



## Improving growth, yield and fruit quality of strawberry by foliar and soil drench applications of humic acid

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**ABSTRACT**-Organic compounds including seaweed extract were applied in organic production system and sustainable agriculture. One of these compounds is humic acid that was used widely in research and commercial programs. Humic acid is an organic acid obtained from humus and other natural resources with hormonal effects and improving nutrient absorption, increasing root and shoot biomass. In order to investigate the effect of foliar and soil drench applications of humic acid on growth responses of strawberry, this research was conducted as a completely randomized design. Well-rooted daughter strawberry cv. Paros plants were potted in 3 liter plastic pots filled with leaf mold, field soil and sand (1:1:1 v:v:v). After the establishment of plants, Greenhum (13.5% humic acid) was sprayed at 300, 600, 900, and 1200 mg L<sup>-1</sup> and soil drench was applied at 300, 450, 600, and 750 mg L<sup>-1</sup> (250 ml pot<sup>-1</sup>). Results showed that foliar application at 600 and 900 mg L<sup>-1</sup> produced the highest dry mass of shoot and root. Total acid of fruit in 750 mg L<sup>-1</sup> soil application and in 300 mg L<sup>-1</sup> foliar application were significantly higher than untreated control plants. The greatest vitamin C and TSS were obtained from 900 and 600 mg L<sup>-1</sup> foliar application treatments, respectively. The highest flower numbers and yield were produced in 900 mg L<sup>-1</sup> foliar application. In general, foliar application of Greenhum, especially at 600 and 900 mg L<sup>-1</sup>, significantly increased most evaluated parameters.

### INTRODUCTION

Adverse impacts of chemicals and pesticides used in agricultural practices induce the idea of a modern way of agriculture without or at least with minimum usage of such compounds (Tan, 2003). This novel method of farming is called organic agriculture. Vast usage of chemical fertilizers and pesticides leads to water, air and soil pollution which, in turn, reduce production each year. The aim of organic agriculture is the protection of soil fertility and augmentation of yield with minimum utilization of chemicals. In other words, organic agriculture is a new and scientific approach, compared to our ancestors' methods of agriculture. Nowadays, organic compounds, such as seaweed extracts or humic acid, have attracted the attention of researchers and fruit growers, the latter, for example, having been used extensively for commercial production and scientific studies.

Humic substances are shown to contain a wide variety of components. Some typical components are: polysaccharides, fatty acids, polypeptides, lignins, esters, phenols, ethers, carbonyls, quinones, lipids, peroxides, various combinations of benzene, acetal, ketal, and lactol, and furan ringed compounds, and aliphatic (carbon chains) compounds. The oxidative degradation of some humic substances produces aliphatic, phenolic, and benzene carboxylic acids in addition to n-alkanes and n-fatty acids. The major phenolic acids released contain approximately 3 hydroxyl (-OH) groups and between 1 and 5 carboxyl (-COOH) groups (Cacco and Dell Agnolla, 1984; Pettit, 2004).

Analyses have shown that, humic substances are composed of oxygen, hydrogen, nitrate and sulfur atoms within a carbon chain (Pertuitet al., 2001; Tan, 2003; Zimmer, 2004). Humic acid has weak acidic plus phenolic functional groups which enable this compound to act as cation exchanger and metal chelator.

Humic substances play a vital role in soil fertility and its application increased the plant growth and nutrient uptake (Ameri and Tehranifar, 2012; Dursun et al., 2002; El-Mohamedy and Ahmed, 2009; Khaled and Hassan, 2011; Turkmen et al., 2004). Also plants grown on soils which contain adequate humic acids are less subject to stress, are healthier, and produce higher yields; In addition, the nutritional quality of fruits, harvested parts and processed foods are superior (Pettit, 2004; Ameri and Tehranifar, 2012).

Humic substances function to buffer the hydrogen ion (H<sup>+</sup>) concentration or pH of the soil. These substances in alkaline soils also liberate carbon dioxide (CO<sub>2</sub>) from calcium carbonates present within the soil (Nardi et al., 2002; Tan, 2003; Zimmer, 2004; Katkat et al., 2009).

Humic substances function to help stabilize soil temperatures and slow the rate of water evaporation. It has also been reported that humic acid application positively affects the plant parameters of plants grown in salinity condition (Turkmen et al., 2004; Turkmen et al., 2005). Improvements of soil properties as soil structure, porosity, permeability and water holding capacity, cation and anion exchange and equilibrium

among plant nutrients chelation of mineral elements are some of the useful characteristics of humic acid (Dursun et al., 2002; Turkmen et al., 2004; Zandonadi et al., 2007; El-Mohamedy and Ahmed, 2009; Khaled and Hassan, 2011). Humic substances are a good source of energy for beneficial soil organisms (Tan, 2003; Pettit, 2004; Zimmer, 2004).

