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Foliar Application of Salicylic Acid and Calcium Chloride Enhances Growth and Productivity of Lettuce (*Lactuca sativa*)

S.M.S. Youssef, S.A. Abd El-Hady, Nashwa A.I. Abu El-Azm, M.Z. El-Shinawy
Department of Horticulture, Faculty of Agriculture, Ain Shams University,Cairo, Egypt.

SALICYLIC ACID (SA) and Ca^{2+} regulate the physiological and biochemical mechanisms in plants. A factorial experiment was designed to evaluate the effect of foliar application of calcium chloride (0, 10, 20, 30 or 40 mM) and salicylic acid (0, 0.5, 1, 1.5 or 2 mM) on the vegetative growth of romaine lettuce cv. Balady during 2013/2014 and 2014/2015 seasons at the Experimental Farm of Horticulture Department, Faculty of Agriculture, Ain Shams University, Qalubia Governorate, Egypt. Results clearly indicated positive effects of foliar applications of calcium chloride and salicylic acid either alone or in combination on lettuce growth, productivity and some physiological parameters. Foliar application of calcium chloride at 20 mM significantly increased vegetative growth parameters (plant length, head diameter, fresh and dry weights of head, number of leaves/head, average leaf area and leaf area index), chlorophyll (*a, b,* and total), leaf relative water content, leaf membrane stability index, and macro- and micro-nutrients. Moreover, salicylic acid spraying at 1.5 mM significantly gave the highest significant values of all aforementioned parameters. Exogenous applications of calcium chloride and salicylic acid either alone or in combination reduced nitrate accumulation in the leaves. Spraying of calcium chloride at 20 mM with salicylic acid at 1.5 mM was the most effective treatment which can be used as an applicable practice in romaine lettuce cv. Balady cultivation.

Keywords: Salicylic acid, Calcium chloride, Membrane stability index, Relative water content, Nitrate content.

Introduction

Lettuce (*Lactuca sativa*), an annual plant of *Asteraceae* family, is considered as one of the most important vegetables in human diet which cultivated since 4500 BC in Egypt. Lettuce is the 26th among 39 vegetables and fruits of nutrition value and is the fourth of consumption. The plant is a source of vitamins and minerals with lots of fiber which facilitates colon peristalsis. Moreover, lettuce contains lactocin and lactucopicrin which improve the sleep. In Egypt, lettuce production area was 4541 ha with a total production of 113185 tons and average yield of 27.48 tons ha-1 in 2014 (FAOSTAT, 2016).

Foliar application of agro-chemicals has widely been used in agriculture as a rapid, low-cost and effective way for enhancing growth and productivity of many vegetable crops especially green leafy vegetables like lettuce which have larger and fleshier leaves within a shorter period of time. Salicylic acid and calcium chloride are considered as important agro-chemicals which can be sprayed and play important roles in physiological and biochemical processes.

Salicylic acid (SA, also known as Ortho-hydroxybenzoic acid) is an endogenous growth regulator of phenolic nature (Hayat et al., 2010), which is normally produced in plants in very small quantities (Raskin, 1992) and regulates various physiological and biochemical processes in plants including seed germination, plant growth, thermogenesis, flower induction, nutrient uptake and transport, membrane permeability, ethylene

Corresponding author E-mail: sabrysoliman@hotmail.com
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biosynthesis, stomatal movements, photosynthesis and enzyme activities (Hayat et al., 2010). Moreover, salicylic acid use is safe with respect to human health and is likely to improve the quality and stress resistance of crops (Peng and Yueming, 2006). In recent years, a number of studies have indicated that application of exogenous salicylic acid at non-toxic concentrations to plants can enhance the plant growth and productivity of many crops. Salicylic acid can move freely in and out of the cells, tissues and organs (Kawano et al., 2004) and this movement is finely regulated by Ca^{2+} (Chen et al., 2001).

Calcium (Ca) is an essential macronutrient for plant growth and development, and is considered as an important intracellular messenger, mediating responses to hormones, stress signals and a variety of developmental processes. In addition, calcium is an important constituent in the structure of cell walls and cell membranes (Hepler and Winship, 2010), as well as resistance to bacterial and viral diseases (Hepler, 2005). However, calcium is considered as an immobile element and the plants need a constant supply of calcium for vigorous leaf and root development (Del Amor and Marcelis, 2003) which can be accomplished through foliar application. The literature on the efficiency of foliar application of calcium is controversial because such efficiency depends on the calcium source and applied dosage. In this respect, foliar application of $CaCl_2$ was more efficient than that of CaO and Ca chelate (Almeida et al., 2016).

Since the combined effects of salicylic and calcium chloride have hardly been reported, the current study was, therefore, designed to evaluate the influence of foliar application of salicylic acid and calcium either alone or in combination on the growth and productivity of romaine lettuce cv. Balady.

Material and Methods

Experimental design

A field experiment was designed to evaluate the effect of foliar application of salicylic acid and calcium chloride on the vegetative growth of romaine lettuce cv. Balady, the main cultivar used commercially in the market in Egypt. The study was carried out during 2013/2014 and 2014/2015 seasons at the Experimental Farm of Horticulture Department, Faculty of Agriculture, Ain Shams University, Qalubia Governorate, Egypt. According to soil analysis results, soil texture of the experimental site was a sandy loam.

Lettuce seeds were sown in the nursery on the 2nd of October. On the 1st of November in both seasons, lettuce seedlings at the 2-3 leaf stage were transplanted when the seedlings were three weeks old at both sides of rows at a distance of 20 cm between plants and 60 cm between rows, giving a plant population of ~160,000 stands per hectare.

The experimental design was a split plot with three replications. The treatments included five foliar applications of calcium chloride (0, 10, 20, 30 or 40 mM) as the main plot and five foliar applications of salicylic acid (0, 0.5, 1, 1.5 or 2 mM) as the sub-plot. Each subplot was composed of five rows of 5 m length.

Calcium chloride ($CaCl_2$) was dissolved in distilled water. Salicylic acid (SA) was dissolved in absolute ethanol then added drop-wise to water (ethanol/water: 1/1000, v/v). The pH of all solutions was set to 6.5-7. A surfactant tween 20 (0.5%) was added with the control (deionized water) and all treatment solutions.

Four weeks after transplanting, lettuce plants were sprayed with the above-mentioned concentrations of calcium chloride. Three days after calcium chloride application, the plants were sprayed with the above-mentioned concentrations of salicylic acid. All foliar sprayings were carried out early in the morning.

All other cultural practices (irrigation, fertilization, weeding, and pest control) were carried out uniformly in all plots as recommended by the Egyptian Ministry of Agriculture for lettuce production during growth season.

