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EFFECT OF ORGANIC REMNANTS AND ZINC APPLICATION ON LOCAL ORANGE SAPLINGS, *CITRUS SINENSIS* (L.) GRAFTED ON SOUR ORANGE ROOT STOCK

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Abstract

This experiment was carried out at Al-Hindia Horticultural Station (Province of Karbala) affiliated by the General company of Horticulture and forestry/ Ministry of agriculture in November 2016 until June 2017, to study the effects of Organic remnant application as well as Zinc foliar application on vegetative parameters for Local orange saplings grafted previously on sour orange root stocks. For this study decomposed animal remnants were used as Organic compost at four levels (0, 0.5, 1.0, 1.5 kg. sapling^{-1}) and Zinc sulfur salts in water form were used as a source of Zinc at three levels (0, 20, 40 mg.L^{-1}). A factorial experiment using a complete randomized block design was carried out with three replicates. Means were compared using least significant differences and results were as follows:

Organic treatment applied at a rate of (1.5 kg/ sapling^{-1}) to sapling growth media exceeded significantly by increasing sapling vegetative growth parameters which recorded the highest results 103.87 cm for sapling height, 1.33 cm sapling stem diameter, 105.27 leaves for total leaf number, 23.27 dsm^2 for sapling leaf area, 48.37 chlorophyll leaf content spad, 128.6 g sapling dry weight, 6.13 secondary branch count, 71.9 secondary root count,%1.88 leaf nitrogen content, %0.34 phosphor content, %1.81 potassium content and finally %10.46 for leaf carbohydrate content. As for Zinc foliar applications applied at a rate of (40 mg.L^{-1}) significantly succeeded in increasing previous parameter means as follows,99.45 cm, 1.3 cm, 101.9 leaves, 22.7 dsm^2, 48.48 spad, 129.3 g, 6.15 branches, 73.28 secondary roots,%1.87, %0.32, %1.69 and %9.85 respectively. As for the best interaction treatment (1.5 kg.sapling^{-1} + 40 mg.L^{-1}) recorded the highest results for the previously mentioned parameters.

Key words : Organic remnants, zinc, orange saplings, sour orange.

Introduction

Citrus sinensis L. orange can be considered a member of the Rutaceae family and of the genus Citrus. Its importance comes from the fact that it makes up a third of Citrus production around the world as well as its great tasting bitter-free fruits which are suitable for human consumption (Aga and Dawood, 1991). These trees are propagated by grafting on seed stocks such as Sour orange stocks, which are popularly used in orange production areas and are suitable for Iraq's climate conditions as well as its resistance to many diseases such as citrus gummosis and root rot, as well as a deep, well-branched root system and its compatibility with other citrus cultivars grafted on to these root stocks (Salman, 1988).

Organic fertilizers have been used for many years in order to improve soil characteristics and to increase soil fertility as well as increasing nutrients availability which is necessary for plant growth, these fertilizers can be applied as a decomposed plant or animal remnants (Organic compost material) (Jhonson, 1980). Mansour and Shaaban (2007) mentioned that application of decomposed Organic remnants to citrus growth media at a rate of 1:1 resulted in an increase in leaf area, vegetative dry weight, and chlorophyll content, as for Madari *et al*. (1998), who pointed out that Olive trees Organically fertilized increased anti-oxidant levels in fruits and oil phenol content and growth medias including Organic remnants were more effective than medias without Organic remnants. When foliar applied Humic acid was used on olive trees significant increases were recorded in means such as primary stem height, leaf number, nitrogen, potassium, phosphor, and carbohydrate content compared to the comparison treatment (Mohamed Ali *et al*., 2012). Chicken remnants compost used in growing media for Anna apple trees resulted in a significant increase in leaf potassium content as well as yield and plant leaf area (Al-Ahbaby, 2011). Al-Abassy et al. (2016) mentioned that the ground application of soaked organic remnants on sour lemon resulted in an increase in sapling height, new leaf number, wet and dry vegetative weight and leaf chlorophyll content.