These substances affect the solubility of nutrient elements by building complex forms or chelating agents of humic matter with metallic cations. Recent studies on the subject summarize the effects of humic substances on seed germination, seedling growth, root initiation, root growth, shoot development and microelements uptake by plants (e.g. Fe, Zn, Mn) (Chen and Aviad, 1990; Varanini and Pinton, 1995; Bohme and ThiLua, 1997; Nardi et al., 2002; Salman et al., 2005; Eyheraguibel et al., 2008).

Also the effects of humic acid derived from various organic wastes on seedling growth of tomato in some growth media have been investigated by David et al. (1994), Loffredo et al. (1997), Pertuit et al. (2001), and Atiyeh et al. (2002).

The positive effects of the humic substances were also observed on the studies such as dry matter yield increases on strawberry (Ameri and Tehranifar, 2012), corn and oat seedling (Celik et al., 2008), yield increases on radish and green bean seedlings (Russo and Berlyn, 1992; Singhvi, 1989). Although much research has well documented the effects of humic substances on numerous plants, such as tomato (Bohme and ThiLua, 1997; Padem and Ocal, 1999), forage turnip (Albayrak and Carna, 2005) and pineapple (Baldotto et al., 2010), the growth response of plants has not been adequately studied under abiotic stress conditions. Under the calcareous soil conditions, application of the humic substances may increase the tolerance of the plants to stress, and promote growth by increasing nutrients uptake (Tan, 2003).

However, the application method of humic acid and its effect on strawberry plants have little been studied. Therefore, the aim of present study was to evaluate the effect of foliar and soil drench applications of humic acid on vegetative and reproductive characteristics of 'Paros' strawberry.

## MATERIALS AND METHODS

### Plant Growth Conditions and Treatments

Well-rooted strawberry plants of cultivar 'Paros', grown under greenhouse conditions, were used to study their responses to humic acid. The plants were potted in 3 L pots, plastic pots, filled with 1:1 ratio of leaf mold and soil.

Light status was  $> 800 \mu\text{mol m}^{-2}\text{S}^{-1}$ , day/night temperatures were adjusted at  $25 \pm 3^\circ\text{C}$  /  $16 \pm 3^\circ\text{C}$  and RH was set at  $50 \pm 5\%$ . Treatments were carried out when plants were well established and each produced 5-6 fully expanded leaves. Treatments included spray (300 ml per plant) of Greenhum (an organic fertilizer with 13.5% humic acid produced from leonardite algae) at

300, 600, 900 and  $1200 \text{ mg L}^{-1}$  and 300, 450, 750 and  $600 \text{ mg L}^{-1}$  as soil-drench treatments of Greenhum.

### Measurements

Leaf chlorophyll index was measured with a SPAD-502 chlorophyll-meter (Minolta, Japan), using 3 fully expanded leaves to find an average for chlorophyll index. For leaf area, three fully expanded leaves of each plant were chosen and total area was measured using a leaf area meter (Delta T Devices Ltd., Burwell, and Cambridge, England) and the data were presented in  $\text{cm}^2$ .

For measuring root and shoot fresh and dry mass, the plants were taken out of their pots and growth media carefully, were oven dried for 48 hours at  $70^\circ\text{C}$  and then were weighed for dry mass determination.

Number of inflorescences in plant and achenes in fruits were counted throughout the experiment period and the averages were reported. Primary fruits were weighed twice a week from the beginning to the end of the experiment.

Length and diameter of fruits were determined using a digital caliper. Yield was determined summing up total fruit mass produced throughout the experiment (60 days). Total soluble solids (TSS) expressed in °Brix was measured using a hand refractometer. Ascorbic acid was determined by the indophenol titration method. Total acid (TA%) was measured by the NaOH titration method in 10 ml of fruit juice, followed by the addition of 5-6 droplets of phenolphthalein; then, the mixture was titrated with 0.3 N NaOH until a color change occurred. TA was calculated by the following formula:  $\% \text{TA} = [\text{ml}(\text{NaOH}) \times \text{N}(\text{NaOH}) \times \text{acid. meq. factor} / \text{ml juice titrated}] \times 100$ .

### Statistical analysis

The experiment was conducted in a completely randomized design with 4 replications, each consisting of three pots and with each pot containing one plant. Data were analyzed by SPSS 17 software and means were compared using LSD test at 5 percent of probability.