Data recorded

A random sample of five plants from the three inner rows of each experimental plot was taken at harvest (60 days after transplanting) and the vegetative growth data were recorded. Plant length was measured from the ground level to the top living point of plant. After then, these plants were harvested and then trimmed according to market standards and head diameter was measured. Marketable head fresh weight was recorded and then dried in an oven at 70°C until constant weight to record the head dry weight. Also, the standing leaves on each individual plant were counted.

Average leaf area was calculated as relation between area unit and fresh weight of leaves (Koller, 1972) using the following equation:

$$\text{Leaf area} = \frac{\text{Disk area x No.of disks x fresh weight of leaves}}{\text{Fresh weight of disks}}$$

Leaf area index (LAI) was calculated as a ratio of foliage area to soil area.

Fresh fully expanded leaves were collected and immediately frozen in liquid nitrogen, pulverized in a liquid nitrogen and then stored at -80°C until used to determine chlorophyll content. Chlorophyll a (*Chl a*) and b (*Chl b*) were extracted with 100 % ethanol several times (x3) until the extract became colourless. Their levels were calculated as previously described by López-Orenes et al. (2013).

Leaf relative water content was determined according to the method developed by Barrs and Weatherley (1962). Second leaf of the randomly selected ten plants was used for determining relative water content. Fresh weight (FW) was immediately recorded, and then leaves were immediately soaked for 4 hours in distilled water at room temperature under a constant light and saturated humidity to record turgid weight (TW). The samples were then dried for 24 hours at 80 ºC for recording dry weight (DW). Relative water content (RWC) was calculated by the following formula:

$$\text{RWC (\%)} = \frac{\text{FW-DW}}{\text{TW-DW}} \times 100$$

Leaf membrane stability index was determined according to Sairam et al. (1997). Leaf disks (200 mg) were taken in two sets of test tubes containing 10 ml of distilled water. One set was kept at 40ºC in a water bath for 30 min and electrical conductivity (C1) was measured. The second set was incubated at 100ºC for 15 min and electrical conductivity (C2) was measured. MSI was calculated according to the following formula:

$$\text{MSI (\%)} = (1 - C1/C2) \times 100$$

For mineral analysis, leaf samples from the fourth outer leaf were taken and oven-dried at 70 °C until constant weight. Then they were ground to pass a 1 mm sieve and 0.1 g of the dry samples was taken and digested using a mixture of sulphuric acid (H_2SO_4 98 %) and hydrogen peroxide (H_2O_2 30 %) as described by Allen (1974). All the studied elements were assayed in the digest of the concerned plant samples. Total nitrogen was determined using Kjeldahl method as described by Piper (1950). Phosphorus content was spectrophotometrically measured according to Watanabe and Olsen (1965). Potassium, calcium, magnesium, iron, zinc, manganese, and copper content were determined as described by Chapman and Pratt (1961).

Nitrate content in leaves was determined according to Al-Moshileh et al. (2004) using the HORIBA LAQUAtwin Nitrate Meter, Spectrum Technologies, Inc., IL, USA.

Statistical analysis

All data were subjected to an analysis of variance using the CoStat package program (version 6.303, CoHort Software, USA). The differences among main effects were compared by Duncan's Multiple Range Test, while the differences among interactions effects were separated using least significance difference (LSD). All statistical determinations were made at $p \leq 0.05$.

Results and Discussion

Vegetative growth parameters

The growth parameters of lettuce plants (plant length, head diameter, fresh and dry weights of head, number of leaves/head, average leaf area and leaf area index) were significantly increased by foliar applications with calcium chloride as compared to control plants in both growing seasons (Tables 1 and 2). The maximum and significant stimulatory effect existed in plants sprayed with 20 mM calcium chloride followed by spraying with 30 mM. Similar stimulatory effects of calcium chloride on vegetative growth parameters were reported in snap bean (El-Tohamy et al., 2001), pepper (El-Tohamy et al., 2006), tomato (Rab and Haq, 2012), cucumber (Kazemi, 2013b), strawberry (Kazemi, 2015), cowpea (Mohamed and Basalah, 2015), and lettuce (Almeida et al., 2016). The stimulatory effect of calcium chloride on lettuce growth may be attributed to the fact that calcium ions (Ca2+) appeared to participate in the regulation of several aspects of cell division. Calcium is a necessary ion in the formation of the mitotic spindle which directly affects cell division (Hepler, 1994).

Also, data presented in Tables 1 and 2 clearly showed that all tested foliar applications of

TABLE1. Effect of foliar application with calcium chloride and salicylic acid on plant length, head diameter, and fresh and dry weights of head of romaine lettuce cv. Balady in 2013/2014 and 2014/2015 seasons.

