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Biochar Combined with Foliar Application of Chelated and Nano Iron Enhanced Qualitative and Quantitative Attributes of *Polianthes tuberosa*, L. Plants

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TWO pot experiments were conducted during the two consecutive growing seasons of 2020 and 2021 at Beni-Suef governorate, Egypt, to compare the influence of biochar treatments (50, 100 and 200 g/pot) combined with foliar application of chelated and nano iron (250 and 500 mg L^{-1}) on growth productivity and chemical composition of tuberose plants. The best result for vegetative attributes (leaf number/ plant, leaf fresh and dry weights/ plant), flowering characteristics (flowering date, florets number/ plant, florets fresh weight/ plant, spike length and diameter in addition to fresh weight), vase life, bulbs productivity (number of new bulblets/ plant in addition to fresh and dry weights of the replacement clumps), oil %, photosynthetic pigments and total carbohydrates% in bulbs were obtained due to high level of biochar (200 g/ pot). Regarding iron fertilization treatments, all of the above mentioned qualitative and quantitative attributes of tuberose plants were considerably augmented due to Fe-chelated at 500 mg L^{-1} followed by 250 mg L^{-1} then nano-iron at 500 mg L^{-1} while nano-iron at 250 mg L^{-1} gave the least values. Spike diameter did not appear to be appreciably changed by any of the four iron fertilization treatments.

In regard to the interaction between the two involved factors, the highest of the prementioned characteristics, as well as qualitative and quantitative characteristics values for the component of volatile oil, were obtained from fertilizing tuberose plants with the Fe-chelated at 500 mg L^{-1} in combination with biochar at 200 g/pot. Finally, adding biochar combined with chelated iron to tuberose plants obviously improved its qualitative and quantitative attributes.

Keywords: Tuberose (*Polianthes tuberosa*), Biochar, Chelated iron, Nano iron, Bulbs productivity.

Introduction

Polianthes tuberosa L. is a bulbous perennial plant belongs to Agavaceae family. It is known under the common name tuberose. It is an ideal cut flower plant and is widely cultivated as a beautiful and glamorous decoration and landscaping plant in parks and gardens. It is successfully grown for the floriculture industry as potted plants, in borders or beds and mass planting (Dahiya et al., 2001).

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Tuberose plants have been widely utilized as a source of volatile oils for the perfume industry (Majid et al., 2012).

Biochar is a biomass pyrolysis like manure, organic and agricultural wastes that is created under limited conditions of oxygen (Lehmann and Joseph, 2009). Food security, degradation of soil fertility, climate change and profitability are the strong reasons for the implementation of modern technology or new agricultural systems. The aim of amending soils for their remediation is to reduce the risk of transfer contaminants to neighboring waters or receptor organisms. For this reason, biochar can serve as a common option because it has a biological source and can be added directly to the soil (Beesley et al., 2011). There are two points that make biochar have an advantage over other organic materials, high stability against degradation and has a greater ability to retain nutrients. Biochar enhances physical and chemical properties of the soil by improving microbial flora, moisture-holding capacity and cation-exchange capacity (Mensah and Frimpong, 2018).

Iron (Fe) is an important micronutrient for plants because it plays a vital role in metabolic processes like for example, processes of uptake, DNA synthesis, photosynthetic activity, N-fixation, chlorophyll synthesis and respiration (Kim and Rees, 1992). Also, it is an effective co-factor for several enzymes essential for the synthesis of plant hormones (Siedow, 1991). Iron is an important nutrient and its deficiency is popular among several different crops (Sánchez-Alcalá et al., 2014). Although the Fe content in soil is generally high, a large amount is retained in soil granules (Mimmo et al., 2014 and Bindraban et al., 2015). Fe addition in the soil is not feasible especially in calcareous soils with a high pH, because it is in the form of insoluble Fe_3^+. Since plants typically absorb iron in its soluble form Fe_2^+ from the soil, the lack of Fe in the soil leads to its deficiency in plants (Kobayashi and Nishizawa, 2012). The use of foliar spray for nutrients may be beneficial in such situations in order to avoid fixing the soil to the micro-nutrients (El-Naggar, 2009). The goal of this study were to explore the effect of different levels of biochar and various foliar application of chelated and nano iron on vegetative, flower and postharvest criteria, essential oil percentage and components in addition to bulbs productivity of tuberose plants**.**

Materials and Methods

This study was performed during two successive seasons (2020 and 2021) at the experimental farm, Faculty of Agriculture, Beni-Suef University, in order to examine the influence of adding four levels of biochar to the soil and foliar spraying with two different concentrations from the two sources of iron (chelated Fe-EDDHA 6 % and nano iron), as well as, their interaction on vegetative, floral and postharvest characters, essential oil percentage and bulbs productivity on tuberose (*Polianthes tuberosa*, L.) plants.

Design of the experiment

The experimental design was laid out with three replicates in a split-plot design. Four biochar levels (0, 50, 100 and 200 g pot^{-1}) were included in the main plots, while five treatments for iron concentrations (0, 250 and 500 mg L^{-1}) as chelated Fe-EDDHA 6 % or nano iron occupied the sub-plots.

Procedures of experiment

In the second week of April, tuberose bulbs were planted as a clump in pots (25 cm), each one containing a mother bulb (diameter 7-8 cm, weight 45-55 g) surrounded by 4-5 bulblets and soaked in a fungicide solution (Mon cut 25% wp), at a rate of 3 g/l for 15 minutes before planting, for both seasons, filled beforehand with a mixture of sand and clay (3:1 v/v). the plants were well established (4 weeks after potting), 5 g of granular fertilizer of N: P: K; 19:19:19 were added to each plant/pot. Two doses were added after 30 and 60 days from the planting date, at last, in the second week of August were added 5 g of potassium sulphate (48% K_2O). Some physiochemical characteristics of the used soil are shown in Table (1) which were assessed before planting bulbs according to Jackson (1973). Two types of plant residues for biochar source, namely wheat and soybean straw were gathered from the experimental farm, Sids research station, Agriculture Research Center, Beni-Suef, Egypt. At 60-70° C, soybean straw was oven-dried and eventually smashed into small particles (1-2 cm). The dried straw was put under minimal oxygen conditions at 450° C for 30 minutes in a muffle furnace for pyrolysis (Lu et al., 2014). Properties of the experimental biochar were revealed in Table (2). All five concentrations of iron were sprayed three times, three-week intervals starting the second week of May for both seasons (2020 and 2021).