## RESULTS AND DISCUSSION

Table 1 indicates the effect of foliar and soil drench application of Greenhum on some vegetative parameters of 'Paros' strawberry. It seems that all these features have been affected significantly and vegetative characteristics of the treated plants increased significantly in comparison to control plants. The maximum amount of shoot and root fresh and dry masses obtained in plants sprayed with 600 and  $900 \text{ mg L}^{-1}$  of Greenhum and leaf area reached its maximum level ( $53.34 \text{ cm}^2$ ) when plants treated with 900 or  $1200 \text{ mg L}^{-1}$  of sprayed Greenhum or  $750 \text{ mg L}^{-1}$  of soil drenched Greenhum.

Table 2 shows the effect of foliar and soil drench application of Greenhum on some generative parameters of 'Paros' strawberry. Generally, the number of

inflorescences, mass and length of primary fruits, the number of achenes and yield in treated fruits were higher than those of untreated control plants. The highest amounts of inflorescences number and achenes,

and length of fruits were obtained from 600 mg L<sup>-1</sup> of Greenhum spray. The greatest yield (g per plant) was obtained from plants foliar sprayed with Greenhum at 900 mg L<sup>-1</sup> (Table 3).

**Table1.**Effect of Greenhum, foliar and soil drench application on vegetative growth responses of strawberry (Means with the same letters are not significantly different at 5% level using LSD).

Greenhum application (mg L <sup>-1</sup> )	Shoot fresh mass (g)	Shoot dry mass(g)	Root fresh mass (g)	Root dry mass (g)	Leaf area (cm <sup>2</sup> )
0 (control)	24.89 g	6.05 f	23.06 f	8.66 d	364.5e
Soil drench(300)	34.93 f	8.91e	32.71 e	14.45c	409.5d
Soil drench(450)	40.38 d	9.82bc	36.72 cd	15.13 b	470c
Soil drench(600)	42.52c	9.9bc	38.5 b	15.45b	470c
Soil drench(750)	40.82 d	9.96b	37.61 bc	13.91 c	527.7a
Foliar application(300)	37.65 e	9.48cd	36.5d	14.19 c	488b
Foliar application(600)	47.86 a	10.83 a	41.77a	16.66 a	487b
Foliar application(900)	46.4 b	10.86 a	41.16a	16.72 a	533.4a
Foliar application(1200)	35.22 f	9.12 de	38.65 b	15.21 b	532.1a

Table 3 indicates the effect of foliar and soil drench application of Greenhum on TSS, total acids, ascorbic acid concentration and chlorophyll content of the leaves. Maximum concentration of vitamin C and chlorophyll content were obtained when plants were sprayed with 900 mg L<sup>-1</sup> of Greenhum. Spraying plants with all levels of Greenhum soil drench application of 750 mgL<sup>-1</sup> of this compound caused elevated amounts of TSS content in fruits. The findings of the study showed that Greenhum had promoting impact on both vegetative and reproductive parameters of 'Paros' strawberry. Foliar application of Greenhum or its soil drench treatment caused improvement of quality features such as TSS or ascorbic acid. Our findings exhibited the beneficial influence of this compound on the growth of strawberry.

Because of high health and nutritional value of strawberry, its production is becoming an ever

increasing industry. Chemical fertilizers as a way to stimulate higher yield levels is common practice but impose problems, such as nitrate accumulation, lower shelf life and quality, and pollution (Tan, 2003; Chen et al.,2004; Pettit, 2004). Humic acid with its unique features, such as supplying more available essential elements and increment of plant resistance to biotic and abiotic stresses, may improve quantity and quality of production (Zimmer, 2004; Zandonadi et al., 2007;Ameri and Tehranifar, 2012). Positive correlations between the humus content of the soil, yields and product quality have been reported (Hartwigson and Evans 2000;Nardi et al., 2002;Chen et al.,2004; Hafez, 2004;Cimrin and Yilmaz,2005; Sangeetha et al., 2006; Baldotto et al.,2010;Khaled and Hassan,2011;Ameri and Tehranifar, 2012).

**Table2.**Effect of Greenhum, foliar and soil drench application on reproductive growth responses of strawberry (Means with the same letters are not significantly different at 5% level using LSD).