Foliar applications		Plant length (cm)		Head diameter (cm)		Head fresh weight (g)		Head dry weight (g)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Calciumchloride[a]									
0.0 mM		36.19D	38.32D	8.26E	8.57D	561.13D	620.53D	35.53D	38.56D
10 mM		40.55C	41.39C	9.42C	9.13C	628.69C	669.98C	39.79C	41.62C
20 mM		44.56A	45.49A	10.22A	10.16A	689.90A	736.32A	43.71A	45.74A
30 mM		43.11B	44.59B	9.78B	9.85B	668.70B	721.15B	42.12B	44.85B
40 mM		40.08C	41.29C	9.21D	9.23C	621.41C	667.90C	39.33C	41.49C
Salicylic acid[a]									
0.0 mM		40.04c	41.17c	9.19b	9.15b	620.73c	666.81c	39.28c	41.43c
0.5 mM		40.55bc	42.01b	9.32b	9.17b	628.62bc	680.46b	39.81bc	42.26b
1.0 mM		41.09b	42.13b	9.43ab	9.41ab	636.09b	681.97b	40.29b	42.37b
1.5 mM		41.72a	43.60a	9.51a	9.76a	647.55a	705.40a	40.79a	43.88a
2.0 mM		41.08b	42.17b	9.44ab	9.44ab	636.85b	681.25b	40.31b	42.31b
Calcium chloride X Salicylic acid interaction									
0.0 mM	0.0 mM	35.07	36.20	8.04	8.19	543.40	586.47	34.39	36.50
	0.5 mM	35.73	38.20	8.07	8.43	554.43	618.93	35.11	38.44
	1.0 mM	37.73	38.73	8.61	8.63	585.13	627.50	37.05	38.97
	1.5 mM	36.27	40.20	8.30	8.88	562.27	651.69	35.60	40.48
	2.0 mM	36.13	38.27	8.26	8.73	560.43	618.07	35.49	38.39
10 mM	0.0 mM	39.60	40.13	9.10	8.78	614.13	649.17	38.87	40.32
	0.5 mM	40.07	41.07	9.52	8.59	622.67	665.33	39.42	41.33
	1.0 mM	40.40	40.67	9.46	9.08	624.00	658.87	39.49	40.91
	1.5 mM	41.33	43.20	9.42	9.75	642.00	699.60	40.64	43.48
	2.0 mM	41.33	41.87	9.58	9.43	640.67	676.93	40.55	42.05
20 mM	0.0 mM	43.87	44.80	10.12	10.00	679.93	725.83	43.04	45.06
	0.5 mM	44.53	45.30	10.19	10.12	688.77	732.70	43.67	45.50
	1.0 mM	44.73	45.50	10.25	10.19	690.97	734.00	43.87	45.66
	1.5 mM	46.20	47.13	10.65	10.63	716.10	763.73	45.32	47.44
	2.0 mM	43.47	44.73	9.87	9.87	673.73	725.33	42.64	45.05
30 mM	0.0 mM	42.40	44.47	9.75	9.83	657.20	720.30	41.56	44.75
	0.5 mM	42.20	44.27	9.58	9.64	654.13	717.00	41.41	44.53
	1.0 mM	43.13	44.80	9.82	9.96	668.60	726.33	42.31	45.11
	1.5 mM	44.40	45.27	9.90	10.00	690.87	728.70	42.76	45.55
	2.0 mM	43.40	44.13	9.87	9.80	672.70	713.43	42.58	44.31
40 mM	0.0 mM	39.27	40.27	8.93	8.94	608.97	652.27	38.55	40.52
	0.5 mM	40.20	41.20	9.23	9.07	623.10	668.33	39.44	41.51
	1.0 mM	39.47	40.93	8.99	9.18	611.73	663.13	38.72	41.19
	1.5 mM	40.40	42.20	9.26	9.56	626.53	683.30	39.65	42.44
	2.0 mM	41.07	41.87	9.63	9.39	636.73	672.47	40.30	41.77
$LSD_{0.05}$ [b]		1.40	1.59	0.38	0.42	24.70	27.60	1.35	1.68

[a]Means into every group within a column for the same factor followed by the same letter are not significantly different (P = 0.05) according to Duncan' s multiple range test.

[b]L.S.D applies to comparison of interactions between calcium chloride and salicylic acid.

salicylic acid generally had a positive effect on vegetative growth parameters in both seasons. Foliar application of salicylic acid at 1.5 mM significantly gave the highest significant values of plant length, head fresh and dry weights and number of leaves. However, there were no significant differences between plants sprayed with 1.0 or 1.5 mM in leaf area and leaf area index. On the contrary, foliar application of salicylic acid at different concentrations was less effective on head diameter. Similar results were reported by Szepesi et al. (2005), and Mady (2009) on tomato, Eraslan et al. (2007) on carrot, Çanakçi (2008) on radish, Yildirim et al. (2008) on cucumber, Elwan and El-

TABLE 2. Effect of foliar application with calcium chloride and salicylic acid on number of leaves/head. average leaf area and leaf area index of romaine lettuce cv. Balady in 2013/2014 and 2014/2015 seasons.

Foliar applications		Number of leaves/head		Average leaf area (cm²)		Leaf area index (LAI)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Calciumchloride[a]							
0.0 mM		26.80D	29.59D	200.47E	208.78E	8.97E	10.32E
10 mM		30.08C	32.09C	232.57C	234.14C	11.66C	12.53C
20 mM		32.87A	35.13A	247.47A	251.83A	13.51A	14.76A
30 mM		31.65B	34.39B	242.74B	247.87B	12.72B	14.10B
40 mM		29.63C	31.84C	221.68D	224.76D	10.96D	11.94D
Salicylic acid[a]							
0.0 mM		29.60d	31.84c	223.17c	226.17c	11.08c	12.08c
0.5 mM		29.95c	32.47b	228.42bc	234.07b	11.40bc	12.65bc
1.0 mM		30.40b	32.59b	232.70ab	237.11ab	11.84ab	13.01ab
1.5 mM		30.67a	33.59a	234.18a	241.41a	11.96a	13.45a
2.0 mM		**30.41b**	**32.55b**	**226.46c**	**228.62c**	**11.54bc**	**12.45bc**
Calcium chloride X Salicylic acid interaction							
0.0 mM	0.0 mM	25.93	28.00	193.2.00	195.14	8.36	9.12
	0.5 mM	26.47	29.47	200.46	211.14	8.85	10.38
	1.0 mM	27.93	29.93	214.08	215.99	9.97	10.78
	1.5 mM	26.87	31.07	199.32	219.21	8.93	11.36
	2.0 mM	26.80	29.47	195.27	202.40	8.73	9.95
10 mM	0.0 mM	29.27	31.07	224.39	223.29	10.95	11.56
	0.5 mM	29.73	31.87	232.57	234.89	11.53	12.48
	1.0 mM	30.13	31.57	236.50	235.77	11.87	12.41
	1.5 mM	30.67	33.40	237.15	245.22	12.12	13.65
	2.0 mM	30.60	32.53	232.23	231.52	11.85	12.56
20 mM	0.0 mM	32.40	34.67	245.33	248.21	13.27	14.35
	0.5 mM	32.67	34.93	250.06	253.03	13.32	14.42
	1.0 mM	32.95	35.00	247.82	255.62	13.61	14.80
	1.5 mM	34.20	36.47	254.72	258.03	14.52	15.68
	2.0 mM	32.13	34.60	239.40	244.28	12.83	14.09
30 mM	0.0 mM	31.33	34.33	232.79	242.01	12.16	13.85
	0.5 mM	31.20	34.13	234.00	243.46	12.17	13.86
	1.0 mM	31.87	34.80	247.09	255.16	13.13	14.80
	1.5 mM	31.73	34.47	258.41	256.46	13.22	14.17
	2.0 mM	32.13	34.20	241.41	242.25	12.93	13.81
40 mM	0.0 mM	29.07	31.13	220.12	222.18	10.67	11.53
	0.5 mM	29.70	31.93	225.02	227.81	11.14	12.13
	1.0 mM	29.13	31.67	218.02	223.00	10.60	11.79
	1.5 mM	29.87	32.53	221.29	228.15	11.02	12.38
	2.0 mM	30.40	31.93	223.97	222.66	11.36	11.86
$LSD_{0.05}$ [b]		1.18	1.36	N.S.	N.S.	0.90	0.80

[a]Means into every group within a column for the same factor followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.
[b]L.S.D applies to comparison of interactions between calcium chloride and salicylic acid.
N.S.: non-significant

Hamahmy (2009) on pepper, Karlidag et al. (2009 a,b), and Metwally et al. (2013) on strawberry, and Shafeek et al. (2014) on snap bean for different vegetative growth characteristics. The stimulatory effect of salicylic acid on vegetative growth could be attributed to the positive effect of salicylic acid upon the endogenous phytohormones specially the growth promoters, i.e. auxins, gibberellins and cytokinins (Shakirova, 2007, Mady, 2009 and Mady, 2014).

