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SOUR ORANGE SEEDLING RESPONSE TO THE TREATMENT OF CARBON DIOXIDE AND NITROGEN SOURCE

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Abstract

Foliar application of organic sources of Carbon dioxide (Carbolizer) and Nitrogen (Vigamino) could enhance the growth of sour seedlings (*Citrus aurantium* L.). One year old uniform sour orange seedlings were planted in 15 L. pots filled with sandy loam + peat moss (2:1), the experiment designed as RCBD, carried out at the department of Hort. And Landscape - Coll. Of Agric. Engine. Sci. – Univ. Of Baghdad, In the 2017-2018 season. Results showed a significant increase in the leaf area, thickness and shoot, root systems and total dry weight, Seedlings leaf content of the chlorophyll (138.59mg.100gm fresh weight) and branches carbohydrates (2.25%) increased significantly, with a higher concentration of carbolizer treatment (CO_2 3), while plants treated with CO_2 (14.71%) Characterized by high protein content, results indicated a decrease in nitrogen concentration by increasing Carbolizer concentrations as a carbon source, despite the increase of amino acids as a source of nitrogen (Vigamino). Treatments did not affect phosphorus concentration in the seedlings, while there was a significant increase in potassium.

Key words : Sour orange, Foliar application, Carbon, Nitrogen, Shoot, Root, Dry weigh, Carbohydrate, Protein.

Introduction

Citrus aurantium L. (Rutaceae), known as sour orange or bitter orange, commonly used for the grafting of Citrus such as lemons, oranges, and mandarins, it is the most desirable rootstock in the world due to its adaptability to a wide range of soils, moderate tolerance of irrigation water and soil salinity (Al–Bayati *et al.*, 2005, AL-Hadithi *et al.*, 2005). Sauls (2008) indicated that most varieties of citrus can be grafted on sour orange rootstock, it produces high quality fruits. Sour orange seedlings Needed to supply with essential mineral elements, to grow naturally and strong to be able to graft them, Khalil (2008) indicated that different types of citrus grafted on sour orange planted in central Iraq, such as orange, lemon and Clementine, differed in their content of major and minor nutrient elements. Plant growth and development controlled by several factors, including environmental conditions, nutritional status, the most specific factors for photosynthesis and carbon assimilation is CO_2 concentration, (Chen *et al.,* 2002). As Carbon is the primary raw material in carbohydrate production in plant tissues, several studies have shown that plant's exposure to a different concentrations of CO_2 gas, higher than its concentration in the air, associated positively with plants physiological actions, which affect its growth and development by cell division, extension and differentiation (Taylor *et al.*, 2003). CO_2 enrichment is a type of fertilization designed to build a strong plant structure, it is the raw material for the formation of carbohydrates and organic matter production during photosynthesis. Plants exposure to higher concentrations of CO2 than the air showed a positive correlation with plant's physiological functions, leads to an increase in plant height, trunk diameter and leaf area (downton et al. 1987, and Koch *et al.*, 2008). Bowse (1993) elucidate that to carbohydrates produced by CO_2 assimilation forms 90% of the dry matter accumulated in the plant. Syvertsen *et al.*, (2000) indicated to a lower leaf content of N, p and K elements in some Citrus species exposed to high CO_2 concentration, attributed to alleviation due to plant growth

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increasing. Idso *et al*., (2002) reported that sour orange trees had a higher ability to respond to high concentrations of CO_2, Kimball *et al.,* (2007) Indicated that CO_2 enrichment led to an increase in sour orange seedling biomass by 70%. High concentration of CO_2 directly affected growth, physiological and chemical processes of the plant (Yoon *et al*., 2009). Allen and Vu, (2009); AL-Falahy, (2014), indicated that growth parameters could be improved by elevated atmospheric CO_2. In a study on *Citrus aurantium* L. grown for 6 years under the conditions of CO_2 enrichment at a concentration of 0.04% and 0.07%, it was found that the leaf content of protein was low in the concentration of 0.07% compared to CO_2 in the air atmosphere of 0.035. The researchers attributed the reason for the decrease in the ratio of protein to the lack of nitrogen processing required for the growth and development of new branches (Idso *et al*., 2008).