Greenhum application (mg L <sup>-1</sup> )	Number of inflorescence	Mass of primary fruits (g)	Number of achenes (primary fruits)	Length of primary fruits (cm)	Diameter of primary fruits (cm)
0 (control)	6.5e	16.5d	189d	3.5c	2.4c
Soil drench(300)	7.7d	22.9bc	203dc	3.67bc	3.25b
Soil drench(450)	8.7c	20.8c	229ab	4.05abc	3.5ab
Soil drench(600)	8.8c	23.5ab	233ab	4.2ab	3.5ab
Soil drench(750)	7.7d	24.4ab	220bc	4.3ab	3.6ab
Foliar application(300)	10.2b	24.6ab	241ab	4.1abc	3.42ab
Foliar application(600)	11.2a	23.8ab	247a	4.1abc	3.87a
Foliar application(900)	10.4b	25.15a	242a	4.45a	3.65ab
Foliar application(1200)	7.7d	24.8ab	237ab	4.4a	3.47ab

**Table 3.** Effect of Greenhum, foliar and soil drench application on growth responses of strawberry (Means with the same letters are not significantly different at 5% level using LSD).

Greenhum application(mg L <sup>-1</sup> )	Vitamin C (mg per 100 ml)	TSS	Chlorophyll content (mgg <sup>-1</sup> FW)	TA	Yield (g per plant)
0 (control)	45.7 f	5 c	0.913 c	0.64b	74.2h
Soil drench(300)	60.5 d	6 bc	0.955 ab	0.81ab	116f
Soil drench(450)	59.67 e	6 bc	0.94 b	0.79ab	109g
Soil drench(600)	66.32 c	6 bc	0.962 ab	0.88ab	119e
Soil drench(750)	60.15 de	7 ab	0.96 ab	0.89a	132c
Foliar application(300)	60.5 d	6 bc	0.973a	0.89a	130c
Foliar application(600)	66.5 c	8 a	0.97a	0.87ab	127d
Foliar application(900)	72.9 a	7 ab	0.975a	0.85ab	150a
Foliar application(1200)	70.22b	7 ab	0.968a	0.86b	135b

In the presence of humic acid, higher activity and density of beneficial microorganisms have been reported. Also chelating capacity of this compound and its effect on cellular membrane might facilitate uptake of elements into plant cells (Dursun et al., 2002; Chen et al., 2004; Zimmer, 2004; Sangeetha et al., 2006; Zandonadi et al., 2007).

Humic acid by chelating of plant nutrients, such as iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg), manganese (Mn), and calcium (Ca), reduces their toxicity as cations, prevents their leaching, and increases their uptake rate by plant roots. Electrostatic attraction of metal cations to anionic sites on the humic substance keeps them from leaching into the subsoil. The metal cation is loosely attached, being released when attracted to another stronger electrical charge. The cation is readily available in the soil environment for transport into the plant roots or exchange for another metal cation (Rengrudkij and Partida, 2003; Chen et al., 2004; Salman et al., 2005; Baldotto et al., 2010; Khaled and Hassan, 2011)

The chelation process also increases the mass flow of micro nutrient mineral elements of the root. When toxic heavy metals, such as mercury (Hg), lead (Pb), and cadmium (Cd), are chelated, these organo-metal complexes become less available for plant uptake (Farouk et al., 2011).

The uptake of major plant nutrients is mediated by humic substances. One stimulative effect of humic substances on plant growth is the enhanced uptake of major plant nutrients: nitrogen (N), phosphorus (P), and potassium (K). When adequate humic substances are present within the soil, the requirement for N-P-K fertilizer application is reduced (Chen et al., 2004; Salman et al., 2005; Baldotto et al., 2010; Khaled and Hassan, 2011; Ameri and Tehranifar, 2012). More availability of micro- and macro-elements may be responsible for higher shoot and root fresh and dry mass in our study (Dursun et al., 2002; Salman et al., 2005; Zandonadi et al., 2007).

The most important function of humic substances within the soil is their ability to hold water. Humic substances help create a desirable soil structure that facilitates water infiltration and help hold water within

the root zone. Water stored within the soil, when needed, by Water bridging simply illustrated by the following formula  $-COO-H_2O-Fe^+$  provides a carrier medium for nutrients required by soil organisms and plant roots. Also soil temperatures and water evaporation rate are stabilized by humic substances (Zandonadi et al., 2007). The insulating properties of humic substances help maintain a more uniform soil temperature, especially during periods of rapid climate changes, such as cold spell or heat waves (Baldotto et al., 2010). Because water is bound within the humic substances and humic substances reduce temperature fluctuations, soil moisture is less likely to be released into the atmosphere (Chen and Aviad, 1990; Chen et al., 2004).

Saruhan et al. (2011) showed that humic acid treatments increased the yield and yield components of common millet significantly. Kaya et al. (Kaya et al., 2005) reported that, as compared to the control, combined zinc and foliar humic acid or zinc and separate applications increased the grain yield of bread wheat.

