.*, 2004; Thygesen *et al.*, 2009). These fertilizers promote plant growth and induce soil microorganisms like bacteria and fungi and provide carbon as a source for the organisms (Leonard, 2008). El-Bassiony *et al.* (2010) stated that growth of green bean organs such as leaf, branch, plant wet and dry weight, height, pods' green yield and their quality such as length and weight, and chlorophyll content of green leaf was increased due to the application of humic acid. Results of a research conducted by Rasaei *et al.* (2012) with regard to the physiological effects of application of humic acid on green peas indicated that use of humic acid exhibited significant effect on value of chlorophyll "a" and "b", compared to those plants that did not receive this substance. Seaweeds are a renewable, competitive local resource along the coastal agricultural area (Hernandez-Herrera *et al.*, 2014). The Southern coast of Iran has abundant seaweed resources with more than 160 species being found in Persian Gulf and Amman Sea. Marine algae are an important source of diverse components, which have beneficial effects in terms of enhancement of plant growth and development (Craigie, 2011; Briceño-Domínguez *et al.*, 2014; Hernández-Herrera *et al.*, 2014), improved tolerance to environment stress (Zhang and Schmidt, 2000; Zhang *et al.*, 2003), and increasing antioxidant properties of plants (Zhang and Schmidt, 2000). SWE contains growth promoting hormones (IAA and IBA, Cytokinins), trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids and also enhances leaf total phenolic, flavonols, and tannin contents and, consequently, increases the antioxidant activity of plants leaf extracts (Krajnc *et al.*, 2012). Also, SWE increases antioxidant

properties of plants. Eris *et al.* (2008) showed that spraying of *Ascophyllum nodosum* extract on pepper plants leads to increase in growth, yield, and concentration of some nutrient elements, significantly. Zhang *et al.* (2003), in a study to test the combined effect of humic acid and SWE on growth and physiology of some creeping herbaceous plants (Bentgrass), found significant positive effects on growth and nutrient content of plants.

The present study was undertaken to investigate the effect of seaweed liquid fertilizers, humic acid, and chemical fertilizer (NPK), on the morphological, physiological, and biochemical characteristics and antioxidant activity of *Trigonella foenum-graecum*.

MATERIALS AND METHODS

A pot experiment was carried out in 2016 at the Agricultural Research Greenhouse at Imam Khomeini International University, Qazvin, Iran. The experimental design was Complete Randomized Design (CRD) with four treatments and seven replications. Treatments included SWE fertilizer (containing *Ascophyllum nodosum* extract), humic acid (containing 15% humic acid and 2% fulvic acid), chemical fertilizers (recommended rates of NPK for fenugreek) and control (without the use of fertilizers). Twenty eight plastic pots (32×30 cm) were prepared and filled with 5 kg of garden soil. The physical and chemical properties of the experimental soil and SWE are shown in Tables 1 and 2, respectively. Seeds were purchased from Isfahan Pakan Bazr Seed Production Company in Iran and sown at a depth of 1.5 cm in each pot. Seedlings were thinned to five plants per pot 10 days after emergence. Irrigation was regularly provided during the vegetative period and all agronomic management practices were performed as needed. In 4 leaves appearance stage; SWE was applied as a foliar spray in concentration of 1 mL L⁻¹ and humic acid was applied dissolved in irrigation water in

Table 1. Physical and chemical properties of experimental soil.

Texture	Clay (%)	Silt (%)	Sand (%)	Available Phosphorus (ppm)	Available Potassium (ppm)	N (%)	OM (%)	PH	EC
Loam	31	35	34	12.7	145	0.08	0.71	7.4	2.31

Table 2. Chemical properties of seaweed extract (SWE) fertilizer.

