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ORIGINAL ARTICLE

Effect of Foliar Application of Humic Acid and Calcium Chloride on tomato growth

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ABSTRACT

The study was carried out to evaluate the effects of foliar application of humic acid and calcium chloride on vegetative and reproductive growth, yield, and quality of tomato plants as a completely randomized block design with 4 replications, each consisting of 3 pots with each pot containing one plant. Humic acid (15 and 30 ppm) and calcium chloride (10 and 15 mM) solutions were applied as foliar sprays either alone or in combination. Data were recorded for plant height, branches per plant, flowers per cluster, fruits per plant, yield, fruit weight, fruit firmness and total soluble solid content of the fruit. Results showed that humic acid (30ppm) and calcium chloride (15 mM) spray either alone or in combination (30 ppm HA+15 mM Ca) affected on vegetative and reproductive growth and chlorophyll content, significantly. Mean comparisons indicated yield, and quality of tomato plants was improved by increasing humic acid and calcium chloride concentration up to 30 ppm and 15 mM. Foliar application of Ca (15 mM) + HA (30 ppm) resulted in the maximum TSS (5.14 °Brix), vitamin C (25.14), nitrate reductase activity (6.4), yield (25.36 t ha^{-1}), fruit firmness (3.91 kg cm^{-2}), fruit lycopene content (2.14) and the lowest blossom end rot incidence (5%). In Finally, humic acid and calcium chloride application can be helpful for yield improvement and prevent of decreasing yield.

Keywords: *tomato, spray, humic acid, yield and yield component, calcium chloride*

Abbreviations: HA, Humic acid; TSS, Total soluble solids; TA, Titratable acidity; Ca, calcium chloride.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is a member of the Solanaceae family. It considered a major vegetable crop in many parts of the world including Iran. Tomato is a rich source of lycopene and vitamins. Lycopene may help counteract the harmful effects of substances called "free radicals", which are thought to contribute to age-related processes and a number of types of cancer, including, but not limited to, those of prostate, lung, stomach, pancreas, breast, cervix, colorectum, mouth and esophagus [1-2]. It is, therefore, highly desirable to explore possible ways and means to enhance the productivity of this important crop employing cost effective and easy to use techniques. The foliar application of macro and micro-nutrients have very important role in improving fruit set, productivity and quality of fruits. It has also beneficial role in recovery of nutritional and physiological disorders in fruit trees. Previous various experiments have been conducted on foliar spray of micro and micro nutrient in different fruit crops and shown significant response to improve yield and quality of fruits [3]. Wojciechowska et al. [4] report that foliar feeding significantly decreased nitrate contents in broccoli heads, as compared to non-feeding plants. When Del Amor et al. [5], were applying foliar feeding of peppers with macro-element, nitrogen total contents in fruit increased. Calcium (Ca^{2+}) is a universal second messenger and it has long been considered as the second messenger in many signaling cascades; including defense signaling [6]. Calcium plays an important role in plant growth and in many physiological activities of bulbous flowers [7]. Direct application of calcium to the fruit is the most effective method for increasing fruit calcium content [8]. It is well known that calcium have an important role in maintaining quality of fruits and vegetable. Calcium treatment helps to retain fruit firmness and increase vitamin C content [9]. Preharvest and postharvest treatments with calcium salts have been effective in controll of several physiological disorders, reducing the incidence of fungal pathogens and maintaining fruit firmness [10]. Humic acid (HA) is a promising natural resource that can be used as an alternative to synthetic fertilizers to increase crop production. It exerts either a direct effect, such as on enzymatic activities and membrane permeability, or an indirect effect, mainly by changing the soil structure [11]. Humic acid application, berry weight, titratable acidity and maturity index values of Italy grape cultivar increased significantly in the full bloom period [12]. Humic acid Application at medium levels (250 g/m^2) increased yield and nutrient content of spinach[13]. They concluded that humic acid application caused increased nitrogen uptake which was the main reason of enhanced vegetation growth of spinach. Zaky et al. [14] found that the number of shoots/plant, average leaf area, total yield, average pod fresh weight and P content were increased by application of humic acid as a foliar fertilizer at a rate of 1 g/L. 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.

