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Effect of Urea and Nano-nitrogen Spray Treatments on Some Citrus Rootstock Seedlings.

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ABSTRACT

A factorial experiment was conducted during two consecutive seasons (2020 –2021) and (2021–2022) using seedlings of Volkamer lemon (*Citrus volkameriana*) and Sour orange (*Citrus aurantium L.*) potted in the citrus seedling nursery of Horticulture Research Institute, Giza, Egypt. Two nitrogen sources (urea & nano particles) each at 2 concentrations (500 & 750 ppm) and water spray were foliar sprayed three times at one month interval. Obtained results revealed that various growth parameters (plant height, stem diameter, no. of leaves, leaf area, fresh & dry weights of three plant parts) and Chemical analysis (leaf total free amino acids, photosynthetic pigments, leaf protein electrophoresis, N, P and K contents) have responded differently to each of the investigated factors (N spray treatments or rootstock) or to both (interaction effect). Anyhow, except for leaf area, Volkamer lemon growth parameters were superior and its leaves had richer chlorophyll, a & b contents. Meanwhile, Sour orange leaf chemical components (carotenoids, free amino acids, N, P, and K %) revealed the superiority. Moreover, four N solutions significantly increased all growth measurements and leaf chemical composition (except P %) over control. The specific effect of both investigated factors reflected directly on their interaction effects. Therefore, higher N concentration regardless of source caused Volkamer lemon growth characteristics to be superior. While the richest leaf chemical contents resulted from Sour orange leaves sprayed with 750 mg/L N. The total number of generated bands across the three treatments of each rootstock were thirteen. Herein, with Sour orange 9 bands were common (monomorphic), while 4 other ones were unique Polymorphic with 30.8% polymorphism, whereas 3 of them were negative and only one was positive. As for Volkamer lemon eleven bands from the 13 generated ones were monomorphic and two other ones were Polymorphic with 15.38% Polymorphism, one of them was negative and other was positive.

Keywords: Sour orange, Volkamer lemon, Urea, Protein electrophoresis, Nano nitrogen.

INTRODUCTION

Commercially citrus rootstock has a crucial role in citrus production in all citrus production regions (**Bowman & Joubert, 2020**).

Sour orange (Citrus aurantium L.) and Volkamer lemon (Citrus volkameriana) have been the primary rootstocks employed in Egypt. The time required to produce citrus seedlings to a suitable size for grafting may be as long as 1 or 2 years (**Castle, *et al.*, 1993**). Citrus nursery plants show slow growth during the rootstocks growth stage, rootstock production stage is an important stage in the production of citrus trees (**Abobatta, 2019**), and the production of the rootstock requires about 60% of the required time (**Fagundes, *et al.*, 2014**). Therefore, accelerating seedling growth could reduce this period and save seedlings' production costs, hence, shortening this period is highly important (**El-Sayed, 2018**).

Citrus seedlings are usually grown in containers, these are containing a small volume of soil, which limits the availability of water and nutrients to plants (**Fagundes, *et al.*, 2014**), and represents a constraint to the roots due to the small space and rapid vegetative growth. In these circumstances, providing more nitrogen fertilizer can be crucial in accelerating the growth of seedlings in containers (**Girardi, *et al.*, 2018 & Girardi, *et al.*, 2005**).

Nitrogen is one of the main elements for plant growth, plants' requirement from N is larger than other nutrients, and nitrogen plays important role in forming dry matter (**Leghari, *et al.*, 2016**).

Using foliar spraying of some nutrients such as nitrogen stimulate plant growth and compensate for the deficiency in these elements, which accelerates the growth of seedling to reach the grafting stage, thus reducing both of time required and seedlings' production costs **(Pal, *et al.*, 2020**).

Numerous modern agriculturalists give more attention to nanotechnology techniques by using nano fertilizers to enhance fertilizer efficiency, reflected in production. But since no enough studies have been carried out to evaluate the effect of Nano nitrogen fertilizers on citrus rootstock so far. This work aims to explore the effect of foliar application of nano N and urea on stimulating the growth of citrus seedlings.

MATERIALS AND METHODS

A factorial experiment was conducted on potted seedlings of two citrus rootstocks Volkamer lemon and Sour orange grown in greenhouse of the citrus nursery, Horticulture Research Institute, Agriculture Research Center, Giza governorate, Egypt during two successive 2020-2021& 2021-2022 experimental seasons.

It was aimed to enhance and accelerate growth of such seedlings rootstock for reaching suitable stage for grafting through investigating their response to foliar spray with two N source solutions.

The investigative factors in this experiment were:

1- Citrus rootstock species

Volkamer lemon and Sour orange seedlings.

2- Nitrogen foliar spray solutions

In this regard, solutions of two nitrogen sources (nano nitrogen and urea fertilizers), each at 2 concentrations, besides water spray as a control, so the following five foliar spray solutions were adopted:

a-water spray as control, b- nitrogen nano particles (N n) at 500 ppm, c- nitrogen nano particles (N n) at 750 ppm, d-urea at 500 ppm and e- urea at 750 ppm.

Nitrogen nano-fertilizer preparation

Nano nitrogen kindly obtained from department of radiation research of polymer chemistry, National Center for Radiation Research and Technology, atomic energy authority, Cairo – Egypt.