Amino acid (%)	C (%)	S	Zn	P ₂ O ₅	K ₂ O	N	Fe	Mn	B	Mg
						(mg kg ⁻¹)				
20	15	4000	50	800	4000	4000	400	150	20	1000

concentration of 500 mL L⁻¹; and repeated every 2 weeks until harvest, according to the manufacturer's directions. All amounts of calcium super-phosphate (15% P₂O₅), potassium sulfate (48% K₂O), and half of Urea (46% N) were applied during soil preparation. The remaining half of urea was applied 30 days after sowing.

Determination of Morphological and Physiological Parameters

Growth parameters including plant height, fresh and dry weight were measured at flowering stage and numbers of fruits per plant, number of seed per fruit, and 1,000 seeds weight at seed harvest stage. Photosynthetic pigments i.e. chl. "a", chl. "b", total chlorophyll, and total carotenoids were determined according to the methods described by Von Wettstein (1957).

Extraction of Plant Essential Oil

Dried seeds of fenugreek (20 g, three times) were subjected to hydro distillation for 3 hours using a Clevenger-type apparatus to produce oil according to the method recommended by the European pharmacopoeia, and essential oil percentage was determined.

Total Phenolic Content (TPC)

The TPC of the fenugreek extract was determined by the Folin-Ciocalteu reagent as previously reported, with minor changes

(Salehi *et al.*, 2013). Results are expressed as mg of gallic acid equivalents per g dry matter of extract (mg GAE g⁻¹ DM).

Total Flavonoid Content (TFC)

TFC was estimated by aluminum chloride colorimetric method as previously described, with a few modification (Marija *et al.*, 2014). The total flavonoid content was expressed as mg quercetin per g dry matter of extracts (mg QE g⁻¹ DM) by comparison with the quercetin standard curve.

DPPH Radical Scavenging Activity

The stable 1,1-DiPhenyl-2-PicrylHydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts. The DPPH radical-scavenging activity was determined using the method described by Zengin *et al.* (2015). The results were expressed as equivalents of trolox (mg TAE g⁻¹ DM).

Total Antioxidant Activity (TAC) by Phosphomolybdenum Method

The TAC of fenugreek extracts was spectrophotometrically determined by the phosphomolybdenum assay using the method described by Zengin *et al.* (2015). The antioxidant activity of the extracts were



expressed as mg ascorbic acid equivalents per g of dry matter (mg AAE g⁻¹ DM).

RESULTS

Alpha-Glucosidase Inhibition Assay

The α -glucosidase inhibitory activity of the extracts was assessed according to an earlier reported bioassay method (Asghari *et al.*, 2015). The results were expressed as percent of α -glucosidase inhibition.

Statistical Analysis

All the data were subjected to statistical analysis using SPSS software (IBM SPSS Statistics, version 22). Differences between the treatments were performed by Duncan's Multiple Range Test at 5% confidence interval. Transformations were applied to the data to ensure that the residuals had normal distribution. Correlations were performed using the Pearson's correlation coefficient (r).

Growth Parameters

From the data presented in Table 3, it is clear that the plants height, fresh and dry weight, number of seeds per plant, and 1,000 seeds weight of fenugreek was highly increased as a result of SWE and chemical fertilizer treatments. SWE and chemical fertilizer exhibited the highest shoot length (24.93 and 22.95 cm), respectively, compared to the control (19.3 cm). The plant height increased 29% and 19% by the application of SWE and chemical fertilizers, respectively. Results showed that SWE fertilization increased plants fresh and dry weights in fenugreek from 11.11 and 7.38 g (control), to 14.66 and 9.73 g, respectively (Table 3).

The highest numbers of pods per plant recorded were 12.12 and 11 in the plants that received SWE and NPK fertilizer, which were 63 and 48% higher than the control, respectively. All fertilizer treatments

Table 3. Means comparison of morphological traits of fenugreek.^a

Treatments	Plant height (cm)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Number of fruit per plant	Number of seed per fruit	1000 Seeds weight (g)
SWE	24.93 a	14.66 a	9.73 a	12.12 a	11.5 a	10.22 a
Humic acid	21.92 b	12.43 bc	8.19 bc	8.37 b	11.62 a	9.17 ab
NPK	22.95 ab	13.47 ab	8.62 b	11 ab	11.28 a	9.64 ab
Control	19.3 c	11.11 c	7.38 c	7.43 b	9.14 b	8.23 b

^a Means in each column followed by similar letter(s), are not significantly different at 5% probability level.

