

## Vegetative and Reproductive Growth of Strawberry Plants cv. 'Pajaro' Affected by Salicylic Acid and Nickel

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### ABSTRACT

The present research was carried out under hydroponic culture to evaluate the interaction between salicylic acid (SA), nickel (Ni) on vegetative and reproductive growth of strawberry plants as a factorial experiment in a completely randomized design. Well-rooted daughter plants of Pajaro cultivar, were potted in 3 L plastic pots and were sprayed with SA at concentrations of 0, 1, 2 and 3 mM and NiSO<sub>4</sub> at 0, 150 and 300 mg.L<sup>-1</sup>, after establishment. Results indicated that SA at 2 mM increased root and shoot fresh weight, number of inflorescences and fruits, fruit nitrogen concentration and yield. The effect of Ni was promoting too, as 150 mg.L<sup>-1</sup> of Ni led to significant increments of fruit number, inflorescences, leaf area per plant and yield. However, the best results were found when SA was applied accompanied by Ni.

**Keywords:** Essential element, Growth parameter, Nickel, Salicylic acid, Strawberry, Yield.

### INTRODUCTION

Strawberry is one of the most delicious, nutritive and refreshing fruits and there has been a high interest in strawberry production over the past decade (Singh *et al.*, 2007). Because plant growth regulators and essential elements are vital components of plant life cycle affecting quality and quantity of fruits, their optimized usage can improve production, increases yield and promote health.

Mineral elements such as nickel (Ni) are essential for a plant to complete its vegetative and reproductive life cycle (Brown *et al.*, 1987; Marschner, 1995). The most important action of Ni within the plant organs is its influence on nitrogen metabolism as a component of ubiquitous phytoenzyme 'Urease' and probably some other enzymes and proteins awaiting discovery (Brown, 2007) especially in Ureid – transporting species (Bai *et al.*, 2006).

Previous studies have shown a general beneficial impact of this element on overall plant growth and development; improved seed germination (Senegar *et al.*, 2008), higher yield (Mishra and Kar, 1974; Welch, 1981; Brown, 2007) more resistant plants against pests and diseases (Wood and Reilly, 2007) and inhibitory effects on ethylene production (Zhang *et al.*, 2006).

Salicylic acid (SA) naturally occurs in plants and various aspects of plant life cycle can be affected by this phytohormone (Raskin, 1992). Previous studies have demonstrated that a wide range of responses might appear after exogenous SA application as follows: yield increases (El-Tayeb, 2005; Khodary, 2004; Yildirim *et al.*, 2008), more photosynthetic activity (Singh and Usha, 2003), higher total anthocyanins (Hernandez and Vargas, 1997), inhibiting ethylene biosynthesis (Huang *et al.*, 2004) and protection against biotic and abiotic stresses (Doares *et al.*, 1995; Karlidag *et al.*, 2009).

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Therefore, both of these compounds can increase crop quality and quantity. Thus, the main aim of this study was to evaluate the interactive effects of these compounds alone or in combination on vegetative and reproductive characteristics of strawberry plants.

## MATERIALS AND METHODS

### Plant Growth Conditions and Treatments

Well-rooted strawberry plants of cultivar Pajaro, grown under greenhouse conditions around Shiraz city were used to study their responses to salicylic acid and nickel. The plants were potted in 3 L. plastic pots, filled with 1:1 ratio of peat moss and perlite. Plants were irrigated twice a day using Melspray solution (10% N, 40% K<sub>2</sub>O, 8% P<sub>2</sub>O<sub>3</sub>, 2% MgO, 1000 mg.L<sup>-1</sup> Fe, 230 mg.L<sup>-1</sup> Zn, 75 mg.L<sup>-1</sup> Cu). Light status was > 800 μmol.m<sup>-2</sup>.S<sup>-1</sup>, 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. Treatments included nickel sulfate (Ni) at 0, 150 and 300 mg.L<sup>-1</sup> and salicylic acid (SA) at 0, 1, 2 and 3 mM. Sprayed materials were used as follows: after plant establishment, at the beginning of flowering, and 15 days after the second time.