As for the interaction effect, data in Tables 1 and 2 revealed that the combination between calcium chloride at 20 mM and salicylic acid at

1.5 mM resulted in significant increases in the aforementioned vegetative growth parameters except for leaf area in both seasons. However, the combined effects had no significant effect on leaf area. These results clearly indicated that the increases in weight and diameter of head (productivity) are mainly caused by increasing in number of leaves, but not by increasing in leaf area. The synergistic effects of calcium chloride and salicylic acid were reported on vegetative growth of strawberry (Kazemi, 2013a).

Chlorophyll content

As shown in Table 3, foliar application of calcium chloride increased chlorophyll (*a, b* and total) as compared with the control plants in both seasons. The highest significant values were obtained with $CaCl_2$ at 20 mM followed by spraying at 30 mM. No significant changes were noted in chlorophyll (*a, b* and total) content when the plants were sprayed with $CaCl_2$ at 10 or 40 mM. Previous studies reported that calcium chloride increased chlorophyll content in leaves of cucumber (Kazemi, 2013b), and cowpea (Mohamed and Basalah, 2015).

Data in Table 3 also showed that the exogenous applications of salicylic acid at different concentrations significantly increased chlorophyll (*a, b* and total) content compared with the control plants in both growing seasons. Spraying at 1.5 mM gave the highest significant values. However, the increments in chlorophyll a and b contents at 1 or 1.5 mM were not significant. In this regard, foliar application of salicylic acid was found to increase the chlorophyll content in cowpea (Chandra and Bhatt, 1998), tomato (Kalarani et al., 2002), cucumber (Yildirim et al., 2008), strawberry (Karlidag et al., 2009 a,b and Jamali et al., 2011).

The combined effect of calcium chloride at 20 mM and salicylic acid at 1 or 1.5 mM gave the highest significant values of chlorophyll *a, b* and total chlorophyll contents without significant differences between both treatments (Table 3).

Leaf relative water content and leaf membrane stability index

As shown in Table 4, foliar application of calcium chloride at 10, 20 or 30 significantly increased leaf relative water content, while all concentrations of calcium chloride (10, 20, 30 or 40 mM) significantly increased leaf membrane stability index as compared with the un-sprayed plants in both seasons. The highest significant values of both physiological parameters were obtained with $CaCl_2$ at 20 mM. The obtained results coincide with those reported by Tabatabaeian (2014) who found that the relative water content in tomato leaves was increased by increasing the concentration of calcium chloride. In addition, the results of leaf membrane stability index coincide with the essential role of calcium in the preservation of the cell wall structures and plasma membrane stabilization (Hepler, 2005).

Data presented in Table 4 also clearly revealed that all concentrations of SA significantly increased leaf relative water content and leaf membrane stability index compared with the control plants in both seasons. However, salicylic acid spraying at 1.5 mM gave the highest and significant values of both parameters. The obtained results coincide with those reported by Szepesi et al. (2005) who found that exogenous salicylic acid increased relative water content in tomato leaves which may be attributed to that SA can increase leaf diffusive resistance and lower transpiration in plants (Yildirim et al., 2008). In addition, improving leaf water content by the application of salicylic acid led to accumulation of different osmolytes such as sugars, sugar alcohol and proline which are responsible for osmotic adjustment (Szepesi et al., 2005, Umebese et al., 2009 and Bidabadi et al., 2012). Moreover, our results also coincide with those of Aldesuquy and Ghanem (2015) who found that salicylic acid increased leaf membrane stability index of drought stressed wheat plants.

As for the interaction effect, exogenous applications of calcium chloride at 20 mM and salicylic acid at 1 or 1.5 mM significantly increased leaf relative water content and leaf membrane stability index over all other treatments (Table 4). The synergistic effects of calcium chloride and salicylic acid on leaf membrane stability are in agreement with the findings of Khan et al. (2010) who found that application of salicylic acid increased the accumulation of Ca^{+2} which can maintain membrane integrity. The increased chlorophyll content in leaves (Table 3) is closely linked with the increased vegetative growth parameters (Tables 1 and 2).

Leaf macro- and micronutrients

Data presented in Table 5 clearly revealed that foliar application of calcium chloride at 20 mM significantly increased nitrogen, phosphorus,

TABLE 3. Effect of foliar application with calcium chloride and salicylic acid on chlorophyll content in leaves of romaine lettuce cv. Balady in 2013/2014 and 2014/2015 seasons.