Nitrogen incorporated nano fertilizer were prepared using zeolite as a carrier material at a laboratory scale as following. Ammonium carbonate and ammonium nitrate in powder form were dissolved in water to reach at concentration of 30 % wt. /wt. The obtained solution was added to the solution of sodium silicate drop with and then solution of $AlCl_3$ (2 wt. %) was added, then the obtained white powder were dried at 40 ºC for 48 hours.

Characterization of Nitrogen nano

The particle size distribution was determent by Dynamic Light Scattering (DLS) supplied from Malvern Analytical

Instruments model M3-PALS zeta potential analysis. The average diameter of nitrogen nano-fertilizer was determined using the TEM images J software. JEOL (JEM-100CXII) Ltd. 1-2, Musashino 3-chome Akishima Tokyo, Japan.

Experimental layout

The complete randomized block design with three replicates was employed for arranging the aforesaid variables of both investigated factors.

Each replicate was represented by five seedlings so, the total number of seedlings needed for conducting this experiment was estimated as follows:

2 citrus rootstocks × 5 N spray solutions×3 replicates×5 seedlings per each replicate = 150 seedlings (75 seedlings from each rootstock).

Such seedlings were obtained by planting the recently extracted seeds from each rootstock in mid-March 2020 and 2021 years then transplanted individually in 50×30 centimeter containers (plastic bags), previously filled with clay and sand mixture at equal of proportion (by volume) through the last third of May 2020 and 2021 years during 1st and 2nd seasons, respectively.

Thereafter, one month later after transplanting had been done, required number seedlings from each citrus rootstock (75) where carefully selected as being healthy and insects or disease free. Thereafter, selected seedlings of each rootstock were classified according to their vigor into three equal categories plots each of 25.

Taking into consideration that seedlings of each category were randomly subjected to spraying with the five investigated N spray solutions (5 seedling per every treatment). Since, foliar sprays with various N solutions was applied three times at one month interval which starts from last third of (June 2020 and 2021) continued (July 2020 and 2021) and (August 2020 and 2021) during both seasons. On the other hand, the experiment was terminated on early September 2021 and 2022 years during 1st and 2nd seasons, respectively.

The response of two citrus rootstock seedlings to the specific and interaction effects of the two studied factors was evaluated through the differences, exhibited in the following growth and chemical determinations:

As the experiment had been terminated on early September 2021 and 2022 years during first and second seasons, respectively the following measurements where determined.

1- Growth measurements:

Plant height (stem length, stem diameter at 30.0 centimeter above ground surface and number of leaves per seedling. Moreover, leaf samples i.e, 10 leaves from each replicate (5 seedlings) were collected from the third and fourth positions for measuring leaf area and chemical determination.

Thereafter, seedlings were removed and each one was individually divided into three plant parts (roots, shoots and leaves) whereas each organ of every individual seedling freshly weighed separately then cleaned and dried at 70°c till constant weight, then an average of five seedlings weights per every replicate was estimated.

2- Chemical determination:

2-a) Leaf total chlorophyll content: it was determined using 80% acetone extraction method described by **Nayek *et al.*, (2014)**.

2-b) Nutritional status

In dried leaves acid hydrolysis solution N, P, and K were determined by using the semi-micro Kjeldahl method after **Plummer, (1971)** total N% was estimated. The **King (1951)** method was used to colorimetrically estimate phosphorus. Flame photometer was used to determine potassium%, and by following the process outlined by (**Jayaraman 1985**), the total free amino acids mg per 100g of dry weight were measured using a spectrophotometer at 570 nm.

3-Protein related index:

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to assess electrophoretic proteins in treated seedlings leaves with nitrogen nano particles. Fresh leaves of Volkamer lemon and Sour orange rootstocks were picked at the end of first season (early September 2021), cleaned and immediately sent to the laboratory for determine electrophoretic proteins. Samples were weighed then processed using the procedure described by **Oresegun *et al.*, (2016).** By comparing with a medium molecular weight protein standard marker given by Ferments com., the number of distinct protein bands and its molecular weight were determined.

Statistical analysis

The obtained data were subjected to analysis of variance using a two-way analysis of variances (ANOVA) with two rootstocks × five treatments and three replications per treatment. The least significant differences were calculated using the MSTAT-C package (**Freed & Scott, 1986)** at a probability of 0.05, capital and small letters were used to compare the means of specific effect for two factors and interaction effect of their combinations, respectively according to **(Wallar** and **Duncan 1969).**

RESULTS AND DISCUSSIONS

1. Vegetative growth measurements:

In this respect the response of the investigated growth parameters i.e, plant height, stem diameter, No. of leaves per seedling, leaf area, fresh & dry weights of three plant organs (shoots, leaves, roots) total plant dry weight and top/root ratio to specific and interaction effects of two studied factors (rootstock & N spray treatments) and their combinations, data obtained during both seasons are presented in **Tables (1), (2)** and **(3).**

A.specific effect:

It is quite evident that all growth parameter responded specifically to both investigated factors (rootstock & nitrogen spray treatments). Herein, Volkamer lemon rootstock surpassed statistically Sour orange in all evaluated growth measurements except the leaf average area whereas the reverse was detected with Sour orange. Such trend was true during both (2020-2021) and (2021-2022) experimental seasons. As for the specific effect of nitrogen foliar spray treatments **Tables (1), (2)** and **(3)** obviously display that all the evaluated growth measurements were impacted specifically by the investigated nitrogen spray treatments, whereas the four sprayed N solutions of either urea or nitrogen nano particles increased them significantly as compared to the analogous ones of tap water spray (control) during two seasons. On the other hand, foliar spray with the higher N nano concentration (750 ppm) was significantly the superior which resulted generally in the greatest values of all growth parameters. Such trend was true during both seasons with very scarce exceptions dealing mainly with some leaves parameters (area & dry weight) and Top / Root ratio, whereas differences

didn't reach level of significance with comparing to some investigated spray treatments. Moreover, foliar spray treatments with either 500 mg/L N nano or 750 mg/L urea solutions ranked statistically second in general particularly for fresh and dry weights of three plant organs (shoots, leaves, roots), as well as the total plant dry weight parameters.

