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Effects of Acidified Irrigation Water and Iron Fertilizers on Shoot Dry Yield, Iron Uptake and Photochemical Efficiency of Corn

FEREIDON NOURGHOLIPOUR[1*], MOHAMMAD PASSANDIDEH[1] AND EMAM GHOLI NOURGHOLIPOUR [2]
1 - Soil and Water Research Institute, Tehran, Iran
2- University of Tehran, College of Abureihan, Tehran, Iran

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[*] Corresponding Author: E-mail: nourfg@yahoo.com

ABSTRACT

Expansion and economical importance of yield reduction due to iron deficiency chlorosis in the world has attracted many researchers attention. Different materials, such as iron sulfate, acidified remediation, wastes, by-products of industries, iron chelate and organic compounds, have been tested to correct iron chlorosis. This experiment studied effect of applying an iron fertilizer for corn (SC 704) consisting sulfur and Thiobacillus bacteria. Experiment was carried out in greenhouse in factorial with complete of randomized design in four replications. The first factor was two kinds of irrigation water, w1= irrigation with ordinary water of soil and water research station of Karaj and w2= irrigation with acidified water of station with pH of 5-5.2 (acidified with sulfuric acid). The second factor was seven iron fertilizer treatments, T1=Control (without iron fertilizer); T2= Iron sequestrine 138 (10 mg. kg^{-1}), T3= Iron sulfate (110 mg.kg^{-1}), T4= Iron oxide powder of iron ore (220 mg.kg^{-1}), T5= T4 + sulfur (250 mg.kg^{-1} powdered sulfur) + Thiobacillus inoculant (10^4 cell.gr soil $^{-1}$), T6= T5 + organic matter, T7= T3 + organic matter. Nitrogen, potassium, triple superphosphate, Mn, Zn, Cu sulfates and acid boric was used 200, 85, 90, 10, 5, 10 and 5 mg. kg^{-1}, respectively. In the end of experiment, Dry shoot and root weight, iron uptake, chlorophyll Index (SPAD 502) and Fv/Fm were measured. Based on results, acidified irrigation water had significant effect on shoot dry weight and P and Fe uptake at five percent probability level, but had no significant effect on chlorophyll index. Effects of different fertilizer treatments on dry weight, P and Fe uptake and chlorophyll index, against Fv/Fm, were significant on five percent level. T6 and T7 produced highest shoot dry weight and T2 produced the lowest. The highest amount of P and Fe uptake was belonging to T7 and the lowest was belonging to T2. The highest amount of chlorophyll index was belonging to T6. Between treatments there was no significant difference on Fv/Fm factor.

Keywords: Iron sources, Corn, Uptake, Acidified water, Chlorophyll and Fv/Fm.

