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HUMIC ACID AND IRON CHELATE FOLIAR APPLICATION INFLUENCE ON GROWTH AND QUALITY OF TWO LETTUCE CULTIVARS (*LACTUCA SATIVA L.*).

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Abstract

The experiment was carried out during 27th October-2018 to 26th April-2019 in the open field on two lettuce cultivars; NADER and Polaris. Two sources of Fe were used for foliar spray; EDDHA (0, 2 and 4 g/l) and Potassium Humate (0, 3 and 6 g/l) to study their effects on longest leaf, leaf number, plant height, head diameter, head fresh weight, plant bolting , flower buds formation Leaf chlorophyll content and Total Soluble Solids (TSS%). The obtained results showed that Fe chelate at the rate of 4 g/l enhanced flower bud formation. While, 3 g/l humic acid caused significant increase of longest leaf, leaf number, plant height, fresh weight and leaf chlorophyll content, 2g/l Fe chelate with 3 and 6g/l humic acid were attributed to highest bolting % and chlorophyll content respectively and the application of highest rates of Fe chelate and humic acid significantly increased the head diameter. The two studied cultivar were varied in their response to the interaction of Fe chelate and humic acid rates, the best chlorophyll and TSS% contents (35.98 and 6.38% respectively) were recorded by the interaction of 2g/l of Fe chelate and 6g/l humic acid foliar spray on NADER cultivar, and significant increase in number of leaves and bolting% were obtained from the application of 2g/l of Fe chelate and 6g/l humic acid in Polaris cultivar.

Key word: Humic acid, Fe chelate, Lettuce cultivars

Introduction

Lettuce (*Lactuca sativa* L.) is a member of the Asteraceae family; it is the most important crop in the group of leafy vegetables. Lettuce has a wide morphological and genetic variation (Køístková *et al.,* 2008). The origin of common cultivated species comes from the Eastern Mediterranean basin (Rubatzky and Yamaguchi 1997). Lettuce is considered as an excellent nutritive source of minerals and vitamins as it is consumed as a fresh green salad (Hanafy, *et al.,* 2000). In horticultural plant fertilization, both mineral and chelated compounds are used as the source of micronutrients which are required by plants in a very low concentration for sufficient production (Kozik *et al.,* 2008, Fouda 2016). The widespread plant disease is iron chlorosis, which affects on plant growth and reduces the yield quality (Cascone *et al.,* 2014). Iron is abundant in the soil as silicate minerals or iron oxides and hydroxides but not available for the plant with a pH greater than 7.5 and can be corrected either by foliar spraying with Fe compounds such as ferrous sulfate or iron chelates or by decreasing soil pH (Ruszkowska and Wojcieska-wyskupajtys 1996, Lucena, 2003, Schulte 2004, Kozik *et al.,* 2011). The plant foliar cuticle and the role of stomata in the process of foliar uptake has been a matter of interest since the beginning of the 20th century, it is theoretically more environmentally friendly than soil fertilization since nutrients can be directly delivered to plant tissues during appropriate stages of plant growth (Fernández, *et al.,* 2013). Humic acid is a principal component of humic substances (that contain high levels of organic Fe) are the major organic constituents of soil reached the final degradation state and their benefits on the growth of plants and seedlings had been documented (Stevenson 1982 , Sani, 2014, Verdi *et al.,* 2015).

Field experiments observed a growth increase of lettuce plants according to foliar application of Fe and humic acid fertilizers. Ylivainio *et al.,* (2004) investigated the effect of soil and foliar application of

