Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 3, 2021, Pages. 4384 - 4391
Received 16 February 2021; Accepted 08 March 2021.

Impact of Nanoparticle and Salicylic Acid Foliar Spraying in the Growth of Sour Orange Seedling

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Abstract:

The effects of foliar spraying with KHARZA nanoparticles (NPs) and Salicylic Acid (SA) on sour orange seedlings in conditions of shade house were surveyed to get the perfect combination sour orange growth. Seedlings of the local cultivar were investigated in a factorial experiment with three replications in 2019. The results showed that spraying with NPs at a concentration of (2g. l^{-1}) led to improve the vegetative growth characteristics, (including, plant height, stem diameter, leaves number and leaf area), chemical characteristics (including, chlorophyll and carbohydrate content) in addition to the leaf mineral content. Also, the foliar spraying with salicylic acid at a concentration of **150 mg.l^{-1}** showed a significant superiority in the vegetative and chemical traits. From the tested interaction between NPs (2g. l^{-1}) and SA (150mg. l^{-1}) it was the best performing by resulting in all studied traits.

Keywords: KHARZA, SA, shade house

INTRODUCTION

Sour orange (*C. aurantium*) is a large shrub tree, reaching a height of 5-15 meters, its evergreen which belongs to the Rutaceae family, their flowers are white in single or small clusters turns to a round-shaped and green fruits then yellow or orange upon ripening. The cultivation of sour orange trees is widespread in Iraq due to its ability to withstand hot weather. Therefore, its juice is widely used in Iraqi dishes as an alternative to lemon juice. Citrus fruits consider as a precious source of carotenoids, flavonoids, vitamins and also active compounds which derived and used to cure and prevent heart disease, hypertension, anticancer and antifungal activity (Abobatta, 2019).

Micronutrient like, Boron, Zink, Manganese...etc. are essential factors for biological systems to biosynthesize primary and secondary compounds, also they play a significant role in flowering, fruit setting, vegetative growth and biomass of yield (Vishekaii *et al.*, 2019). The world market offers a wide variety of micro-nutrient compounds, and Nano-products are already becoming incredibly common among them and made a powerful innovation in agriculture (Sharma *et al*. 2018). NPs are engineered to make leaves more accessible to nutrients, thereby increasing the efficiency of nutritional absorption by the plant (Akintelu *et al.*, 2021).

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Salicylic acid exists naturally in trace levels in plants and considered one of the natural plant hormones, such as auxins, cytokinins, gibberellins. Pirbalouti *et al*., (2019) showed that this compound play a role in activation and synthesis of antioxidants, phenolic, anthocyanin, in addition to release a signal molecule which plays an important role in regulating plants' response to environmental stress conditions.

In general, nutritional products are added to the soil. However, the micro-nutrients foliar spray is tested to be more reliable, in addition the exogenous application seems to have the disadvantage of high concentrations, consistent nutrient distribution and a quicker uptake stimulation by the plant (Umar *et al*., 1999).

The objective of this research is to use a number of combinations of NPs (KHARZA) and SA foliar application to reveal their impact on growth vegetative traits of sour orange seedlings.

MATERIALS AND METHODS

The shade house experiment was implemented during the vegetative growth season of 2019 (from April till October) located in agriculture college/AL-Qasim green university, Babil governorate in the middle of Iraq. To find out some of their physical and chemical characteristics, the experiment soil was analyzed and the results are shown in table 1.

Table 1: Physical and chemical characteristics of the sour orange experiment soil

Texture	**Loam**	**Clay**	**Sand**	**EC**	**pH**	**Available nutrients g. kg^{-1}**			
	g. kg^{-1} soil			**dS. m^{-1}**	**soil paste**	**N**	**P**	**K**	**Organic material**
Loamy sandy	**115**	**75**	**810**	**1.4**	**7.30**	**3.6**	**6.1**	**3.46**	**7.1**

In this study, (144) uniform vigor and healthy seedlings included 6 months old sour orange seedlings, planted in black plastic pots (60cm height and 30cm diameter) and obtained standardized botanical practices involved, irrigation; weeding and pest control.

In spring season, various NPs concentrations (0, 1.0, 1.5, 2) g. l^{-1} were used as foliar spray thrice in the course of the season, 1st spray was on 15/3/2019, 2nd on 1/4/2019 and 3rd on 15/4/2019. After 2 days from every previous foliar spray, SA (0, 50, 100, and 150) mg. l^{-1} was applied. In fall season, the same treatments (NPs and SA) were repeated on 15/8/2019, 1/9/2019 and 15/9/2019. In early morning and for greater uptake and lasting impact, uniform spraying was applied on every plant using hand sprayer on each sides of the leaves (Hamza. Al Dabagh, 2020). The control treatment consisted of only distilled water.
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Table 2: NPs (KHARZA) content