Regarding the important physiological role of N nutrition on stimulating vegetative growth of various fruit species, especially during juvenile stage. Moreover, the beneficial effect of nitrogen spray solutions exhibited in both citrus rootstock seedlings could be logically explained on nitrogen necessity as an essentially constituents of various metabolites and complexes like as proteins, amino acids, nucleotides and Coenzymes. Moreover, the N vital role it plays for other essential nutrient elements absorption.

Such results go generally with the findings of **(Sharma *et. al.*, 2022, Midde *et. al.*, 2021, Atwia, 1992, Havlin *et al.*, 2005, Gough *et al.*, 2012** and **Rajasekar, *et al.*, 2017)** on citrus seedling rootstocks and nitrogen addition.

B. Interaction effect:

It was quite clear that the distinct effect of each investigated factor (rootstock & nitrogen foliar spray treatments) reflected directly on the interaction effect of their combinations. Since, the superiority of either rootstock i.e, Volkamer lemon over Sour orange in all growth parameters (except leaf area) from one hand and/or foliar spray with the higher nano nitrogen concentration (750 mg/L) from the other pointed out that highest values of the evaluated growth measurements were significantly in closed relationship to the 750 mg/L N nano sprayed seedlings of Volkamer lemon rootstock. Such trend had true during both seasons with most measurements particularly stem diameter, (fresh & dry weights), of three plant organs (shoots, leaves, roots) total plant dry weight and Top/Root ratio. However, only three exceptions were observed, i.e those dealing with the differences between the abovementioned superior combination and the sprayed Volkamer lemon seedlings with either 500 mg/L nano nitrogen or 750 mg/L urea were too slight to be significant as stem height or number of leaves / seedling were concerned, respectively. Besides both combinations of sprayed Sour orange seedlings with urea at either 750 or 500 mg/L exhibited statistically the greatest leaf area during both seasons. On the contrary, water sprayed seedlings of both citrus rootstocks especially Sour orange were significantly the inferior. In addition, other combinations were in between the aforesaid two extremes with a relative variance of tendency, revealed the exceeding of the Volkamer lemon seedlings of such intermediate category over the analogous Sour orange combinations sprayed with the same nitrogen spray solution. This may be mainly attributed to the higher rate of variance between two rootstock rather than that exhibited between nitrogen foliar spray solutions.

2. Chemical composition:

Leaf photosynthetic pigments chlorophyll (a, b & carotenoids), total free amino acids, N, P and K contents were the seven investigated chemical composition in response to specific and interaction effects of two studied factors and their combination: Data obtained during both seasons are presented in **Tables (4)** and **(5).**

Table(1): Plant height, stem diameter, number of leaves/ seedling and average leaf area of Volkamer lemon and Sour orange seedlings as influenced by some urea and (N n) nitrogen nano spray solutions during (2020-2021) and (2021-2022) seasons.

Parameters	Stem length (cm)			Stem diameter (mm)			Number of leaves/ seedling			Leaf area (mm)		
Rootstock / Spray treatments	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*
2020-2021 season												
Control	53.33f	46.33g	**49.83E**	5.80e	4.47g	**5.12E**	33.74d	26.50f	**30.12D**	22.54e	31.04c	**26.78C**
Urea 500 ppm	87.00c	57.97e	**72.49D**	6.63c	5.13f	**5.88D**	42.07b	32.00e	**37.03B**	26.98d	33.84ab	**30.42B**
Urea 750 ppm	90.00b	62.77d	**76.39C**	7.47b	5.20f	**6.33C**	43.53a	33.13d	**38.33A**	27.02d	34.32a	**30.66B**
N n 500 ppm	97.90a	62.00d	**79.95B**	7.57b	5.60e	**6.58B**	38.77c	33.57d	**36.17C**	30.62c	33.20b	**31.90A**
N n 750 ppm	100.10a	63.20d	**81.65A**	7.93a	6.20d	**7.07A**	43.87a	34.20d	**39.03A**	30.48c	33.36b	**31.92A**
Mean**	**85.67A**	**58.45B**		**7.08A**	**5.32B**		**40.40A**	**31.88B**		**27.53B**	**33.15A**	
2021-2022 season												
Control	58.67f	50.97g	**54.82E**	6.20e	5.00g	**5.60E**	38.81de	30.48g	**34.64E**	23.66e	32.59c	**28.13C**
Urea 500 ppm	95.70c	63.76e	**79.73D**	7.20c	5.70f	**6.45D**	48.38b	36.80f	**42.59C**	28.34d	35.53ab	**31.93B**
Urea 750 ppm	99.00b	69.04d	**84.02C**	8.37b	5.83f	**7.10C**	50.07a	38.11e	**44.09B**	28.36d	36.04a	**32.20B**
N n 500 ppm	107.70a	68.20d	**87.94B**	8.33b	6.33e	**7.33B**	44.58c	38.60de	**41.59D**	32.14c	34.86b	**33.50A**
N n 750 ppm	110.10a	69.52d	**89.81A**	8.83a	6.67d	**7.75A**	50.45a	39.33d	**44.89A**	32.00c	35.03b	**33.52A**
Mean**	**94.23A**	**64.30B**		**7.79A**	**5.91B**		**46.46A**	**36.66B**		**28.90B**	**34.81A**	

*, ** Refer to specific effect of N spray treatments and citrus rootstock, respectively. Means followed by the same small or capital letter/s within each season are not significant at 0.05 level.