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different Fe compounds ($FeSO_4$, Fe EDTA, Fe EDDS, and Fe EDDHA) on nutrient contents in (*Lactuca sativa cv.* Australian gelber) and (*Lolium perenne cv.* Prego) cultivated in pots under greenhouse. Spraying of Fe EDDS on the leaves increased Fe content in both lettuce and ryegrass, however the form of Fe EDDHA minimized Mn and Zn content in lettuce more than others. Tyksinski and Komosa (2008) conducted comparison study among three iron chelates (Fe-DTPA, Fe-EDTA+DTPA, Fe-AM-4) in the following doses (mg $Fe{\cdot}dm^{-3}$): 20 (control), 50, 75, 100 and 125. Greenhouse lettuce 'Michalina' cultivar in the years 2005 and 2006, spring and autumn experiments were carried out. Lettuce yields obtained after the application of all four Fe levels were higher than in the control. Lettuce yields at the levels of 50, 75 and 100 mg Fe·dm-3 were similar. On the level 125 mg Fe·dm-3 were significantly higher than those obtained with the remaining Fe levels. The effects of soil and foliar fertigation of humic substances (humic and fulvic acid at the rates of 0, 7, 10 and 13 kg fed^{-1}) on the plant growth and some nutrient elements uptake of lettuce were examined. The results showed significant effects of different application rates of humic substances on fresh and dry weights, plant height, N%, P% and K%. It was indicated that highest values of fresh and dry weight of shoot as well as plant height (cm) were at foliar spraying of fulvic acid at 13 kg/ fed treatment, but humic substances gave the best significant results of N, P and K content of leaves (Taha, *et al.,* 2016). Raheem *et al.,* (2018) demonstrated the effect of humic acid; soil treatment (0, 1.5, 2.5 and 3.5 ml/l) and foliar spraying (0, 2.5, 3.5 and 4.5 ml/l) on yield and yield components of *Lactuca sativa* L.var Romaine. Highest total yield (47.863t/ha) was obtained with 1.5 ml/l of soil treatment, followed by treatment 2.5 ml/l foliar spray, however the foliage treatment with high levels resulted highest plant height and total soluble solids as compared with control. Wojciechowska*, et al.,* (2019) applied six sources of iron (two sulfates and four chelated forms) to determine of N, P, K, Ca, Mg, S and Na total content in lettuce leaves, the plants cultivated in spring and autumn season for three years. All tested fertilizers significantly influenced the content of these macronutrients, while negative correlation was observed between the iron in the fertilizers and phosphorus and calcium content, however with sodium content it was positive.

The purpose of the study was to compare the influence of different Fe fertilizers (organic and chemical) on some growth characters of lettuce heads until reaching the flower bud formation stage in the open field.

Materials and Methods

Plant material and growth conditions

The experiment was carried out during 27th October to 26th April on two lettuce cultivars; NADER (the origin is Romaine with 85% germination) and Polaris (from California with 90% germination) to study the effect of two sources of Fe; EDDHA and Potassium Humate on some growth characteristics. Polystyrene trays with 77 cells were used for seed germination, every cell is 2.5cm in width and 6.5cm in height, filled with sterilized peat moss (Pokon Naturado BV, Veenendaal, Holland), contain 50% organic matter and (14:16:18) NPK with pH about 5.2-6.2. Two seeds were sown in each cell after germination the seedlings were thinned to one, after eight weeks uniformly plants were transferred to black polyethylene bags (30 × 40 cm) filled with sandy loamy soil. The results of soil chemical and physical tests are shown in table 1. Some metrological data through the condition of experiment period are shown in table 2.

Preparation of spray solutions and treatments

Table 1: Some physical and chemical properties of the soil used in the study*.

Properties	Field Soil
pH	7.79
Cat ion exchange capacity (mol.kg $^{-1}$)	19.68
Organic matter (g. kg $^{-1}$)	16.5
Total $CaCO_3$ (g. kg $^{-1}$)	270
K^+ (m.mol.l^{-1})	0.72
Mg^+ (m.mol.l^{-1})	0.80
Bulk density (µg.m^{-3})	1.3
Silt (%)	70
Sand (%)	25
Clay (%)	5
Soil texture	Sandy Loam

*Laboratory Collage of Agriculture / Soil and Water Department.

Table 2: The metrological data during the study periods*.

Years	Months	Average air temp.°C		Average relative Hum. %		Total rain (mm)
		Min.	Max.	Min.	Max.	
2018	October	11.4	38.7	26.81	94.00	22.6
	November	12.06	28.2	45.31	98.3	113.5
	December	8.07	14.74	58.48	91.64	160.8
2019	January	5.03	13.11	49.32	86.24	96.3
	February	5.82	15.04	45.77	85.12	42.4
	March	7.52	16.16	50.81	88.08	215.9
	April	10.28	21.34	41.68	86.51	125.7

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Two sources of Fe were used; EDDHA 6% Fe and Potassium Humate (Humic -acid 80% and 11% K_2O. Solutions with the concentrations 2 and 4 g/l of EDDHA and 3 and 6 g/l of potassium humate were prepared by dissolving them in the distilled water for foliar application, while in the control treatment plants were sprayed with distilled water (Karakurt *et al.,* 2009). The Solutions were sprayed two times according to their treatments during the evening hours began on 15th December with one month interval. The experiment contain three studied factors, two lettuce cultivars (NADER and Polaris), Fe chelate at levels (0, 2 and 4 $ml.l^{-1}$) and the third was different concentrations of humic acid (0, 3 and 6 $ml.l^{-1}$).