Table 4. Means comparison of biochemical traits of Fenugreek.^a

Treatments	Total chlorophyll (mg g ⁻¹ FW)	Chlorophyll "a" (mg g ⁻¹ FW)	Chlorophyll "b" (mg g ⁻¹ FW)	Ascorbic acid (mg g ⁻¹ FW)	Total carotenoids (mg g ⁻¹ FW)	Essential oil (%)
SWE	1.98 a	1.30 a	0.68 a	0.0040 a	0.27 a	0.97 a
Humic acid	1.70 b	1.18 a	0.51 b	0.0034 ab	0.20 b	0.85 a
NPK	1.35 c	0.93 b	0.41 b	0.0032 b	0.16 b	0.88 a
Control	0.96 d	0.78 c	0.18 c	0.0030 b	0.08 c	0.69 b

^a Means in each column followed by similar letter(s), are not significantly different at 5% probability level.

increased number of seeds per pod but there were no significant differences between treatments. SWE also caused 24% increase in 1,000 seeds weight over the control.

Biochemical Traits

The obtained results showed that treatments had significant effect on total chlorophyll, chlorophyll “a” and “b”, total carotenoids, and essential oil content of fenugreek ($P \leq 0.05$) (Table 4). The maximum chlorophyll “a” content was obtained in plants that were treated with SWE and humic acid, with the values of 1.30 and 1.18 mg g^{-1} fresh weight, respectively, which were 66 and 51% increase over the control. The highest chlorophyll “b”, total chlorophyll, ascorbic acid and carotenoids contents were obtained as 0.68, 1.98, 0.0040, and 0.27 mg g^{-1} FW, respectively, in plants that received SWE. An important point is that NPK treatment exhibited significant positive effect on the amount of total chlorophyll, chlorophyll “a”, chlorophyll “b” and total carotenoids of the plant, compared to the control, while its effect on ascorbic acid amount was not significant (Table 4). All of applied nutrition treatments enhanced significantly the essential oil percentage in the plants. As can be seen in Table 4, the highest amount of essential oil is related to SWE with the value

of 0.97%.

Phytochemical Contents and Antioxidant Activity

As shown in Figure 1, total phenolic and flavonoids contents of fenugreek extract were influenced by fertilizer source ($P \leq 0.05$). It was observed that application of SWE increased the production of total phenolics and flavonoids contents in fenugreek. The TPC in the *T. foenum-graceum* leaf extract were expressed as mg GAE g^{-1} extract and indicated that plants treated with SWE and NPK had the highest amount of phenolic content (158.47 and 146.67 mg GAE g^{-1} extract, respectively). Also, humic acid treatment had no significant effect on TPC in comparison with the control. According to the obtained results, plants that received SWE, had the maximum amount of flavonoids (177.77 mg QE g^{-1} DM). So, total flavonoids were enhanced 125% in the SWE treatment compared to the control. The TFC in the plants cultivated with NPK treatment (89.23 mg QE g^{-1} extract) was significantly lower than SWE, however, this amount was statistically higher than the control (52.33 mg QE g^{-1} DM). In order to evaluate the antioxidant activity of fenugreek extracts, DPPH and phosphomolybdenum assays were conducted. As can be seen in Figure 2,