Zinc can be considered a necessary micro nutrient in plant growth and a deficiency can cause problems in growth, as it comprises the amino acid Tryptophan then forming Indole acetic acid (IAA), which is necessary for cell elongation and plays a role in nuclear acid synthesis (RNA, DNA) and the genetic code (Vallce and Valehuk, 1991). It was mentioned by Swietlik and Zhang (1994) that the use of three levels of foliar applied Zinc to fertilize Sour orange saplings resulted in a significant increase in leaf area means and vegetative shoot dry weight. Sheall *et al*. (2009) noticed that zinc sulfide foliar applied to citrus saplings grafted on Sour orange root stocks such as local orange, significantly increased sapling growth, plant leaf area, number of secondary branches, Nitrogen and Phosphor content in leaves. Al-Aaraji *et al*. (2006) noticed an increase in leaf Nitrogen, Potassium and Zinc contents, leaf area, stem height, and diameter as well as leaf dry weight when chelated Zinc was applied to Olive saplings cv. (Khudairy, Durmlaly and Surany). Foliar-applied Zinc to Orange trees by Al-Hamdany and Al-Bayatee (2015) resulted in a significant increase in leaf number, leaf area, chlorophyll leaf content, length, diameter, and weight of fruits which reflected on overall yield for zinc treated trees.

The aim of this study is to investigate the response of Local Orange saplings grafted on Sour orange seed stocks to Organic fertilizer applications and foliar applied Zinc.

Materials and Methods

This experiment was carried out in Al-Hindia horticultural station (Province of Karbala) affiliated by the General company of Horticulture and forestry/ Ministry of agriculture in November 2016 until June 2017, to study the effects of Organic material application and foliar applied zinc on vegetative parameters for Local orange saplings grafted previously on sour orange root stocks. For this study decomposed animal remnants were used as Organic compost at four levels (0, 0.5, 1.0, 1.5 $kg. sapling^{-1}$) and Zinc sulfur salts in water form were used as a source of Zinc at three levels (0, 20, 40 $mg.L^{-1}$). A factorial experiment using a complete randomized block design was carried out with three replicates, means were compared using least significant differences under 0.05% as mentioned by Al-Rawy and Khalfallah (1980) and analyzed using SAS statistics program. 180 homogenous one-year-old saplings were chosen for this experiment and every five saplings represented one experimental unit, each sapling was planted in 10 kg plastic bag. Organic fertilizers were applied before planting blended with the soil as for Zinc foliar applications which were applied six times starting from 15/11/2016 at 30 day intervals using a hand-held sprayer with a 2 liter capacity and with every concentration (1 cm^3) of cleaner as a surfactant to decrease surface tension of water (Al-Jumaily and Al-daily, 1989). Foliar application was carried out in the early mornings with a uniform coverage until saplings were completely wet. The saplings were previously irrigated a day before foliar applications, which dilutes the cell solute concentration causing a net influx of water molecules into the cell promoting cell turgidity and the opening of stomata, as a result, increasing penetration of foliar applied ions into leaf tissue (Al-Sahaf, 1989) the comparison treatment was sprayed with distilled water. Chemical fertilizers used were DAP at a rate of 25 g per sapling, irrigation, and weeding of planting media was done as needed. Samples were taken randomly from the planting media and analyzed to determinate physiochemical characteristics (table 1) as mentioned by Jaskson (1950), Black (1965). Table 2 contains components of Organic fertilizers used.

At the end of the experiment three saplings were chosen in order to study means of the following parameters :

1) Sapling height (cm) : measurements were taken from crown to meristem tip using a tape measure.
2) Stem diameter (cm) : sapling stem diameter was measured at the fifth leaf of the main stem
3) Total leaf number/sapling : number of leaves on each sapling and as a total mean for each experimental unit.
4) Leaf area (Dcm^2) : leaf area was measured for specified saplings for each experimental unit by measuring the area of 3 fully expanded leaves from tip, middle and bottom of sapling using an area measurement device Planimeter, the leaves were copied on a copy machine, and leaf area means of one leaf was multiplied by number of leaves on each sapling.
5) A number of secondary branches for each sapling were counted and a mean for every experimental unit was calculated.
6) Chlorophyll content (SPAD Unit) : was measured using a Chlorophyll meter (SPAD) directly from

Table 1 : Physical and chemical parameters of nursery soil used in the experiment.