	Element				
	Boron	**Zinc**	**Manganese**	**Copper**	**Iron**
Percentage %	**0.5**	**1.5**	**1.5**	**0.5**	**8**

Plant growth variables data were assessed five months after the last spray, including:

1. Plant height: by using the measuring tape (cm).
2. Stem diameter: measured with the vernier calipers (mm).
3. Leaf number.
4. Leaf area: by randomly taking four leaves from shoot center and measuring the leaf area (cm^2) by digital planimeter then calculating the total leaf area: (number of leaves X leaf area).
5. Chlorophyll: the leaf greenness of sour orange seedlings was measured by a portable chlorophyll meter (SPAD-502, Japan).
6. % Carbohydrates: determined by digestion method outlined by Joslyn (1970) (Joslyn, 1970).
7. Leaf mineral content: the micro Kieldahl procedure was applied for determination of N, spectrophotometer was digested to calculate P content and the flame photometry was used to calculate the content of K in leaf.

Statistical design:

The shade house experiment was carried out as a factorial experiment in the Completely Randomized Block Design (CRBD) which involved 16 treatments, 3 replicates, each replicate containing 3 seedlings per unit. The findings were subjected by variance analysis using ANOVA table, and the statistical differences between variables were compared using the Least Significant Difference (LSD) at the 5% probability level.

RESULTS AND DISCUSSION:

The vegetative parameters:

- **Plant height:**

Table 3 results show the impact of SA and NPs in the means of sour orange plant height, the interaction between them gave the maximum height reached 53.26 cm for the treatment of SA (150)mg. l^{-1} and NPs (2) g. l^{-1}, whereas the control treatment, which is the lowest value, was observed 24.76cm.

- **Stem diameter:**

From the findings in table 3, it is evident that application of SA (150) mg. l^{-1} via interaction with NPs (2) g. l^{-1} showed a significant difference in the average of stem diameter amounted to 4.90 mm compared to the control treatment which showed the lowest mean (1.40mm).

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-Leaf number and leaf area:

Seedlings with no SA or NPs application had the minims average of leaf number and leaf area (table 3). In contrast, the foliar application of both of SA (150 mg. l^{-1}) and NPs (2 g. l^{-1}) significantly increased the average of leaf number and leaf area, 72.85 leaf/plant and 37.81cm^2 respectively, in comparison with control seedlings (41.67 leaf/plant and 14.70cm^2) respectively.

Plant organ's shape and size is detected via a controlled sequence, begins with cell division passing through proliferation, differentiation, and ending with elongation. Plant growth regulator exogenous like SA impacts significantly in mentioned parameters (Qi Wang *et al*., 2020).

NPs foliar application could resulted in controlling and regulating the growth characteristic's via its key role in metabolism, transport, and plant hormone synthesis (Shireen *et al*., 2018). This result is in line with prior studies that recorded the plant absorbs nanoparticles more effectively and easily to fulfill nutritional requirement (Vishekaii *et al.*, 2019 and Akintelu *et al*., 2021)

Table 3: Effect of foliar fertilization of SA and NPs at various concentrations in some vegetative growth indices of sour orange seedlings

NPs (g. l^{-1})	Salicylic acid (mg. l^{-1})	Plant height (cm)	Stem diameter (mm)	Leaves number	Leaf area (cm^2)	Chlorophyll (SPAD)
0.0	0.00	24.76	1.40	41.67	14.70	31.71
	50.00	31.59	2.29	46.25	19.18	34.87
	100.00	32.34	2.33	52.81	20.57	40.10
	150.00	33.65	2.49	60.34	22.75	44.27
1.0	0.00	32.97	2.66	45.78	21.20	44.94
	50.0	36.90	2.90	54.83	24.61	45.51
	100.0	37.44	3.08	60.76	24.11	46.55
	150.0	47.05	3.54	66.52	26.77	46.11
1.5	0.0	34.81	2.68	58.02	24.21	46.43
	50.0	38.83	3.34	58.16	26.62	47.84
	100.0	39.40	3.15	65.20	28.27	48.47
	150.0	41.84	3.63	68.44	29.33	49.60
2.0	0.0	35.56	3.29	66.84	26.67	49.37

	50.0	39.95	3.55	68.66	29.00	50.39
	100.0	42.40	3.86	71.33	34.44	51.10
	150.0	53.26	4.90	72.85	37.81	61.63
LSD at 5% level		**NPs (A)= 2.624** **SA (B)= 2.624** **A x B= 5.248**	**A= 0.29** **B= 0.29** **A x B=0.57**	**A=2.965** **B= 2.965** **A x B=5.930**	**A=2.272** **B=2.272** **A x B=4.544**	**A=3.145** **B= 3.145** **A x B=6.290**

The chemical parameters:

- **Chlorophyll content of the leaves (SPAD):**

A significant impact on chlorophyll content caused by addition of SA and NPs to the combined seedlings (table 4). When the treatments are placed on a comparative scale, the control led to decrease average chlorophyll content in leaves (31.71 SPAD), whereas the combination between SA (150 mg. l^{-1}) and NPs (2g.l^{-1}) had a significant effect which gave 61.63 SPAD.