The experiment parameters:

The data collected for all plants at the end of the growing season. The morphological parameters included: longest fully expanded leaf (cm), leaf number, plant height (cm), head diameter (cm), head fresh weight (g), plant bolting and the flower buds formation. The quantitative parameters included: Leaf chlorophyll concentration was estimated using a portable SPAD 502 meter (Minolta, Japan). Readings were taken from the three youngest fully expanded leaves of each replicate (Incesu *et al.*, 2015). However Total Soluble Solid (TSS%) of mature leaves were measured by using an ATC-1E hand-held Refractometer (Shahmaleki *et al.,* 2014).

Statistical analysis

The experiment was laid in a Factorial Complete randomized design (F-CRD), it comprised of three treatments with four replications., analysis of variance (ANOVA) and statistical calculations were performed by SAS software and Excel (SAS institute, 2005). Significant differences between means were calculated by using Duncan's multiple range test at a significance level of $P \leq 0.05$.

Results and Discussion

Data in Fig. 1 clearly indicated the significant influences of NADER and Polaris lettuce cultivars on studied parameters except head diameter and TSS%. NADER was better than Polaris in longest leaf, plant height, fresh weight and chlorophyll content. However, number of leaves, bolting and flower buds formation % were preferable in Polaris. The flower buds formation% were recorded significant value among growth parameters in a response to different concentrations of Fe chelate, the best result was (54.17%) obtained from 4 g/l as compared with control Fig. 2. While the chosen humic acid levels caused significant effects on all parameters except head diameter and flower buds formation% Fig. 3. The level of 3 g/l humic acid had more significant effects on longest leaf, leaf number, plant height, fresh weight and chlorophyll content, but plant bolting was better with the level of 3 g/l compared with the control. Otherwise, TSS% reduced with humic acid application. These results may be due to that the lettuce leaves accumulate Fe in selected tissues, and the leaves of Fe- deficiency may contain Fe similar higher than those of the leaves of Fe- sufficiency, and was termed the "Fe-chlorosis paradox" (Morales *et al.,* 1998, Römheld 2000), some related studies proved that the leaves of Fe-deficiency their mesophyll areas contain lower amount of Fe and collected it in veins and midribs, whereas these investigations improved Fe immobilization appearance in areas close to the vascular tissues in the leaves of Fe-deficiency (Jiménez *et al.,* 2009 on peach, Tomasi *et al.,*

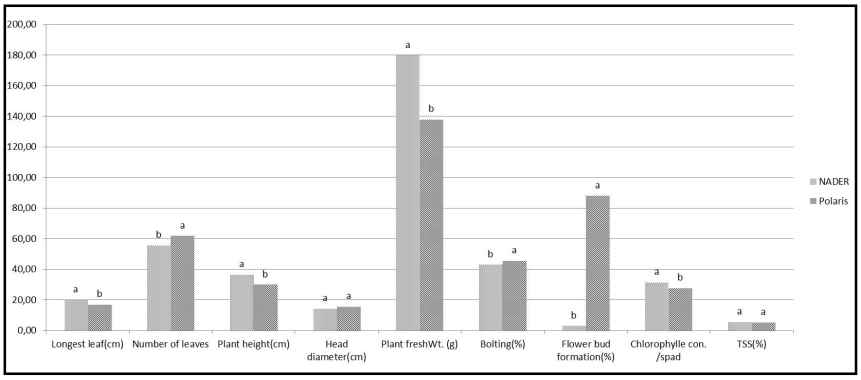

Fig. 1: Response of *Lactuca sativa L.* cultivars studied characteristics.
*(columns with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level).