Units of measure	Value	Analysis type
	Clay loam	Soil texture
g. kg^{-1}	336.3	Clay
g. kg^{-1}	368.4	Silt
g. kg^{-1}	295.3	Sand
	7.4	pH
Decicimens/m	2.46	Electrical conductivity (Ec)
Mg m^{-3}	1.38	Bulk density
$cmol_c$/kg	19.4	Cationic exchange capacity
g. kg^{-1}	8.7	Organic matter
g. kg^{-1}	186.6	Calcium carbonate
mg. kg^{-1}	10.7	Total nitrogen
mg. kg^{-1}	4.2	Available Phosphorus
mg. kg^{-1}	16.2	Available potassium

Table 2 : Lab analysis of organic remnants used in the experiment.

Total potassium g. kg^{-1}	Total phosphorus g. kg^{-1}	Total nitrogen g. kg^{-1}	C: N	Organic carbon g. kg^{-1}	Organic matter g. kg^{-1}	Salinity g. kg^{-1}	pH
2.4	0.23	14.6	20.8	330.2	479.6	5.82	6.32

plant leaves by taking three readings for every leaf.

7) Leaf dry weight percentage: Saplings total wet weights were recognized and Five leaves from each sapling were taken and dried, in an electric oven at 65-70^{0}C until a consistent weight was acquired, dry weight was then recorded using a sensitive scale.

8) A number of Secondary roots: was calculated as a mean including specified saplings for each experimental unit.

9) Nutrient content in leaves: Randomly chosen leaf samples from all parts of the plant were taken for every replicate, washed with distilled water in order to get rid of clinging soil particles, then placed in perforated paper bags and dried in an electric oven at 70^{0}C until a constant dry weight was acquired. Samples were then grinded and 0.2 g from each sample were taken and digested by adding 4 ml Sulfuric acid and 2 ml of concentrated Perchloric acid using the method mentioned by Jones and Styne (1973). Nitrogen content was then estimated using Micro-Kijldahl method (Haynes, 1980), Phosphor content was estimated using Ammonia molybdate and ascorbic acid with a spectrophotometer (Jhon, 1970) and Potassium content was estimated with a flame photometer as mentioned by Hesse (1971).

10) Leaf carbohydrate content: Total soluble carbohydrate content was measured using Phenol and Sulfuric acid by the method mentioned by Cresser and Parson (1979).

Results and Discussion

Sapling height mean (cm) and sapling stem diameter (cm)

Significant differences are noticed from the table 3 between Organic fertilizer levels applied on sapling height means and stem diameter, reaching 103.87 cm and 1.33 cm respectively compared to the comparison treatment (no application), which recorded the lowest levels 83.43 cm and 0.90 cm, respectively. As shown in table 3, Zinc applications resulted in a significant increase in sapling height and sapling stem diameter, Zn at 40 mg.L^{-1} recorded the highest levels 99.45 cm and 1.3 cm respectively, as for the comparison treatment gave the lowest levels reaching 85.55 cm and 0.90 cm, respectively. Interactions between both factors had a significant effect on increasing sapling height means and sapling stem diameters, the interaction treatment (1.5 kg.$sapling^{-1}$ + 40 mg Zn.L^{-1}) recorded the highest levels reaching 112.6 cm and 1.5 cm respectively, the lowest means were recorded by the interaction between no organic fertilizer treatment and no Zinc foliar application.

Total sapling leaf number mean and leaf area dcm^{-2}

Significant differences are noticed from the effect of organic treatments on leaf number mean, organic fertilizer applied at 1.5 kg. $sapling^{-1}$ recorded the highest results for both parameters reaching 105.27 leaves, 23.27 dcm^{-2}, respectively. Zinc foliar applications resulted in a significant increase in both parameter means, 40 mg Zn.L^{-1} level recorded the highest values reaching 101.90 leaf and 22.7 dcm^{-2}, respectively. The comparison treatment (no foliar applications) recorded the lowest values reaching 90.98 leaves and 20.45 dcm^{-2}, respectively. The interaction between both experimental factors resulted in a significant increase in sapling height and stem diameter. The interaction treatment 1.5 kg.$sapling^{-1}$ organic fertilizer +40 mg Zn.L^{-1} gave recorded the highest values reaching 113.4 leaves and 24.8 dcm^{-2}, respectively, the least value recorded was a result of the interaction between no organic applications and no zinc foliar applications.