Foliar applications		Chlorophyll content (µg/g DW)					
		Chl*a*		Chl*b*		Total chlorophyll	
		1st season	2nd season	1st season	2nd season	1st season	2nd season
Calciumchloride[a]							
0.0 mM		1292.28D	1350.40D	850.32D	888.57D	2142.60D	2238.97D
10 mM		1449.56C	1459.43C	953.81C	960.31C	2403.37C	2419.74C
20 mM		1600.42A	1609.23A	1053.08A	1058.87A	2653.50A	2668.10A
30 mM		1533.09B	1572.26B	1015.09B	1034.55B	2548.18B	2606.81B
40 mM		1432.61C	1454.84C	942.66C	957.29C	2375.26C	2412.13C
Salicylic acid[a]							
0.0 mM		1375.01c	1381.24c	904.76c	908.86c	2279.77d	2290.09c
0.5 mM		1463.39bc	1500.33b	962.91bc	985.22b	2426.30c	2485.56b
1.0 mM		1489.15ab	1507.19b	979.86ab	993.73b	2469.01b	2500.92b
1.5 mM		1497.86a	1555.34a	991.91a	1023.41a	2489.77a	2578.75a
2.0 mM		1482.55bc	1502.07b	975.52bc	988.36b	2458.07b	2490.44b
Calcium chloride X Salicylic acid interaction							
0.0 mM	0.0 mM	1195.00	1204.10	786.31	792.30	1981.31	1996.40
	0.5 mM	1290.68	1364.67	849.27	897.96	2139.95	2262.63
	1.0 mM	1362.15	1383.57	896.29	910.39	2258.44	2293.96
	1.5 mM	1308.93	1436.90	861.28	945.48	2170.21	2382.38
	2.0 mM	1304.65	1362.78	858.46	896.71	2163.11	2259.49
10 mM	0.0 mM	1359.66	1342.35	894.65	883.27	2254.31	2225.62
	0.5 mM	1449.54	1466.98	953.79	965.27	2403.33	2432.25
	1.0 mM	1452.63	1452.74	955.83	955.90	2408.46	2408.64
	1.5 mM	1494.53	1542.54	983.40	1014.99	2477.93	2557.53
	2.0 mM	1491.44	1492.56	981.37	982.10	2472.81	2474.66
20 mM	0.0 mM	1512.83	1511.38	995.44	994.49	2508.27	2505.87
	0.5 mM	1603.41	1615.52	1055.05	1053.01	2658.46	2668.53
	1.0 mM	1650.44	1636.03	1085.99	1086.51	2736.43	2722.54
	1.5 mM	1667.03	1683.94	1096.91	1108.03	2763.94	2791.97
	2.0 mM	1568.40	1599.27	1032.01	1052.32	2600.41	2651.59
30 mM	0.0 mM	1459.92	1499.18	960.63	986.46	2420.55	2485.64
	0.5 mM	1522.77	1580.91	1001.98	1040.24	2524.75	2621.15
	1.0 mM	1556.46	1601.48	1024.15	1053.77	2580.61	2655.25
	1.5 mM	1560.30	1606.70	1058.26	1057.21	2618.56	2663.91
	2.0 mM	1566.00	1573.04	1030.43	1035.06	2596.43	2608.10
40 mM	0.0 mM	1347.64	1349.18	886.75	887.76	2234.39	2236.94
	0.5 mM	1450.54	1473.59	954.45	969.63	2404.99	2443.22
	1.0 mM	1424.07	1462.13	937.04	962.08	2361.11	2424.21
	1.5 mM	1458.52	1506.60	959.71	991.34	2418.23	2497.94
	2.0 mM	1482.27	1482.72	975.33	975.63	2457.60	2458.35
$LSD_{0.05}$[b]		**18.25**	**20.35**	**25.18**	**28.25**	**25.88**	**30.79**

[a]Means into every group within a column for the same factor followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.
[b]L.S.D applies to comparison of interactions between calcium chloride and salicylic acid.

TABLE 4. Effect of foliar application with calcium chloride and salicylic acid on leaf relative water content and membrane stability index of romaine lettuce cv. Balady in 2013/2014 and 2014/2015 seasons.

Foliar applications		Leaf relative water content (%)		Membrane stability index (%)	
		1st season	2nd season	1st season	2nd season
Calciumchloride[a]					
0.0 mM		79.62C	80.34C	61.28D	61.65D
10 mM		85.43B	86.01B	68.89C	69.50C
20 mM		89.34A	89.58A	76.00A	75.34A
30 mM		84.88B	85.39B	72.34B	72.37B
40 mM		79.47C	79.90C	66.93C	67.03C
Salicylic acid[a]					
0.0 mM		81.09d	81.99d	66.75c	67.17d
0.5 mM		83.54c	83.62c	69.00b	68.93c
1.0 mM		84.65b	85.19b	69.71b	69.82b
1.5 mM		85.72a	86.37a	70.78a	70.40a
2.0 mM		83.73c	84.04c	69.19b	69.56b
Calcium chloride X Salicylic acid interaction					
0.0 mM	0.0 mM	77.38	78.40	59.84	60.23
	0.5 mM	79.00	79.50	60.46	60.32
	1.0 mM	80.36	81.38	61.82	62.44
	1.5 mM	80.81	81.83	62.27	62.69
	2.0 mM	80.56	80.58	62.02	62.59
10 mM	0.0 mM	80.25	80.99	63.71	64.11
	0.5 mM	84.75	84.79	68.21	69.35
	1.0 mM	86.13	86.79	69.59	70.25
	1.5 mM	87.58	88.62	71.04	71.44
	2.0 mM	88.42	88.84	71.88	72.34
20 mM	0.0 mM	86.38	87.44	73.84	74.32
	0.5 mM	88.73	88.73	76.19	75.00
	1.0 mM	92.27	92.25	77.73	76.17
	1.5 mM	92.92	92.99	78.38	76.89
	2.0 mM	86.41	86.48	73.87	74.31
30 mM	0.0 mM	84.18	84.84	71.64	72.09
	0.5 mM	85.09	84.92	72.55	73.00
	1.0 mM	85.89	85.91	73.35	73.79
	1.5 mM	86.80	87.84	74.26	72.66
	2.0 mM	82.45	83.46	69.91	70.32
40 mM	0.0 mM	77.28	78.29	64.74	65.12
	0.5 mM	80.14	80.15	67.60	66.98
	1.0 mM	78.60	79.62	66.06	66.44
	1.5 mM	80.49	80.58	67.95	68.34
	2.0 mM	80.82	80.85	68.28	68.25
$LSD_{0.05}$ [b]		1.25	1.22	0.80	0.99

[a]Means into every group within a column for the same factor followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.
[b]L.S.D applies to comparison of interactions between calcium chloride and salicylic acid.

potassium and magnesium concentrations in lettuce leaves as compared with the control plants in both growing seasons. The increases of N, P and K were non-significant when the plants were sprayed with 10, 30 or 40 mM. On the contrary, increasing of calcium chloride sprayings led to significant increases in calcium concentrations in the leaves. As for the micronutrients content, data in Table 6 indicated that all concentrations of calcium chloride significantly increased iron, zinc, manganese and copper contents compared with the control plants in both seasons. Spraying at 20 or 30 mM gave the highest and significant values without significant differences between both concentrations. These results are in a good accordance with those obtained by Del Amor and Marcelis (2003) who found that calcium significantly increased mineral-nutrients uptake.