CO_2 enrichment leads to the formation of carbohydrate compounds and many carbon structures in plants as substrates, to converted them to other compounds need an elements such as Nitrogen. Nitrogen is an essential nutrient of the plant, it is the main component of amino acids, protein-building units, It also involves in a large number of important organic compounds, such as DNA and RNA (Taiz and Zeiger, 2010). So, decreasing N in plant tissue results in a significant imbalance in plant nutritional status and growth, (Taub, 2010). Amino acids are an important Nitrogen source used in plant processing, they are basic constituents of a protein that consists of a chain of amino acids that are linked to each other by covalent bonds. Amino acids are used to treat shortage of N by leaf spraying due to the small size of the its molecules which are easily absorbed by the surface layer of leaves, Plants treated with Free amino acids, as a natural nitrogen source, stimulate shoot and root growth (Abdel-Aziz and Balbaa, 2007). Junxi *et al.*, (2010) mentioned that, preventing nitrogen loss from the soil, and N uptake efficiency, was improved by amino acids foliar application. Hussein *et al*., (2015) observed, improving tomato's vegetative growth and production when treated with Agrosol and Enraizal as Carbon and Nitrogen organic source. During CO_2 enrichment to enhance photosynthetic processes and to keep on plant growth, Nitrogen (N) must be available continuously. Availability of a strong growth, root stock seedlings is the aim of this study by treat seedlings with carbon dioxide and nitrogen source, and their response to these compounds.

Materials and Methods

The study was carried out in the lath house of the research station in the Faculty of Agricultural Engineering Sciences- University of Baghdad - Al-Jadriyah for the 2017- 2018 growing season, to study the effect of a source of Carbon Dioxide (Carbolizer), which CO_2 would be release when this compound absorbed within plant tissues, to promote carbon essimilation and offset atmospheric carbon dioxide deficiencies, and a source of Nitrogen (a group of amino acids, Vigamino) in Sour Orange seedling growth, to obtain strong growth seedlings Before grafting or Planting in the field. One year old uniform sour orange seedlings were planted in 15 L. pots filled with sandy loam + peat moss (2:1). A complete Randomized Blook experiment, designed with three replicates to investigate the effect of Carbolizer (as CO_2 source) with concentrations (0, 2, 2.5, 3) ml. L^{-1} and Amino Acids Vigamino (as Nitrogen source) with concentration (0, 2, 3) ml. L^{-1}. Seedlings were treated with Carbolizer and Vigamino by foliar application four times periodically 10 day intervals, plants were watered using drip irrigation. Growth parameters measurements: At the end of the experiment (after eight months), Plant height increasing rates was obtained using the following equations: Increase rate %= T2 - T1 / T1 × 100, leaf area, leaf thickness and leaf content of total chlorophyll were measured, then seedlings were uprooted carefully from the soil and separated to shoot and roots, rinsed with distilled water, dried in oven at 60 ºC, until a constant dry weight obtained, to measure and determine shoot, root and total dry weight biomass (gm), S:R ratio. Plant samples were used to determine the concentration of nitrogen, phosphorus, potassium, carbohydrates, protein and Carbo: N ratio, Plant Nitrogen Uptake (PNU) as the production of a biomass per unit of fertilizer (Lea-Cox *et al.,* 2001) were determined, data were analyzed by analysis of variance (ANOVA) using the Genstat statistical program and mean were compared using LSD test at 5 % of probability.

Results and Discussion

Results

Vegetative characteristic indicators

Results in table 1 showed a significant increase in leaf area, thickness and dry weight of shoot, root systems and total dry weight of seedlings when treated with Carbolizar and Vegamino (amino acids) at a concentration of 2 ml. L^{-1} when compared to comparative and other concentrations treatment. Seedlings treated with Carbolizer (CO_2 2ml.L^{-1}) and Vigamino (AA 3 ml. L^{-1}) both separately recorded the highest plant height, S: R ratio varied, significantly by treatments (CO_2 2.5 ml. L^{-1}) and Vigamino (AA 2 ml. L^{-1}) as values are 3.62 and 3.49

respectively.

