Effect of Liquid organic fertilizer, Nano-zinc and Triacontanol on Vegetative Growth and leaf Mineral Composition of lemon Trees

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Abstract

This experiment was conducted in one of private orchards located in Alexandria district, affiliated with Babylon (45 km south of Baghdad), for 2024 growing season on lemon trees to study effect of adding liquid organic fertilizer (proteon) and spraying with Triacontanol and Nano-zinc on vegetative growth and leaves mineral content of local lemon trees. Treatments were designed with a factorial experiment within RCBD design, that included adding liquid organic fertilizer with three levels 0 (O_0), 50 (O_1) 100 (O_2) ml.L^{-1} as 5 liters per tree, Nano-zinc spray with three levels 0(Z_0), 50(Z_1) and 100 (Z_2) mg.L^{-1} and Triacontanol spray with three levels 0 (T_0), 10 (T_1) and 20 (T_2) mg.L^{-1} . Number of trees was 81. Results showed that addition of liquid organic fertilizers at a 100 ml.L^{-1} (O_2) led to a significant increase in stem diameter of 4.063 cm, shoot length of 6.615cm, leaf area 41.78 cm^2 , leaves nitrogen content of 1.718%, phosphorus of 0.407 %, potassium 1.740 %. Results showed also superiority of spraying Nano-zinc at a 100 mg.L^{-1} (Z_2) in stem diameter of 3.922 cm, leaf area 36.74 cm^2, leaves nitrogen content of 1.615 %, phosphorus of 0.402 %, potassium of 1.697 % . Results also showed superiority of Triacontanol spraying especially at a concentration of 20 mg.L^{-1} (T_2) in shoot length of 5.559 cm, leaf area 37.64cm^2, leaves nitrogen content of 1.611 %, phosphorus of 0.409 %, potassium of 1.742 %. Effect of twice and triple interactions between study factors were significant in all studied traits.

Keywords: citrus, lemon, sustainability, Nano fertilizer, growth

***part of Ph.D. Dissertation for first authors.**

Introduction

Citrus is considered one of evergreen fruit trees that belong to Rutaceae family most important genera to which it belongs are Citrus, Fortunella, and Poncirus because of their adaptation to environmental conditions they are

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widely spread in different parts of world especially genus Citrus which includes oranges, tangerines, and Indian lemons as it is divided into several breeds and cultivars, extent of spread of citrus fruits and their high production has made them important for agriculture and global economy as they flourish and grow in subtropical (dry), subtropical, and tropical regions located between latitudes 40 north and south which are characterized by their appropriate climate and good soil that retains moisture that is important for survival and trees growth. Soil deterioration in terms of chemical and physical properties as a result of traditional fertilizers impact use is reflected in productivity per unit area add to environmental pollution challenges and climate change that affect horticultural citrus production [1,20,31]. Globally production reached 20,049,630 tons India comes first then Mexico, China, Argentina and Brazil in terms of production quantity Locally Wasit Governorate comes first then Salah al-Din and Diyala, number of fruitful trees lemon throughout Iraq is estimated at 291,487 trees with a production quantity of 5,375 tons with an average of 18.4 kg per tree [12,19] . Lemon fruits Nutritional value is summed up in their nutritional, medical, economic and environmental importance as they are an important vitamin C source, B1 and contain mineral such as Fe, P, K, Mg, S, Na and other elements [3].

Liquid organic fertilizer is often reasonably priced and well available in local markets and being environmentally friendly it's a good effect on soil and its properties, as it increases ability to retain water and this has a major impact on resistance to dry conditions. It also works on making soil crumbly and improving its aeration which reflects well on root process spread and growth add to role it plays in aforementioned readiness nutrients. It also works to increase various microorganisms activities and their secretions which can be secretions rich in plants growth regulators necessary and are fertilizers considered that are health safe [6,14]. Many studies have been carried out to determine liquid organic fertilizers role on growth characteristics and mineral fruit trees leaves content, [26**]** found in an experiment in which used three citrus rootstocks to which liquid organic fertilizer was added at three levels 0, 2.5, and 5 $ml.L^{-1}$ where he found that 5 $ml.L^{-1}$ had a significant effect on stem diameter , leaf area ,N, P, K leaves content for both growing seasons superior to control treatment. [24] pointed out in a study in which liquid organic fertilizer vit-org was added to three citrus rootstocks transplants to soil at 10,20 $ml.L^{-1}$ through data obtained it was noted that 10 $ml.L^{-1}$ led to a significant difference in stem diameter , leaf area and N,P leaves content were superior to control treatment which gave least significant difference.