Data recorded

About 3 weeks after iron foliar spraying, when flowering started, following characteristics were measured, Leaf number/ plant, Spike length (from top of inflorescence to the lowest floret), flowering spike diameter, fresh weight of flowering spike and photosynthetic pigments.

The following data were collected:

Vegetative characters:
- Leaf number / plant.
- Leaf fresh and dry weights / plant (g).

Floral characters:
- Flowering date (from planting date till the opening of the first floret).
- Florets number / spike.
- Florets fresh weight / spike (g).
- Spike length (cm).
- Spike diameter (mm).
- Spike fresh weight (g).

Postharvest characters:

Early in the morning, monthly from July and August, spikes were cut when 2-3 pairs of florets from the bottom of the spike opened at 70-75 cm, trimmed to 60 cm, and the lower third of the stem's leaves were removed before being placed in containers filled with tap water and held at room temperature (Khimani et al., 2005). Vase life (days) of tuberose cut flowers were terminated when the number of senesced florets exceeded the number of open ones/ spike (The loss or wilting of 50% of florets indicates that the vase's life is coming to an end) as defined by Dole et al., (1999).

Productivity of bulbs:

In the second week of November new bulbs and bulblets were dug out and the following data were recorded:

- Number of new bulblets / plant.
- Fresh and dry weights of the replacement clumps (g).

Chemical composition

Photosynthetic pigments

The three photosynthetic pigments, for tuberose fresh leaf, were determined according to Moran (1982).

- Total carbohydrates percentage in bulbs

Total carbohydrates including poly-saccharides in dry bulbs were colorimetrically determined with anthrone sulphuric acid method (Fales, 1951).

Essential oil percentage

Hexane solvent (250 ml) was used to soak 50 grams of fresh florets picked were gathered, weighed and exposed to the solvent before they fully opened for one hour. The solvent was evaporated after the debris were removed, leaving the concrete behind. Tuberose absolute was extracted from concrete using alcohol according to Rakthaworn et al. (2009).

TABLE1 . Physical and chemical characterizations of the used soil.

Particle size distribution*			Texture class	Chemical properties**											
Clay %	Silt %	Sand %		OM %	EC, dSm^{-1}	pH	Available (ppm)								
			Sand clay				N	P	K	Ca++	Fe	Zn	Mn	Cu
13.20	8.30	78.50		0.75	3.25	8.1	18.70	6.50	15.6	21.3	2.45	0.31	0.68	0.25

TABLE2 . The physical and chemical properties of the experimental biochar.

Properties	EC, dSm^{-1}*	pH*	Organic carbon (g kg^{-1})	CEC (cmol+ kg^{-1})	Total N (%)	Total P (%)	Total K (%)	Total Ca (%)	Total Mg (%)	Bulk density (g cm^{-3})	Total Fe, Mn, Zn (mg kg^{-1})
Values	2.66	8.1	512	44.7	1.34	0.68	1.33	0.76	0.42	0.55	56.3, 28.3, 46.5

Essential oil components

The G.C. analysis of the volatile oil samples extracted from florets of tuberose plants taken from the second season for some treatments which have the highest effect upon vegetative growth and floral and oil yield characteristics. Essential oil absolutes were determined using a GC-MS analysis system in EL mode, with a capillary column HP-5MS 30 mx 0.25 mm, film thickness 0.25 un, and a temperature program ranging from 60^0 C (5 min) to 280^0 C at a rate of 3^0 C/min. GC-MS analysis was also performed using a trap mass spectrometer at a temperature of 200^0 C. According to Van den Dool and Kratz (1963), Massada (1976) and Adams (2001) the components were identified by comparing their mass specters to those of Willey and NBS.

Statistical Analysis

According to MSTAT-C (1986) the collected data were analyzed statistically using variance analysis (ANOVA) and least significant difference (LSD) to differentiate means at a 5% level of probability.

Results

Vegetative characters

Leaf number besides fresh and dry weights were all significantly augmented due to tested biochar treatments in comparison with that produced by control plants. The highest value of leaf number in addition to fresh and dry weights were obtained as a result of application with high level of biochar (200 g/pot) in the two seasons. While, the lowest values were obtained from the untreated plants (controls). Numerically, as shown in Table (3) the increase in the abovementioned two parameters due to biochar at 200 g/pot, over that of control treatment (24.50 and 18.51% respectively, for leaf number), (49.94 and 47.60% for leaf fresh weight and 53.59 and 49.23% for leaf dry weight) in the first and second seasons, respectively.

Data presented in Table (3) show also significant improvement in the three vegetative criteria (Leaf number in addition to fresh and dry weights) due to the foliar iron fertilization treatments, except Fe-nano at 250 mg L^{-1}, in both seasons, in comparison with untreated plants. The highest values were obtained from supplying tuberose plants with chelated Fe 500 mg L^{-1}, chelated Fe 250 mg L^{-1} and Fe-nano 500 mg L^{-1}, respectively.

Concerning the interaction between biochar and Fe fertilization treatments, it was significant, in both seasons, as indicated in Table (3). The majority of the interacting treatments gave significantly best values of leaf number in addition to fresh and dry weights, in comparison with the control treatment. Among all other treatments, the plants receiving biochar at 200 g pot^{-1} in combination with chelated Fe 500 mg L^{-1} could be the best choice. The numerical increase in the abovementioned three parameters, in comparison with that of control treatment, reached 52.58, 96.43 and 101.34%, in the first season and 39.59, 87.17 and 88.22% in the second one for the three tested parameters, respectively.

Floral characters

It was obvious from the recorded data in Table (4) that biochar treatments significantly affected flowering date, florets number and flower fresh weight over the control treatment in both experimental seasons. The only exception was in the low level of biochar treatment (50 g pot^{-1}). Application of biochar treatments caused the earliest flowering date (82.9 and 77.2 days) followed by (85.9 and 80 days) then (93.2 and 87.3 days) for 200,100 and 50 g pot^{-1} in the first and second seasons, respectively.

