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ORIGINAL ARTICLE

Vegetative and Reproductive Growth of Tomato Plants Affected by Calcium and Humic Acid

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ABSTRACT
In order to study the effect of calcium and humic acid foliar application on growth, yield and yield components of tomato plants as a completely randomized experimental design with four replications. These factors included humic acid in 3 levels (5, 10 and 20 ppm) and calcium in 2 levels (5 and 10 mM) spray on tomato. Results indicated that humic acid (20 ppm) and calcium (10 mM) either alone or in combination (20 ppm HA+10 mM Ca) increased vegetative and reproductive growth, yield and chlorophyll content. The application 20 ppm HA+10 mM Ca significantly increased the leaves-NK content and dry weight; in the other hand it decreased the incidence of blossom end rot. The TSS, TA, and vitamin C content of tomato fruit had significantly affected by the application of 20 ppm HA+10 mM Ca. Foliar application of 20 ppm HA+10 mM Ca resulted in the maximum TSS (6.64 °Brix), TA (3.5 and vitamine C (15.1). In conclusion, application of calcium and humic acid improved the yield contributing factors that resulted in significant increase in tomato fruit yield.
***Key words**: tomato, growth, yield, fruit quality, humic acid, calcium*
Abbreviations: HA, Humic acid; Ca, Calcium; TSS, Total soluble solids; TA, Titr atable acidity.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is important crop in many countries. According to FAO, tomato has a main role in human nutrition because of its rich source of lycopene, minerals and vitamins such as ascorbic acid and *b*-carotene which are anti-oxidants and promote good health [1]. Foliar application is based on the principle that the nutrients are quickly absorbed by leaves and transported to different parts of the plant to fulfill the functional requirement of nutrition. Foliar application of the nutrients is obviously an ideal way of evading the problems of nutrient availability. Refaat and Saleh [2] demonstrated that foliar application of macro and micro components at 3000 ppm in monthly time intervals, advantageously affected basil yield quantity and the quality of essential oil.

Calcium ions perform multiple roles in plant cell physiology. They are important intracellular messengers, mediating responses to hormones, biotic and abiotic stress signals and a variety of developmental processes [3]. In most fruits, firmness retention is an important quality parameter in fresh-cut fruits and vegetable products. The preharvest nutritional status of fruit, especially with respect to calcium, is an important factor affecting potential storage life [4]. Foliar applications of calcium chloride have been reported to delay ripening and retard fungal growth on strawberries [5].

Humic acid (HA) is a heterogeneous mixture of many compounds with generally similar chemical properties; it performs various functions in the soil and on plant growth. The effect of HA on the availability of P and micronutrients has been given particular attention because of considerable increases in uptake rates of HA application [6]. The objective about the effect of humic acid on plant growth [7] means that humic substances affect the ion exchange of plant nutrients which are useful in microbial activity by increasing conversions directly as well as indirectly as a result of the stimulating plant growth hormones. Albayrak and Camas [8] found that increasing application of humic acid has significantly promoted root and leaf yield of forage turnip (*Brassica rape* L.). Soil pH increased with rising levels of HA addition and the same trend was also observed for organic C and CEC of the soils by Sharif *et al.,* [9]. The application of Humic reduces the requirement of other fertilizers. It also increases crop yield, soil aeration, and drainage. It can also be improved by Humic, the establishment of desirable environment for the development of microorganisms. The aim of the present study was the effects of foliar spraying of HA and Ca either alone or in combination on the growth, yield, fruit quality characteristics and blossom end rot incidence of tomato fruit.