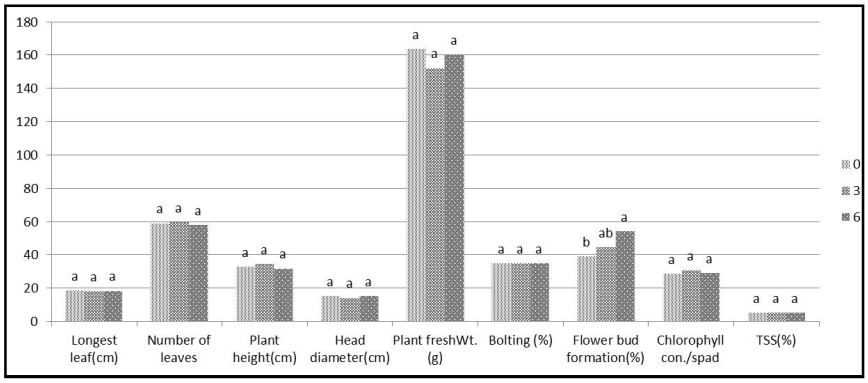

Fig. 2: Effect of Fe chelate studied characteristics.
*(columns with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level).

Table 3: Response of *Lactuca sativa L.* cultivars to Fe chelate on studied characteristics.

Cultivars	Fechelate ($g.l^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Head diameter (cm)	Plant fresh Wt.(g)	Bolting (%)	Flower bud forma-ation(%)	Chloroph -yllcon./ spad	TSS (%)
NADER	0	20.46a	59.52ab	38.83a	13.94a	159.00a	36.36a	0.00c	30.71ab	5.05a
	2	19.55a	53.58b	35.13ab	13.83a	160.64a	50.00a	0.00c	32.81a	5.48a
	4	19.61a	53.99b	35.21ab	14.60a	179.96a	41.67a	8.33c	30.60ab	5.47a
Polaris	0	16.61b	58.42ab	27.28c	16.21a	129.62a	33.33a	75.00b	26.47c	5.26a
	2	16.84b	65.20a	33.78abc	14.53a	143.66a	66.67a	88.89ab	28.57bc	5.03a
	4	17.11b	61.56ab	28.28bc	15.86a	140.17a	36.11a	100.00a	27.59bc	4.91a

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level.

2009 on tomato).

In table 3 are shown that longest leaf and plant height of NADER cultivar influenced negatively to Fe chelate levels when the best value was recorded in the control treatment, while plant height was responded significantly to Fe levels compared with control in Polaris cultivar, 2g/l Fe was recorded best results of number of leaves and leaf chlorophyll content in Polaris and NADER cultivars respectively. The obtained data are confirmed by similar studies conducted on pepper plants in aquaponic system (Roosta and Mohsenian 2012) and green bean (Moshtagh and Aminpanah 2015). Also there was a positive effect of Fe chelate on flower bud formation of Polaris cultivar plants, where the more effective level of Fe chelate was 4g/l. Data in table 4 show that, the highest amount of longest leaf, leaf number, plant height, head fresh weight, flower buds formation and chlorophyll content.found by using foliar spray of humic acid at a rate of 3g/l, on the other hand, the highest flower bud formation was given by 6g/l of humic acid when compared with other treatments. These results agree with those reported by Fawzy (2010), Shahein *et al.,* (2014), Shahein *et al.,* (2015) on other two lettuce cultivars (combined application of humic substances with mineral fertilization) and Raheem *et al.,* (2018) on lettuce. The foundation of some researches referred to that Fe should be translocated inside the plant by organic acids to prevent Fe precipitation with phosphate or hydroxide in the cell it is make complexes with organic acids and founded mostly in plastids (Hochmuth 2019). The interaction of Fe chelate and humic acid rates affected significantly on head

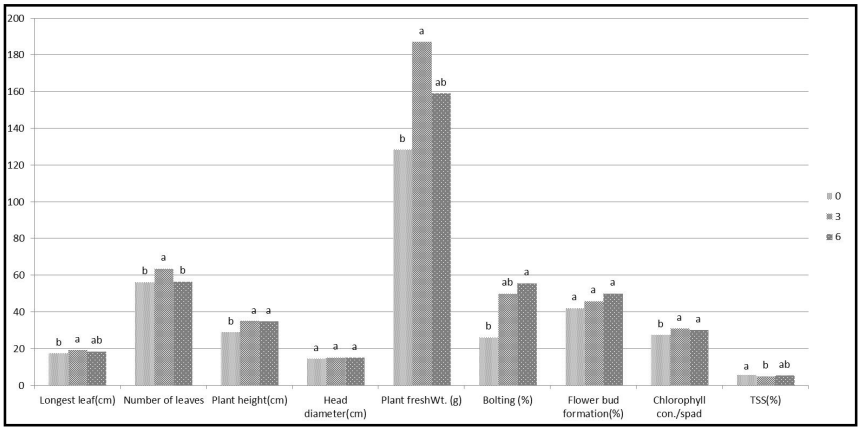

Fig. 3: Effect of humic acid studied characteristics.
*(columns with the same letter are not significantly different from each other according to Duncan's Multiple Range test at 0.05 level).