Supplying tuberose plants with chelated and nano iron treatments caused the earliest in flowering date in the two consecutive seasons as shown in Table (4). Such earliness in flowering date was significantly affected due to chelated and Nano treatments over control one with exception nano-Fe (250 mg L^{-1}). The application of Fe-chelated (500 mg L^{-1}) treatment resulted in the earliest flowering 81.3 and 76.6 days followed by Fe-chelated (250 mg L^{-1}) treatment 86.3 and 80.1 days then Fe-nano (500 mg L^{-1}) treatment 98.9 and 84.2 days, comparing with the untreated plants which flowering after 95.4 and 89.2 days from planting in both seasons, respectively.

In relation to the biochar treatments, the three tested levels had a positive and significant increase in spike length, diameter and fresh weight over that given by control treatment in both seasons as shown in Table (5). The highest values concerning the three aforementioned characteristics were obtained due to treatment with high level of biochar which augmented the spike length, diameter and fresh weight over the untreated plants by27.56 ,19.63 and 24.87% in the first season and by 20.94, 28.41 and 19.38% in the second one, respectively.

In respect to the other flowering traits, namely florets number and fresh weight, spike length

TABLE 3. Influence of biochar, iron foliar application and their interactions on leaf number/plant and leaf fresh and dry weights/plant (g) of tuberose plants during the two seasons 2020 and 2021.

Iron concentrations (B) mg L^{-1}	Biochar (A) g pot^{-1}									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	leaf number / plant									
Control (water)	32.33	34.13	38.67	41.51	36.66	34.15	36.44	38.92	40.33	37.46
Fe nano 250	33.82	35.33	42.33	42.67	38.54	35.85	38.33	39.41	41.95	38.89
Fe nano 500	35.67	37.25	44.11	45.18	40.55	37.33	39.87	40.67	43.55	40.36
Fe chelated 250	37.15	39.76	46.75	47.67	42.83	38.67	42.33	44.15	46.05	42.80
Fe chelated500	42.85	44.31	48.22	49.33	46.18	39.25	44.17	47.45	47.67	44.64
Mean (A)	36.36	38.16	44.02	45.27		37.05	40.23	42.12	43.91	
L.S.D. at 5%	A: 1.33		B: 2.17		AB: 4.34		A: 2.05	B: 2.23	AB: 4.46	
	Leaf fresh weights / plant (g)									
Control (water)	46.25	52.66	74.45	82.15	63.88	49.33	54.35	75.95	80.75	65.10
Fe nano 250	48.65	61.35	77.14	84.65	67.95	52.07	59.44	77.56	84.05	68.28
Fe nano 500	57.02	64.22	82.80	83.57	71.90	58.64	66.75	84.64	85.65	73.92
Fe chelated 250	62.88	72.90	84.58	86.33	76.67	65.44	73.29	85.67	90.88	78.82
Fe chelated500	70.33	77.65	88.07	90.85	81.73	68.33	74.25	88.55	92.33	80.87
Mean (A)	57.03	65.76	81.41	85.51		58.76	65.62	82.47	86.73	
L.S.D. at 5%	A: 4.35		B: 5.63		AB: 11.26		A: 5.03	B: 4.82	AB: 9.64	
	Leaf dry weights / plant (g)									
Control (water)	7.46	8.63	12.62	13.58	10.57	8.15	9.74	12.62	13.80	11.08
Fe nano 250	7.85	10.06	13.07	13.99	11.24	8.61	10.52	12.88	13.96	11.49
Fe nano 500	9.20	10.53	14.03	13.81	11.89	9.69	11.81	14.06	14.23	12.45
Fe chelated 250	10.14	11.95	14.34	14.27	12.67	10.82	12.97	14.63	15.10	13.38
Fe chelated500	11.34	12.73	14.93	15.02	13.50	11.29	13.14	15.71	15.34	13.87
Mean (A)	9.20	10.78	13.80	14.13		9.71	11.64	13.98	14.49	
L.S.D. at 5%	A: 0.46		B: 1.26		AB: 2.52		A: 0.63	B: 1.12	AB: 2.24	

and fresh weight, they were all significantly increased in the two experimental seasons due to Fe-chelated (500 and 250 mg L^{-1}) and nano-iron at 500 mg L^{-1} treatments, in comparison with the control. No significant differences were detected among such four treatments for spike diameter in both seasons.

The Fe-chelated 500 mg L^{-1} gave the highest values for the four traits in the two seasons as show in Table (5). Numerically, the increment in these four traits due to the high concentration of Fe-chelated in comparison with control treatment recorded 19.53, 19.66, 11.50 and 16.91% in the first season and 22.79, 22.58, 13.12 and 15.17% in the second one. Spike diameter seemed to be unaffected significantly by any one of the four examined iron fertilization treatments, Table (5).

In relation to the combined effects of biochar and foliar application of Fe fertilization on studied flowering traits, data presented in Table (5) indicated that all these traits were significantly and considerably respondent to the investigated interaction treatments in both seasons. The interaction between biochar at 200 g pot-1 and chelated iron at 500 mg L-1 showed the superiority over the other combination treatments in the two growing seasons.

TABLE 4. Influence of biochar, iron foliar application and their interactions on flowering date (days), florets number and fresh weight/spike of tuberose plants during 2020 and 2021.