MATERIAL AND METHODS

The field experiment was conducted in 2012-2013 at the greenhouse complex in Ilam (Elevation 1339 m, Latitude East 33.638, Longitude North 46.431), Iran. The soil of the experimental field was silty loam in texture with a pH of 6.5, containing total N (2.4%), total C (1.35%), a C/N ratio of 0.45, 77, 114 mg·kg^{-1} of P, and K, respectively, and with an EC of 0.078 ds·cm^{-1}. The aim of the experiment was to study the effect of 3 concentrations of HA (5, 10 and 20 ppm) and 2 concentrations of Ca (5 and 10 mM) spray, on plant height, number of fruits per cluster, total yield, mean fruit weight, soluble solid content, total titrable acid, vitamine C, total nitrogen and potassium in fruits of tomato. \`Rada\` cultivar seeds were sown on 5 April 2012 in single plastic pots (12 × 11 cm) filled with white peat. Transplantation took place on 1 May 2012 into greenhouse, at a plant density of 3 plants per m^2 for the remainder of the experiment after soil plowing and disking. Plants were grown vertically, allowing the principal stem to grow. Spraying solutions were prepared with distilled water and sprayed three times during plant growth by 20 days between treatments with a sprayer. Temperature inside the greenhouse was controlled using automatic activation of the aerial heating fan with a TCL split type air condition-indoor unit system to maintain temperature between 25 and 16°C (day and night). Harvesting took place from 3 July to 18 September 2012 (7 times). The recorded growth traits were: During the trial three plants per experimental unit were sampled and fruit numbers and weight were determined. Fresh and dry weight were measured for the fruits after drying in a thermo-ventilated oven at 70°C. Plant height was determined for 5 plants in the middle row in each treatment after the first picking. For this purpose, the plant height from the soil line to the top was determined with a measuring tape and averaged to represent corresponding treatments.
Total nitrogen of the sample was determined by Kjeldahl method [10]. Leaf K was measured with the help of a flame photometer. Lycopene in fruits was estimated as described by Sadasivam and Manikam [11]. The number of flower clusters per plant, number of fruits per cluster, and number of fruits per plant were determined for 5 plants in the middle row of the replication. For this purpose, the number of flower clusters per plant, number of fruits per cluster, and number of fruits per plant were counted and divided by the total number of plants. Nitrate Reductase Activity (NRA) was determined by the method of Silveira *et al.,* [12]. Leaf disc from the second youngest fully expanded leaves (200 mg fresh mass) were infiltrated twice for two minutes with 5 ml of reaction mixture containing 100 mmol/l Potassium Phosphate buffer (pH 7.5) ; 25 mmol/l KNO3 ; and 1% isopropanol. The reaction mixture was incubated at 35°C for 30 minutes in the dark. NR activity was estimated from the amount of NO^{2-} formed during the incubation period and released from the leaf discs to the medium after boiling for 5 minutes. Aliquots were mixed with 2 ml of (1:1) 1% sulfanilamide in 2.4 mol /l HCl ; 0.02% N-1-naphtyl-ethylenediamine and the absorbance was taken at 540 nm. The total yield for each treatment was calculated by weighing the fruit picked in each replication and converting the weight to Mg. ha^{-1}. The average fruit weight was estimated by weighing 10 fruits in each treatment, with the help of an electronic balance measuring in grams to the third decimal place, and then converting to average fruit weight. Sub-samples (10 g) were pressed through cheese cloth to extract the juice. Total soluble solids were determined on a portable refractometer (NC-1, Atago Co., Japan) standardized with distilled water. Total titrable acid and vitamine C was measured by NaOH (0.1 M) titration and indophenol's method according to Horvitz *et al.,* [13]. Blossom end rot incidence (%) was estimated by counting the total number of fruits and fruits showing symptoms of blossom end rot in each treatment. The blossom end rot incidence is expressed as a percentage of total fruits. The fruit firmness was recorded with the help of a penetrometer. For this purpose, 5 fruits from each treatment were taken and penetration force was measured by gently inserting the probe into the equatorial region of the fruit. The readings for all 5 fruits were averaged to represent the corresponding treatments. Photosynthetic pigments chlorophyll was determined using chlorophyll meter (SPAD-502, Minolta Co. Japan), which is presented by SPAD value. Average of 3 measurements from different spots of a single leave was considered.

Statistical Analysis

The experiment was completely randomized experimental design (CRD) with three replications.
Data were analyzed by SPSS 16 software and and comparing averages was done by Duncan's test and a probability value of %5.

