Vegetative and Reproductive Parameters of 'Selva' Strawberry as Influenced by Algaren, Drin and Green Hum Foliar Application.

SAEID ESHGHI1*, MASoud ZARE1, BABAK JAMALI1, ALI GHARAGHANI1 and MEHDI HOSEINI FARAH2
1 Department of Horticultural Science, College of Agriculture, Shiraz University, Shiraz, Iran
2 Young Researchers and Elite Club, Yasooj Branch, Islamic Azad University, Yasooj, Iran.
*Corresponding Author: eshghi@shirazu.ac.ir
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ABSTRACT
In order to evaluate the effects of foliar application of seaweed extracts including Algaren, Drin and Humic acid on vegetative and reproductive growth of strawberry cv. ‘Selva’ the present study was carried out in a completely randomized block design. Well-rooted daughter strawberry plants were potted in 5 liter plastic pots filled with Perlite and Cocopeat (1:1 v/v), and were grown hydroponically in greenhouse. When plants well established, they were sprayed with Algaren at 0, 3, 6 and 9 g l⁻¹, Drin at 0, 0.5, 1 and 2 g l⁻¹ and green hum at 0, 1.5, 3 and 4.5 g l⁻¹ concentration. Results indicated that 3 g l⁻¹ of green hum significantly increased leaf area, primary fruit weight, dry weights of shoot and root and yield. Algaren at 6 g l⁻¹ concentration produced the highest chlorophyll content. The lowest percentage of malformed fruit production and the highest vitamin C concentration were obtained when plants treated with 0.5 g l⁻¹ of Drin. Generally it can be concluded that green hum and seaweeds extracts are capable of influencing vegetative and reproductive growth of strawberry cv. ‘Selva’ in a promoting and beneficial way but for elucidating the details of this impact more studies are required.

Keywords: Ascorbic acid, growth, fruit quality, seaweeds extracts, soilless culture.

Abbreviations:
TA: Total Acids; TTS: Total Soluble Solids.

INTRODUCTION
Strawberry production, as a delicious fruit with high health and nutritional value is an ever-increasing industry. A vast range of compounds has been used exogenously by researchers in order to increase the quality and quantity of yield (Eshghi and Jamali, 2009).

Green hum is the commercial name of an organic fertilizer with 13.5% content of Humic acid which is obtained from Leonardite Algae. Humic acid is a principal component of Humic substances, which are the major organic constituents of humus, peat, coal, many upland streams, dystrophic, lakes and ocean water. Humic acid is produced by biodegradation of dead organic matter (Stevens, 1994).

There is a direct correlation between soil fertility and the organic fraction of the soil (Liu and Cooper, 2000). Previous studies reported a beneficial impact of this compound on plant growth and development (David et al., 1994; Adani et al., 1998; Nikbakht et al., 2008). Humic acid improves the absorption of mineral elements such as N, P, K, Ca and Mg (Bohme and Thi-Lua, 1997; Atieyeh et al., 2002). Sharif et al. (2002) reported higher accumulation of nitrogen in maize plants treated with Humic acid. Humic acid is not a single acid, rather it is a complex mixture of many different acids containing carboxyl and phenolate groups, therefore Humic acid can form complexes with ions such as Mg²⁺, Ca²⁺, Fe²⁺ and Fe³⁺. In addition hormone-like activity has been mentioned as one of the characteristics of Humic acid (Stevens, 1994).

Algaren is organic fertilizer of vegetal origin and contains Ecklonia maxima brown seaweed extracts. Ecklonia maxima is a seaweed that grows in the Southern Hemisphere: they are hand-harvested when ripe, that is when they feature the highest ratio between Auxins and Cytochinins. The cytoplasmic contents of the cells are extracted through a process during which high differential pressures are applied. For this reason, this organic fertilizer does not alter the nature and the contents of the active principles, especially of natural Auxines and Cytochinins, of vitamin B and of micro-nutrients (Robertson-Andersson et al., 2006).