Iron concentrations (B) mg L-1	Biochar (A) g pot-1									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	Flowering date (days)									
Control (water)	102.5	100.7	90.4	88.1	95.4	94.6	92.7	85.1	84.4	89.2
Fe nano 250	99.3	97.5	88.7	86.4	93.0	93.3	90.4	83.3	81.5	87.1
Fe nano 500	94.6	93.3	86.8	84.7	89.9	89.5	87.9	81.7	77.6	84.2
Fe chelated 250	91.4	90.1	83.7	79.8	86.3	86.4	84.7	75.2	74.2	80.1
Fe chelated500	85.5	84.2	79.8	75.6	81.3	82.7	80.6	74.5	68.4	76.6
Mean (A)	94.7	93.2	85.9	82.9		89.3	87.3	80.0	77.2	
L.S.D. at 5%	A: 3.3		B: 4.3		AB: 8.6	A: 2.7		B: 3.1		AB: 6.2
	Florets number / spike									
Control (water)	26.25	29.15	34.16	34.95	31.13	24.67	26.35	31.75	33.95	29.18
Fe nano 250	27.67	30.45	35.33	36.05	32.38	25.45	28.75	33.44	34.07	30.43
Fe nano 500	31.45	32.67	36.55	37.33	34.50	27.85	31.27	35.67	36.88	32.92
Fe chelated 250	33.44	34.20	37.08	38.56	35.82	31.62	33.67	36.09	37.55	34.73
Fe chelated500	34.67	35.85	37.66	40.67	37.21	32.77	34.05	37.33	39.15	35.83
Mean (A)	30.70	32.46	36.16	37.51		28.47	30.82	34.86	36.32	
L.S.D. at 5%	A: 2.15		B: 3.11		AB: 6.22	A: 2.61		B: 2.93		AB: 5.86
	Florets fresh weight / spike (g).									
Control (water)	74.81	81.62	93.94	96.11	86.62	73.67	75.10	89.85	94.04	83.17
Fe nano 250	78.86	85.26	97.16	99.14	90.10	75.88	81.94	94.64	94.37	86.71
Fe nano 500	89.63	91.48	100.51	102.66	96.07	82.16	89.12	100.95	102.16	93.60
Fe chelated 250	95.30	95.76	101.97	106.04	99.77	94.28	95.96	102.13	104.01	99.10
Fe chelated500	98.81	100.38	103.57	111.84	103.65	96.67	97.04	105.64	108.45	101.95
Mean (A)	87.48	90.90	99.43	103.16		84.53	87.83	98.64	100.61	
L.S.D. at 5%	A: 4.25		B: 7.72		AB: 15.44	A: 3.85		B: 6.27		AB: 12.54

Postharvest characters

Regarding to the effect of biochar level treatments on vase life, data presented in Table (6) outlined that, all treatments increased vase life of tuberose florets. Biochar at 200 g pot^{-1} was the best treatment for extending the vase life of cut fresh florets (7.78 and 8.68 days) compared to untreated plants (5.92 and 6.22 days) in the first and second seasons, respectively. It was revealed from the recorded data that vase life of tuberose florets was significantly and noticeably enhanced due to all iron fertilization treatments except Fe-nano at 250 mg L^{-1} in comparison with control treatment in the two growing seasons. The longest days recorded were from the following treatments in descending order; Fe-chelated 500 followed by 250 mg L^{-1} then Fe-nano at 500 mg L^{-1}.

In connection with the interaction between biochar levels and iron fertilization treatments, data proved that the vase life of tuberose florets were significantly respondent to the interaction between the treatments either in the first season -effective interaction treatments which resulted in the longest days of florets vase life were obtained as a result of the following interaction treatments; biochar at 200 g pot^{-1} plus Fe-chelated at 500 mg L^{-1} followed by biochar at 200 g pot^{-1} plus Fe-chelated at 250 mg L^{-1} then biochar at 100 g pot^{-1} plus Fe-chelated at 500 mg L^{-1}, and at the same time no significant differences were recorded between these treatments. A similar trend was found in the second season.

Productivity of bulbs

All tested bulbs productivity aspects of tuberose plants were significantly affected by all biochar treatments. The increase of these aspects was gradual with the gradual increase of biochar levels in the two growing seasons. Data tabulated

TABLE 5. Influence of biochar, iron foliar application and their interactions on spike length (cm), diameter (mm) and fresh weight (g) of tuberose plants during 2020 and 2021.

Iron concentrations (B) mg L^{-1}	Biochar (A) g pot^{-1}									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	Spike length (cm)									
Control (water)	56.45	58.67	65.44	67.35	61.98	52.33	54.85	62.75	65.12	58.76
Fe nano 250	57.33	61.75	67.12	71.05	64.31	54.28	56.77	64.67	66.45	60.54
Fe nano 500	60.15	62.64	70.23	72.38	66.35	56.07	60.42	65.90	67.33	62.43
Fe chelated 250	62.05	64.85	71.65	73.25	67.95	59.15	61.35	66.72	70.67	64.47
Fe chelated500	63.77	65.25	72.85	74.55	69.11	60.85	64.44	68.25	72.35	66.47
Mean (A)	59.95	62.63	69.46	71.72		56.54	59.57	65.66	68.38	
L.S.D. at 5%	A: 2.44		B: 2.71		AB: 5.42	A: 2.85		B: 3.34		AB: 6.68
	Spike diameter (mm)									
Control (water)	8.25	9.25	10.33	10.85	9.67	8.33	9.42	10.55	10.75	9.76
Fe nano 250	8.33	9.29	10.45	11.13	9.80	8.62	9.65	10.52	10.92	9.93
Fe nano 500	8.85	9.37	10.67	11.25	10.04	8.85	9.75	10.59	11.33	10.13
Fe chelated 250	9.05	9.67	10.88	11.33	10.23	9.05	10.12	10.88	11.67	10.43
Fe chelated500	9.44	10.11	10.05	11.45	10.26	9.15	10.11	11.05	11.85	10.54
Mean (A)	8.78	9.54	10.48	11.20		8.80	9.81	10.72	11.30	
L.S.D. at 5%	A: 0.75		B: N.S		AB: 1.32	A: 0.87		B: N.S		AB: 1.66
	Spike fresh weight (g)									
Control (water)	42.45	45.67	52.65	54.33	48.78	43.17	46.15	51.05	53.72	48.52
Fe nano 250	43.11	50.02	53.36	58.62	51.28	44.78	48.33	52.45	54.82	50.10
Fe nano 500	45.23	51.74	56.83	60.05	53.46	46.26	50.62	53.04	55.55	51.37
Fe chelated 250	52.53	54.35	57.35	60.43	56.16	50.62	52.65	55.75	58.30	54.33
Fe chelated500	52.85	55.85	57.92	61.50	57.03	51.45	55.02	57.35	59.69	55.88
Mean (A)	47.24	51.53	55.62	58.99		47.26	50.55	53.93	56.42	
L.S.D. at 5%	A: 3.73		B: 4.53		AB: 9.06	A: 2.92		B: 2.67		AB: 5.34

TABLE 6. Influence of biochar, iron foliar application and their interactions on vase life (days) of tuberose plants during 2020 and 2021.