Table (2): Shoots and leaves (fresh & dry weights) of Volkamer lemon and Sour orange seedlings as influenced by some urea and (N n) nitrogen nano spray solutions during (2020-2021) and (2021-2022) seasons.

Parameters	Shoot fresh weight (g)			Shoot dry weight (g)			Leaves fresh weight (g)			Leaves dry weight (g)		
Rootstock / Spray treatments	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*
2020-2021 season												
Control	9.10 f	7.70 g	**8.40 D**	6.07g	5.20h	**5.63D**	13.49e	8.83h	**11.16E**	8.47c	6.53f	**7.50C**
Urea 500 ppm	13.33 c	11.30 e	**12.32 C**	8.57d	6.73f	**7.65C**	16.89c	11.76f	**14.32C**	10.10b	7.67e	**8.88B**
Urea 750 ppm	14.47 b	12.10 d	**13.28 B**	9.00c	7.37e	**8.18B**	18.37b	13.87e	**16.12B**	10.67a	8.03de	**9.35A**
Nn 500 ppm	14.87 b	11.00 e	**12.93 B**	9.67b	6.53f	**8.10B**	16.23d	10.93g	**13.58D**	10.40ab	8.23cd	**9.32A**
Nn 750 ppm	16.30 a	12.30 d	**14.30 A**	10.83a	6.80f	**8.82A**	19.47a	13.77e	**16.62A**	10.73a	8.43c	**9.58A**
Mean**	**13.61 A**	**10.88 B**		**8.83A**	**6.53B**		**16.89A**	**11.83B**		**10.07A**	**7.78B**	
2021-2022 season												
Control	10.19 f	8.62 g	**9.41 D**	6.98g	5.98b	**6.48D**	14.84e	9.72h	**12.28E**	10.16c	7.84f	**9.00D**
Urea 500 ppm	14.93 c	12.66 e	**13.80 C**	9.85d	7.75f	**8.80C**	18.58c	12.94f	**15.76C**	12.12b	9.20e	**10.66C**
Urea 750 ppm	16.20 b	13.55 d	**14.88 B**	10.35c	8.47e	**9.41B**	20.20b	15.25e	**17.73B**	12.80a	9.64d	**11.22AB**
Nn 500 ppm	16.65 b	12.32 e	**14.48 B**	11.12b	7.51f	**9.32B**	17.86d	12.03g	**14.94D**	12.48ab	9.88cd	**11.18B**
Nn 750 ppm	18.26 a	13.78 d	**16.02 A**	12.46a	7.82f	**10.14A**	21.41a	15.14e	**18.28A**	12.88a	10.12c	**11.50A**
Mean**	**15.25 A**	**12.19 B**		**10.15A**	**7.51B**		**18.58A**	**13.02B**		**12.09A**	**9.34B**	

*, ** Refer to specific effect of N spray treatments and citrus rootstock, respectively. Means followed by the same small or capital letter/s within each season are not significant at 0.05 level.

Table (3): Root (fresh & dry weights), total plant dry weight and top/root ratio for Volkamer lemon and Sour orange seedlings as influenced by some urea and (N n) nitrogen nano spray solutions during (2020-2021) and (2021-2022) seasons.

Parameters	Root fresh weight (g)			Root dry weight (g)			Total plant dry weight (g)			Top/Root ratio		
Rootstock / Spray treatments	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*
2020-2021 season												
Control	14.13f	12.77g	**13.45D**	8.80e	7.93f	**8.37D**	23.33f	19.67g	**21.50D**	1.657de	1.483g	**1.570B**
Urea 500 ppm	17.63bc	14.93e	**16.28C**	10.70b	9.17de	**9.93C**	29.37c	23.57f	**26.47C**	1.750c	1.570f	**1.660A**
Urea 750 ppm	18.20b	16.03d	**17.12B**	11.43a	9.47cd	**10.45B**	31.10b	24.87e	**27.99B**	1.720cd	1.630ef	**1.675A**
Nn 500 ppm	17.93b	17.17c	**17.55B**	10.70b	9.90c	**10.30B**	30.77b	24.67e	**27.72B**	1.880b	1.493g	**1.687A**
Nn 750 ppm	19.63a	18.20b	**18.92A**	10.93b	10.67b	**10.80A**	32.50a	25.90d	**29.20A**	1.973a	1.427g	**1.700A**
Mean**	**17.51A**	**15.82B**		**10.51A**	**9.43B**		**29.41A**	**23.74B**		**1.796A**	**1.521B**	
2021-2022 season												
Control	16.96f	15.32g	**16.14D**	11.00e	9.92f	**10.46D**	28.14g	23.74h	**25.94D**	1.563de	1.397h	**1.480B**
Urea 500 ppm	21.16bc	17.92e	**19.54C**	13.38b	11.46de	**12.42C**	35.35d	28.40g	**31.88C**	1.647c	1.477fg	**1.562A**
Urea 750 ppm	21.84b	19.24d	**20.54B**	14.30a	11.84d	**13.07B**	37.45b	29.95f	**33.70B**	1.620cd	1.533ef	**1.577A**
Nn 500 ppm	21.52b	20.60c	**21.06B**	13.38b	12.38c	**12.88B**	36.98c	29.77f	**33.38B**	1.770b	1.407gh	**1.589A**
Nn 750 ppm	23.56a	21.84b	**22.70A**	13.67b	13.33b	**13.50A**	39.01a	31.28e	**35.15A**	1.853a	1.343h	**1.598A**
Mean**	**21.01A**	**18.98B**		**13.14A**	**11.78B**		**35.39A**	**28.43B**		**1.691A**	**1.431B**	

*, ** Refer to specific effect of N spray treatments and citrus rootstock, respectively. Means followed by the same small or capital letter/s within each season are not significant at 0.05 level.