### Measurements

Leaf chlorophyll content was measured with a SPAD-502 chlorophyll-meter using 3 fully expanded leaves to find an average for 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 chemical method. Extraction was performed with 80% ethanol and the yielded solution was centrifuged at 8000 rpm for 10 min. Chlorophyll content was colorimetrically

determined using the following formula (A.O.A.C. 1975):

$$\text{Chlorophyll (mg.g}^{-1} \text{ fresh weight)} = [20.2 (A 645) + 8.02 (a 663) \times V] / (1000 w)$$

where A is absorption value, V is ultimate volume of extract and W is leaf fresh weight. A regression line and equation were obtained using Excel software, considering the SPAD-502 readings for the mentioned 14 leaves and colorimetrically determined chlorophyll contents. This equation was later used to estimate other chlorophyll readings from SPAD-502.

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, and Cambridge, England) and data were presented in cm<sup>2</sup>.

Number of fruits, inflorescences and flowers per inflorescence were counted throughout the experiment period and the averages were reported. Primary and secondary 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 was determined summing up total fruit weight (g) produced throughout the experiment.

Leaf and fruit total nitrogen concentrations were determined using micro-kjeldahl method and data were presented in percents. Atomic absorption spectrophotometer (Hitachi Z-2000 Polarized Zeeman Atomic Absorption Spectrophotometer) was utilized to determine fruits nickel concentration (Sparks, 1996).

Finally, plants were taken out from the pots and separated into different parts. Root and shoot were weighed to find fresh weights of each and data were presented in grams.

### Statistical Analysis

The experiment was conducted in a factorial completely randomized design with 4 replications, each consisting of 3 pots with each pot containing one plant. Data were

analyzed by SPSS 16 software and means were compared using Tukey's test.

## RESULTS

Application of Ni alone significantly influenced leaf area and root fresh weight (Table 1). The highest rates of these variables were found at 150 mg.L<sup>-1</sup> of nickel. In other words, application of lower concentrations of this element increased leaf area and root growth as compared to control (Table 1). It is evident that increase in nickel concentration (from 150 to 300 mg.L<sup>-1</sup>) reduced leaf area and root fresh weight (Table 1). Salicylic acid significantly affected leaf area, chlorophyll content, shoot and root fresh weights and leaf nitrogen content (Table 2). The highest chlorophyll content and root fresh weight were obtained at 2 mM SA. Results indicated a rise in leaf area as SA concentration increased. The highest and lowest leaf nitrogen concentrations were resulted in at 1 and 3 mM SA, respectively with noticeable reduction of this parameter at higher SA concentrations (Table 2). Salicylic acid application significantly increased leaf area, root fresh weight and leaf nitrogen concentration when accompanied by nickel treatment (Table 3). As data showed, lower concentrations of SA must be used together with higher nickel concentrations and vice versa, to yield higher leaf area (Table 3). SA and Ni interaction had no significant effect on chlorophyll content and shoot fresh weight. The highest root fresh weight was observed when 2mM SA and 150 mg.L<sup>-1</sup> were used together (Table

3). Increase in SA concentration significantly reduced leaf nitrogen concentration when accompanied by Ni, and the highest and lowest values of this parameter were found at three levels of Ni accompanied by 1 mM SA and 300 mg.L<sup>-1</sup> Ni accompanied by 3 mM SA, respectively (Table 3). Nickel application increased fruit number, primary and secondary average fruit weight, nitrogen concentration of fruit, total yield and fruits nickel concentration compared with control, however, higher Ni concentrations (from 150 to 300 mg.L<sup>-1</sup>) reduced rates of both fruit number and total yield (Table 4). Our data showed the lowest inflorescence and fruit numbers, average flower number, average weight of primary and secondary fruits and total yield in treatments without SA (Table 4). SA application increased inflorescence number, fruit number, flower number per inflorescence, average weight of primary and secondary fruits, nitrogen concentration per fruit and total yield, however, the highest rate of SA significantly reduced all mentioned parameters when compared with lower concentrations. Also the fruits nickel concentration was reduced as the higher rate of SA was applied (Table 4). Using 1 and/or 2mM SA when accompanied by 150 mg.L<sup>-1</sup> Ni led to the increments in inflorescence number, flower number per inflorescence, fruit number, average weight of primary and secondary fruits, nitrogen concentration of fruits and total yield, compared to other treatments. Fruits nickel concentration did not show a significant difference in comparison with control samples (Table 5). The lowest rates of mentioned parameters were