Iron concentrations (B) mg L^{-1}	Biochar (A) g pot^{-1}									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	Vase life (days)									
Control (water)	5.14	5.95	6.78	7.32	6.30	5.40	6.37	7.20	8.17	6.78
Fe nano 250	5.25	6.27	7.09	7.55	6.54	5.51	6.45	7.33	8.42	6.93
Fe nano 500	5.85	6.88	7.48	7.68	6.97	6.25	7.36	7.98	8.57	7.54
Fe chelated 250	6.60	7.05	7.82	8.1	7.39	6.85	7.75	8.40	9.04	8.01
Fe chelated500	6.77	7.45	8.08	8.25	7.64	7.11	7.97	8.74	9.20	8.26
Mean (A)	5.92	6.72	7.45	7.78		6.22	7.18	7.93	8.68	
L.S.D. at 5%	A: 0.57		B: 0.63		AB: 1.26	A: 0.78		B: 0.67		AB: 1.34

in Table (7) demonstrated that increasing biochar levels improved number of new bulblets/ plant, fresh and dry weights of the replacement clump›s by 45.21 and 40.91%, 27.72 and 34.10% and 29.63 and 30.71% for high level of biochar (200 g pot^{-1}) treatment compared with the control check treatment in the first and second season, respectively.

Data presented in Table (7) indicate that number of new bulblets/ plant, fresh and dry weights of the replacement clumps were significantly enhanced in the two experimental seasons in response to different iron fertilization treatments except for Nano iron at 250 mg L^{-1} in comparison with those of untreated control plants. The highest values concerning these characters were resulted from the Fe-chelated at 500 or 250 mg L^{-1} treatments without any significant differences between them.

The interaction between biochar levels and iron fertilization treatments was significant for the three tested bulb productivity parameters for both growing seasons. The highest values of these parameters were obtained due to the treatment of high level of biochar at 200 g pot^{-1} in combination with Fe-chelated at 500 mg L^{-1}

Chemical composition

Photosynthetic pigments

The three photosynthetic pigments were significantly affected by the various biochar levels treatments as indicated in Table (8), in both growing seasons. Biochar treatment at 200 g pot^{-1} showed the highest values (1.028 and 1.051 for chlorophyll a), (0.740 and 0.731 for chlorophyll b) and (0.675 and 0.683 for carotenoids) in the two growing seasons, respectively.

TABLE 7. Influence of biochar, iron foliar application and their interactions on number of new bulblets / plant, fresh and dry weights of the replacement clump's (g) of tuberose plants during 2020 and 2021.

Iron concentrations (B) mg L^{-1}	Biochar (A) g pot^{-1}									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	Number of new bulblets / plant									
Control (water)	7.33	8.50	9.82	11.22	9.22	7.92	9.01	10.66	11.78	9.84
Fe nano 250	7.85	8.58	10.32	11.40	9.54	8.48	9.09	11.25	11.97	10.20
Fe nano 500	8.18	8.95	10.68	11.77	9.90	8.83	9.49	11.66	12.36	10.58
Fe chelated 250	8.64	9.53	11.22	12.05	10.36	9.33	10.10	12.18	12.65	11.07
Fe chelated500	8.72	9.75	11.35	12.67	10.62	9.42	10.34	12.44	13.25	11.36
Mean (A)	8.14	9.06	10.68	11.82		8.80	9.61	11.64	12.40	
L.S.D. at 5%	A: 0.88		B: 0.64		AB: 1.28	A: 0.72		B: 0.68	AB: 1.36	
	Fresh weights of the replacement clump's (g)									
Control (water)	33.97	35.22	40.54	43.05	38.19	30.69	34.08	37.55	42.56	36.22
Fe nano 250	34.41	36.52	42.35	43.38	39.17	31.99	34.16	39.19	42.89	37.06
Fe nano 500	35.22	38.79	43.33	45.79	40.78	33.16	35.85	40.10	45.28	38.60
Fe chelated 250	36.65	39.95	45.07	46.50	42.04	34.67	37.52	41.71	45.97	39.97
Fe chelated500	37.05	40.85	46.39	47.74	43.01	35.74	38.77	42.93	46.25	40.92
Mean (A)	35.46	38.27	43.53	45.29		33.25	36.08	40.29	44.59	
L.S.D. at 5%	A: 1.85		B: 1.31		AB: 2.62	A: 2.26		B: 1.75	AB: 3.50	
	Dry weights of the replacement clump's (g)									
Control (water)	10.05	10.38	11.65	12.85	11.23	9.16	9.71	11.07	12.34	10.57
Fe nano 250	10.18	10.55	12.17	12.95	11.46	9.55	9.86	11.56	12.43	10.85
Fe nano 500	10.42	11.02	12.45	13.67	11.89	9.90	10.44	11.83	13.12	11.32
Fe chelated 250	10.65	11.35	12.95	13.88	12.21	10.35	10.69	12.30	13.32	11.67
Fe chelated500	10.84	11.62	13.33	14.25	12.51	10.67	11.33	12.66	13.68	12.09
Mean (A)	10.43	10.98	12.51	13.52		9.93	10.41	11.88	12.98	
L.S.D. at 5%		A: 0.46		B: 0.28	AB: 0.56	A: 0.38		B: 0.45	AB:0.90	

The effect of iron treatments here caused a significant increase for the photosynthetic pigments compared to the control check treatment in the two experimental seasons, except, for Fe-nano treatment at 250 mg L^{-1} for carotenoids in both seasons. The Fe chelated (500 mg L^{-1}) treatment had highest chlorophyll a, b and carotenoids compared to all other treatments and the control, as showed in Table (8).

There was a significant difference among the interaction between two experimental factors regarding to photosynthetic pigments compared to the control check treatment. Among these treatments, foliar application with Fe-chelated at high concentration in combination with biochar at high level had the greatest values for the three photosynthetic pigments, Table (8).

Total carbohydrates percentage:

As seen in Table (9), a significant increase in total carbohydrates percentage in bulbs was observed as response to all biochar treatments compared to the control. The only exceptional biochar treatment was at 50 g pot^{-1} in the second season. The high level of biochar gave the highest values over the control by 23.81 and 22.51% for the two growing seasons, respectively. Conversely, the control plants recorded the lowest values.

Foliar application of iron treatments were positively enhanced total carbohydrates % in corms, in both seasons, in comparison with untreated plants. Maximum percentage of total carbohydrates was obtained from plants receiving Fe-chelated at 500 mg L^{-1}. The magnitude of the increase compared to the control was 11.58 and 9.44 % in the two growing seasons, respectively.