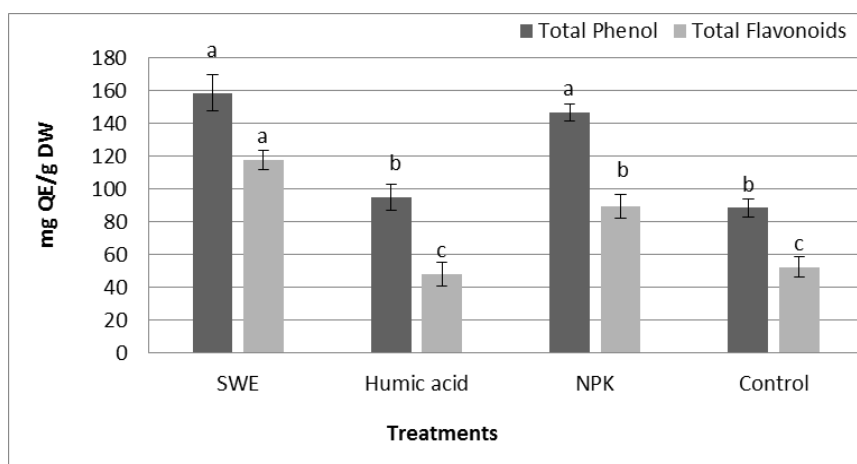


Figure 1. Effect of treatments on total phenol and flavonoids of Fenugreek.

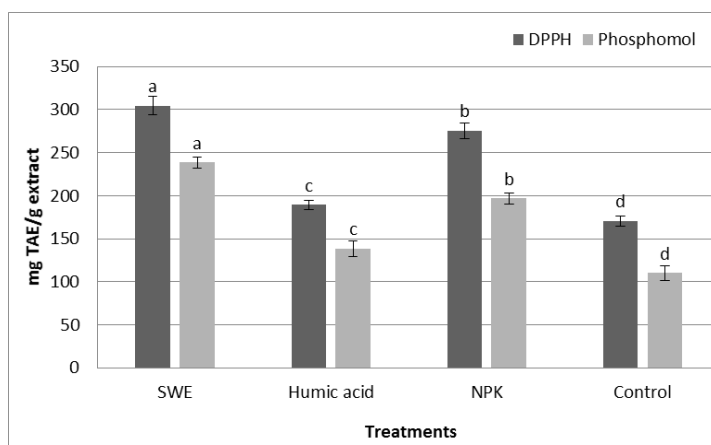


Figure 2. Effect of treatments on DPPH radical scavenging total antioxidant activities of fenugreek extract.

all fertilizer treatments increased significantly DPPH radical scavenging activity of the plants by 11 to 78.5% in comparison to the control. The highest DPPH radical scavenging activity was revealed to occur in plants that were treated with SWE indicating 304.33 $\mu\text{g TE g}^{-1}$ extract.

Enzyme Inhibitory Activity

Inhibitory activity of fenugreek extract on α -glucosidase is presented in Figure 3. Alpha-glucosidase inhibitors could be used as the remedies of diabetes. According to the obtained data, plants treated with SWE showed significantly stronger activity against α -glucosidase (IC_{50} values of 171.37

$\mu\text{g mL}^{-1}$) in comparison with the control (IC_{50} = 38.07 $\mu\text{g mL}^{-1}$).

Simple Correlation

Simple correlation results showed that plant height had significant positive correlation with fresh weight ($r=0.810^{**}$), dry weight ($r=0.786^{**}$), number of pods per plant ($r=0.426^{*}$), number of seeds per pod ($r=0.448^{*}$), total chlorophyll ($r=0.545^{**}$), chlorophyll “a” and “b” ($r=0.551^{**}$ and $r=0.480^{**}$), total carotenoids ($r=0.519^{**}$), essential oil percentage ($r=0.541^{**}$), DPPH ($r=0.866^{**}$), phosphomol ($r=0.905^{**}$), and glucosidase ($r=0.883^{**}$). Other correlation results can be seen in Table 5.