Chlorophyll leaf content mean and sapling dry weight g

It can be noticed from table 5 significant differences in organic treatment results. Organic fertilizer applied at 1.5 kg. $sapling^{-1}$ recorded the highest value for these

Table 3 : Effect of organic remnants and zinc foliar applications and the interaction between them on sapling height mean and stem diameter.

Sapling stem diameter cm				Organic remnants kg.sapling^{-1}	Sapling height cm				Organic remnants kg.sapling^{-1}
Mean	Zinc mg Zn. L^{-1}				Mean	Zinc mg Zn. L^{-1}			
	40	20	0			40	20	0	
0.90	1.1	20	0.7	0	83.43	87.3	85.7	77.3	0
1.03	1.3	0.9	0.8	0.5	86.33	90.5	85.9	82.6	0.5
1.17	1.3	1.0	1.0	1	97.00	107.4	94.7	88.9	1
1.33	1.5	1.2	1.1	1.5	103.87	112.6	105.6	93.4	1.5
	1.3	1.4	0.9	Mean		99.45	92.98	85.55	Mean
Organic matter 0.14 Zn 0.09 interaction 0.21					Organic matter 4.86 Zn 3.94 Interaction 7.93				LSD *.05*

Table 4 : Effect of organic remnants and zinc foliar applications and the interactions between them on leaf number mean and leaf area.

Sapling stem diameter cm				Organic remnants kg.sapling^{-1}	Sapling height cm				Organic remnants kg.sapling^{-1}
Mean	Zinc mg Zn. L^{-1}				Mean	Zinc mg Zn. L^{-1}			
	40	20	0			40	20	0	
20.47	21.1	20.8	19.5	0	90.27	92.5	91.1	87.2	0
21.10	21.8	21.4	20.1	0.5	93.63	96.5	94.8	89.6	0.5
22.20	23.1	22.6	20.9	1	100.30	105.2	103.4	92.3	1
23.27	24.8	23.7	21.3	1.5	105.27	113.4	107.6	94.8	1.5
	22.70	22.13	20.45	Mean		101.90	99.23	90.98	Mean
Organic matter 1.02 Zn 0.67 interaction 1.58					Organic matter 4.22 Zn 3.82 Interaction 7.68				LSD *.05*

parameters reaching 48.37 spads and 128.6 g respectively compared to the lowest values given from the comparison treatment reaching 42.43 spads, 112.8g, respectively. As for Zinc foliar applications, both parameter means were significantly increased, 40 mg.L^{-1} application levels gave the highest values reaching 48.48 spads and 129.30 g respectively the interaction between both factors also gave a significant increase in the parameters mentioned above, the interaction treatment 1.5 kg.sapling of organic fertilizer +40 mg Zn.L^{-1} of zinc foliar application gave the highest values reaching 51.4 spad and138.2 g, respectively. The lowest interaction treatment was recorded with no organic fertilizer and no foliar applied Zinc treatments.

Branch number mean and number of secondary roots

Table 6 reveals significant differences between organic fertilizer application treatments on a number of branches and secondary roots for every sapling. Organic fertilizer treatment level 1.5 kg.sapling^{-1} gave the highest rates for the previously mentioned parameters reaching 6.13 and 71.9, respectively in compared to the comparison treatment (no application) which gave the lowest rates reaching 5.40 and 24.60 respectively, table 6 also refers to a significant difference in Zinc foliar applied treatments on the previously mentioned parameters, treatment 40 mg Zn. L^{-1} gave the highest rates at 6.15 and 73.28 respectively, the comparison treatment recorded the lowest rates at 5.20 and 59.75, respectively, as for interaction treatments between both factors statistical analysis revealed a significant effect on increasing both parameters previously mentioned, the highest rates were recorded by the interaction treatment 1.5 kg.sapling^{-1} Organic Fertilizer+ 40 mg Zn. L^{-1} reaching 6.5 and 76.1 respectively, and the lowest rates were recorded by the no application treatments of both organic and zinc interaction.