Zinc is important and necessary microelements for all plants types it has been found to be involved in protein

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synthesis processes through its incorporation into enzymes which affects cellular membranes integrity, It has been found to be important in energy, seeds production and various plant organs , deficiency has a negative impact on plant growth changes in leaf colors and elements absorption lack, in extreme deficiency cases may lead to plant death. In recent years researchers interested using Nano-zinc due to its positive effect on plant growth through its absorption into plants which leads to increase in vegetative growth, flowering and yield while improving product and increasing fruits shelf life [15,32,34] . There are some experiments that have indicated Nano-zinc role in leaves minerals content, [24] showed a study in which two Nano-zinc concentrations1,2 gm.L^{-1} were sprayed on apple trees where both gave highest rate in stem diameter , leaf area, shoot lenght and N, P, K leaves content. [4] explained in a study conducted on one-year-old local lemon transplants in which zinc multiple sources were used sprayed at 50 mg.L^{-1} Nano-zinc was superior in increasing of stem diameter , leaf area and N, P leaves content superior to other zinc types as well as control treatment.

Tricontanol is a natural growth regulators which represents a long chain of primary alcohols , chemical formula $C_{30}H_{62}O$ and molecular $CH_3(CH_2)_{28}CH_2OH$ it consists of 30 carbon atoms, molecular weight is 438.8192 gm.mol melting point is 86.5°C, Studies have shown that triacontanol is a great importance in plant growth, Yield, photosynthesis, protein synthesis, water and nutrient absorption, free amino acid content and soluble protein in different crops [29,35]. There are a few experiments that have indicated triacontanol role in vegetative growth and leaves minerals content studies conducted results by [28] on "Sharabi" apple cultivar were sprayed on trees 5 and 10 g.L^{-1} where it was observed that higher concentration gave N,P,K highest percentage in leaves compared to control treatment that gave least. [21] showed in an experiment of Ber trees when sprayed at 10 mg.l^{-1} where a significant difference was obtained in N leaves content compared to control treatment. In a study conducted by [25] on “Le-Conte” pear cultivar was used triacontanol three levels 25, 50 and 100 mg.L^{-1} sprayed on trees where it was noted that second level was superior in some characteristics including stem diameter , leaf area and N,P leaves branches content were superior to control treatment that gave lowest values. Aim of our study is to know adding liquid organic fertilizer positive effect to soil and spraying with Nano zinc and triacontanol on leaves mineral content.

Materials And Methods

This experiment was conducted in one of citrus orchards in Alexandria city within Babylon Governorate (45 km south of Baghdad) for 2024 growing season on 7-year-old local lemon trees. 81 trees homogeneous in vegetative growth were selected to study adding

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liquid organic fertilizer effect (Proteon) and spraying with Nano-zinc and Tricontanol on growth and leaves mineral content of local lemon trees. Replications were placed on trees at beginning of March 2024 to begin experiment. It was implemented in form of a factorial experiment within a randomized complete block design R.C.B.D with three replicates and one tree for experimental unit. The spring dose of liquid organic fertilizer was added 5 times starting from March and period between one spray and another is 20 days at concentrations 0 (O_0), 50 (O_1) 100 (O_2) $ml.L^{-1}$ as 5 liters per tree. Spraying date with Nano-zinc was also in March with three levels at 0(Z_0), 50(Z_1) and 100 (Z_2) $mg.L^{-1}$. Two days later Tricontanol was sprayed with three levels at 0(T_0), 10 (T_1) and 20 (T_2) $mg.L^{-1}$. The following traits were studies:

Vegetative growth characteristics :

- Stem diameter (cm): 25/2/2024 stem diameter was measured by metric tape before start of experiment, as well as at growing season end on 31/12/2024, and difference between two measurements which represents increase in stem diameter.
- Shoot length (cm) : Three shoots were selected from each tree and their lengths were measured by metric tape before starting experiment in 25/2/2024 and same branches were also re-measured at end of season in 31/12/2024 and difference between two measurements was calculated which represents increased in shoot length.
- Leaf area (cm^2): Leaves samples were taken from experimental units at growing season end and leaf area was calculated using computer program Digimizer.

Leaves minerals content (N, P and K):

In last week of December on 25/12/2024 five fully-grown leaves were collected from each experimental unit. They were characterized by being physiologically active, newly mature and fully expanded and from recent growth they were washed with plain water acidified water (0.1 HCL) and then with distilled water. In order to remove any plankton from them such as dust and plant extracts remains, after drying they were placed in perforated paper bags and placed in an electric oven at a 70°C for three days. After this process they were crushed manually and using a sensitive scale 0.4 gm of them were weighed and digested using concentrated sulfuric acid H_2SO_4 and $HClO_4$ in a ratio of 4:1 respectively for each. After these plant extracts were ready according to [11] elements were estimated as follows; nitrogen percentage calculated according to [13], phosphorus percentage calculated according to [30]. Obtained results were subjected to variance analysis according to [17] using L.S.D 0.05 for comparing

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differences between various treatment means.