TABLE 8. Influence of biochar, iron foliar application and their interactions on chlorophyll "A", "B" and carotenoids (mg/ g F.W.) of tuberose plants during 2020 and 2021.

Iron concentrations (B) mg L^{-1}	Biochar (A)									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	Chlorophyll "A" (mg/ g F.W.)									
Control (water)	0.825	0.852	0.885	0.955	0.879	0.790	0.865	0.878	0.976	0.877
Fe nano 250	0.846	0.866	0.925	0.972	0.902	0.814	0.884	0.897	0.995	0.898
Fe nano 500	0.865	0.877	0.946	1.035	0.931	0.833	0.890	0.904	1.058	0.921
Fe chelated 250	0.872	0.915	0.966	1.082	0.959	0.835	0.929	0.943	1.106	0.953
Fe chelated500	0.895	0.944	1.022	1.095	0.989	0.875	0.947	0.961	1.119	0.976
Mean (A)	0.861	0.891	0.949	1.028		0.829	0.903	0.916	1.051	
L.S.D. at 5%	A: 0.021		B: 0.018		AB: 0.036	A: 0.045		B: 0.017		AB: 0.034
	Chlorophyll "B" (mg/ g F.W.)									
Control (water)	0.577	0.588	0.665	0.695	0.631	0.568	0.612	0.677	0.689	0.637
Fe nano 250	0.585	0.605	0.688	0.733	0.653	0.588	0.618	0.705	0.718	0.657
Fe nano 500	0.596	0.652	0.718	0.748	0.679	0.615	0.664	0.731	0.744	0.689
Fe chelated 250	0.615	0.675	0.723	0.757	0.693	0.624	0.689	0.736	0.749	0.700
Fe chelated500	0.633	0.695	0.736	0.765	0.707	0.642	0.710	0.749	0.754	0.714
Mean (A)	0.601	0.643	0.706	0.740		0.608	0.658	0.720	0.731	
L.S.D. at 5%	A: 0.027		B: 0.009		AB: 0.018	A: 0.031		B: 0.011		AB: 0.022
	Carotenoids (mg/ g F.W.)									
Control (water)	0.617	0.638	0.648	0.665	0.642	0.624	0.633	0.660	0.673	0.648
Fe nano 250	0.622	0.644	0.657	0.672	0.649	0.633	0.643	0.669	0.680	0.656
Fe nano 500	0.635	0.647	0.668	0.676	0.657	0.646	0.656	0.681	0.684	0.667
Fe chelated 250	0.652	0.656	0.663	0.680	0.663	0.664	0.674	0.676	0.688	0.675
Fe chelated500	0.658	0.665	0.674	0.682	0.670	0.670	0.680	0.687	0.690	0.682
Mean (A)	0.637	0.650	0.662	0.675		0.647	0.657	0.675	0.683	
L.S.D. at 5%	A: 0.008		B: 0.008		AB: 0.016	A: 0.007		B: 0.009		AB: 0.018

The effect of interaction between biochar and iron fertilization treatments was significant, in the two consecutive seasons. The best overall results were due to the interaction between biochar 200 g pot^{-1} plus Fe-chelated 500 mg L^{-1} (31.07 and 30.45 in the two experimental seasons, respectively).

Essential oil percentage

Essential oil percentage in tuberose plants was significantly increased due to the use of biochar level treatments compared to the control check treatment in the two experimental seasons except low biochar level as shown in Table (10). The highest essential oil percentage was obtained from high biochar level followed by medium biochar level. These two treatments recorded 0.0252 and 0.0248%, respectively, in the first season and 0.0263 and 0.0258%, in the second one respectively.

With regard to the other factor, the tested iron treatments caused a significant increase in essential oil % over that given by control treatment in the two growing seasons, except the Nano iron (250 mg L^{-1}) treatment as shown in Table (10). The essential oil percentage in tuberose florets due to the three iron treatments over those of control treatment recorded 11.84, 9.65 and 7.90% in first season and 12.93, 11.21 and 9.05% in the second one, respectively.

The interaction between biochar at 200 g pot^{-1} and chelated iron at 500 mg L^{-1} treatments was significant during both seasons.

Essential oil components

Data presented in Fig. (1) showed the presence of 16 components in the essential oil of tuberose. The major component of volatile oil was tuberons with concentration range from 23.45 to 25.23 %

TABLE 9. Influence of biochar, iron foliar application and their interactions on total carbohydrates percentage in bulbs of tuberose plants during 2020 and 2021.

Iron concentrations (B) mg L^{-1}	Biochar (A) g pot^{-1}									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	Total carbohydrates (%)									
Control (water)	22.17	23.50	24.92	27.85	24.61	22.72	23.03	25.54	28.29	24.90
Fe nano 250	23.45	23.95	25.63	28.44	25.37	23.57	23.47	26.27	28.87	25.55
Fe nano 500	24.05	24.85	26.15	29.65	26.18	23.70	24.73	26.80	29.26	26.12
Fe chelated 250	24.18	25.17	27.35	29.33	26.51	23.87	24.67	28.03	28.74	26.33
Fe chelated 500	24.36	26.65	27.77	31.07	27.46	24.97	25.12	28.46	30.45	27.25
Mean (A)	23.64	24.82	26.36	29.27		23.77	24.20	27.02	29.12	
L.S.D. at 5%	A: 1.05		B: 0.87		AB: 1.74	A: 1.33		B: 0.92		AB:1.84

TABLE 10. Influence of biochar, iron foliar application and their interactions on oil percentage of tuberose plants during 2020 and 2021.