Average nitrogen and phosphorous percentage in leaves %

Table 7 reveals significant differences between organic treatment levels applied on Nitrogen and Phosphor leaf content, treatment level 1.5 kg.sapling^{-1}

Table 5 : Effect of organic remnant and zinc foliar applications and the interaction between them on chlorophyll content and sapling dry weight.

Sapling stem diameter cm				Organic remnants kg.sapling^{-1}	Sapling height cm				Organic remnants kg.sapling^{-1}
Mean	Zinc mg Zn. L^{-1}				Mean	Zinc mg Zn. L^{-1}			
	40	20	0			40	20	0	
112.80	120.1	115.7	102.6	0	42.43	46.1	44.3	36.9	0
117.97	125.4	122.6	105.9	0.5	44.10	47.3	45.8	39.2	0.5
124.20	133.5	129.7	109.4	1	46.67	49.1	47.7	43.2	1
128.60	138.2	135.4	112.2	1.5	48.37	51.4	48.9	44.8	1.5
	129.30	125.85	107.53	Mean		48.48	46.68	41.03	Mean
Organic matter 4.09 Zn 3.64 interaction 7.42					Organic matter 1.38 Zn 1.21 Interaction 2.26				LSD *.05*

Table 6 : Effect of organic remnant and foliar applications and the interaction between them on a number of branches and secondary roots means.

Sapling stem count. sampling^{-1}				Organic remnants kg.sapling^{-1}	Number of secondary roots.sapling^{-1}				Organic remnants kg.sapling^{-1}
Mean	Zinc mg Zn. L^{-1}				Mean	Zinc mg Zn. L^{-1}			
	40	20	0			40	20	0	
5.40	5.8	5.6	4.8	0	64.20	69.5	68.9	54.2	0
5.63	6.1	5.7	5.1	0.5	67.07	73.2	69.4	58.6	0.5
5.80	6.2	5.9	5.3	1	69.43	74.3	72.6	61.4	1
6.13	6.5	6.3	5.6	1.5	71.90	76.1	74.8	64.8	1.5
	6.15	5.88	5.20	Mean		73.28	71.43	59.75	Mean
Organic matter 0.26 Zn 0.18 interaction 0.34					Organic matter 2.35 Zn 2.11 Interaction 3.68				LSD *.05*

Table 7 : Effect of organic remnant and zinc foliar application and the interaction between them on average nitrogen and phosphor content.

Phosphorus in leaves %				Organic remnants kg.sapling^{-1}	Nitrogen in leaves %				Organic remnants kg.sapling^{-1}
Mean	Zinc mg Zn. L^{-1}				Mean	Zinc mg Zn. L^{-1}			
	40	20	0			40	20	0	
0.27	0.29	0.28	0.23	0	1.61	1.73	1.68	1.42	0
0.28	0.30	0.31	0.24	0.5	1.71	1.85	1.79	1.48	0.5
0.32	0.34	0.32	0.29	1	1.78	1.88	1.83	1.62	1
0.34	0.36	0.34	0.31	1.5	1.88	2.03	1.91	1.69	1.5
	0.32	0.31	0.27	Mean		1.87	1.80	1.55	Mean
Organic matter 0.03 Zn 0.02 interaction 0.04					Organic matter 0.07 Zn 0.05 Interaction 0.11				LSD *.05*

recorded the highest percentages reaching 1.88% and 0.34%, respectively compared to the comparison treatment which recorded the lowest rates reaching 1.61% and 0.27%, respectively. Zinc foliar applications increased previously mentioned parameters significantly, foliar applications at a level of 40 mg Zn. L^{-1} gave the highest rates reaching 1.87% and 0.32% respectively, the comparison treatment gave the lowest rates reaching 1.55% and 50.27%, respectively. As for the interaction treatments, significant differences were also noticed for the previously mentioned parameters the interaction treatment (1.5 kg. sapling^{-1} gave the highest percentages

Table 8 : Effect of organic remnant and zinc foliar applications and the interaction between them on average potassium and carbohydrate content