**Table 1.** The effect of nickel treatments on vegetative characteristics of strawberry plants.

Treatment	Leaf area (cm <sup>2</sup> )	Leaf chlorophyll content (mg.g <sup>-1</sup> fresh weight)	Shoot fresh weight (g)	Root fresh weight (g)	Leaf nitrogen concentration (%)
0	52.58 B	1.00 A	130.02 A	57.54 C	2.19 A
150	54.38 A	1.03 A	129.43 A	61.88 A	2.22 A
300	52.91 B	0.99 A	123.96 A	59.18 B	2.18 A

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

**Table 2.** The effect of salicylic acid treatments on vegetative characteristics of strawberry plants.

	Leaf area (cm <sup>2</sup> )	Leaf chlorophyll content (mg.g <sup>-1</sup> fresh weight)	Shoot fresh weight (g)	Root fresh weight (g)	Leaf nitrogen concentration (%)
0	51.88 C	0.99 B	128.75 D	55.77 D	2.20 B
1	53.92 AB	1.01 B	129.53 CD	60.87 B	2.32 A
2	53.32 B	1.04 A	132.40 A	63.00 A	2.22 AB
3	54.05 A	0.98 B	130.53 BC	58.50 C	2.04 C

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

obtained for control compared with others (Table 5).

### DISCUSSION

According to the results, Ni, SA and their interaction had increasing effects on leaf area. This was in agreement with Mordy and Ally (1999) who demonstrated that adding 25 and 50 mg Ni kg<sup>-1</sup> soil, increased leaf surface of parsley plants. Moreover, Gad *et al.* (2007) reported that application of 30 and 45 mg Ni kg<sup>-1</sup> soil caused a 40% increase in tomato leaf area. As mentioned earlier, the most important role of Ni within plants is its influence on nitrogen metabolism and therefore due to the importance of nitrogen availability for plant growth and development, Ni might increase vegetative parameters such as leaf area. Results

indicated a positive influence of SA on leaf area which was in agreement with Amin *et al.* (2007) on onion; El-Tayeb, (2005) on barley; Khodary, (2004), Szepesi *et al.* (2005), Stevens *et al.* (2006) on tomato, Gunes *et al.* (2005) on maize and Yildirim *et al.* (2008) on cucumber. It is suggested that because SA has anti-senescence influence on plant organs, vegetative growth may be prolonged following its application consequently leading to higher leaf area.

Ni did not result in a significant change in chlorophyll content, whereas SA alone and in combination with Ni caused an 11% increase in this parameter. Ni ion prevents the chlorophyll destruction of Indian mustard during senescence of leaves (Singh *et al.*, 2001). SA also shows this effect (Farriduddin *et al.*, 2003; Kaya *et al.*, 2007), and its inhibition of chlorophyll degradation is probably via prevention of

**Table 3.** Interaction of salicylic acid and nickel on vegetative characteristics of strawberry plants.

Treatment SA (mM)	Ni (mg.L <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )	Leaf chlorophyll content (mg.g <sup>-1</sup> fresh weight)	Shoot fresh weight (g)	Root fresh weight (g)	Nitrogen concentration of leaves (%)
0	0	50.4 d	0.95 de	124.40 c	55.03 e	2.14 de
	150	51.86 c	0.99 cde	126.53 bc	56.06 de	2.22 bc
	300	53.38 b	1.03 abc	129.34 bc	56.22 de	2.26 b
1	0	51.57 cd	0.99 cde	126.29 bc	56.77 de	2.27a
	150	55.12 a	1.06 ab	131.84 ab	63.06 b	2.34 a
	300	55.09 a	1.00 bcd	130.46 abc	63.78 b	2.34a
2	0	53.38 b	1.08 a	137.07 a	60.47 c	2.25 b
	150	55.39 a	1.06 ab	129.46 bc	67.40 a	2.26 b
	300	51.19 cd	1.00 bcd	130.69 abc	61.13 c	2.15 cd
3	0	54.99 a	0.99 cde	132.34 ab	57.91 d	2.09 e
	150	55.17 a	1.01 bcd	129.91 bc	61.00 bc	2.07 e
	300	52.01 bc	0.94 de	129.35 bc	56.59 de	1.97 f