RESULTS AND DISCUSSIONS

Vegetative factors, chlorophyll and leaf NK content

Our results showed that application of HA and Ca either alone or combination significantly influenced plant height and dry weight (Table 1). The highest rates of these variables were found at 20 ppm HA+10 mM Ca. In other words, application of higher concentrations of these factors increased plant height and dry weight as other treatments (Table 1). It is evident that increase in HA (from 5 and 10 to 20 ppm) and Ca concentration (from 5 to 10mM) increased plant height and dry weight (Table 1). HA and Ca either alone or combination significantly affected chlorophyll and leaf-NK content (Table 1). The highest chlorophyll and leaf-NK content were obtained at 20 ppm HA+10 mM Ca. Results indicated a rise in chlorophyll and leaf-NK content as HA and Ca concentration increased. Foliar application of 20 ppm HA+10 mM Ca resulted in the maximum plant height (122 cm) and dry weight (6.79 g). Fernández-Escobar *et al.,* [14] found that application of HA and Ca stimulated chlorophyll content and accumulation of K, B, Mg, Ca and Fe in leaves. Ayas and Gulser [15] reported that HA application was the main reason of enhanced nitrogen uptake in spinach. These results are in agreement with Del-Amor and Marcelis [16] and Celik *et al.,* [17]. They reported that, HA and Ca significantly increased mineral -nutrients uptake. The results are accordance to Abdel Fatah *et al.,* [18], Yaseen *et al*., [19] and Kashif *et al*., [20] who observed that application of humic acid and Ca improved growth parameters. Yousef *et al.,* [21] indicated that treated Chemlali olive seedlings with HA was the most effective compared with the other treatment since this gave the best results concerning plant height, brunch numbers, leaf numbers. Also it increased plant diameter and leaves area comparing with control. Deficiency of Ca decreases plant height by mitotic activity reduction in the terminal meristem [22]. Thus, the application of calcium raises plant height [23]. Smolen and Sady [24] reported that Ca application caused an increase in the concentration of N-total in storage roots in comparison with control plants. Similar results were obtained in our research that total NK in fruits increased when sprayed with higher level of Ca. Since HA enhances plant height (Table 1), the Ca and HA combinations were more effective than individual application on climbing the number of branches per plant and plant height. The present findings are in agreement with the results obtained by Siddiq *et al.,* [25] and Mahmoud and Hafez [26] who reported vegetative growth parameters of soybean improve at high concentration of HA and Ca than low.

Table 1. Effect of HA and Ca on leaves-NK content, yield, number of fruits per plant,mean fruit weight, number of flower branch per plant, plant height of tomato

Treatments	Plant height (cm)	Number of flower branch per plant	Number of fruits per plant	Mean fruit weight (g)	Yield (Mg. ha-1)	Chlorophyll (SPAD)	D. W. (g/100g F.W.)	N(%)	K(%)
Control	83.14c	3.14c	16.12c	56.42c	87.15cc	16.21c	4c	1c	1.87c
5 ppm HA	97.14b	4.12b	20.14ab	78.35b	100b	18.1b	4.79b	2b	2.1b
10 ppm HA	100.12b	4.36b	20.21ab	80.14b	101.1b	20.14b	5.1b	2.1b	2.14b
20 ppm HA	120.14a	5.14ab	25.14ab	90.14ab	122.14b	27.14ab	6a	2.41a	3.2a
5 mM Ca	90.4b	4.25b	18.2b	72.1b	91.1b	21.4b	5b	2b	3.1ab
10 mM Ca	100.1b	5.1ab	21.14ab	91.2ab	101.14b	25.3ab	5.71a	2.34a	3.34a
5 ppm HA+5 mM Ca	94.89b	4.41b	17.6b	81.8b	128.6b	20b	3.7b	1.8bc	2.7b
5 ppm HA+10 mM Ca	95.36b	4.61b	17.86b	88.6ab	127.3b	19.89b	3.8b	1.79bc	2.49b
10 ppm HA+5 mM Ca	97b	4.5b	18b	90ab	131.14b	21.7b	4.1b	1.89bc	2.89b
10 ppm HA+10 mM Ca	97.1b	4.89ab	18.36b	87.14ab	140.1ab	22.1b	4.8ab	2.1b	3b
20 ppm HA+5 mM Ca	100.14b	5.1ab	21.14ab	90.14ab	142.1ab	24.17b	5.1ab	2.12b	3.12ab
20 ppm HA+10 mM Ca	122a	6a	30.1a	95.14a	160.14a	32.1a	6.79a	2.61a	3.4a

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Reproductive growth, Mean fruit weight and Yield

HA application significantly increased reproductive growth and yield when accompanied by nickel treatment (Table 1). HA and Ca interaction had significant effect on number of flower branch per plant, number of fruits per plant, mean fruit weight and yield. The highest number of flower branch per plant (6), number of fruits per plant (30.1) observed when 10 mM Ca and 20 ppm HA were used together (Table 1). Increase in HA concentration significantly increased mean fruit weight and yield when accompanied by Ca, and the highest values of this parameter (95.14 g and 160.14 Mg. ha^{-1})were obtained at 20 ppm HA+10 mM Ca (Table 1). Our results were in agreement with that of Muromtsev *et al*., [27] and