Obsuwan et al. (2011) investigated the effect of various concentrations of HA obtained from the compost, and derived from mix manures and some agricultural wastes, on the growth of eggplant seedlings (*Solanum melongena* L. cv. Chao Praya) in tissue cultures. They showed that seedlings grown on ¼MS supplemented with HAs at the concentration of 25 and 50 mg L<sup>-1</sup> had the average plant heights higher than the other treatments. Both treatments significantly showed the maximum average leaf fresh and dry masses although the latter yielded the highest average number of leaves and the longest average root length.

Humic substances have a very pronounced influence on the growth of plant roots and it is an effective plant root growth stimulator. When humic acids are applied to soil, enhancement of root initiation and increased root growth are observed (Zandonadi et al., 2007).

Humic acids are excellent foliar fertilizer carriers and activators. By increasing plant growth processes within the leaves, an increase in carbohydrates content of leaves and stems occurs. These carbohydrates are then transported down the stems into the roots where

they are in part released from the root to provide nutrients for various soil microorganisms on the rhizoplane and in the rhizosphere (Zandonadi et al., 2007; Baldotto et al., 2010). Also plants respond more slowly to soil applications of humic substances because a large percentage of the humic substances are retained within the roots during plant growth. In most plants, less than 30% of the humic substances present within the roots is translocated up the stems into the plant leaves. When adequate humic substances are present within the

soil, the requirement for nitrogen, phosphorus and potassium fertilizer applications may be reduced (Pettit, 2004).

## CONCLUSIONS

In general, Greenhum application increased the strawberry plant growth and yield. Overall, Greenhum was very effective when applied as foliar spray especially at 600 and 900 mgL<sup>-1</sup>.

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## بهبود رشد رویشی و زایشی توت فرنگی رقم پاروس با محلول پاشی و کاربرد خاکی هیومیک اسید

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هیومیک اسید

عصاره جلبک دریایی

**چکیده** - ترکیبات آلی از جمله عصاره جلبک های دریایی در کشاورزی ارگانیک و پایدار به کار برده می شوند. یکی از این ترکیبات هیومیک اسید است که به طور گسترده در پروژه های پژوهشی و تجاری کاربرد دارد. هیومیک اسید یک اسید آلی است که از هوموس و ترکیبات طبیعی به دست می آید و اثرات هورمونی داشته و باعث افزایش جذب عناصر غذایی و زیست توده ریشه و اندام هوایی می شود. به منظور بررسی اثر محلول پاشی و کاربرد خاکی هیومیک اسید بر پاسخ های توت فرنگی، پژوهش حاضر به صورت طرح کاملاً تصادفی انجام شد. گیاهان دختری ریشه دار شده توت فرنگی رقم پاروس در گلدان های پلاستیکی ۳ لیتری پر شده با خاک برگ، خاک مزرعه و ماسه، (۱:۱:۱ حجمی) کاشته شدند. پس از استقرار گیاهان ترکیب Greenhum (حاوی ۱۳/۵٪ هیومیک اسید به عنوان ماده موثره) با غلظت های ۰، ۳۰۰، ۶۰۰، ۹۰۰ و ۱۲۰۰ میلی گرم در لیتر به صورت محلول پاشی و ۰، ۳۰۰، ۴۵۰، ۶۰۰ و ۷۵۰ میلی گرم در لیتر به صورت خاکی به کار برده شدند. نتایج نشان داد که محلول پاشی این ترکیب در غلظت های ۶۰۰ و ۹۰۰ میلی گرم در لیتر بالاترین وزن خشک ریشه و شاخساره را ایجاد کرد. اسید کل میوه در کاربرد خاکی هیومیک اسید به میزان ۷۵۰ میلی گرم در لیتر و محلول پاشی ۳۰۰ میلی گرم در لیتر به طور معنی دار بیشتر از گیاهان شاهد بود. بالاترین ویتامین ث و TSS میوه به ترتیب از گیاهان محلول پاشی شده با ۹۰۰ و ۶۰۰ میلی گرم در لیتر به دست آمد. بالاترین تعداد گل و عملکرد را گیاهان تیمار شده با محلول پاشی ۹۰۰ میلی گرم در لیتر تولید کردند. به طور کلی کاربرد گرین هام به ویژه در غلظت های ۶۰۰ و ۹۰۰ میلی گرم در لیتر به صورت محلول پاشی بیشتر پارامترهای بررسی شده را به طور معنی دار افزایش دادند.