Drin is another organic seaweed obtained fertilizer, containing amino acids, organic carbon
Seaweeds extracts such as Algaren or Drin contain amino acids, minerals, vitamins and phytohormones such as Auxin or Auxin-like substances, cytokinines or cytokinine-like substances, so it is possible to use them as growth stimulators or fertilizers (Zhang and Ervin, 2008). Utilization of these compounds, might lead to growth and yield increment (Crouch et al., 1992; Reitz and Trumble, 1996; Stirk et al., 2003; Ordog et al., 2004). Seaweeds extracts contain uncommercial carbohydrates such as Laminaran, Fucoidan and Alginate (Lane et al., 2006; Duarte et al., 2001). Laminaran induces defense responses of plants (Fritig et al., 1998, Van-Loon and Van-Strien, 1999), and Fucoidan may influence a vast range of biological activities (Rioux et al., 2007). Betaines and Betaine-like substances are another group of compounds found in seaweeds extracts (Blunden et al., 1986), they can act as metabolism compatible osmolite and a source of nitrogen (Naide et al., 1987). Seaweeds extracts application increase the mineral elements uptake (Metting et al., 1990, Jeanin et al., 1991), have promoting effect on chlorophyll content and photosynthesis (Blunden et al., 1997), improve the yield (Arthur et al., 2003) and optimized responses to biotic and abiotic stresses (Wu et al., 1997; Fike et al., 2001; Zhang and Ervin, 2004). Since both of these two groups; Humic acid and seaweeds extracts (Algaren and Drin), optimize growth of plants, the aim of this study was to evaluate their influence on growth and quality of strawberry cv. 'Selva'.

**MATERIALS AND METHODS**

**Plant Growth Conditions and Treatments:**

Well-rooted strawberry (*Fragaria ananassa*) plants of cultivar 'Selva', grown under greenhouse conditions were used to study their responses to Green hum, Algaren and Drin. The plants were potted in 5 l plastic pots, filled with 1:1 ratio of Cocopeat and Perlite. Plants were irrigated twice a day using Melspray solution (10% N, 40% K₂O, 8% P₂O₅, 2% MgO, 1000 mg l⁻¹ Fe, 230 mg l⁻¹ Zn, 75 mg l⁻¹ Cu). Light status was > 800 μmol m⁻² S⁻¹, day/night temperatures were adjusted at 25±2°C / 16±2°C and RH was set at 50±5%. Treatments were carried out when plants were well established and each produced 5-6 fully expanded leaves, and then repeated 15 days later. Treatments included Green hum at 0, 1.5, 3 and 4.5 g l⁻¹, Algaren and Drin at 0, 1.5 and 2 g l⁻¹.

**Measurements:**

1. **Plant Vegetative Characteristics:**

   - **Leaf chlorophyll content:** Leaf chlorophyll content was measured with a SPAD-502 chlorophyll-meter using 3 fully expanded leaves to obtain average leaf chlorophyll content. Subsequently, 14 leaves with their chlorophyll content already measured by SPAD-502 were randomly detached from the plants to extract chlorophyll using the destructive method. Extraction was performed with 80% ethanol and the obtained solution was centrifuged at 8000 rpm for 10 min. Chlorophyll content was determined using a colorimeter and acquiring the following formula (Horwitz et al., 1975):

   \[
   \text{Chlorophyll} = \frac{20.2 \times A_{645} + 8.02 \times A_{663} \times V}{1000 \times W}
   \]

   Where Chlorophyll content is given as: mg g⁻¹ fresh weight; A: absorption value; V: ultimate volume of extract; and W: leaf fresh weight.

   A regression line and equation were obtained using Excel software, considering the SPAD-502 readings for the mentioned 14 leaves and the destructively determined chlorophyll contents. This equation was later used to estimate other chlorophyll readings from SPAD-502.

   - **Leaf Area:** For leaf area, 3 fully expanded leaves of each plant were chosen and total area was measured using a leaf area meter (Delta-T Devices Ltd., Burwell, Cambridge, England) and data were presented in cm².

   - **Plant Dry Weight:** For measuring root and shoot dry weight, the plants were taken out of their pots and growth media carefully, then oven dried for 48 hours at 70°C and dry weights were determined.

2. **Fruit Quantitative Characteristics:**

   - **Inflorescence and Fruit Quantity:** Number of inflorescences and percentage of malformed 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 experiment to find an average and data were presented in grams. Yield per plant was determined summing up total fruit weight (g) produced throughout the experiment.

3. **Fruit Qualitative Characteristics:**

   - **Total Soluble Solids:** Total Soluble Solids (TSS) expressed in ºBrix was measured using a hand refractometer.

   - **Ascorbic acid:** Ascorbic acid was determined by the indophenol titration method (Ting and Russeff, 1981) then was expressed in (mg 100 g⁻¹ Fresh Weight).

   - **Total acid:** Total acid (TA) % was measured by the NaOH titration method: in 10 ml of fruit juice, 5-6 droplets of phenolphthalein were added, then the mixture was titrated with 0.3 N NaOH until a color change occurred. Then following formula was used:

   \[
   \text{TA} \% = \frac{\text{ml (NaOH) \times N (NaOH) \times acid meq factor / ml juice titrated} \times 100}{100}
   \]

   **Statistical Analysis:**

   The experiment was conducted in a completely randomized block design with 3 replications, each consisting of 5 pots and each pot containing one plant. Data were analyzed by SPSS 17 software and
means were compared using Duncan’s multiple range test (DMRT).