A. Specific effect

Regarding the specific effect of rootstock on chemical compositions, it is quite clear that two conflicted trends were detected. Herein, Sour orange seedlings exceeded significantly Volkamer lemon as their leaves total amino acids, carotenoids, N, P and K contents were concerned. Meanwhile, the reverse was true with both leaf chlorophyll a & b contents where Volkamer lemon leaves were the richest during both experimental seasons.

As for the specific effect of foliar spray solutions **Tables (4)** and **(5)** display obviously that all studied leaf chemical constituents didn't follow the same trend of response to the four urea and nano spray solutions as compared to water spray (control). Herein, each / some leaf chemical compound/s follow its/ their own trend. Anyhow, leaf chlorophyll, N and K contents followed a firm trend of response where such four leaf chemical components were increased significantly by any of the four urea and N nano spray solutions with comparison to control (water spray) during both seasons.

However, the 750 mg/L urea solution was the superior for both chlorophyll pigments (A&B) and leaf N %, while as for leaf K% was concerned the nano nitrogen at 750 mg/L was the most effective from the statistical point of view. On the contrary, the response of leaf P% to specific effect of N foliar spray solutions took the other way around, whereas all N solutions resulted in significant redaction below control.

In addition, the response of two other leaf chemical constituents' i.e, total free amino acids and carotenoids compounds to the specific effect of N spray solutions was greatly varied from one measurement to another. Generally it could be observed that differences in leaf total free amino acids were not so pronounced and didn't reach level of significance as compared with each other except with the 750 mg/L N nano solution which resulted in significant increase over control. Meanwhile, **Table (4)** shows that the 500 gm/L urea solution increased significantly the leaf total carotenoids compounds over control, while three other investigated N solutions i.e, nano nitrogen at either 500 or 750 mg/L and 750 mg/L urea decreased exhibited significant reduction below control.

Because of the evaluated leaf chemical composition are estimated as ratio on the base of either day or fresh matter. Thus, the concentrations of such components are certainly controlled by the accumulation and depletion rates of both dry matter (mainly carbohydrates) from one hand and the concerned leaf chemical components, like as nutrient elements from the other. Accordingly, differences exhibited in level of evaluated leaf chemical compositions by the nitrogen foliar spray solutions may be attributed to dilution effect. Since, increase leaf N and K% of nitrogen sprayed seedlings over control may be reflected the relative higher depletion rate of carbohydrates needed to meet further growth in such seedlings which in the same time accompanied with the a relative higher accumulation rate of both N and K resulted by the outer N supply which stimulates also K absorption. Moreover, the decrease in leaf P% of nitrogen sprayed seedlings below control could be also discussed as a dilution effect. Herein, the depressed growth of control minimized the upward movement of such highly mobile element towards the active growing tips and consequently in higher P accumulation rate than the analogous one of dry matter.

B. Interaction effect:

As for the interaction effect of two studied factors on leaf photosynthetic pigments (chlorophyll a, b and carotenoids), **Table (4)** shows that three pigments didn't follow the same trend. Anyhow, both

chlorophyll a & b followed to same extent the same trend, whereas leaves of two combinations of urea 750 mg/L sprayed seedlings of both citrus rootstocks had the highest values. However, differences were not so pronounced with chlorophyll (a) and in most cases didn't reach level of significance except as compared to other Sour orange combinations. However, with chlorophyll (b) variances were considerably observed, whereas leaves of sprayed Sour orange seedlings with either water or lower concentration of urea & N nano solution (500 mg/L) were significantly the poorest in their chlorophyll b content. This may be due to the more pronounced response of chlorophyll (b) to nitrogen spray solutions rather than chlorophyll (a). In addition, other combinations were in between the aforesaid two extremes.

As for the leaf carotenoids content, data obtained revealed that the 500 mg/L urea sprayed Volkamer lemon seedlings were significantly the richest followed by those sprayed Sour orange seedlings with 500 ppm nano nitrogen solution. On the contrary, leaves of 500 mg/L nitrogen nano sprayed seedling of Volkamer lemon and those of both rootstocks subjected to 750 mg/L urea were the inferior. In addition, other combinations were in between.

Referring, the response of the leaf total free amino acids to interaction effect of two studied factors, **Table (5)** reveals that the 750 mg/L urea sprayed Sour orange seedlings were significantly the richest, while other combinations were equally the same from the statistical point of view.

As for the leaf N content, **Table (5)** displays clearly that 750 mg/L urea sprayed seedlings of Sour orange were significantly the superior. However, other combinations of other N sprayed Sour orange seedlings ranked second. On the contrary, water sprayed seedlings of Volkamer lemon were the inferior. Other combinations were in between.