As for the effect of salicylic acid, data in Table 5 clearly showed that foliar spraying of salicylic acid at 1.5 mM gave the highest and significant values of nitrogen, phosphorus, potassium, calcium and magnesium concentrations in lettuce leaves as compared with the un-sprayed plants in both growing seasons. However, the increases in calcium concentration obtained by spraying at 1.0, 1.5 or 2.0 mM were not significant. Concerning the micronutrients content, data from Table 6 clearly showed that exogenous application of salicylic acid at 1.5 mM significantly increased iron, zinc, manganese and copper contents in lettuce leaves in both seasons. In this respect, many studies demonstrated that salicylic acid applications increased the concentrations of macro- and microelement concentrations in the leaves of strawberry (Karlidag et al., 2009a, b). Similar results were also obtained by Szepsi et al. (2005) for tomato and Yildirim et al., (2008) for cucumber, who found out that exogenous SA applications stimulated N, P, K, Mg, Fe, Mn and Cu uptake. These increases in macronutrients and micronutrients accumulation may be attributed to that salicylic stimulated root formation and consequently increased mineral uptake by plants (Yildirim et al., 2008 and Khan et al., 2010). The increased nutrients content in leaves coupled with the increased values of leaf membrane stability index (Table 4). In this respect, previous studies indicated that salicylic acid increased membrane permeability which would facilitate absorption and utilization of mineral nutrients and transport of assimilates (Gunes et al., 2005 and Aftab et al., 2010).

Concerning the interaction effect, foliar applications of calcium chloride at 20 mM and salicylic acid at 1 or 1.5 mM significantly gave the highest values of the macronutrients over all other treatments (Table 5), while the combined effect of calcium chloride and salicylic acid did show any significant difference on the studied micronutrients (Table 6).

The increased nutrients content in leaves especially nitrogen and magnesium concentrations (Table 5) are in harmony with the increased chlorophyll content in leaves (Table 3) and closely linked to the increased vegetative growth characteristics (Tables 1 and 2).

Nitrate content in leaves

As shown in Fig.1, foliar application of calcium chloride decreased nitrate content in lettuce leaves as compared with the control plants in both growing seasons. The lowest significant values were obtained with CaCl2 at 30 mM followed by spraying at 40 mM. This result agrees with that reported by Maynard et al. (1976) who suggested that chloride application could be used as a strategy to decrease nitrate content of vegetables, particularly of those such as spinach, lettuce and cabbage.

Exogenous applications of salicylic acid at different concentrations significantly decreased nitrate content in lettuce leaves compared with the control plants in both growing seasons. Spraying at 1.5 or 2.0 mM gave the lowest significant contents without significant differences (Fig.1). The obtained results are in agreement with those obtained by Cao et al. (2009) who found that nitrate contents of Chinese chives were significantly decreased with various concentrations of salicylic acid.

As for the interaction effect, Figure 2 showed that exogenous applications of calcium chloride at 30 mM and salicylic acid at 1.0 or 1.5 mM significantly decreased nitrate contents in both seasons. These reductions in nitrate content with combined calcium chloride and salicylic acid treatments could be attributed to their individual effects on the activity of nitrate reductase and nitrite reductase enzymes. Nitrate reductase activity was increased with calcium chloride in tomato (Kazemi, 2014) and with salicylic acid in mungbean (Dar et al., 2007), and cucumber (Singh el al., 2010). Also, Jain and Srivastava (1981) found that salicylic acid increased nitrite reductase in maiz. It is well known that nitrate reductase reduces nitrate (NO-3) to nitrite in the cytosol (Campbell, 1988) and nitrite moves into plastids where subsequently reduced to NH4+ by nitrite reductase enzyme.

Conclusion

In conclusion, this study indicated positive effects of foliar applications of calcium chloride and salicylic acid either alone or in combination on lettuce growth, productivity and some physiological parameters. Moreover, foliar applications with both chemicals decreased nitrate contents in the

TABLE 5. Effect of foliar application with calcium chloride and salicylic acid on nitrogen, phosphorus, potassium, calcium and magnesium concentrations in leaves of romaine lettuce cv. Balady in 2013/2014 and 2014/2015 seasons.

Foliar applications		Nitrogen (%)		Phosphorus (%)		Potassium (%)		Calcium (%)		Magnesium (%)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Calciumchloride[a]											
0.0 mM		2.84C	3.12C	0.61C	0.54C	3.63C	4.23C	1.92E	1.95E	0.569C	0.593C
10 mM		3.32B	3.60B	0.72B	0.67B	4.86B	5.44B	2.01D	2.04D	0.579B	0.603B
20 mM		3.95A	4.19A	0.86A	0.80A	5.32A	5.73A	2.10C	2.13C	0.623A	0.647A
30 mM		3.36B	3.64B	0.73B	0.67B	4.84B	5.39B	2.14B	2.18B	0.579B	0.603B
40 mM		3.35B	3.63B	0.73B	0.67B	4.81B	5.46B	2.21A	2.26A	0.569C	0.593C
Salicylic acid[a]											
0.0 mM		3.22d	3.50d	0.70c	0.64c	4.41c	4.96d	1.97c	2.02c	0.496d	0.519d
0.5 mM		3.27c	3.54c	0.71c	0.65c	4.48c	5.18c	2.07b	2.09b	0.594c	0.618c
1.0 mM		3.42b	3.70b	0.75ab	0.68b	4.82b	5.34b	2.10ab	2.13ab	0.607b	0.631b
1.5 mM		3.49a	3.76a	0.76a	0.70a	4.98a	5.46a	2.14a	2.17a	0.622a	0.646a
2.0 mM		3.42b	3.69b	0.74b	0.68b	4.77b	5.31b	2.11ab	2.14ab	0.600c	0.625b
Calcium chloride X Salicylic acid interaction											
0.0 mM	0.0 mM	2.55	2.84	0.55	0.48	3.01	3.49	1.83	1.87	0.481	0.505
	0.5 mM	2.73	3.01	0.59	0.52	3.36	4.21	1.92	1.94	0.577	0.601
	1.0 mM	2.86	3.14	0.62	0.55	3.67	4.22	1.92	1.96	0.603	0.627
	1.5 mM	3.05	3.33	0.66	0.59	4.12	4.68	1.97	1.99	0.600	0.624
	2.0 mM	2.99	3.28	0.65	0.58	3.99	4.56	1.94	1.97	0.585	0.609
10 mM	0.0 mM	3.25	3.53	0.71	0.65	4.69	5.28	1.93	1.94	0.493	0.516
	0.5 mM	3.27	3.55	0.71	0.66	4.74	5.41	1.98	2.01	0.587	0.612
	1.0 mM	3.34	3.62	0.73	0.67	4.89	5.48	2.00	2.04	0.598	0.622
	1.5 mM	3.39	3.67	0.74	0.68	5.01	5.48	2.13	2.17	0.616	0.640
	2.0 mM	3.36	3.64	0.73	0.67	4.95	5.54	2.03	2.06	0.602	0.626
20 mM	0.0 mM	3.77	4.03	0.82	0.76	5.16	5.58	1.98	2.01	0.537	0.561
	0.5 mM	3.86	4.09	0.84	0.78	5.20	5.57	2.07	2.10	0.640	0.664
	1.0 mM	4.07	4.33	0.89	0.81	5.55	5.94	2.14	2.16	0.643	0.667
	1.5 mM	4.10	4.34	0.89	0.83	5.63	6.15	2.16	2.20	0.665	0.689
	2.0 mM	3.94	4.17	0.86	0.80	5.07	5.40	2.15	2.17	0.628	0.652
30 mM	0.0 mM	3.23	3.51	0.70	0.64	4.54	5.12	2.04	2.07	0.485	0.508
	0.5 mM	3.15	3.44	0.69	0.62	4.36	5.15	2.14	2.19	0.584	0.609
	1.0 mM	3.49	3.78	0.76	0.70	5.15	5.57	2.17	2.21	0.601	0.626
	1.5 mM	3.50	3.78	0.76	0.70	5.18	5.54	2.18	2.21	0.625	0.649
	2.0 mM	3.42	3.70	0.74	0.68	4.97	5.57	2.16	2.20	0.599	0.624
40 mM	0.0 mM	3.28	3.57	0.71	0.66	4.66	5.34	2.05	2.20	0.484	0.507
	0.5 mM	3.32	3.60	0.72	0.67	4.75	5.54	2.22	2.23	0.582	0.606
	1.0 mM	3.35	3.63	0.73	0.67	4.82	5.50	2.26	2.28	0.589	0.614
	1.5 mM	3.41	3.70	0.74	0.69	4.97	5.47	2.28	2.30	0.603	0.627
	2.0 mM	3.37	3.65	0.73	0.68	4.87	5.47	2.26	2.30	0.587	0.611
$LSD_{0.05}$[b]		0.12	0.15	0.02	0.02	0.33	0.34	0.07	0.08	0.020	0.020