RESULTS

Plant Vegetative Characteristics:
Table 1 indicates the effect of green hum, Algaren and Drin on some vegetative features of strawberry cv. ‘Selva’. All vegetative characteristics were affected significantly by treatments. Shoot and root dry weights of plants treated with green hum, Algaren and Drin increased significantly in comparison to control plants up to about two folds. Similarly higher ... obtained in treated plants; about 13% increment in leaf chlorophyll content was observed in plants treated with 6 g l⁻¹ of Algaren and about 36% increase in leaf area was obtained when plants treated with 3 g l⁻¹ of green hum.

<table>
<thead>
<tr>
<th>Treatments (g l⁻¹)</th>
<th>Shoot Dry Weight (g)</th>
<th>Root Dry Weight (g)</th>
<th>Leaf area (cm²)</th>
<th>Leaf chlorophyll content (mg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green hum 1.5</td>
<td>3.10 d†</td>
<td>11.83 bc</td>
<td>32.4 abc</td>
<td>1.00 bc</td>
</tr>
<tr>
<td>3</td>
<td>7.46 a</td>
<td>19.91 a</td>
<td>37.8 a</td>
<td>1.06 ab</td>
</tr>
<tr>
<td>4.5</td>
<td>4.72 bcd</td>
<td>9.75 bc</td>
<td>33.4 ab</td>
<td>0.99 c</td>
</tr>
<tr>
<td>3</td>
<td>3.98 cd</td>
<td>7.00 bc</td>
<td>28.7 bc</td>
<td>0.94 c</td>
</tr>
<tr>
<td>Algaren 6</td>
<td>6.77 ab</td>
<td>9.66 bc</td>
<td>32.5 ab</td>
<td>1.08 a</td>
</tr>
<tr>
<td>9</td>
<td>5.93 abc</td>
<td>12.21 b</td>
<td>35.9 ab</td>
<td>0.99 c</td>
</tr>
<tr>
<td>0.5</td>
<td>5.05 bcd</td>
<td>8.16 bc</td>
<td>28.7 bc</td>
<td>0.94 c</td>
</tr>
<tr>
<td>Drin 1</td>
<td>5.36abcd</td>
<td>12.17 b</td>
<td>33.2 abc</td>
<td>1.08 a</td>
</tr>
<tr>
<td>2</td>
<td>6.89 ab</td>
<td>8.51 bc</td>
<td>28.2 bc</td>
<td>0.96 c</td>
</tr>
<tr>
<td>Control</td>
<td>3.51 d</td>
<td>4.70 c</td>
<td>27.7 c</td>
<td>0.95 c</td>
</tr>
</tbody>
</table>

† Means followed by same letter in each column are not significantly different at 5% probability using Duncan’s multiple range test (DMRT).

Fruit Quantitative Characteristics:
Table 2 shows the effect of green hum, Algaren and Drin on some generative features of strawberry cv. ‘Selva’. More inflorescences obtained in plants treated with green hum, Algaren or Drin. The maximum for this parameter obtained when plants treated with 1.5 g l⁻¹ of green humor 2 g l⁻¹ of Drin. Primary fruit weight increased significantly after application of green humor Algaren, about 50% increment of weight observed in plants treated with 3 g l⁻¹ of green hum and about 43% increase of this parameter was obtained when plants treated with 9 g l⁻¹ of Algaren. Except for 6 g l⁻¹ of Algaren, in all treated
plants yield increased significantly. Treatments did not change the percentage of malformed fruits significantly.

**Fruit Qualitative Characteristics:**

Table 3 shows the effect of green hum, Algaren and Drin on TSS, total acids and ascorbic acid concentration of the fruits. The only fruit quality characteristic which has been affected significantly was ascorbic acid, whose concentration increased when plants treated with one of the applied compounds.