INTRODUCTION

Among micronutrients, Fe is the first element which its necessity has been demonstrated for plant growth (Hills, 1995). Though Fe is the fourth element found in lithosphere and is needed in very small quantity for plant requirements, many plant species show iron deficiency (Bindra, 1983; Mortvedt *et al.,* 1982). Iron chlorosis is available in many fields of Iran such as: Khorasan, Isfahan, Karaj, Azerbaijan, etc (Salardini, 1991). Due to extension and economic importance of iron deficiency in the world, Fe deficiency has attracted attention of many researchers. Some different materials such as iron inorganic salts, acidified improvers, byproducts of industries, iron chelates, organic compounds, blood powder and blood powder combined with iron sulfate have been examined for remediation of iron deficiency chlorosis (Hagstrom, 1984). An iron artificial chelate (Fe-EDDHA) is the best but expensive iron fertilizers (Mortvedt, 1986). Therefore, it is necessary to find other replaceable fertilizers. Iron oxide powder from iron ore is the first material of iron melting industries and iron fertilizers. Due to alkalinity, high pH, low organic mater and high bicarbonate in irrigation water, direct use of iron oxide powder in farmlands of Iran is impossible. However, many researchers have examined many methods. In a greenhouse experiment, using of high amounts of pyrite (400 $tons.ha^{-1}$) obtained from a mine in Colorado, was effective on eliminating of iron chlorosis in soybean. In next steps, it was recognized that the banding use of Fe_2O_3 in rice, corn and soybean was ineffective, but this method with powdered sulfur can be effective. At the end, some amount of improvers, containing sulfur and a byproduct of iron melting with crystals of sulfuric acid was added. Combination of sulfur with acidify materials was more effective than acidified material alone. Researchers suggest that sulfur oxidizing bacteria can be active in alkaline soils which have been partially acidified (Wallace *et al.*,1984 ; Wallace *et al.*,1986). In another study, a byproduct of Cu (jarosite) which contains iron was combined with different amounts of sulfuric acid and used for sorghum. Combination of 2:1 ratio of jarosite to acid, significantly improved plant growth and chlorophyll amount rather than jarosite or H_2SO_4 alone (Ryan and Stroehlein, 1976). In other experiment, using of 30 $mg.kg^{-1}$ Fe-EDDHA improved growth of inefficient variety of corn but reduced plant upper part growth in efficient variety and increased Fe concentration in them (Zaharieva, 1982). Sulfur or iron oxidizing microorganisms such as *Thiobacillus ferroxidans* can be used to improve iron availability of pyrite by oxidization (Mishra *et al*., 1984). In a greenhouse experiment, using of acidic iron oxidized powder in amount of 8 percent of soil weight, increased Fe, P and Zn concentration and uptake in soybean. Combination of sulfur and *Thiobacillus* increased Fe concentration and uptake, too. Adding of fresh organic matter to Fe oxidized powder increased Fe concentration compared to control treatment but reduced uptake of it (Foruhar, 1999). Some researchers have tested using of iron oxidized powder with sulfur oxidizing microorganisms (Barau and Berg, 1977; Besharati kelaye, 1998). Researchers in many countries are trying to reduce iron chlorosis of plants with cheap resources.

The other hand, measuring iron concentration in leaves with existing methods, as a criterion for assessing the iron status of the plant, has its own specific problems (Samar, 1998). However, other methods have been used to assessment of iron condition in plant, such as chlorophyll index and fluorescent of chlorophyll (Maxwell and Johnson, 2000; Samar, 1998). Capacity of plants for photosynthesis is restricted and depends on some factors such as stresses which have induced from environmental conditions. Due to this restriction, additional energy which has not been used in photosynthesis process must be repelled. These processes are diffusion of heat or rays of light energies which is known as chlorophyll fluorescence (Hansatech Institutes Ltd., 1993). The peak of fluorescence obtains in red light radiation (685 nm) and extends to infra red region (800 nm). If a healthy leaf is exposed to illumination after a darkness period, a time dependent fluorescence induction could be observed in it. Key parameters of fluoresces are F_0 (amount of fluorescence in beginning of lightening which first permanent receiver of electron of photosystem II which we know it as Q_a, fully oxidized). F_m (This factor achieves when maximum fluorescence for a same intense of light gets. Light intense must be saturated and electron receiver must completely be reduced). F_v (This factor is variable component of fluorescence. It is obtained by subtraction of F_0 from the F_m value). $T_{1/2}$ (The half time at which the maximum fluorescence occurs based on mili second). F_V/F_m (A ratio of the variable fluorescence divided by the maximal fluorescence). This is a useful ratio which has been shown to be proportional to the quantum yield of photochemistry and shows a high degree of correlation with the quantum yield of net photosynthesis (Hansatech Institutes Ltd., 1993; Ibarak and Murakami, 2007; Maxwell and Johnson, 2000).

Balance and sufficient nutrition increases photosynthesis and reducesnutrient stress in plant because of essentiality of them for plant growth. Investigation of the mechanisms improving chlorophyll fluorescence distribution can be used as a tool for studying on the physiological behavior of plants which are induced of nutrient deficiency or excess (Val *et al.,* 1995). Researchers indicated that key parameters of fluorescence can be used for studying on nutrient deficiency or excess in plants (Abadia *et al.,* 1998 Adams *et al.,* 2000; Belkhoja *et al.,* 1998 Val *et al.,* 1995). In this research, the effects of two kinds of irrigation water and different treatments of Fe fertilizers on dry weight of corn shoot, Fe uptake, correlation of them with chlorophyll index and F_V/F_m parameter were studied.