Celik *et al.,* [17] and Albayrak and Camas [8] who observed that, application of Ca and HA can significantly increased the reproductive growth and yield. In the full bloom period of humic acid application, berry weight, reproductive growth and maturity index values of Italy grape cultivar increased significantly [28]. Application of humic acid enhanced head weight of lettuce (*Lactuca sativa* L. var. longifolia) by increasing the availability of phosphorus and nitrogen [29]. The positive effects of the humic substances were also observed on the studies such as dry matter yield increases on corn and oat seedling [16], yield that increases on radish and green bean seedlings [30]. Similar results were also obtained from pepper fruit treated with HA [31]. However, in another study, Yildirim [32] have reported a significant enhancement in fruit diameter and length as a result of exogenous HA application in tomato. Muromtsev *et al*., [26] reported that Ca application during growth stage of 3-4 true leaves of tomatoes increased the yield by 30-50% along with accelerated ripening and improved quality. Application of Calcium carbide also stimulates root growth and early onset of flowering in agronomic and vegetable crops [19-20]. Zaky *et al.,* [33] found that the number of shoots/plant, average leaf area, total yield and average pod fresh weight were increased by application of humic acid as a foliar fertilizer at a rate of 1 g/L. Our results is supported by the finding of Russo and Berlyn [30] and Hao and Papadopoulos [34] who reported that HA and Ca sprays increased fruit yield and reproductive growth of tomato.

Table 2. Effect of HA and Ca on TSS, TA, vitamine C and Blossom end rot of tomato

Treatments	TSS (°Brix)	TA (%)	Vit. C (mg. 100 g fresh fruit-1)	Blossom end rot (%)
Control	3.12c	1c	7.36c	32.14a
5 ppm HA	4.1b	2.7b	9.45b	14.51a
10 ppm HA	4b	3.1ab	10.1b	14.3a
20 ppm HA	5.15ab	3.41a	12.1a	10.12b
5 mM Ca	5.1b	2.4b	10.3b	7.1a
10 mM Ca	6.1a	3.14a	12.14a	5.1b
5 ppm HA+5 mM Ca	4b	1.6b	9.79b	6.2a
5 ppm HA+10 mM Ca	4.01b	1.89b	10b	6.12a
10 ppm HA+5 mM Ca	3.89b	2.64ab	9.8b	6.71a
10 ppm HA+10 mM Ca	4b	2.5ab	10.1b	6a
20 ppm HA+5 mM Ca	4.12b	2.87ab	11.49ab	6.8a
20 ppm HA+10 mM Ca	6.64a	3.5a	15.1a	4.14b

Means followed by same letter are not significantly different at 5% probability using Duncan's test.

Fruit quality

Different HA and Ca levels alone or in combination had significant effect on fruit quality (Table 2). TSS, TA and vitamin C content increased when plants were sprayed with 20 ppm HA and 15 mM Ca (Table 2). Interaction between factors was significant in TSS, TA and vitamin C contents. Highest amount of TSS (6.64 °Brix), TA (3.5 %) and vitamin C (15.1 mg. 100 g fresh fruit^{-1}) were observed from sprayed at 20 ppm HA+10 mM Ca. The blossom end rot was affected by application of HA and Ca alone or in combination. Lowest end rot (4.14%) was obtained from 20 ppm HA+10 mM Ca (Table 2). The blossom end rot of tomato fruit is a physiological disorder resulting from calcium deficiency [16]. It reduces fruit quality and market value [35]. The blossom end rot incidence can be aggravated by the deficiency of other nutrients such as NH_4-N, K, and Mg [36]. These results support the conclusion that the application of calcium combination as growth media reduces disease of plants which was exposed to foliar pathogens [37]. Similar findings were reported by Samant *et al.,* [38]. Preharvest and postharvest treatments with calcium salts have been effective in control of several physiological disorders, reducing the incidence of fungal pathogens and maintaining fruit firmness [39]. It may also be due to micro-nutrients which are known to impart direct and indirect effects on fruit yield and quality. Similarly, Kumar and Shukla [40] also reported that fruit quality of litchi increased by spray of macro and micro element. Increasing the Ca^{2+} content of apples maintains fruit firmness, decreases the incidence of disorders such as water core, bitter pit and internal breakdown [41]. Calcium was found to increase cell membrane integrity by binding to the polar head groups of phospholipids. Obviously, many studies have been focusing on the role of calcium in maintaining fruit apple quality since it binds to the cell wall polymers. In general, TSS is an important quality factor which influences the palatability and acceptability of fruit. The presented data in this study agreed with that found by Kadir [42] who showed that preharvest tree sprays with Ca have been used commercially to improve ratio of soluble solid concentration to titralable acidity of apple fruits. Saleh *et al.,* [43] and Yildirim [32] have reported a significant enhancement fruit quality as a result of exogenous HA application in tomato. The same results were noticed when humic acid applied on 'Canino' apricot when it enhanced T.S.S and decreased acidy [44]. According to the results significant improvement on tomato quality characteristics was observed. It seems that under higher concentration of Ca and HA could find a better relation between spraying and quality.

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