The results elucidate table 1 that the best treatment for both Carbon and Nitrogen Source was ($CO_2$2 AA2) in their effect on most studied vegetative characters, such as leaf area, thickness, dry character of shoot system and total dry, however, treatment ($CO_2$2 AA3) had the highest root system dry weight, treatment ($CO_2$2 AA3) was distinguished by its superiority in S: R ratio compared to other treatments.

Mineral elements Concentration, and PNU

Seedlings in $CO_2$0 and $CO_2$2 which treated with (Carbolizer) as Carbon source, had the highest concentrations of Nitrogen (2.35%) compared with the lowest concentrations (2.22%) in $CO_2$2 and $CO_2$3 seedlings, while plants treated with amino acids as a source of Nitrogen achieved the highest concentration of N in ($CO_2$0AA3) treatment seedlings, which amounted to 2.50%, with a slight decrease in N concentration in seedlings treated with both Carbon and Nitrogen source (Carbolizer + Vigamino), results indicate a decrease in nitrogen concentration by increasing Carbolizer oncentrations as a carbon source, despite the increase of amino acid concentrations as a source of Nitrogen, (Table 2).The results showed that Carbolizer and Vigamino treatments as Carbon and Nitrogen source, and their interaction did not affect significantly the phosphorus concentration in the seedlings, while there was a significant increase in potassium with foliar application of Carbolizer and Vigamino table 2, the highest potassium concentration (2.25%) was found in the treatment ($CO_2$2AA3) compared to other treatments. The ability of sour orange seedlings for Nitrogen uptake was increased by Carbolizer treatment (2 ml. L^{-1}), as well as seedlings treated with Vigamino (2ml.L^{-1}), both treatments recorded 4.5 mg. g^{-1}, while the treatment of ($CO_2$2 AA2) distinguished in its ability to absorb Nitrogen superiority (5.1mg. g^{-1}).

Table 1: Carbon dioxide and Nitrogen Sources affect plant leaf area, leaf thickness, Plant height, Shoot, Root, Total dry weight and S: R ratio.

Treat		Leaf area Dc^2. $Plant^{-1}$	Leaf thickness mm	Plant height Rate %	Shoot dry Weight (gm)	Root dry Weight (gm)	Total dry Weight (gm)	S: R ratio
CO_2								
CO_2 0		26.36	0.10	10.83	104.76	29.94	134.70	3.54
CO_2 2		58.66	0.13	17.96	146.57	44.80	191.37	3.27
CO_2 2.5		44.05	0.11	18.69	144.51	39.87	184.38	3.62
CO_2 3		43.76	0.12	16.48	142.57	42.22	184.79	3.38
Lsd 5%		2.261	0.0079	0.4322	0.4201	0.082	0.4230	0.0179
AA								
AA 0		31.70	0.10	10.11	114.56	33.27	147.82	3.47
AA 2		54.15	0.13	17.31	147.84	42.29	190.13	3.49
AA 3		43.78	0.11	20.55	141.41	42.06	183.46	3.41
Lsd 5%		1.958	0.0068	0.3743	0.3638	0.0707	0.3663	0.0156
CO_2 * AA								
CO_2 0	AA 0	9.20	0.11	5.33	70.38	19.40	89.78	3.63
	AA 2	37.90	0.10	9.17	120.88	38.78	159.66	3.12
	AA 3	31.98	0.10	18.00	123.01	31.62	154.64	3.89
CO_2 2	AA 0	38.05	0.10	10.83	124.99	38.92	163.91	3.21
	AA 2	76.91	0.17	19.63	166.51	46.91	213.42	3.55
	AA 3	61.03	0.11	23.40	148.20	48.58	196.78	3.05
CO_2 2.5	AA 0	40.78	0.10	13.80	130.28	35.08	165.37	3.71
	AA 2	46.73	0.11	20.67	154.40	40.24	194.64	3.84
	AA 3	44.64	0.12	21.60	148.84	44.29	193.12	3.36
CO_2 3	AA 0	38.76	0.10	10.47	132.56	39.65	172.22	3.34
	AA 2	55.05	0.14	19.77	149.55	43.24	192.78	3.46
	AA 3	37.46	0.12	19.20	145.61	43.76	189.36	3.33
Lsd 5%		3.916	0.0136	0.7486	0.7276	0.1414	0.7327	0.03110