**DISCUSSION**

**Plant Vegetative Characteristics:**

In our experiment, vegetative parameters were affected after application of green hum, Algaren or Drin. As it has been mentioned before, all three compounds have promoting influence on nitrogen absorption and metabolism, so more availability of nitrogenous resources might be one of the factors responsible for augmentation of vegetative characteristics. Nikbakht *et al.* (2008) concluded that Humic acid might increase the plant growth by improving the minerals nutrition uptake. Similarly, Wang *et al.* (1995) showed that P uptake by the roots increased (25%) significantly after application of Humic acid to soil. Activation of ATPase and proton pumps in the root cellular membranes are one of the primary responses to Humic acid application (Pinton *et al.*, 1999). Increment of root dry weight in our study was in accordance with previous experiments, Kelting *et al.* (1998) reported higher root growth in red maple after treatment with Humic acid. Similar results were obtained with sugar beets (Sanchez *et al.*, 1972) and maize (Alexandrova, 1977). Liu *et al.* (1998) showed that 400 mg l\(^{-1}\) of Humic acid increased photosynthesis rate and root development in bentgrass. 500 mg l\(^{-1}\) of Humic acid induced the root elongation in *Pisum sativum* (Vaughan, 1974). 50% increment of root length and 22% increase in root dry matters accumulation in wheat root was obtained when plants treated with Humic acid (Kauser and Azam, 1985). Generally increase in root weight is a direct effect of soil fertility and accessibility of roots to essential elements and Humic acid is capable of playing both roles and increasing the growth (Liu and Cooper, 2000).

On the other hand, there are many reports implying the beneficial impact of seaweeds extracts on vegetative parameters of the plants. More lateral roots (Atzmon and Van-staden, 1994; Vernieri *et al.*, 2005) and higher root mass (Thompson, 2004; Mancuso *et al.*, 2006) have been reported in plants treated with various seaweeds extracts. Plant growth regulators such as Zeatin, Isopentenyl, aminopurin and Topolin have been detected in seaweeds extracts (Stirk *et al.*, 2003 and 2004), so presence of higher concentration of these growth stimulators might be another reasons responsible for higher growth of the treated plants. All three compounds in our experiment increased the chlorophyll content of the leaves. Similarly Xudan (1986) reported higher chlorophyll content in wheat leaves treated with Humic acid. As mentioned above plant growth regulators such as cytokinines or cytokinine-like substances in seaweeds extracts cause delay or inhibition of chlorophyll degradation, as postharvest application of seaweeds extract on peppers increased their shelf life and delayed their chlorophyll degradation (Blunden, 1991). In addition, seaweeds extracts contain Betaine which as a nitrogen source may increase this parameter (Genard *et al.*, 1991; Whapham *et al.*, 1993; Blunden *et al.*, 1997).

**Fruit Qualitative Characteristics:**

Like vegetative features, reproductive characteristics were also affected significantly in our study and this was in accordance with previous studies. Sanchez *et al.* (1968) reported augmentation of flowers in pepper flowers after application of Humic acid. In another study 500 mg l\(^{-1}\) of Humic acid caused about 52% increase in number of flowers in gerbera (Nikbakht *et al.*, 2008). One of the main factors influencing flowering is Phytohormones such as Auxin s, gibberellins, and Cytokinines whose presences have been detected in seaweeds extracts (Chen and Aviad, 1990; Atiyeh *et al.*, 2003). Auxin or Auxin-like activities have been reported for seaweeds extracts (Crouch and Van-Staden, 1993). In 1 g of dry extracts of *Ascophyllum nodosum* over 50 mg of IAA has been detected by Kingman and Moore (1982). So their usage might lead to more flowers which occurred in our study. As it has been shown in table 2 weight and length of fruits increased after application of Humic acid, Algaren or Drin. Since strawberry fruit growth is directly related to IAA production by Achenes, and seaweeds extract might increase the concentration of plant growth regulators that stimulate the growth, bigger fruits would be expected with their usage, our results are in accordance with the previous mentioned hypothesis. On the other hand, gibberellins and cytokinin-like activities have been reported for Humic acid (Zhang and Ervin, 2004). Atiyeh *et al.* (2003) reported 250 mg l\(^{-1}\) of Humic acid caused tomato fruit increment, they included hormone like activity for this compound as one the reasons responsible for bigger fruits. The presence of hormones or hormone like substances in Humic acid, Algaren or Drin may induce transport of higher amounts of assimilates or nutrients into fruits (Davey and Van-Staden, 1978).

**Fruit Qualitative Characteristics:**

TA and TSS did not significantly change in this investigation but highest amount of ascorbic acid
concentration occurred when plants treated with 1.5 g l\(^{-1}\) Humic acid, 3 g l\(^{-1}\) of Algaren or 1 g l\(^{-1}\) of Drin. This accumulation of vitamin C may be due to mentioned hormone activity of these three compounds. In fact they might protect the fragile structure of ascorbic acid and prevent its degradation.

**CONCLUSION**

Humic acid and seaweeds extracts are capable of influencing vegetative and reproductive growth of strawberry cv. 'Selva' in a promoting and beneficial way. They increased production quality and quantity parameters, although there are still question about the details of exact mechanisms of manipulation of these compounds. Studies aimed to elucidate the effect of these compounds on photosynthesis, carbohydrates metabolism and their interaction with endogenous hormones could be recommended for further studies.

**REFERENCES**