Concerning the response of leaf P% to interaction effect it was quite evidence that both combinations of water sprayed seedlings of either Sour orange or Volkamer lemon had the highest values, however those of former rootstock was significantly the superior. Moreover, both combinations of water sprayed Volkamer lemon seedlings and sprayed Sour orange with 500 mg/L solution ranked statistically second. On the contrary, both Combinations of sprayed Volkamer lemon seedlings with either 500 on 750 mg/L nano nitrogen solutions, besides, 750 ppm nano N sprayed Sour orange were significantly the poorest in their leaf P%. In addition, other combinations were in between.

Regarding the response of leaf K %, **Table (5)** obviously declares that the interaction effect represented typically the direct reflection of both investigated factors. Herein, the highest leaf k% was significantly coupled with leaves of Sour orange seedlings subjected to N nano solutions at either 500 or 750 mg/L. Moreover, three combinations of sprayed Sour orange seedlings with urea (regardless of concentration) and Volkamer lemon seedlings sprayed with 750 mg/l ranked statistically 2nd as their leaf K% was concerned. The least leaf K% was significantly detected by the tap water sprayed seedlings of Volkamer lemon. In addition other combinations were in between. Our results are go partially with findings of **Bondada, 2003, Rajasekar *et al.*, 2017** and **Hayyawi & Estabraq, (2020).**

3- SDS-Protein electrophoresis.

In this respect leaf SDS-protein electrophoresis of both Volkamer lemon and Sour orange rootstock seedlings as affected by N nano spray solution at either 500 or 750 ppm compared with water spray (control)

Table (4): Photosynthetic pigments of Volkamer lemon and Sour orange seedlings leaves as influenced by some urea and (N n) nitrogen nano spray solutions during (2020-2021) and (2021-2022).

Parameters	Chlorophyll a (mg/ml^{-1})			Chlorophyll B (mg/ml^{-1})			Total carotenoids (mg/ml^{-1})		
Rootstock / Spray treatments	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*
2020-2021 season									
Control	18.59ab	15.65d	**17.12C**	6.39f	4.91i	**5.65D**	6.21cd	6.04 d	**6.13B**
Urea 500 ppm	18.35b	17.45c	**17.90B**	8.40d	6.07g	**7.24C**	7.44 a	6.51c	**6.98A**
Urea 750 ppm	19.00a	19.07a	**19.04A**	11.93a	9.38b	**10.66A**	5.29 e	5.21e	**5.25D**
Nn 500 ppm	18.24b	17.30c	**17.77B**	8.41d	5.85h	**7.13C**	4.67 f	6.97 b	**5.82C**
Nn 750 ppm	18.60ab	17.27c	**17.94B**	8.62c	6.83e	**7.73B**	6.07 d	6.07 d	**6.07BC**
Mean**	**18.56A**	**17.35B**		**8.75A**	**6.61B**		**5.94B**	**6.16A**	
2021-2022 season									
Control	16.90a	14.23c	**15.57C**	5.68f	4.37i	**5.03E**	5.65cd	5.49d	**5.57B**
Urea 500 ppm	16.68a	15.87b	**16.28B**	7.47d	5.39g	**6.43C**	6.76a	5.92c	**6.34A**
Urea 750 ppm	17.27a	17.33a	**17.30A**	10.61a	8.33b	**9.47A**	4.1e	4.74e	**4.78D**
Nn 500 ppm	16.58a	15.73b	**16.16B**	7.47d	5.20h	**6.34D**	4.24f	6.34b	**5.29C**
Nn 750 ppm	16.91a	15.70b	**16.31B**	7.66c	6.07e	**6.87B**	5.52d	5.52d	**5.52BC**
Mean**	**16.87A**	**15.77B**		**7.78A**	**5.87B**		**5.40B**	**5.60A**	

*, ** Refer to specific effect of N spray treatments and citrus rootstock, respectively. Means followed by the same small or capital letter/s within each season are not significant at 0.05 level.

Table (5):Nutritional status of Volkamer lemon and Sour orange seedlings leaves as influenced by some urea and (N n) nitrogen nano spray solutions during (2020-2021) and (2021-2022).

Parameters	Total free amino acids (mg/100 gm)			N (%)			P (%)			K (%)		
Rootstock / Spray treatments	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*	Volkamer lemon	Sour orange	Mean*
2020-2021 season												
Control	3.01b	3.31b	**3.16B**	2.09 f	2.30 e	**2.20D**	0.350b	0.380a	**0.365A**	1.57f	1.93de	**1.75D**
Urea 500 ppm	3.30b	3.38b	**3.34B**	2.43cd	2.48bc	**2.46BC**	0.308c	0.331bc	**0.320B**	1.79e	2.23bc	**2.01C**
Urea 750 ppm	3.43b	3.88b	**3.66AB**	2.48bc	2.61a	**2.55A**	0.283d	0.319c	**0.301C**	2.08cd	2.29b	**2.19B**
Nn 500 ppm	2.93b	3.83b	**3.38B**	2.36de	2.47bc	**2.42C**	0.245e	0.280d	**0.263D**	2.00d	2.38ab	**2.19B**
Nn 750 ppm	3.25b	5.40a	**4.33A**	2.46bc	2.54b	**2.50AB**	0.228e	0.251e	**0.240E**	2.26bc	2.52a	**2.39A**
Mean**	**3.18B**	**3.96A**		**2.36 B**	**2.48A**		**0.283B**	**0.312A**		**1.94B**	**2.27A**	
2021-2022 season												
Control	2.78b	3.06b	**0.292B**	1.98f	2.18e	**2.08D**	0.346b	0.376a	**0.361A**	1.31f	1.61de	**1.46D**
Urea 500 ppm	3.05b	3.13b	**0.309AB**	2.30c	2.36bc	**2.33C**	0.305d	0.328c	**0.317B**	1.49e	1.86bc	**1.60C**
Urea 750 ppm	3.18b	3.59b	**0.339AB**	2.35c	2.48a	**2.42A**	0.280e	0.316cd	**0.29C**	1.73cd	1.91b	**1.82B**
Nn 500 ppm	2.72b	3.55b	**0.314AB**	2.24d	2.34c	**2.29C**	0.243fg	0.277e	**0.260D**	1.67d	1.98ab	**1.83B**
Nn 750 ppm	3.01b	5.00a	**0.401A**	2.33c	2.41b	**2.37B**	0.226g	0.249f	**0.238E**	1.88bc	2.10a	**1.99A**
Mean**	**2.95B**	**3.67A**		**2.24B**	**2.35A**		**0.280B**	**0.309A**		**1.62B**	**1.89A**	