Results and Discussion

Role of Liquid Organic Fertilizer and Spraying with Nano-Zinc and Tricontanol on Increased in Stem Diameter, Shoots Length and Leaf Area

Data concerning effect of treatments on increased in stem diameter shoot length and leaf area are listed in Tables (1, 2 and 3). The findings showed that stem diameter reached to 4.063 cm, shoot length 6.615 cm and leaf area increased to 41.78 cm^2 when treated with 100 $ml.L^{-1}$ of liquid organic fertilizers (O_2) compared to lower values of these features in (O_0) treatment. Tricontanol spray, especially at 20 $mg.L^{-1}$ is shown to be effective in tables 2 and 3 .shoot length was up 5.559 cm and leaf area was up 37.64 cm^2 (T_2) . Both stem diameter and leaf area were increased significantly by Nano-zinc spray, especially at 100 $mg.L^{-1}$ and resulted in greatest increases in stem diameter 3.922cm and leaf area 36.74 cm^2 whereas (Z_0) resulted in least significant changes in these variables. Interaction treatment (O_2Z_2) had greatest changes in stem diameter 5.356 cm and leaf area 46.05 cm^2, (O_2T_2) in shoot length 7.400 cm among those exposed to liquid organic fertilizers, tricontanol and nano-zinc spray. These characteristics were significantly affected by triple interactions between study treatments.

Table 1. Role of liquid organic fertilizer, triacontanol and nano-zinc spray in stem diameter (cm) of local lemon trees for season 2024.

Tricontanol (T)	Nano-zinc (Z)	Organic Fertilizer (O)			T × Z
		O_0	O_1	O_2	
T_0	Z_0	2.867	3.833	3.967	3.556
	Z_1	2.700	2.967	3.867	3.178
	Z_2	2.733	2.900	5.167	3.600
T_1	Z_0	2.267	3.300	3.367	2.978
	Z_1	3.333	3.600	3.100	3.344
	Z_2	2.833	2.967	6.700	4.167
T_2	Z_0	2.333	2.800	3.167	2.767
	Z_1	2.967	3.067	3.033	3.022
	Z_2	3.233	4.567	4.200	4.000
L.S.D 5 %		0.510			0.295
T × O					T
T_0		2.767	3.233	4.333	3.444
T_1		2.811	3.289	4.389	3.496
T_2		2.844	3.478	3.467	3.263
L.S.D 5 %		0.295			0.170
Z × O					Z
Z_0		2.489	3.311	3.500	0.373
Z_1		3.000	3.211	3.333	0.388

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Z_2	2.933	3.478	5.356	0.402
L.S.D 5 %	0.295			0.170
O	2.807	3.333	4.063	
L.S.D 5 %	0.170			

Table 2. Role of liquid organic fertilizer, triacontanol and nano-zinc spray in shoot length (cm) of local lemon trees for season 2024.

Tricontanol (T)	Nano-zinc (Z)	Organic Fertilizer (O)			T × Z
		O_0	O_1	O_2	
T_0	Z_0	3.533	4.900	5.233	4.556
	Z_1	4.100	3.700	6.967	4.922
	Z_2	3.833	5.967	5.833	5.211
T_1	Z_0	4.800	5.200	5.700	5.233
	Z_1	4.067	3.467	8.367	5.300
	Z_2	3.500	4.367	5.233	4.367
T_2	Z_0	3.533	4.433	7.033	5.000
	Z_1	6.100	4.467	6.433	5.667
	Z_2	4.033	5.267	8.733	6.011
L.S.D 5 %		0.692			0.399
T × O					T
T_0		3.822	4.586	6.011	4.896
T_1		4.122	4.344	6.433	4.967
T_2		4.556	4.722	7.400	5.559
L.S.D 5 %		0.399			0.230
Z × O					Z
Z_0		3.956	4.844	5.989	4.930
Z_1		4.756	3.878	7.256	5.296
Z_2		3.789	5.200	6.600	5.196
L.S.D 5 %		0.399			0.230
O		4.167	4.641	6.615	
L.S.D 5 %		0.230			

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Table 3. Role of liquid organic fertilizer, triacontanol and nano-zinc spray in leaf area (cm^2) of local lemon trees for season 2024.