Iron concentrations (B) mg L^{-1}	Biochar (A) g pot^{-1}									
	Control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)	control (zero)	Biochar 50	Biochar 100	Biochar 200	Mean (B)
	1st season					2nd season				
	Oil %									
Control (water)	0.0215	0.0222	0.0235	0.0241	0.0228	0.0205	0.0225	0.0248	0.0250	0.0232
Fe nano 250	0.0228	0.0235	0.0238	0.0245	0.0237	0.0234	0.0239	0.0252	0.0258	0.0246
Fe nano 500	0.0233	0.0245	0.0251	0.0254	0.0246	0.0240	0.0249	0.0259	0.0267	0.0253
Fe chelated 250	0.0237	0.0248	0.0257	0.0258	0.0250	0.0245	0.0252	0.0265	0.0269	0.0258
Fe chelated500	0.0242	0.0252	0.026	0.0264	0.0255	0.0249	0.0261	0.0268	0.0271	0.0262
Mean (A)	0.0231	0.0240	0.0248	0.0252		0.0235	0.0245	0.0258	0.0263	
L.S.D. at 5%	A: 0.001		B: 0.001		AB:0.002	A: 0.001		B: 0.002		AB:0.004

followed by benzyl benzoate (22.64 to 24.24 %) in all the treatments. The minor compounds were furfural and hexanol.

Discussion

Biochar has been found to promote physical, chemical and biological features of farmed soils, resulting in improved plant growth, development and production. Biochar treatments also improve soil fertility through both direct and indirect effects. The nutrients contained on the surfaces of biochar particles in mineral forms have a direct effect (Lehmann et al., 2005 and Yamato et al., 2006). Furthermore, biochar increased the physical features of the soil, particularly the soil water holding capacity and decreased soil compaction, resulting in improved root proliferation and crop growth and development. It also has a significant impact on plant nutrient availability, and it resembles an ion exchange matrix, catching a huge number of cations K+, Ca++, and Mg++ (Glaser et al., 2002, Laird et al., 2010 and Olmo et al., 2014). Hafez et al. (2020) on barley showed that the number of leaves increased significantly after the application of biochar and Dad et al. (2021) on tomato declared that leaf fresh and dry weights significantly increased by application of biochar. The favorable reactions of floral characteristics and yield from biochar treatments in this research study could be attributable to the potential contribution of biochar through improved root growth, better uptake of nutrients and water, and increased photosynthetic activity, which encouraged higher food accumulation that leads and possibly increased production (Seleem and Khalil, 2019). A similar trend was found by Osman et al. (2019) on *Vigna unguiculata*. Guo et al. (2021) found that the tomato's content of chlorophyll a, b and a + b was significantly impacted by biochar. Ali and Mjeed (2017) on chrysanthemum and Seleem and Khalil (2019) on *Dimorphotheca ecklonis.* Nassour, et al. (2022) found that comparing biochar treatments to control, they produced the best results for every qualitative flowering parameter (flower stalk length and diameter, number of flowers per spike, percentage of dry matter and vase life) on gladiolus plants. The amount of total carbohydrates in corms depends on the ions and organic solutes that are transported into the corm and converted into glucose, and the use of biochar enhances glucose biosynthesis and produces corms of tuberose plants with higher quality characteristics. Firmansyah et al. (2020) on shallot and Abdelrasheed et al. (2021) on *Allium cepa* both illustrated that there were significant effects of biochar, organic fertilizer and hormone on the dry weight of bulbs/clumps, number of bulbs/10 clumps, number of bulbs splitting and diameter of bulbs of shallot plants. Our results on total carbohydrates matched those found by Youseef et al. (2017) on potato and Abdelrasheed et al. (2021) on onion. The results showed that biochar treatments increased the percentage of essential

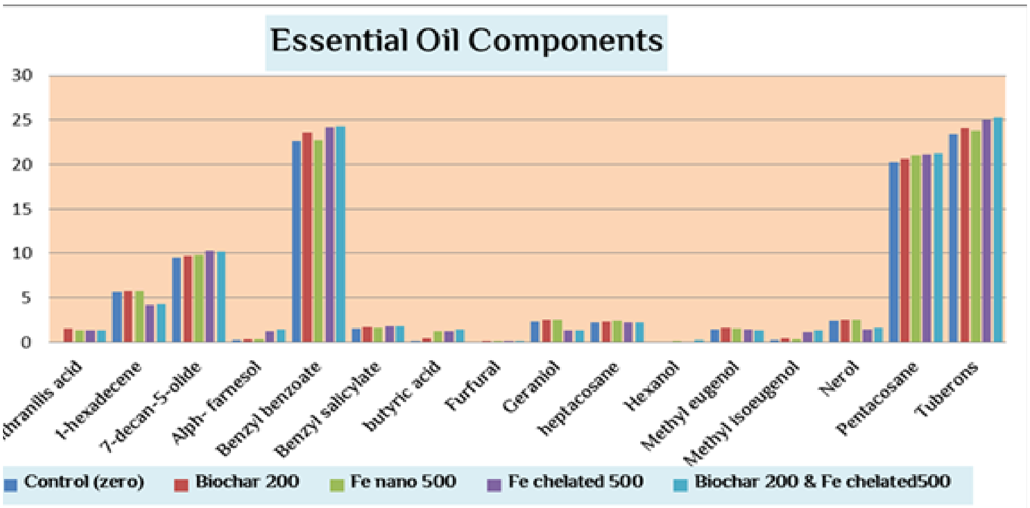

Fig.1. Influence of biochar, iron foliar application on essential oil components of tuberose plants during 2020 and 2021.

oil of tuberose compared to control. The rise in essential oil could be the result of either increased vegetative growth or monoterpene production (Gharib et al., 2008). Essential oil production is reliant on the physiology of the entire plant, particularly the stage of tissue synthesis and metabolic functions (Tawfeeq et al., 2016). In line with our results, concerning biochar, were the findings of Calamai et al. (2019) on *Pelargonium graveolens* and Tavali (2022) on marjoram.