[a]Means into every group within a column for the same factor followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

[b]L.S.D applies to comparison of interactions between calcium chloride and salicylic acid.

leaves. Spraying of calcium chloride at 20 mM and salicylic acid at 1.5 mM was the most effective treatment which can be used as an applicable practice in romaine lettuce cv. Balady cultivation.

TABLE 6. Effect of foliar application with calcium chloride and salicylic acid on iron, zinc, manganese and copper contents in leaves of romaine lettuce cv. Balady in 2013/2014 and 2014/2015 seasons.

Foliar applications		Iron (ppm)		Zinc (ppm)		Manganese (ppm)		Copper (ppm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Calciumchloride[a]									
0.0 mM		460.3C	524.3C	111.4C	127.3C	75.68C	85.81C	7.85C	8.37C
10 mM		513.5B	565.8B	124.3B	137.3B	84.46B	92.60B	8.76B	9.04B
20 mM		564.7A	612.4A	136.7A	148.6A	92.88A	100.23A	9.63A	9.78A
30 mM		548.1A	609.0A	132.7A	147.8A	90.15A	99.67A	9.35A	9.73A
40 mM		507.8B	563.2B	123.0B	136.7B	83.52B	92.18B	8.66B	9.00B
Salicylic acid[a]									
0.0 mM		507.3c	559.7c	122.8c	135.9c	83.43c	91.61c	8.65c	8.94c
0.5 mM		516.3bc	574.2b	125.0b	139.4b	84.90bc	93.98b	8.80b	9.17b
1.0 mM		519.6b	574.8b	125.8b	139.5b	85.43b	94.07b	8.86b	9.18b
1.5 mM		531.3a	593.5a	128.7a	144.1a	87.39a	97.14a	9.06a	9.48a
2.0 mM		519.9b	572.5b	125.9b	138.9b	85.53b	93.70b	8.87b	9.14b
Calcium chloride X Salicylic acid interaction									
0.0 mM	0.0 mM	446.0	496.2	108.0	120.5	73.36	81.22	7.61	7.92
	0.5 mM	455.0	522.9	110.2	126.9	74.83	85.59	7.76	8.35
	1.0 mM	480.0	530.1	116.0	128.7	78.80	86.76	8.17	8.47
	1.5 mM	461.0	549.8	111.6	133.5	75.82	89.98	7.86	8.78
	2.0 mM	459.5	522.3	111.3	126.8	75.58	85.48	7.84	8.34
10 mM	0.0 mM	502.1	547.8	121.6	133.0	82.57	89.65	8.56	8.75
	0.5 mM	508.8	561.2	123.2	136.2	83.68	91.84	8.68	8.96
	1.0 mM	509.8	555.7	123.4	134.9	83.85	90.95	8.69	8.87
	1.5 mM	524.0	589.1	126.9	143.0	86.19	96.42	8.94	9.41
	2.0 mM	523.0	575.3	126.6	139.6	86.02	94.15	8.92	9.19
20 mM	0.0 mM	554.1	597.9	134.2	145.1	91.14	97.86	9.45	9.55
	0.5 mM	574.6	620.0	139.1	150.5	94.50	101.47	9.80	9.90
	1.0 mM	562.8	617.6	136.3	149.9	92.57	101.09	9.60	9.86
	1.5 mM	582.7	629.0	141.1	152.7	95.84	102.95	9.94	10.05
	2.0 mM	549.2	597.5	133.0	145.0	90.33	97.79	9.37	9.54
30 mM	0.0 mM	536.1	606.1	129.8	147.1	88.17	99.20	9.14	9.68
	0.5 mM	533.7	603.4	129.2	146.5	87.77	98.75	9.10	9.64
	1.0 mM	545.1	611.1	132.0	148.3	89.66	100.02	9.30	9.76
	1.5 mM	577.1	624.0	139.7	151.5	94.93	102.12	9.84	9.96
	2.0 mM	548.3	600.5	132.8	145.7	90.20	98.28	9.35	9.59
40 mM	0.0 mM	498.0	550.5	120.6	133.6	81.90	90.10	8.49	8.79
	0.5 mM	509.2	563.5	123.3	136.8	83.74	92.23	8.68	9.00
	1.0 mM	500.1	559.3	121.1	135.8	82.25	91.54	8.53	8.93
	1.5 mM	511.8	575.8	123.9	139.8	84.18	94.24	8.73	9.20
	2.0 mM	519.9	567.1	125.9	137.6	85.52	92.81	8.87	9.06
$LSD_{0.05}$ [b]		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

[a]Means into every group within a column for the same factor followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.
[b]L.S.D applies to comparison of interactions between calcium chloride and salicylic acid.