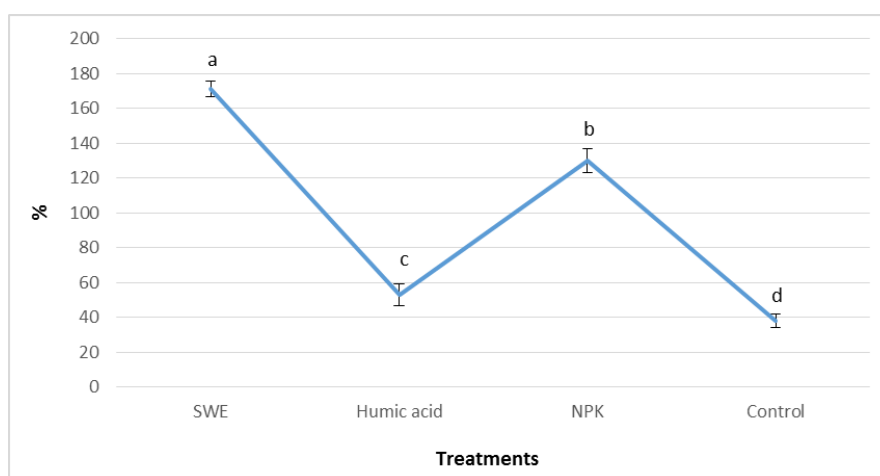


Figure 3. Effect of treatments on α -glucosidase inhibitory effect of fenugreek extract.

Table 5. Correlation coefficient between traits in fenugreek.

Variables	Plant height	Fresh weight	Dry weight	Number of pod per plant	Number seed per pod	1000 Seeds weight	Total chlorophyll	Chlorophyll "a"	Chlorophyll "b"	Ascorbic acid	Total carotenoids	Essencial Oil (%)	Total phenol	Total Flavonoids	DPPH	Phosphomol	Glucosidase
Plant height	1																
Fresh weight	.810**	1															
Dry weight	.786**	.912**	1														
Number of pod per plant	.426*	.492**	.513**	1													
Number seed per pod	.448*	.208	.174	.371*	1												
1000 Seeds weight	.348	.387*	.432*	.316	.202	1											
Total chlorophyll	.545**	.447*	.474**	.209	.427*	.341	1										
Chlorophyll "a"	.551**	.439*	.467**	.185	.381*	.347	.958**	1									
Chlorophyll "b"	.480**	.409*	.431*	.215	.435*	.298	.943**	.807**	1								
Ascorbic acid	.160	.404*	.459*	.346	-.182	.348	.441*	.466**	.365*	1							
Total carotenoids	.519**	.429*	.437*	.207	.405*	.283	.911**	.783**	.962**	.351	1						
Essencial Oil (%)	.541**	.553**	.574**	.239	.438*	.396*	.555**	.497**	.563**	.373*	.517**	1					
Total phenol	-.162	-.057	.129	.242	-.013	.067	.325	.191	.443*	.160	.383	.054	1				
Total flavonoids	-.070	.017	.207	.237	.023	.110	.364	.243	.462*	.181	.415*	.106	.981**	1			
DPPH	.866**	.815**	.888**	.480	.434	.314	.539	.564	.477	.033	.533	.436	.980**	.939**	1		
Phosphomol	.905**	.827**	.886**	.470	.490	.283	.671*	.675*	.620*	.162	.660*	.530	.959**	.948**	.966**	1	
Glucosidase	.883**	.811**	.879**	.452	.417	.274	.578*	.594*	.521	.086	.583*	.484	.977**	.965**	.983**	.988**	1