MATERIALS AND METHODS

In order to determine, the effect of different levels of HA and Ca used in spraying solution on the yield and quality of tomato during 2012-2013 growth seasons 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 7, containing total N (2.5%), total C (1%), a C/N ratio of 0.46, 75, 107 mg·kg^{-1} of P, and K, respectively, and with an EC of 0.071 ds·cm^{-1}. Seeds of tomato cultivar Tivi F1 were sown in the month of January and were transplanted during the month of March. There were 3 ridges in 1 subplot and 10 plants in each ridge. Row-to-row distance and plant-to-plant distance was 75 cm and 30 cm, respectively. HA (15 and 30 ppm) and Ca (10 and 15 ppm), alone or in combination, were applied as foliar spray 30 days after transplanting and when the fruits were berry-sized. A back-held spray pump was used for foliar application of the chemicals. After each treatment, the pump was washed thoroughly. A teaspoon of commercial washing powder was added as a wetting agent. Distilled water containing a comparable amount of wetting agent was sprayed on the plants in the controlled treatment. All foliar spraying was carried out early in the morning. The following characteristics were recorded, 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 leaves. Total nitrogen of the sample was determined by Kjeldahl method [15]. For determination of K contents of leaf, plant samples were air-dried and were then ground. K was determined after dry digestion of dry and sub-samples in a HCL preparation. Potassium was determined by flame photometry. Lycopene in fruits was estimated as described by Sadasivam and Manikam [16]. Nitrate Reductase Activity (NRA) was determined by the method of Silveira et al. [17]. Total soluble solids were determined on a portable refractometer (Sper Scientific Ltd., Scottsdale, Ariz.) 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. [18]. Blossom end rot incidence (%) was estimated by counting the total number of fruits and fruits showing symptoms of blossom end rot in each plot. The blossom end rot incidence is expressed as a percentage of total fruits. The fruit firmness was recorded with the help of a pressure meter (OSK 10576 CO., Japan). 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 laid out in randomized complete block design with 4 replications, each consisting of 3 pots with each pot containing one plant. 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 DISCUSSION

Chlorophyll, Nitrate reductase activity and leaf NK content

Our results clearly indicate that leaves -NK content of tomato was not affected by application of Ca alone, but HA application resulted in a significant increase in the leaves -NK content. Also, it could be noticed that HA at 30 ppm + Ca at 15 mM (2.51 and 2 %, respectivly) was superior in this respect. Foliar application of HA alone significantly increased the nitrate reductase activity. The nitrate reductase activity increased from 4.14 in the control to a maximum of 6.4 with 30 ppm HA and 30 ppm HA+15 mM Ca application (Table 1). The nitrate reductase activity was not significantly affected by the foliar application Ca. Data in Table (1) indicate that chlorophyll content positively responded to the different foliar applications with HA or Ca. The chlorophyll content increased from 13.12 in the control to 22.41 and 21.14 (SPAD) with 30 ppm HA and 15 mM Ca application. Also, the interaction between HA or Ca (30 ppm HA+15 mM Ca) gave the highest value (25.14) in the respect, comparing with the control plants. Fernández-Escobar et al. [19] and Ilias et al. [20] found that application of HA and Ca stimulated chlorophyll content and promoted the accumulation of K, B, Mg, Ca and Fe in leaves. Ayas and Gulser [13] reported that HA application was the main reason of enhanced nitrogen uptake in spinach. Smolen and Sady [21] 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 so that total NK content increased when sprayed with higher level of Ca. These results are in agreement with those obtained by Del-Amor and Marcelis [22] and Celik et al. [23]. They reported HA and Ca significantly increased mineral -nutrients uptake.