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

**Table 4.** The Effect of nickel and salicylic acid treatments on reproductive characteristics of strawberry plants.

Treatment	Concentration	Number of Inflorescence	Average number of flowers per Inflorescence	Number of fruits	Average weight of primary and secondary fruits (g)	Nitrogen concentration of fruits (%)	Yield	Fruit nickel concentration (mg kg <sup>-1</sup> dry weight)
Ni (mg.L <sup>-1</sup> )	0	4.87 AB	5.52 A	9.88 B	15.66 B	0.81 B	137.40 C	0.01 B
	150	5.52 A	5.79 A	12.01 A	16.59 A	0.89 A	162.60 A	0.06 AB
	300	4.81 B	5.76 A	10.53 B	16.21 A	0.88 A	158.30 B	0.12 A
SA (m.M)	0	4.09 C	4.35 C	7.48 C	14.29 C	0.80 B	84.06 C	0.13 A
	1	5.27 B	6.57 A	12.87 A	16.67 AB	0.93 A	166.49 B	0.07 B
	2	5.76 A	6.24 A	13.64 A	17.09 A	0.96 A	191.05 A	0.05 BC
	3	4.80 B	5.61 B	9.19 B	16.23 B	0.75 B	169.47 B	0.02 C

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

**Table 5.** Interaction of salicylic acid and nickel on reproductive characteristics of strawberry plants.

SA (mM)	Treatment		Number of inflorescence	Average number of flowers within inflorescence	Number of fruits	Average weight of primary and secondary fruits (g)	Nitrogen concentration of fruits (%)	Yield (g)	Fruit nickel concentration (mg kg <sup>-1</sup> dry weight)
	Ni (mg.L <sup>-1</sup> )								
0	0		3.37 f	4.25 g	5.98 h	13.43 f	0.65 e	70.94 i	0.02 d
	150		4.26 e	4.31 g	7.72 g	14.98 e	0.85 c	87.88 h	0.12 b
	300		4.64 ed	4.50 g	8.76 ef	14.46 e	0.91 bc	93.36 g	0.25 a
1	0		4.17 c	5.57 ef	9.73 d	15.87 d	0.85 c	123.36 f	0.01 d
	150		5.64 ab	6.94 ab	15.11 a	17.37 ab	0.97 ab	194.74 b	0.08 bc
	300		5.01 cd	7.22 a	13.79 b	16.77 c	0.98 ab	181.37 c	0.12 b
2	0		6.09 a	6.21 cd	14.19 b	16.87 bc	0.91 bc	182.66 c	0.02 d
	150		6.00 a	6.53 bc	15.70 a	17.42 a	1.00 a	197.74 a	0.05 cd
	300		5.19 bc	5.92 ed	11.13 c	16.99 abc	0.99 ab	192.76 b	0.08 bc
3	0		4.88 cd	6.07 cde	9.62 c	16.47 c	0.86 c	172.64 d	0.01 d
	150		5.11 c	5.41 f	9.51 de	16.61 c	0.75 d	170.05 d	0.01 d
	300		4.43 e	5.36 f	8.46 fg	15.62 d	0.65 e	165.73 e	0.04 cd

ethylene production and accumulation (Huang *et al.*, 2004).

Ni and SA application alone and their interaction were capable to increase root fresh weight. Previously conducted studies have shown a positive effect of SA on root growth and development (Riov and Young, 1989; Kaya *et al.*, 2002; Pirlak and Esitken, 2004; Saied *et al.*, 2005; Karlidag *et al.*, 2009). Martin-Mex *et al.* (2005) also reported that SA treatment was able to increase root fresh weight of African violet. Addition of Ni to SA treatments led to the maximum root weight which was in agreement with the results of Gad *et al.* (2007) and Rao and Shantaram (2000). Wood and Reilly (2007) reported the positive effects of Ni on nitrogen metabolism, integrity of proteins and availability of carbohydrates.

SA application increased shoot fresh weight, which was in accordance with Hathout and Kord (1992) in tomato and Canakci (2008) in radish plants. As mentioned previously, it is clear that both SA and Ni have positive effects on shoot fresh weight, which may be the reason for higher values of this parameter when they are applied together.