Table 4: Response of *Lactuca sativa L.* cultivars to humic acid on studied characteristics.

Cultivars	Humic acid ($g.l^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Head diameter (cm)	Plant fresh Wt.(g)	Bolting (%)	Flower bud forma-ation(%)	Chloroph -yllcon./ spad	TSS (%)
NADER	0	18.20bc	51.91bc	31.29bc	13.12a	127.50b	18.18a	9.09c	30.06ab	5.60a
	3	21.53a	63.78a	39.64a	14.99a	227.10a	50.00a	0.00c	31.73a	4.93a
	6	19.71ab	50.77c	37.61ab	14.19a	180.43ab	58.33a	0.00c	32.28a	5.51a
Polaris	0	16.60c	59.97ab	26.73c	15.70a	128.93b	33.33a	72.22b	24.92c	5.48a
	3	16.82c	63.17a	30.71bc	15.29a	146.96b	50.00a	91.67ab	29.94ab	4.74a
	6	17.14c	62.03a	31.89bc	15.61a	137.57b	52.78a	100.00a	27.77bc	4.97a

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

diameter, bolting% and the chlorophyll content in the leaves. The positive effect of applying 2g/l Fe chelate with 3 and 6g/l humic acid were attributed to highest bolting % and chlorophyll content respectively table 5. In the same table results also indicated that the application of highest rates of Fe chelate and humic acid significantly increased the head diameter (17.50cm). The efficient response to iron deficiency comes from various responses; the ability of the plant to activate most efficient activities it will be in iron gaining. These responses expression and regulation could be different among species or genotypes providing them with a different level of efficiency (Zocchi *et al.,* 2007). The interaction between Fe chelate and humic acid rates applied on the two studied cultivars was significant for all parameters table 6. The highest longest leaf, plant height and fresh weight were produced by using humic acid at a rate of 3 g/l on NADER cultivar plants without Fe chelate. On the contrary, the best chlorophyll and TSS% contents (35.98 and 6.38% respectively) were recorded by the interaction of 2g/l of Fe chelate and 6g/l humic acid foliar spray on NADER cultivar too. However, the best head diameter was obtained from the control of Polaris cultivar, and significant

Table 5: Interaction effects of Fe chelate and humic acid on studied characteristics.

Fe chelate ($ml.l^{-1}$)	Humic acid ($g.l^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Head diameter (cm)	Plant fresh Wt.(g)	Bolting (%)	Flower bud forma-ation(%)	Chloroph -yllcon./ spad	TSS (%)
0	0	17.27a	57.14a	29.01a	16.03ab	134.47ab	14.29b	28.57a	27.23c	5.74a
	3	19.75a	63.58a	36.40a	15.24abc	216.94 a	14.29ab	37.50a	29.90abc	4.53a
	6	18.19a	55.88a	32.51a	14.21abc	135.54ab	37.50ab	50.00a	28.20bc	5.28a
2	0	17.85a	55.08a	28.33a	15.18abc	133.38ab	12.50b	33.33a	26.34c	5.26a
	3	19.12a	65.33a	38.15a	14.38abc	181.77ab	87.50a	50.00a	32.55ab	4.96a
	6	17.63a	57.75a	36.89a	12.99bc	141.30ab	75.00a	50.00a	33.19a	5.54a
4	0	16.96a	56.25a	29.40a	12.39c	117.66b	50.00ab	62.50a	28.55abc	5.64a
	3	18.67a	61.50a	30.98a	15.80abc	162.38ab	12.50b	50.00a	30.05abc	5.02a
	6	19.45a	55.57a	34.85a	17.50a	200.15ab	54.17ab	50.00a	28.68abc	4.90a

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

Table 6: Response of *Lactuca sativa L.* cultivars to Fe chelate and humic acid on studied characteristics.