Vegetative growth

Our results showed that growth parameters (number of branches, plant height and dry weight) were significantly increased by HA or Ca foliar applications (Table1). The interaction effect between HA and Ca foliar application at 30 ppm HA+15 mM Ca concentration gave the highest values of growth parameters as compared with either individual foliar application or control plants. Foliar application of 30 ppm HA+15 mM Ca resulted in the maximum plant height (123.14 cm), number of branches (7) and dry weight (6.51 g). The results found are in agreement with the findings of Abdel Fatah et al. [24], Yaseen et al. [25]; Kashif et al. [26] who observed that application of humic acid and Ca improved growth parameters. Yousef et al. [27] indicated that treated Chemlali olive seedlings with HA treatments was the most effective one compared with the other treatment since this treatment gave the best results concerning plant height, brunch numbers, dry weight, leaf numbers, also it increased plant diameter and leaves area comparing with control. El-Ghozoli [28] and L-Ghanam and El-Ghozoli [29] indicated that the application of humic acid significantly increased the dry matter production of faba bean plants. Deficiency of Ca decreases plant height by decreasing mitotic activity in the terminal meristem [30]. Thus, the application of calcium increases plant height [31]. Since HA enhances plant height (Table 2). Ca and HA combinations were more effective than individual application in increasing the number of branches per plant and plant height. The present findings are in agreement with the results obtained by Siddiq et al. [32] and Mahmoud and Hafez [33] who reported HA and Ca application increase vegetative growth parameters of soybean.

Reproductive growth and Yield

Reproductive growth and yield of tomato as affected by HA or Ca foliar application(Table 1). According to these results, flowers per cluster, fruits per cluster, fruits per plant, fruit weight and yield were improved by application of HA and Ca either alone or in combination. The maximum number of flowers per cluster (32.14) and fruits per cluster (6.65) was recorded at 30 ppm HA+15 mM Ca application. The combination of HA+ Ca significantly increased the fruits per plant and fruit weight from 58.74 and 68.8 in the control to 89.14 and 95.14 with 30 ppm HA+15 mM Ca application. Data in Table (1) indicate that significant increases in total yield were existed with foliar applications of HA and Ca either alone or in combination compared to control treatment. The combination treatments gave the highest values especially HA at 30 ppm and Ca at 15 mM which ranked the first in this respect. The maximum number of yield (25.36) was recorded at 30 ppm HA+15 mM Ca. Such increments in flowering and fruit yield due to treating the plants with HA and Ca treatments might be connected with their effect on increasing the vegetative growth parameters, photosynthetic pigments and leaf-NK content (Table 1) which affects plant growth and in turn increased its productivity. Our results were in agreement with Muromtsev et al. [34], Celik et al. [23] and Albayrak and Camas [35] who observed 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 [12]. Application of humic acid increased head weight of lettuce (*Lactuca sativa* L. var. longifolia) due to increasing the availability of phosphorus and nitrogen [36]. The positive effects of the humic substances were also observed on the studies such as dry matter yield that increases on corn and oat seedling [23]; yield increases on radish and green bean seedlings [1]. Similar results were also obtained from pepper fruit treated with HA [37]. However, in another study, Yildirim [38] have reported a significant enhancement in fruit diameter and length as a result of exogenous HA application in tomato. Muromtsev et al. [34] 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 [25]. Zaky et al. [14] 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 [39] and Hao and Papadopoulos [40] who reported that HA and Ca sprays increased fruit yield and reproductive growth of tomato.

Fruit quality

Our results indicate that the different sprayed treatments increased fruit quality (TSS, TA, pH, vitamine C, fruit firmness and fruit lycopene content) of tomato fruits. The maximum fruit firmness of 3.91 kg cm^{-2} was, however, recorded at 30 ppm HA+15 mM Ca application. The HA and Ca application also resulted in a significant increase in firmness of tomato fruit as compared to control. The differences among fruit firmness 15 ppm HA+10 mM Ca, 15 ppm HA+15 mM Ca and 30 ppm HA+10 mM Ca were not significant (Table 2). The TA and PH of tomato fruit was significantly affected by the application of HA and Ca alone, but interaction HA+Ca were not significant. The TA and PH increased from 1.98 and 1.38 (%) in the control to a maximum of 3.12 and 3 with 15 mM Ca application, followed by 3.1 and 2.79 with 15 mM Ca, respectively (Table 2). TSS, vitamin C and fruit lycopene content increased significantly with foliar application of HA and Ca either alone or in combination. The TSS, vitamin C and fruit lycopene content increased from 3.89 (°Brix), 17.36 (mg. 100 g fresh fruit^{-1}) and 1.1 (mg/100 g) (respectively) in the control to a maximum of 5.14 (°Brix), 25.14 (mg. 100 g fresh fruit^{-1}) and 2.14 (mg/100 g) at 30 ppm HA+15 mM Ca application, respectively (Table 2). The blossom end rot was affected by application of HA and Ca alone or in combination. The incidence of blossom end rot was the highest (18.14 %) in the control and decreased significantly to 5, 5.12 and 5.14 % at 30 ppm HA+15 mM Ca, 15 mM Ca and 30 ppm HA application (Table 2). The blossom end rot of tomato fruit is a physiological disorder resulting of calcium deficiency[22]. It reduces fruit quality and market value [40]. The blossom end rot incidence can be aggravated by the deficiency of other nutrients such as NH_4-N, K, and Mg [41]. These results support the conclusion that the application of calcium combination as growth media reduces disease of plants which was exposed to foliar pathogens [42]. Similar findings were reported by Samant et al. [43]. 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 [10]. It may also be due to micro-nutrients which are known to impart direct and indirect effects on fruit yield and quality. Similarly, Kumar et al. [3] 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 [44].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 [45] 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. [46] and Yildirim [38] 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 [47]. 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.