Iron plays a critical role in the formation of amino acids, proteins, and enzymes such as catalase, peroxidase etc., that significantly increase raise tissue growth, cell division, and cambium activity, which in turn keeps increasing plant height, stem diameter, and branch length (Fakhraie, 2012). Our results are in agreement with Kabota (2005) and Hasson (2012) in their studies on mango and ziziphus; where they claimed that the use of chelated iron had improved vegetative growth. Alternatively, it may cause an increasing in chlorophyll content and leaf area, which enhances dry plant nutrients and subsequently increases vegetative growth (Taiz & Zeiger, 2006 and Kabota, 2005). Likewise, the impact of the iron element on photosynthesis and ATP synthesis is crucial for plant bio-processes, encouraging the development of foliar buds and subsequently increasing leaf number (Abu Dahi and Yunus, 1988). Many investigators came to similar results on photosynthetic pigments, such as Al-Hchami et al. (2020) and Abbass et al. (2020) on freesia plants and El-Gioushy et al. (2021) on *Citrus sinensis*. Additionally, the iron element plays a vital role in the biochemical reactions that speed up the transition of some vegetative buds into flowers, leading to a rise in flower buds and flowers number on the plant (Mengel, 2002). Iron deficiency in sandy soil causes tuberose plants to produce low-quality spikes. Many authors also reached conclusions that were comparable to those of the current study, such as Bashir et al. (2013) on *Gerbera jamesonii***,** Salem et al. (2019) on rose and Abdulhadi et al. (2022) on *Antirrhinum majus*. Foliar application of iron to leaves is a very effective and speedy method for enabling plants to absorb nutrients, protecting plants from nutritional insufficiency circumstances (Niu et al., 2020). Tuberose flowers have a short vase life due to internal carbohydrates deteriorating and flower spikes losing their turgidity after harvest (Vidhya and Bhattacharjee, 2002). The above-mentioned results produced in this trial coincided with the findings of Kakade et al. (2009) on Chinese aster, Fahad et al. (2014) on gladiolus for flower fresh weight, Patel et al. (2017) on *Polianthes tuberosa* L. for spike length and Abbass et al. (2020) on freesia for spike length, spike diameter, flowering date and vase life. Through a faster rate of photosynthesis, iron also boosted the storage of carbohydrates, it also plays a significant part in the production of plant hormones and chlorophyll, which improves the flower vase life (Ganesh et al. 2013 and Fahad et al. 2014). Vijayakumar (2009) demonstrated that the use of iron contributed to the physiological development of the flowers and decreased the levels of ethylene and abscisic acid, which enhanced the flowers' visual appeal and vase life. Our results on effect of iron fertilization on postharvest were in line with those of Singh et al. (2015) on Lily and Hajizadeh et al. (2020) on *Gerbera jamesonii*. The increase in leaf chlorophyll content may be explained by iron's function in raising the number and size of chloroplasts (Prism et al., 2011) or this might be as a result of the influence of iron on plant activities as a cofactor in the creation of chlorophyll and cytochromes, which are crucial to the processes of respiration and photosynthesis (Focus, 2003). The positive influence of foliar application with iron on volatile oil of tuberose plant might be due to the promotion of plant growth characteristics and metabolism, which would then increase the number of secondary metabolites, such as volatile oil (Singh and Dwivedi 2019). The role of iron fertilizers on volatile oil was given by Pirzad and Barin (2018) on *Pimpinella anisum* and Khalid and El-Gohary (2020) *on Artemisia abrotanum.* Mishra et al. (2018) found that spraying gladiolus plants with $FeSO_4$ 0.5% + $ZnSO_4$ 0.25% demonstrated most effective for number of corm/ plant, number of corm/ha. and weight of corm/plant. Abdulazeez et al. (2020) on freesia plants found that 0.25 g/L nano iron had a significant influence on corms number/plant, corms diameter, cormels number/ corm and cormels diameter.

Conclusion

Under the environmental conditions of the same study, the important consideration of this study is the use of biochar and iron fertilization was found useful to enhance vegetative, floral characteristics, bulb productivity and chemical composition of tuberose plants. The highest growth, flowering and chemical composition values, in most cases, were given by supplementing tuberose plants with the high level of biochar (200 g pot^{-1}) in combination with spraying chelated iron at 500 g L^{-1}.

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Conflicts of interest

We would like to inform you that this manuscript has no recognized conflicts of interest

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تعزيز الصفات النوعية والكمية لنباتات التبروز باضافة البيوشار مقرونا بالرش الورقي بالحديد المخلبي والنانوى

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اجريت تجربة اصص خلال موسمي النمو المتتاليين عامي ٢٠٢٠ و ٢٠٢١ في محافظة بني سويف ، مصر ، لمقارنة تأثير معاملات البيوشار (٥٠ ، ١٠٠ ، ٢٠٠ جم / اصيص) وكذلك الرش الورقي بالحديد المخلبي والنانومترى (٢٥٠ ، ٥٠٠ ملجم / لتر) على خصائص النمو والصفات الزهرية وانتاج الابصال والمكونات الكيميائية لنباتات التبروز. واوضحت النتائج أفضل الصفات الخضرية (عدد الأوراق / نبات ، الوزن الطازج والجاف للأوراق/ نبات) ، صفات الإزهار (موعد التزهير ، عدد الزهيرات لكل نبات والوزن الطازج والجاف للزهيرات/ نبات وكذلك طول وسمك ووزن الشمراخ الطازج) ، عمر الازهار في الفاز، إنتاجية الابصال (عدد البصيلات الجديدة لكل نبات والأوزان الطازجة والجافة للابصال الجديدة المتكونة) ، ونسبة الزيت الطيار، والمكونات الكيميائية متمثلة في (صبغات كلوروفيل A، B والكاروتينيدات) والنسبة المئوية للكربوهيدرات في الابصال نتيجة استخدام مستوى البيوشار (٢٠٠ جم / اصيص). فيما يتعلق بمعاملات التسميد بالحديد ، تم زيادة جميع الصفات النوعية والكمية المذكورة أعلاه لنباتات التبروز بشكل كبير نتيجة المعاملة بالحديد المخلبي بمعدل ٥٠٠ تليها ٢٥٠ مجم / لتر ثم الحديد في صورة النانو عند ٥٠٠ مجم / لتر بينما معدل الحديد النانوي عند ٢٥٠ مجم / لتر اعطت أقل القيم. لم يحدث تغير ملحوظ فى سمك الشمراخ الزهرى عن طريق أي من معاملات الرش بالحديد الأربعة التي تم اختبارها.

فيما يتعلق بمعاملات التداخل، تم إعطاء أعلى قيم للصفات المشار اليها بالاضافة الى الخصائص النوعية والكمية لمكونات الزيت الطيار ، عن طريق رش نباتات التبروز بالحديد المخلبي عند ٥٠٠ مجم /لتر مع البيوشار بمعدل ٢٠٠ جم / اصيص. أخيرًا ، من الواضح أن معاملة نباتات التبروز بالفحم الحيوي (البيوشار) وكذلك الحديد المخلّبي يحسن من الصفات النوعية والكمية لنباتات التبروز.