*, ** Refer to specific effect of N spray treatments and citrus rootstock, respectively. Means followed by the same small or capital letter/s within each season are not significant at 0.05 level.

was investigated in sampled leaves from each rootstock on early September 2021 during 1st season.

The electrophoretic banding pattern of protein extracted from leaves of each citrus rootstock treated with N nano solutions (500 &750 ppm), beside control (water spray) are shown in **Figure (1**). Analysis of tabulated data in **Table (6)** for the leaves SDS- PAGE of two citrus rootstocks revealed a total number of 13 bands with molecular weight ranging from 13.0 - 198.0 KDa.

On the other side, as the SDS leaf protein electrophoretic banding pattern of control seedlings (water spray), **Table (6)** displays that only twelve bands were observed for each rootstock. Taking into consideration that however two rootstocks showed the same number of total bands, but both varied in absence of one band of those with the molecular weight at 31.0 and 13.0 bp for Sour orange and Volkamer lemon, respectively.

As for the influence of the N nano spray **Table (6)** reveals that each rootstock followed its own trend. Herein, with the Volkamer lemon rootstock analysis of data showed that eleven bands from the total thirteen, ones generated across the leaves of three Volkamer lemon treatments (control, 500 and 750 ppm N nano spray) were common (monomorphic) i.e, presented in leaves of three Volkamer lemon treatments. However, two remaining bands were polymorphic with Polymorphism percentage 15.38%. Both polymorphic bands were unique, one of them was negative (characterized by absence of the band with the molecular, size at 37.0 bp across the leaves of 750 ppm N nano sprayed seedlings. Other unique polymorphic band was positive and appeared within leaves of the 500 ppm N nano sprayed Volkamer seedlings which represented the presence of the band of molecular weight at 13.0 bp.

Referring the influence on Sour orange rootstock analysis of tabulated data in **Table (6)** declared that from the total thirteen bands generated across the SDS leaf protein banding patterns of the three Sour orange treatments (control & two N nano spray solutions) nine of them were common (monomorphic) while others i.e four were polymorphic with polymorphism percentage 30.8%. These four polymorphic bands were unique, whereas three of them were negative which represented by lacking of both bands with molecular weight at 62.0 bp and 22.0 bp within the leaf profile of the 500 ppm N nano sprayed Sour orange seedlings, as well as absence of band with molecular size at 81.0 bp for the 750 ppm N nano sprayed ones. However, the for 4th polymorphic unique band was positive, which represented by the presence of the band with the molecular weight at 31.0 bp within the SDS- leaf protein banding pattern of the 500 ppm nano sprayed Sour orange seedlings.

The changes exhibited in the leaf protein analysis are generally in line with findings of **Hafez (2006).**

However, our results in this regard shed some shadows of doubtfulness about the suitability of application mineral nutritive sources in the nano forms, especially with fruitful trees whereas further studies are needed in this respect.

According to the findings of this study, we can suggest spraying Sour orange and Volkamer lemon seedlings with urea or nano-nitrogen solutions at a concentration of 750 mg/L as an useful and additional way of enhancing growth of seedling rootstocks under study and thus reducing the time and cost of seedlings production.

Table (6): Densitometric analysis for SDS leaf proteins of Volkamer lemon and Sour orange rootstocks seedlings sprayed with 500 ppm, 750 ppm of (N n) nitrogen nano solutions and water (control).

Band No.	M.W bp	Volkamer lemon rootstock			Sour orange rootstock		
		Control	500 ppm of (N n)	750 ppm of (N n)	Control	500 ppm of (N n)	750 ppm of (N n)
1	198	1	1	1	1	1	1
2	136	1	1	1	1	1	1
3	117	1	1	1	1	1	1
4	95	1	1	1	1	1	1
5	81	1	1	1	1	1	0
6	62	1	1	1	1	0	1
7	51	1	1	1	1	1	1
8	41	1	1	1	1	1	1
9	37	1	1	0	1	1	1
10	31	1	1	1	0	1	0
11	22	1	1	1	1	0	1
12	15	1	1	1	1	1	1
13	13	0	1	0	1	1	1
Total		**12**	**13**	**11**	**12**	**11**	**11**