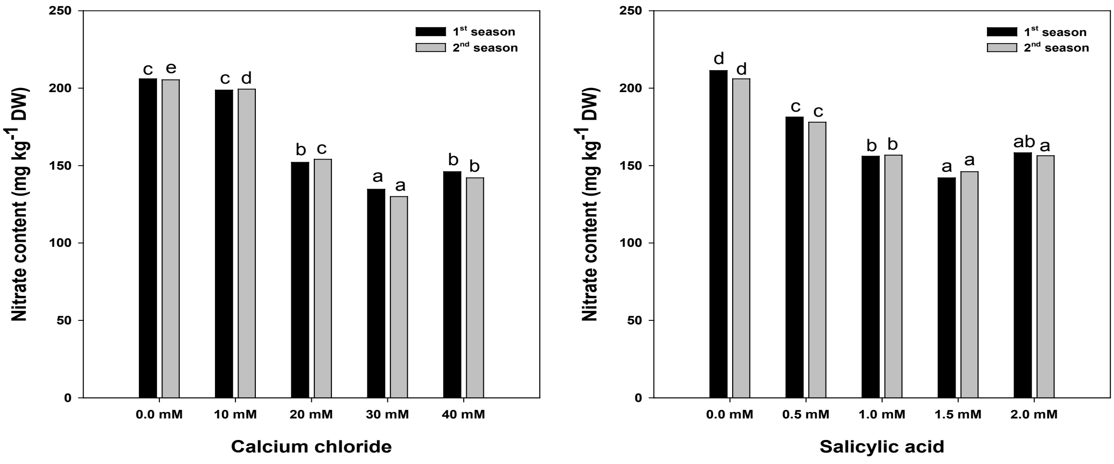

Fig. 1. Effect of calcium chloride or salicylic acid on nitrate content in leaves of romaine lettuce cv. Balady. Different letters on top of bars indicate significant differences according to Duncan's Multiple Range Test at $p \leq 0.05$.

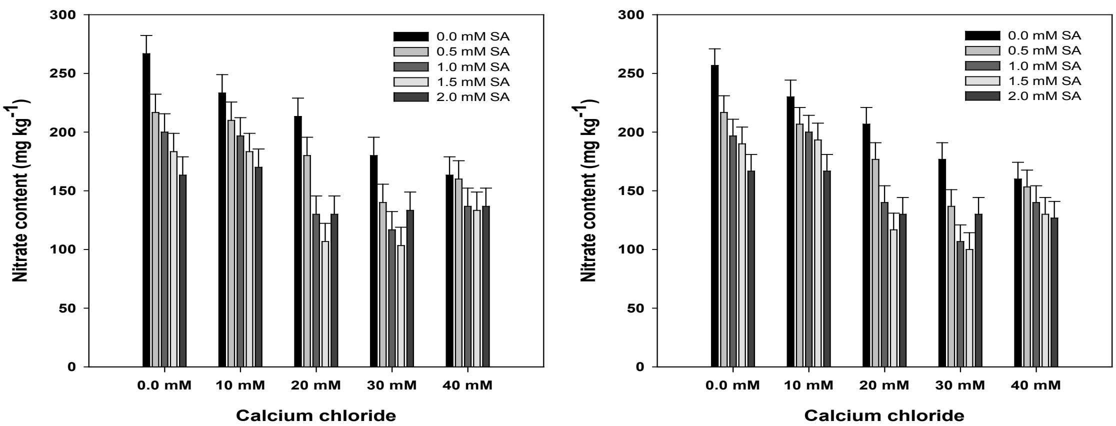

Fig. 2. Effect of calcium chloride and salicylic acid on nitrate content in leaves of romaine lettuce cv. Balady. Vertical bars indicate the LSD value at $p \leq 0.05$.

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الرش الورقى بحمض الساليسيلك وكلوريد الكالسيوم يحسن نمو وانتاجية الخس

صبري موسى سليمان يوسف، سلامه عبد الحميد عبد الهادي، نشوه عطية إبراهيم أبو العزم ومحمد زكي الشناوي
قسم البساتين - كلية الزراعة - جامعة عين شمس - القاهرة - مصر.

حمض الساليسيلك والكالسيوم ينظمان العمليات الفسيولوجية والبيوكيمياوية فى النبات لذا أجريت تجربه عاملية لتقييم تأثير الرش الورقي بكلوريد الكالسيوم بتركيزات صفر، ١٠، ٢٠ ، ٣٠ ، ٤٠ ملليمول وكذلك الرش الورقي بحمض السالسيليك بتركيزات صفر، ٠,٥ ، ١,٠ ، ١,٥ ، ٢,٠ ملليمول على نمو نباتات خس الرومين الصنف البلدي وذلك خلال موسمى الزراعه ٢٠١٤/٢٠١٣ و ٢٠١٥/٢٠١٤ وأقيمت التجربة فى المزرعة التجريبية الخاصة بقسم البساتين بكلية الزراعه جامعة عين شمس بمحافظة القليوبيه - مصر. وأوضحت النتائج التأثيرات الإيجابية للرش بكل من كلوريد الكالسيوم وحمض الساليسيليك إما كلا بمفرده أو رشهما معاً على نمو وانتاجية نباتات الخس وبعض الخصائص الفسيولوجيه. وقد أدى الرش بكلوريد الكالسيوم بتركيز ٢٠ ملليمول إلى زيادة معنوية فى صفات النمو الخضري (طول النبات وقطر الرأس والوزن الطازج والجاف للرؤس وعدد الأوراق لكل رأس ومتوسط مساحة الورقه ودليل مساحة الأوراق) ومحتوى الأوراق من الكلوروفيل أ ، ب والكلي وبعض الخصائص الفسيولوجيه للأوراق مثل المحتوى النسبى للرطوبة ودليل ثبات الأغشية الخلوية وكذلك محتوى الأوراق من العناصر الكبرى والصغرى. علاوة على ذلك فقد أدى الرش الورقي بحمض السليسيليك بتركيز ١,٥ ملليمول إلى أعلى زيادة معنوية فى جميع القياسات السابق الإشارة إليها. من ناحية أخرى فقد أدى الرش الورقي بكل من كلوريد الكالسيوم وحمض الساليسيلك منفردين أو رشهما معاً إلى تقليل تراكم النترات فى أوراق الخس. ويستخلص من النتائج أن الرش الورقي بكلوريد الكالسيوم بتركيز ٢٠ ملليمول وحمض الساليسيلك بتركيز ١,٥ ملليمول معا قد أظهر أعلى تأثير إيجابي على جميع الصفات والخصائص التى تمت دراستها على نباتات خس الرومين الصنف البلدي وبالتالى يمكن التوصية بها لتحسين إنتاجية وجودة محصول الرووس لهذا الصنف وتحت ظروف مشابهة لظروف هذه الدراسة.