Tricontanol (T)	Nano-zinc (Z)	Organic Fertilizer (O)			T × Z
		O_0	O_1	O_2	
T_0	Z_0	24.11	27.61	35.32	29.01
	Z_1	25.12	30.94	40.12	32.06
	Z_2	24.98	32.88	41.03	32.96
T_1	Z_0	25.81	29.35	37.96	31.04
	Z_1	25.02	39.13	41.10	35.08
	Z_2	25.20	37.99	45.92	36.37
T_2	Z_0	28.99	41.62	44.10	38.24
	Z_1	27.83	34.30	39.26	33.80
	Z_2	29.10	42.34	51.21	40.88
L.S.D 5 %		1.56			0.90
T × O					T
T_0		24.74	30.48	38.82	31.35
T_1		25.34	35.49	41.66	34.16
T_2		28.64	39.42	44.86	37.64
L.S.D 5 %		0.90			0.52
Z × O					Z
Z_0		26.30	32.86	39.13	32.76
Z_1		25.99	34.79	40.16	33.65
Z_2		26.43	37.74	46.05	36.74
L.S.D 5 %		0.90			0.52
O		26.24	35.13	41.78	
L.S.D 5 %		0.52			

These results are due to role played by liquid organic fertilizer in soil chemical and physical properties which is positively reflected in plant vegetative growth characteristics, as it facilitates roots spread and aerates soil in addition to retaining water, as it enhances presence of microorganisms and their secretions [36]. These results are consistent with what was found by [27] when adding liquid organic fertilizer to mandarin seedlings soil as it significantly affected stem diameter and leaf area characteristics . As for role played by nano zinc and tricontanol, it may be due to their role in photosynthesis and protein synthesis process and providing sufficient space for various metabolic processes within plant in addition to ability to absorb water and nutrients and facilitate their movement in plant, as well as useful enzymes release [33,35]. These data are consistent with what was found by [4] when treating local lemon transplants with nano zinc, which significantly affected stem diameter and leaf area, and are consistent with what was found

by [2] when using triacontanol on pomegranate trees, which significantly affected stem diameter and leaf area.

Role of Liquid Organic Fertilizer and Spraying with Nano-Zinc and Tricontanol on Leaves minerals content (N, P and K)

Data concerning treatments effect on leaves nitrogen, phosphorus and potassium content are listed in Tables (4, 5 and 6). Findings showed that leaves nitrogen content reached to 1.718 %, phosphorus 0.407 % and potassium increased to 1.740 % when treated with 100 ml.L^{-1} of liquid organic fertilizers (O_2) compared to lower values of these features in (O_0) treatment. All leaves nitrogen content, phosphor and potassium were increased significantly by Nano-zinc spray, especially at 100 mg.L^{-1} and resulted in greatest increases in leaves nitrogen content of 1.615 %, phosphorus of 0.402 % and potassium 1.697 % whereas (Z_0) resulted in least significant changes in these variables. Tricontanol spray, especially at 20 mg.L^{-1} is shown to be effective in leaves nitrogen content was up 1.611 %, phosphorus 0.409 % and potassium was up 1.742 % (T_2). Twice interactions also had significant effects on leaf content of these elements. When interaction between adding liquid organic fertilizer and spraying Nano zinc was combined, (O_2Z_2) interaction had a significant effect and gave highest increase in leaf nitrogen content of 1.771 %, interaction (O_2T_2) phosphorus of 0.428 % and potassium of 1.841 % compared to (O_0Z_0) interaction that gave lowest values for these elements.

Table 4. Role of liquid organic fertilizer , Nano-zinc and triacontanol spray in leaves nitrogen content (%) of local lemon trees for season 2024.

Tricontanol (T)	Nano-zinc (Z)	Organic Fertilizer (O)			T × Z
		O_0	O_1	O_2	
T_0	Z_0	1.249	1.417	1.610	1.425
	Z_1	1.286	1.469	1.671	1.475
	Z_2	1.320	1.493	1.703	1.505
T_1	Z_0	1.294	1.475	1.683	1.484
	Z_1	1.326	1.502	1.718	1.515
	Z_2	1.421	1.794	1.786	1.667
T_2	Z_0	1.383	1.523	1.675	1.527
	Z_1	1.437	1.669	1.790	1.632
	Z_2	1.442	1.757	1.824	1.674
L.S.D 5 %		0.102			0.059
T × O					T
T_0		1.285	1.460	1.661	1.469
T_1		1.347	1.590	1.729	1.555
T_2		1.421	1.650	1.763	1.611
L.S.D 5 %		0.059			0.034

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Z × O				Z
Z_0	1.309	1.472	1.656	1.479
Z_1	1.350	1.547	1.726	1.541
Z_2	1.394	1.681	1.771	1.615
L.S.D 5 %	0.059			0.034
O	1.351	1.567	1.718	
L.S.D 5 %	0.034			

Table 5. Role of liquid organic fertilizer and Nano-zinc and triacontanol spray in leaves phosphorus content (%) of local lemon trees for season 2024.