Table 1. Influence of HA and Ca application on vegetative and reproductive growth of tomato plants.

Treatments	Number of branches	Plant height (cm)	D. W. (g/100g F.W.)	Chlorophyll (SPAD)	N (%)	K (%)	Flowers per cluster	Fruits per cluster	Fruits per plant	Fruit weight (g)	Yield (t ha^{-1})	Nitrate reductase activity µmole NO2- (g. f.w.hr)-1
Control	4.14c	72.13cd	3.41c	13.12c	1.7c	1c	18.36cd	3.89c	58.74c	68.8c	16.12c	4.14c
15 ppm HA	6b	92.11b	5.12b	15.14b	2.17b	1.8ab	24.1b	5.46b	76.1b	86.1ab	19b	5b
30 ppm HA	6.74a	118.45a	6.32a	22.41a	2.45a	1.95a	30.41a	6.44a	83.45a	90.14a	23.68a	6.4a
10 mM Ca	6.04b	90.14b	5b	15b	2.1b	1.23ac	23.1b	5b	71.36b	86.47b	17.63b	5.16b
15 mM Ca	6.61a	118.14a	6.30a	21.14a	2.12b	1.2ac	27.16a	6a	85.14a	92.33a	22.47a	5.23b
15 ppm HA+10 mM Ca	6.12b	95.36b	5.71ab	15.3b	2.13b	1.65ab	24b	6ab	75.14b	85.4ab	20b	5.14b
15 ppm HA+15 mM Ca	6.12b	100.14b	5.8ab	15.49b	2.14b	1.6ab	25.16b	5.41b	63.14bc	78.6b	20.14b	5.12b
30 ppm HA+10 mM Ca	6b	93.14bc	5.85ab	15.46b	2.1b	1.2ac	21.14ac	5.5b	60.14bc	79b	19.65b	5b
30 ppm HA+15 mM Ca	7a	123.14a	6.51a	25.14a	2.51a	2a	32.14a	6.65a	89.14a	95.14a	25.36a	6.4a

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

Table 2. Effect of HA and Ca application on yield and quality of tomato.

Treatments	TSS (°Brix)	vitamine C (mg. 100 g fresh fruit^{-1})	TA (%)	pH	Blossom end rot (%)	Fruit firmness (kg cm^{-2})	Fruit lycopene content (mg/100 g)
Control	3.89b	17.36c	1.98c	1.38c	18.14a	2.14c	1.1c
15 ppm HA	4.59ab	19.46b	2.3ab	2.4b	6.79b	3.1ab	1.59b
30 ppm HA	5a	19.76b	3.1a	2.79a	5.14c	3.51a	2.1a
10 mM Ca	4.47ab	19.36b	2.29ab	2.41b	6.2b	3.6a	1.79b
15 mM Ca	4.89a	19.49b	3.12a	3a	5.12c	3.56a	1.7b
15 ppm HA+10 mM Ca	4.39ab	19.69b	2.45ab	2.38b	6.89b	3.14ab	1.65b
15 ppm HA+15 mM Ca	4.45ab	20.1b	2.3ab	2.1bc	6b	3.17ab	1.69b
30 ppm HA+10 mM Ca	4.69ab	20.14b	2.14ab	2.14bc	6.14b	3.16ab	1.72b
30 ppm HA+15 mM Ca	5.14a	25.14a	2.38ab	2bc	5c	3.91a	2.14a

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