Total chlorophyll (mg. $100g^{-1}$), Carbohydrate%, Protein % and Carb: N ratio

Seedlings leaf content of the chlorophyll (138.59 mg.100gm fresh weight) and branches carbohydrates (2.25%) increased significantly, with a higher concentration of Carbolizer treatment (CO_2 3), while plants treated with (CO_2 2) characterized by high protein content (14.71%), compared to the lowest concentration of chlorophyll, carbohydrates and proteins in plants of treatment $CO_2$0, (Table 3). The plants in treatment AA2 were marked by the highest chlorophyll concentration, carbohydrates and proteins (138.88 mg.$100g^{-1}$, 2.16% and 14.83%) respectively. Carbolizer and Vigamino as Carbon and Nitrogen source interaction in ($CO_2$2AA2) treatment due to a significant increasing in chlorophyll (141.20 mg, 100 g^{-1}) and carbohydrate (2.34%),

Table 2: Carbon dioxide and Nitrogen Sources affect N, P and K concentration in Sour orange seedlings, and plant Nitrogen uptake.

Treat		N %	P %	K %	PNU (mg.g^{-1})
CO_2					
CO_2 0		2.35	0.63	1.91	3.2
CO_2 2		2.35	0.70	2.05	4.5
CO_2 2.5		2.27	0.62	2.05	4.2
CO_2 3		2.22	0.68	2.08	4.1
Lsd 5%		0.0098	Ns	0.0288	0.0216
AA					
AA 0		2.20	0.65	1.94	3.3
AA 2		2.37	0.67	2.03	4.5
AA 3		2.33	0.65	2.10	4.3
Lsd 5%		0.0085	Ns	0.0249	0.0187
CO_2 * AA					
CO_2 0	AA 0	2.05	0.56	1.90	1.8
	AA 2	2.49	0.72	1.91	4.0
	AA 3	2.50	0.60	1.91	3.9
CO_2 2	AA 0	2.40	0.72	1.88	3.9
	AA 2	2.41	0.65	2.01	5.1
	AA 3	2.25	0.73	2.25	4.4
CO_2 2.5	AA 0	2.18	0.58	1.98	3.6
	AA 2	2.30	0.65	2.05	4.5
	AA 3	2.33	0.63	2.11	4.5
CO_2 3	AA 0	2.14	0.72	1.98	3.7
	AA 2	2.29	0.67	2.14	4.4
	AA 3	2.23	0.64	2.11	4.2
Lsd 5%		0.0170	NS	0.0499	0.0375

Table 3: Carbon dioxide and Nitrogen Sources affect total chlorophyll (mg. 100g^{-1}), carbohydrate%, protein % and Carb: N ratio.

Treat		Total chlorophyll mg.100g^{-1}	Carbo hydrate %	Protein %	Carb: N ratio
CO_2					
CO_2 0		136.16	1.92	14.66	0.82
CO_2 2		137.88	2.23	14.71	0.95
CO_2 2.5		137.99	2.16	14.19	0.96
CO_2 3		138.59	2.25	13.88	1.03
Lsd 5%		0.4776	0.0097	0.0615	0.0187
AA					
AA 0		136.20	2.13	13.70	0.99
AA 2		138.88	2.16	14.83	0.91
AA 3		137.88	2.14	14.55	0.92
Lsd 5%		0.4136	0.0084	0.0532	0.0162
CO_2 * AA					
CO_2 0	AA 0	134.80	1.81	12.79	0.88
	AA 2	137.90	1.95	15.56	0.78
	AA 3	135.77	2.01	15.63	0.80
CO_2 2	AA 0	135.00	2.23	15.00	0.93
	AA 2	141.20	2.34	15.06	0.97
	AA 3	137.43	2.14	14.06	0.95
CO_2 2.5	AA 0	136.37	2.18	13.63	1.03
	AA 2	139.10	2.11	14.36	0.92
	AA 3	138.50	2.19	14.56	0.94
CO_2 3	AA 0	138.63	2.32	13.38	1.10
	AA 2	137.30	2.23	14.31	0.98
	AA 3	139.83	2.21	13.94	1.01
Lsd 5%	0.8271	0.0169	0.1065	0.0323	