Carbohydrates in leaves %				Organic remnants kg.sapling^{-1}	Potassium in leaves %				Organic remnants kg.sapling^{-1}
Mean	Zinc mg Zn. L^{-1}				Mean	Zinc mg Zn. L^{-1}			
	40	20	0			40	20	0	
8.35	8.67	8.56	7.82	0	1.47	1.55	1.49	1.36	0
9.13	9.62	9.43	8.33	0.5	1.52	1.62	1.53	1.41	0.5
9.78	10.03	9.89	9.42	1	1.71	1.73	1.71	1.68	1
10.46	11.07	10.43	9.87	1.5	1.81	1.87	1.83	1.72	1.5
	9.85	9.58	8.86	Mean		1.69	1.64	1.54	Mean
Organic matter 0.46 Zn 0.33 interaction 0.68					Organic matter 0.08 Zn 0.05 Interaction 0.11				LSD *.05*

reaching 2.03% and 0.36% respectively, the least percentages were recorded by the interaction comparison treatment (No Organic and no zinc applications).

Average potassium and carbohydrate percentage in leaves %

Significant differences were recorded between organic treatment levels on average potassium and carbohydrate content, treatment level 1.5 kg.sapling^{-1} gave the highest results reaching 1.81% and 10.46% respectively compared to the comparison treatment which recorded the lowest percentages reaching 1.47% and 8.35%, respectively. Zinc foliar applications also significantly increased previously mentioned parameters, 40 mg Zn. L^{-1} gave the highest levels reaching 1.69% and 9.85% respectively, the comparison treatment gave the least average reaching 1.54% and 8.86%, respectively. Interaction treatments between both factors also recorded significant increases in mentioned parameters, the interaction treatment 1.5 kg.sapling^{-1} of Organic fertilizer +40 mg Zn.L^{-1} of Zinc foliar application gave the highest values reaching 1.87% and 11.07%, respectively. The lowest interaction treatment was recorded with no organic fertilizer and no foliar applied Zinc treatments.

Tables 3, 4, 5, 6, 7, 8 reveal a significant effect from both studied factors (Organic matter and Zinc foliar applications) on average vegetative parameters of local orange saplings grafted on sour orange root stocks. Organic treatment level 1.5 kg.sapling^{-1} was distinctly significant in increasing sapling height, stem diameter, sapling leaf number, sapling leaf area, vegetative dry weight, number of secondary branches and secondary roots, chlorophyll leaf content, nitrogen, potassium phosphorus and finally carbohydrate content, this could be explained by the role played by Organic matter and its components such as amino acids and their role in increasing cellular membrane permeability and nutrient transportation throughout the plant which contributes to increasing cell size, elongation and cell division reflecting positively on vegetative growth (Wample *et al*., 1991) or this could also be explained as Organic fertilizers containing Humic and fulvic acids which consist of a large nitrogen percentage which plays a role In increasing stored carbohydrates and increasing vegetative growth rates and photosynthesis levels reflecting positively on plant growth parameters (Keller and Kolet, 1995), humates also play a positive role in nutrient absorption by increasing nutrient availability mobility in the growing media especially micronutrients. As amino groups in Humic acids have the capability to adsorb negatively charged ions such as phosphor and increase their availability to plants (Tatini, *et al*., 1991) Humic acids also play a role in activating IAA Oxidase as a result increasing Auxin activity which stimulates plant and root growth also Humic acids increase soil capacity and capability to hold on to soil elements (Nardi *et al*., 2002). As well as loading soil with nutrients when applying Humic acids to the soil, these acids also play a large role in plant resistance to drought and high temperatures and increasing root growth, potassium added by organic fertilizers is considered a necessary element playing a role in cell regulation and stimulation, regulating osmotic pressure, respiration, protein synthesis, enzyme stimulation, regulation of guard cell osmotic pressure and opening and closing of stomata (Barakat *et al*., 2012).

Zinc treatment level 40 mg Zn. L^{-1} exceeded significantly in increasing previously mentioned parameters, this may be explained as the elements role in chlorophyll synthesis and stimulation of photosynthetic enzymes in leaves which stimulates biological synthesis of photosynthetic products in leaves which is used to build plant vegetative systems (Abdulrahman, 2010). Zinc also plays a role in carbonic anhydrase enzyme stimulation in green plastids which protects proteins from decomposing, This all ends up increasing vegetative growth and carbohydrate accumulation in plants leaves (Downton *et al*., 1987).

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