Culti vars	Fe chelate ($ml.l^{-1}$)	Humic acid ($g.l^{-1}$)	Longest leaf (cm)	Number of leaves	Plant height (cm)	Head diameter (cm)	Plant fresh Wt.(g)	Bolting (%)	Flower bud forma-ation(%)	Chloroph -yllcon./ spad	TSS (%)
NADER	0	0	17.00bc	54.33abc	30.40bcde	13.17abcd	117.04c	0.00b	0.00d	30.80abcd	5.77ab
		3	23.30a	64.00ab	44.13a	15.53abcd	303.10a	50.00ab	0.00d	31.20abcd	5.77ab
		6	20.23abc	55.25abc	39.85ab	12.93bcd	160.55bc	50.00ab	0.00d	30.15abcd	4.85ab
	2	0	19.83abc	52.50bc	33.28abcde	14.73abcd	160.05bc	25.00ab	0.00d	29.18 bcde	5.05ab
		3	21.10ab	64.00ab	39.00abc	14.55abcd	198.82abc	25.00ab	0.00d	33.28ab	5.03ab
		6	17.73bc	44.25c	33.13abcde	12.23cd	123.04bc	50.00ab	0.00d	35.98a	6.38a
	4	0	17.48bc	49.50bc	29.98bcde	11.48d	102.78c	25.00ab	25.00cd	30.40abcd	6.03ab
		3	20.20abc	59.67abc	35.80abcde	14.90abcd	179.39bc	25.00ab	0.00d	30.70abcd	5.08ab
		6	21.17ab	52.80bc	39.87ab	17.43ab	257.70ab	50.00ab	0.00d	30.70abcd	5.30ab
Polaris	0	0	17.48bc	59.25abc	28.98bcde	18.16a	147.55bc	25.00ab	50.00bc	24.55 de	5.73ab
		3	16.20c	59.50abc	28.68bcde	14.95abcd	130.79bc	50.00ab	75.00ab	28.60bcde	4.35b
		6	16.15c	56.50abc	25.18de	15.50abcd	110.53c	25.00ab	100.00a	26.25cde	4.35ab
	2	0	15.87c	57.67abc	23.39e	15.63abcd	106.70c	0.00b	66.67ab	23.50e	5.48ab
		3	17.13bc	66.67ab	37.30abcd	14.22abcd	164.72bc	100.00a	100.00a	31.82abc	4.90ab
		6	17.53bc	71.25a	40.65ab	13.75abcd	159.57bc	100.00a	100.00a	30.40abcd	4.70ab
	4	0	16.45c	63.00ab	28.83bcde	13.30abcd	132.54bc	75.00ab	100.00a	26.70bcde	5.25ab
		3	17.13bc	63.33abc	26.17cde	16.70abc	145.38bc	0.00b	100.00a	29.40abcde	4.97ab
		6	17.73bc	58.33abc	29.83bcde	17.57ab	142.60bc	33.33ab	100.00a	26.67bcde	4.50ab

*Values within each column followed with the same latter are not significantly different from each other according to Duncan's Multiple Range test at the 0.05 level.

increase in number of leaves and bolting% were obtained from the application of 2g/l of Fe chelate and 6g/l humic acid on Polaris cultivar. While, flower bud formation was mostly depended on cultivar type more than other two factors, when Polaris cultivar had a superior response. The obtained results were in accordance with EL-gamal *et al.,* (2016) on sugar beet. These results may be attributed to that iron is a fraction of important physiological process compounds in the plants; functions of certain enzymes, chlorophyll manufacturing and biosynthesis process, part of heme–proteins (eg. cytochromes) functions in significant cellular events; as respiration, cell division, transpiration and photosynthesis (Zocchi *et al.,* 2007). Moeover, the foundation of some researches referred to that Fe should be translocated inside the plant by organic acids to prevent Fe precipitation with phosphate or hydroxide in the cell it is make complexes with organic acids and founded mostly in plastids (Hochmuth 2019).

Conclusions

Foliar spray of humic acid was more effective than Fe chelate on lettuce plants; most of studied parameters were recorded significant values with the rate of 3g/l humic acid, moreover NADER cultivar show higher response than Polaris. Finally, it could be concluded with the triple interaction of the two cultivars and the rates of Fe chelate and humic acid that the best treatment for longest leaf, plant height and fresh weight was in NADER cultivar sprayed with 3g/l humic acid without Fe chelate. While, chlorophyll content and TSS% were recorded in the same cultivar was sprayed with the combination of 2 and 6 g/l of Fe chelate and humic acid respectively. Consequently we recommend more studies using other fertilizers that delayed access to reach bolting stage of lettuce plants to provide long period for marketable yield.

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