Increases in nitrogen concentration in both leaves and fruits after the application of nickel were in agreement with findings of Gad *et al.* (2007) on tomato plants. It is proposed that because nickel has an effect on nitrogen metabolism, and also it has an anti-ethylene effect that postpones leaf senescence, it may also increase nitrogen content in leaves (Bai *et al.*, 2006; Wood and Reilly, 2007; Brown, 2007). SA can delay senescence because of its anti-ethylene effect, therefore when both of these compounds are applied on plants, they show higher leaf and fruit nitrogen concentrations.

Ni did not affect the average number of fruits per inflorescence, but number of inflorescence and total number of fruits were increased by Ni treatment. SA alone and combined with Ni caused an augmentation of these three parameters which was in

agreement with Shetti *et al.* (1992) and Yakito (2001) findings.

Nickel has an indirect effect on internal amino acids and non-protein nitrogen compounds located in shoots and seeds (Bai *et al.*, 2006), and disruption in amino acid distribution by Ni deficiency indicates such an existing disruption of shikimic acid pathway in chloroplast and cytosol. This bio-pathway influences the production of some phytohormones (Wood and Reilly, 2007). Thus, the presence of Ni is required for the production of sufficient plant growth regulators needed for flowering phenomenon. All treatments increased total number of fruits. Number of inflorescences increased after Ni treatment, and number of inflorescences and fruits per inflorescence were higher after SA application alone or in combination with Ni.

All treatments were able to make plants produce higher yields which were in agreement with other studies (Mishra and Kar, 1974; Welch, 1981; Takashi *et al.*, 2002; Gharib, 2006; Hegazi and El-Sharaiy, 2007; Amin *et al.*, 2007; Karlidag *et al.*, 2009).

Because the plants were grown in a hydroponic greenhouse, and their fertigation formula did not contain nickel as a nutrient, the concentration of this element increased when plants received Ni treatments. According to a study carried out by Freeman and Georgia (2006), SA has the ability to reduce the accumulation of nickel within plants by inducing the production of Glutathion, a phenomenon in accordance with our findings.

## CONCLUSION

Generally Ni as an essential element for plant life cycle and SA as a phytohormone has beneficial effects on overall plant growth and development. However, the crucial point is to find their proper concentration to be used in treatments. Because Ni can accumulate at toxic levels within plants organs and induce artificial



deficiency of other microelements such as iron and manganese, the importance of right concentrations is more vital when SA is going to be applied as a hormone where completely inverse, results may be obtained when precise concentrations are not used.

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## تأثیر نیکل و سالیسیلیک اسید بر رشد رویشی و زایشی توت فرنگی رقم 'پاجرو'

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### چکیده

این مطالعه برای ارزیابی برهم کنش سالیسیلیک اسید و عنصر نیکل روی رشد رویشی و زایشی گیاه توت فرنگی به صورت هیدروپونیک و در قالب طرح فاکتوریل کاملاً تصادفی در شرایط گلخانه اجرا گردید. گیاهان دختری رقم 'پاجرو' که به خوبی ریشه دار شده بودند، در گلدان های پلاستیکی ۳ لیتری کاشته شدند و بعد از استقرار یافتن، با محلول سالیسیلیک اسید به غلظت های ۰، ۱، ۲ و ۳ میلی مولار و سولفات نیکل در غلظت های ۰، ۱۵۰ و ۳۰۰ میلی گرم بر لیتر محلول پاشی شدند. نتایج نشان می دهد که سالیسیلیک اسید در نرخ ۲ میلی مولار باعث افزایش وزن تازه ریشه و شاخساره، تعداد گل آذین ها و میوه ها، غلظت نیتروژن میوه و عملکرد گردید. اثر عنصر نیکل نیز افزایش دهنده بود. محلول ۱۵۰ میلی گرم بر لیتر سولفات نیکل باعث افزایش معنادار تعداد میوه ها، تعداد گل آذین ها، سطح برگ و عملکرد گردید. ولی بهترین نتایج وقتی به دست آمد که سالیسیلیک اسید به همراه نیکل به کار رفت.