Tricontanol (T)	Nano-zinc (Z)	Organic Fertilizer (O)			T × Z
		O_0	O_1	O_2	
T_0	Z_0	0.339	0.366	0.374	0.360
	Z_1	0.344	0.379	0.379	0.367
	Z_2	0.353	0.389	0.404	0.382
T_1	Z_0	0.350	0.381	0.392	0.374
	Z_1	0.359	0.391	0.400	0.383
	Z_2	0.357	0.412	0.427	0.399
T_2	Z_0	0.362	0.388	0.406	0.385
	Z_1	0.395	0.416	0.433	0.415
	Z_2	0.406	0.428	0.445	0.426
L.S.D 5 %		0.039			0.023
T × O					T
T_0		0.345	0.378	0.386	0.370
T_1		0.355	0.395	0.406	0.385
T_2		0.388	0.411	0.428	0.409
L.S.D 5 %		0.023			0.013
Z × O					Z
Z_0		0.350	0.378	0.391	0.373
Z_1		0.366	0.395	0.404	0.388
Z_2		0.372	0.410	0.425	0.402
L.S.D 5 %		0.023			0.013
O		0.363	0.394	0.407	
L.S.D 5 %		0.013			

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Table 6. Role of liquid organic fertilizer and Nano-zinc and triacontanol spray in leaves potassium content (%) of local lemon trees for season 2024.

Tricontanol (T)	Nano-zinc (Z)	Organic Fertilizer (O)			T × Z
		O_0	O_1	O_2	
T_0	Z_0	1.549	1.578	1.628	1.585
	Z_1	1.561	1.593	1.646	1.600
	Z_2	1.573	1.636	1.660	1.623
T_1	Z_0	1.577	1.615	1.690	1.627
	Z_1	1.596	1.642	1.746	1.661
	Z_2	1.604	1.673	1.765	1.681
T_2	Z_0	1.625	1.711	1.796	1.711
	Z_1	1.602	1.739	1.841	1.727
	Z_2	1.658	1.822	1.885	1.788
L.S.D 5 %		0.084			0.042
T × O					T
T_0		1.561	1.602	1.645	1.603
T_1		1.592	1.643	1.734	1.656
T_2		1.628	1.757	1.841	1.742
L.S.D 5 %		0.042			0.028
Z × O					Z
Z_0		1.584	1.635	1.705	1.641
Z_1		1.586	1.658	1.744	1.663
Z_2		1.612	1.710	1.770	1.697
L.S.D 5 %		0.042			0.028
O		1.594	1.668	1.740	
L.S.D 5 %		0.028			

These results shown in Tables (4,5,6) are due to liquid organic fertilizer content of compounds rich in polysaccharides and its high nitrogen nitrogen concentration , organic potassium and amino acids important in photosynthesis and nutritional processes, which is reflected in nutritional trees status by absorbing macro and micro elements and increasing soil content of organic matter important in various growth processes which affected increase in elements leaf content [5]. These results are consistent with what [10] found in a study on adding liquid organic fertilizer to "Qaisi" apricot transplants. As for Nano zinc fertilizer effect it may be due to some properties that Nano materials possess such as their small size, high solubility, absorption speed, and high vegetative properties under various conditions which is reflected in efficiency of photosynthesis, nutrition, gene expression, and many other vital processes that have a clear role in nutrients accumulation in leaves [7,8,23]. These results are consistent with what [18] found in a study on "Ewais" mango trees and with what [9]

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found when spraying Nano zinc on pomegranate trees. As for triacontanol effect on macro nutrients content in leaves it may be due to it being an internal growth regulator that facilitates many metabolic and enzymatic plant activities and provides a good internal space for various vital reactions within plant cells [22] These results are consistent with what [28] found in an experiment on "Sharabi" apple cultivar and [16] found in an experiment on strawberry transplants.

Conclusion

According to obtained results it can be concluded that high concentration of study factors $T_2Z_2O_2$ as an interaction treatment gave best results in many vegetative and chemical characteristics during growing seasons,it is possible to recommend increasing their concentration on other fruit trees and other location.

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