while the highest protein was recorded in the treatment $CO_2$0AA3 (15.63%) compared to other treatments. The results of table 3 indicate a significant increase in Carbohydrate : Nitrogen ratio of (0.96) when plants treated with Carbon source (Carbolizer) at 2.5 ml.L^{-1} concentration and in AA0 treatment (0.99), while the highest ratio of carbohydrates to Nitrogen (1.10) was recorded in $CO_2$3AA0 Compared to the lowest ratio in $CO_2$0AA0 amounted to 0.88.

Discussion

The experiment results suggested stimulating sour orange seedlings by Carbolizer and Vigamino as organic sources of Carbon and Nitrogen, that confirmed by a significant increase in vegetative studied Indicators especially leaf area, shoot, root, and total dry weight, which represents plant biomass, resulting from the growth and development of seedlings. Increases in vegetative growth may be due to the effect of Carbon dioxide in building plant's structure through its effect on activities of cell division, expansion and differentiation by providing basic compounds as a substrate for building cells, tissues and organs (Taylor, 1997), which leds to an increase in plant biomass represented as a dry weight of shoot, root and total dry matter, the results were consistent with Idso *et al.*, (2002) who observed increasing *Citrus aurantium* biomass in response to a 75 % increment in atmospheric CO2 concentration, as well as Jifon *et al.*, (2002) pointed out that both citrus species, under consideration responded to high levels of Carbon dioxide, which noted by photosynthesis enhancement and the biomass increasing. The significant increase in vegetative properties (leaf area, leaf thickness, Plant height, Shoot, Root, Total dry weight and S: R ratio) of seedlings when treated with amino acids may be due to Nitrogen effects, as is an essential macro element for plant growth due to its involvement in many cellular vital compound formations (Taiz and Zeiger, 2010). Decreasing Nitrogen concentrations in plants treated with high concentrations of Carbolizer as well as treatment with a combination of Carbon and Nitrogen source, may be due to the dilution resulting from increasing in vegetative growth, it is obvious that the plant assimilated Nitrogen within cell components during construction of tissues and organs, as the results demonstrated in Table 1 with a significant increase in leaf area and accumulated plant biomass, these observations coincided with Kimball *et al*., (2001) and Kimball *et al*., (2007) who confirmed in his experience results in decreasing Nitrogen concentration coupled with plant biomass increasing under elevated CO_2 levels. Chlorophyll rates increased significantly in sour orange seedlings leaves, with increasing Carbon and Nitrogen source concentration separately in the treatments, as well as treatments interference, may resulted from Nitrogen fertilization with amino acids and its availability, which increased the formation of chlorophyll, It is also known that about 70% of the chlorophyll molecule is made up of Nitrogen, the results agreed with AL-Falahy, (2014), who indicated leaf chlorophyll content increasing by foliar application of urea and CO_2 elevated. Increasing leaf chlorophyll content led to photosynthesis enhancement, which increased the products, such as carbohydrates, and proteins, as shown by the results of the study, these products improved the growth and development of sour orange seedlings under study conditions.

Conclusion

The most important conclusion of my study is the possibility of using some organic compounds to enrich plants with carbon dioxide, the foliar application of Carbolizer plus Vigamino as a Carbon and Nitrogen source improved sour orange seedlings vegetative growth, therefore, the recommendation of this study is, instructing seedling producers in nurseries, to use such compounds to obtain strong plants.

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