* **, Significant at $P=0.05$ and $P=0.01$ respectively



DISCUSSION

In the present investigation, plants treated with SWE showed better response in terms of vegetative parameters. These results are in good agreement with previous studies where growth and seedling vigor of maize, mungbean, and cabbage were enhanced by SWE treatment (Rengasamy *et al.*, 2015; Kannan *et al.*, 2016). The stimulatory effect of SWE on cabbage plant may be the result of synergistic interactions between SWE and endogenous growth hormones (Teixeira da Silva *et al.*, 2013). It was speculated that the increase in plant height may have been a result of macronutrients found in NPK fertilizer and N-containing plant growth regulators (auxins and cytokinins) within the SWE that were absorbed by the plants. An alternative explanation is that organic molecules such as organic acids, methionine and even aminoacids in SWE can increase nutrient absorption in plants by chelating the available nutrients, thereby increasing their absorbance (Papenfus *et al.*, 2013). Seaweed extracts can equilibrate growth as a result of auxin and gibberellin acid presence, which will increase vitamins and hormones producing in the treated plants (O' Dell, 2003). This increase in shoots characteristics might be also due to the macronutrients content in seaweed extracts (Table 2). Macronutrients like nitrogen, potassium, and phosphorous are very essential for growth and development of the plant (Attememe, 2009). The observation of SWE treated *Arachis hypogea* plants suggested that the growth and biochemical characteristics might be promoted by micro and macro elements and growth promoting hormones present in the SWE (Ganapathy-selvam and Sivakumar, 2014). Our findings coincide with those of earlier studies carried out on soybean (Rathore *et al.*, 2009) where there was an increase in vegetative growth by the application of SWE. Similar results were also observed in *Cajanus cajan* (L.) Millsp. (Mohan *et al.*, 1994), *Vigna sinensis* L. (Sivasankari *et al.*, 2006), and *Abelmoschus*

esculentus (Thirumaran *et al.*, 2009). The increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the SWE (Whapham *et al.*, 1993). Seaweed extracts contain cytokinins as well, which induce the physiological activities and increase the total chlorophyll in the plant. Also, this will positively reflect on the activity of photosynthesis and the synthesized materials, which will show on shoots characteristics (Janick and Whipkey, 2002). Regarding the biochemical traits, application of SWE showed a significant effect on the content of plant essential oil compared to the control. This result is in a good line with the findings of Golzadeh *et al.* (2011) on *Matricaria recutita* and Rafiee *et al.* (2013) on *Calendula officinalis* L.. Ardebili *et al.* (2012) indicated that foliar application of SWE as a source of amino acids, at suitable concentrations, had positive effects on the content of secondary metabolites, antioxidants, and antioxidant activity. They reported that increasing the phytochemical constituents of *Aloe vera* plants by using amino acids could demonstrate which of these compounds was involved in stimulating plant metabolic pathways. El-Sharabasy *et al.* (2012) reported that amino acids have a significant effect on the production of secondary metabolites. SWE applications significantly influenced the phenolic and flavonoids contents and antioxidant potential of the studied fenugreek leaves. The contents of phenolic and flavonoids in leaves obtained in the treated plants were higher than for the controls. Phenolic compounds are a class of antioxidant agents that act as free radical scavengers and are responsible for antioxidant activity in medicinal plants. Free radicals may cause many disease conditions such as cancer and coronary heart disease in human (Alizadeh *et al.*, 2010; Javanmardi *et al.*, 2003). Many plants extracts containing bioactive compounds, including phenolics and flavonoid, exhibit efficient antioxidant properties and prevent free radical damages (Koleva *et al.*, 2002). High antioxidant