One of the key factors that plant photosynthesis-related with is chlorophyll content which is usually influenced by nitrogen and phosphorus and that's also essential for the purpose of chlorophyll biosynthesis in the form of ATP (Adenosine Tri Phosphate). In 2020, Tombuloglu *et al*. reported that the elements contained in the NPs may separate and instead meet the biomolecules and biological systems, so the catalytic function of enzymes is thus increased, and improved the photosynthetic machinery which certainly increase the phonological variables of the plant, such as the level of chlorophyll. This is in agreement with findings noted on sweet cherry and olive trees where foliar application by NPs resulted in an increment in chlorophyll pigment (Tombuloglu *et al*., 2020 and Vishekaii *et al.*, 2019).

- **Carbohydrate (%):**

Table 4 presents the details related with the carbohydrate content, which expressed as a percentage. The findings show significant variations between the treatments with the highest average of carbohydrate (40.13%) at the interaction of SA (150mg.l^{-1}) and NPs (2g. l^{-1}), while the lowest percent of same indices was achieved with control seedlings (9.62%).

Carbohydrates aggregation in leaves is a predictor of the positive reaction to NPs foliar application due to its significant role in the metabolism and photosyntates distribution (Vishekaii *et al.*, 2019).

In 2005, El-Tayeb reported that metabolic intake of simple sugar can be enabled by SA foliar application to shape new cell respondents as a mechanism to promote development. The metabolic intake of simple sugar can be enabled by SA application to shape new cell respondents as a mechanism to stimulate development, in addition, SA is required to suppress enzymes of poly saccharide hydrolyzing and/or speed up the conversion of simple sugars into poly saccharides.

Final results dealing with the percentage of carbohydrate are in harmony with this gained by Vishekaii *et al.*, 2019.

Table 4: Effect of foliar fertilization of SA and NPs at various concentrations in some Chemical characteristics of sour orange seedlings

NPs (g. l^{-1})	**Salicylic acid (mg. l^{-1})**	**Chlorophyll (SPAD)**	**Carbohydrate (%)**
0.0	0.00	31.71	9.62
	50.00	34.87	12.15
	100.00	40.10	15.80
	150.00	44.27	17.73
1.0	0.00	44.94	15.86
	50.0	45.51	18.39
	100.0	46.55	19.33
	150.0	46.11	20.10
1.5	0.0	46.43	21.33
	50.0	47.84	20.34
	100.0	48.47	23.00
	150.0	49.60	24.36
2.0	0.0	49.37	26.43
	50.0	50.39	30.89
	100.0	51.10	36.92
	150.0	61.63	40.13
LSD at 5% level		**NPs (A)=3.145** **SA (B)= 3.145** **A x B= 6.290**	**A= 2.793** **B= 2.793** **A x B= 5.586**

Leaf mineral content:

Table 5 outlining the leaf mineral content (percentage of nitrogen, phosphorus, and potassium), the data obviously indicate that the interaction between SA (150 mg. l^{-1}) and NPs (2 g. l^{-1}) showed a considerable rise in leaves percentages of N(1.07%), P(0.54%), and K(1.85%) in comparison with the control seedlings which obtained the lowest average of this trait: N(1.07%), P(0.11%), and K(0.72%).

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NPs has been recommended to affect nutrient and makes an obvious support in N, P, and K translocation, and after NPs foliar spraying, the increase incidents can be noted particularly in leaves as it reported in olive (Vishekaii *et al.,* 2019).

Table 5: Effect of foliar fertilization of SA and NPs at various concentrations in Leaf mineral content of sour orange seedlings

NPs ($g.\ l^{-1}$)	**Salicylic acid ($mg.\ l^{-1}$)**	**Mineral leaf content**		
		N%	**P%**	**K%**
0.0	0.0	1.07	0.11	0.72
	50.0	1.30	0.13	0.95
	100.0	1.46	0.16	1.15
	150.0	1.62	0.18	1.22
1.0	0.0	1.80	0.22	1.20
	50.0	1.83	0.26	1.25
	100.0	1.90	0.28	1.26
	150.0	1.92	0.31	1.29
1.5	0.0	2.12	0.24	1.31
	50.0	2.18	0.36	1.37
	100.0	2.26	0.39	1.40
	150.0	2.27	0.40	1.41
2.0	0.0	2.16	0.27	1.37
	50.0	2.43	0.44	1.51
	100.0	2.79	0.47	1.65
	150.0	2.85	0.54	1.85
LSD at 5% level		**NPs (A)= 0.123** **SA (B)= 0.123** **A x B= 0.247**	**A= 0.042** **B= 0.042** **A x B=0.084**	**A= 0.104** **B= 0.103** **A x B= 0.207**

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