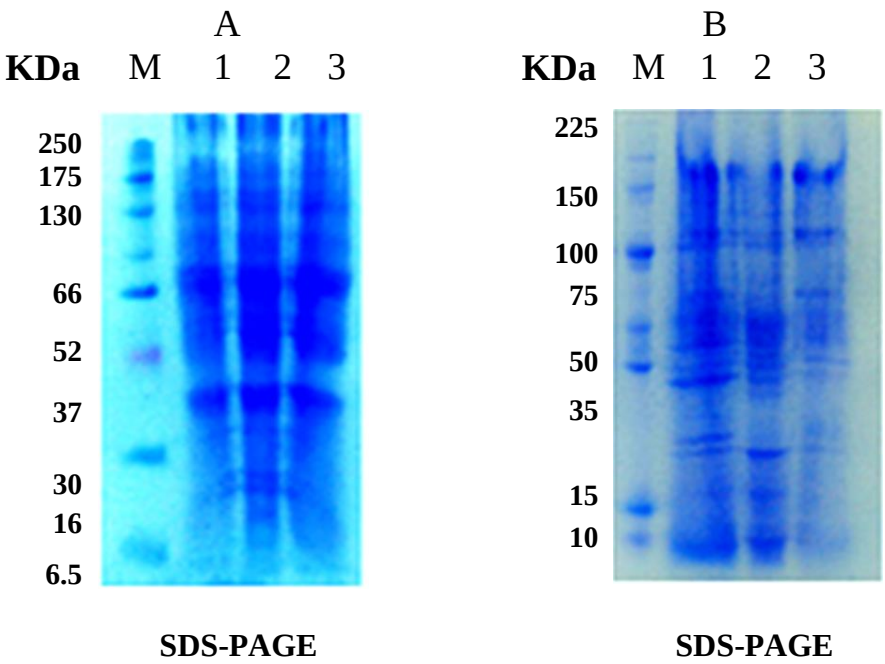

Fig. (1): SDS - leaf protein banding patterns of Volkamer lemon (A) and Sour orange (B) citrus rootstocks seedlings sprayed with water (control) and two (N n) nitrogen nano solutions at 500 & 750 ppm (1, 2 and 3 respectively).

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تأثير معاملات الرش باليوريا والنانو نيتروجين على البادرات البذرية لبعض أصول الموالح

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***قسم بحوث الموالح- معهد بحوث البساتين – مركز البحوث الزراعية - الجيزة – مصر.**

أجريت تجربة عاملية خلال موسمين متتاليين (2020-2021) و (2021-2022) باستخدام شتلات فولكاماريانا ونارنج بمشتل الموالح بمعهد بحوث البساتين - بالجيزة، مصر. حيث تم رش مصدرين من النيتروجين (اليوريا ،وجزيئات النانو) بتركيزين (500 و 750 جزء في المليون) بالإضافة إلى الماء للمقارنه ثلاث مرات بفاصل شهر واحد. أظهرت النتائج المتحصل عليها أن مؤشرات النمو (طول النبات ، قطر الساق ، عدد الأوراق ، مساحة الورقة ، الأوزان الرطبة والجافة للأجزاء النباتية الثلاثة) والتحليل الكيميائي (محتوى الأوراق من الأحماض الأمينية الكلية الحرة ، صبغات التمثيل الضوئي ، التفريد الكهربائي لبروتين الأوراق ، و النيتروجين و الفوسفور و البوتاسيوم) إستجابت لتأثير كل من العاملين المختبرين سواء منفردا (معاملات الرش بالنيتروجين أو الأصل المستخدم) أو لكليهما (تأثير التفاعل بين العاملين المختبرين).على أي حال ، تفوقت قياسات النمو لأصل الفولكاماريانا بإستثناء مساحة الورقة حيث تفوق النارنج، وكانت أوراق الفولكاماريانا هى الأغنى بمحتواها من الكلوروفيل أ ، ب، من جهه أخري أظهرت المكونات الكيميائية لأوراق النارنج (الكاروتينات ، الأحماض الأمينية الحرة ، النيتروجين و الفسفور و البوتاسيوم) تفوقاً. علاوة على ذلك ، زادت محاليل الرش الأربعة بالنيتروجين بشكل كبير من جميع قياسات النمو والتركيب الكيميائي للأوراق (باستثناء النسبة المئوية للفسفور) عن المقارنه. انعكس التأثير النوعى لكل من المعاملات التي تم فحصها بشكل مباشر على تأثيرات تفاعلها. كذلك أدى التركيز الأعلى من النيتروجين بغض النظر عن المصدر إلى تفوق خصائص نمو أصل الفولكاماريانا. بينما كانت الأوراق ألأغنى فى محتوياتها الكيميائية هى أوراق النارنج التي تم رشها بـ 750 ملجم / لتر من النيتروجين. أظهرت نتائج تحليل التفريد الكهربائي للبروتين تأثيراً للرش بالنانونيتروجين، حيث كان عدد الحزم الكليه المنتجه (13) بكل من الأصلين، و تميز النارنج بوجود تسعة حزم متماثله (موجوده في شتلات المعاملات الثلاثه) والأربعه الباقيه منها كانت متباينه بنسبه تباين 30.8% جميعها وحيده (ثلاثه منها سالبه و واحده فقط موجبه) أما بالنسبه للفولكاماريانا فكان عدد الحزم المتماثله (11) موجوده في شتلات معاملات الرش الثلاثه المختبره وحزمتان فقط متباينتين (نسبه تباين 15.38%) إحداهما وحيده سالبه والأخرى وحيده موجبه.