amounts/activities following SWE treatment are important for improving the nutritional value of medicinal plants as well as prolonging their shelf life, thus enhance the overall quality and marketable value of fresh produce. Increases in the phenolic content of the leaves of *Pelargonium* were observed in plants treated with SWE (Krajnc *et al.*, 2012). The increase in the phenolic content of treated plant can be due to increase in plant hormone activities caused by SWE application. These results are in agreement with Fan *et al.* (2011), who also reported an increase in total phenolic content in spinach leaves when applying SWE fertilizer. In broccoli, sprouts treated with sucrose and mannitol, the latter being a component of SWE, were found to be significantly higher in phenolics (Guo *et al.*, 2011). In the present study, the increase in flavonoid content in seaweed treated plants was more than twice as high compared to the control. In the another study on broccoli, it was concluded that biofertilizers (50% *Azotobacter chroococcum* and 50% *Bacillus megaterium*) increased the amount of total phenolics and flavonoids compared to treatments that received chemical fertilizers (Naguib *et al.*, 2012). Several classes of natural products could inhibit the activity of α -glucosidase such as phenolic compounds and flavonoids, which occur too much in fenugreek. SWE samples have high amount of phenolic and flavonoid compounds, which may be the agents responsible for the observed activity. A very good accordance between phenolic and flavonoid contents with α -glucosidase inhibitory activity of SWE ($r=0.977$, $P<0.01$; $r=0.965$, $P<0.01$) established that these metabolites could be the compounds responsible for the observed inhibitory activities. This hypothesis is in agreement with some previous studies that reported phenolic compounds as potent α -glucosidase inhibitors (Salehi *et al.*, 2013; Bahadori *et al.*, 2015; Bahadori *et al.*, 2017).

CONCLUSIONS

SWE treatments significantly enhanced plant's vegetative growth as well as production of bioactive molecules such as the phenolics and flavonoids, which ultimately enhance the antioxidant activity

of the leaf extracts. Hence, the application of SWE on Fenugreek might be useful in increasing the yield and medicinal value of these plants and help produce an essential oil content from fenugreek seeds.

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تأثیر کاربرد عصاره جلبک، هیومیک اسید و کود شیمیایی بر صفات مورفولوژیکی،
فیزیولوژیکی و بیوشیمیایی شنبلیله (*Trigonella foenum-graecum*)

س. مفاخری، و ب. اصغری

چکیده

کشاورزی مدرن به دنبال دستیابی به روش‌های زیستی و مدرنی است که به واسطه آن بتوان مصرف کودهای شیمیایی را بدون تأثیر منفی در میزان محصول و درآمد کشاورزان، کاهش داد. عصاره جلبک دریایی و اسید هیومیک به عنوان مکمل‌های تغذیه‌ای و یا کودهای بیولوژیک در کشاورزی و باغبانی به منظور افزایش رشد و عملکرد گیاه استفاده می‌شود. به منظور بررسی تأثیر عصاره جلبک، هیومیک اسید و کود شیمیایی بر رشد و نمو و فاکتورهای فیزیولوژیکی و بیوشیمیایی شنبلیله، آزمایشی گلخانه‌ای در سال ۱۳۹۵ اجرا گردید. نتایج نشان داد که، محلول‌پاشی با عصاره جلبک، فاکتورهای رشدی گیاه را بهبود بخشید. در بین تیمارهای مختلف، گیاهانی که با عصاره جلبک تیمار شدند، بیشترین مقدار ارتفاع بوته، وزن تر و خشک، تعداد نیام در گیاه، مقدار کلروفیل‌های *a* و *b*، کلروفیل کل و کارتنوئیل کل را دارا بودند. همچنین کاربرد عصاره جلبک مقدار ترکیبات فنولی و فلاونوئیدی را نیز افزایش داد. همه تیمارهای کودی در مقایسه با شاهد، به طور معنی‌داری سبب افزایش قدرت ضد رادیکالی گیاهان در مقابل رادیکال‌های DDPH شدند. گیاهانی که با عصاره جلبک محلول‌پاشی شدند، فعالیت قوی‌تری در مقابله با آنزیم آلفا-گلوکوزیداز با مقدار IC_{50} ۱۷۱/۳۷ میکروگرم در میلی‌لیتر در مقایسه با آکاربوز (با IC_{50} ۱۹/۷ میکروگرم در میلی‌لیتر) به عنوان مهارکننده آلفا گلیکوزیداز رفرنس، داشته‌اند. داده‌های به دست آمده از این مطالعه، نشان داد که می‌توان از محلول‌پاشی عصاره جلبک، برای به دست آوردن حداکثر کمیت و کیفیت محصول شنبلیله استفاده نمود.