MATERIALS AND METHODS

The studied soil was achieved from topsoil of agricultural lands of Ghazvin, Iran. The soil was air dried, sieved with 4 mm sieve and then filled to 3.5 kg pots. Soil properties were determined (Ali Ehyai and Behbehanizadeh, 1992) in mixed soil sample (Table 1). The factorial experiment with completely randomized design was carried out in greenhouse conditions with two factors (irrigation water and fertilizer) in four replications. First factor was irrigation water in two levels: W_1 (ordinary water of soil and water research station of Karaj), W_2 (acidified water of station with sulfuric acid with pH of 5-5.2). Irrigation was carried out after field capacity reached to about 80 percent. Second factor was fertilizer in seven treatments: T_1= Control (without iron fertilizer application), T_2= Iron sequesterin, T_3= Iron sulfate, T_4= Iron oxide powdered of iron ore, $T_5 = T_4$ + powdered sulfur + *Thiobacillus* inoculants, $T_6 = T_5$ + Organic matter, $T_7 = T_3$ + organic matter. Sulfur was used in amount of 250 $mg.kg^{-1}$ (powdered Sulfur particles with less than 0.1 mm diameter).

Triple super phosphate in amount of 90 $mg.kg^{-1}$, Nitrogen in amount of 200 $mgN.kg^{-1}$ (Ammonium Nitrate which 30 mg $N.kg^{-1}$ was used before planting and other amounts was added in seven times), Potassium in amount of 85 $mg.kg^{-1}$ (potassium chloride), Iron sequestrine in amount of 10 $mg.kg^{-1}$, iron sulfate in amount of 110 $mg.kg^{-1}$,cupper sulfate in amount of 10 $mg.kg^{-1}$,manganese sulfate in amount of 10 $mg.kg^{-1}$, zinc sulfate in amount of 5 $mg.kg^{-1}$ and Boric acid in amount of 5 $mg.kg^{-1}$ were used. Powdered iron oxide of iron ore was used in amount of 220 $mg.kg^{-1}$ and organic matter (composted cow manure) in amount of 10 $gr.pot^{-1}$. We added 10^4 $cells.gr^{-1}$ soil *Thiobacillus* inoculant for related treatments. Corn seeds (variety sc704) were sterilized with sodium hypochlorite and replicately was washed by distilled water then was emerged on water- agar. Five emerged seeds were planted in each pot. Two emerged seeds were cut after one week and remained 3 plants in each pot. All pots were moved randomly every four days to prepare identical environmental conditions. After 80 days of planting, two plants of each pot were cut, washed with distilled water, dried in oven at 75 centigrade degree temperature and weighted which its data has been shown as "double shoot after 80 days" in results section. After 120 days of planting, the last plant was cut too which its data has been shown as "single shoot after 120 days" in results section. Roots were separated from soil, and their weights were measured after washing and drying.

Plant extract was prepared in method of dry ashing and with chloridric acid (2 normal), from plant powder (Emami, 1996). Fe concentration in samples was measured with atomic absorption spectrophotometer (Emami, 1996). Chlorophyll index was measured with SPAD 502 (Piekielek *et al*., 1997) 80 days after planting and factors of F_0, F_V, $T_{1/2}$, F_m, F_V/F_m were measured with handy PEA, 90 days after planting (Hansatech Institutes Ltd, 1993). Statistical analysis and means comparison were done with SAS (V9.1) software and Duncan's test, respectively.

RESULTS AND DISCUSSION

Properties of soil, water and iron oxide powder of iron ore which was used in this experiment were shown in Tables 1, 2 and 3.

Table 1. Physical and chemical properties of soil from Ghazvin region (0-30 cm)

Texture	B	Mn	Cu	Zn	Fe	K	P	T.N	O.C	T.N.V	SP	EC_e	pH
	$mg.kg^{-1}$							%				$dS.m^{-1}$	
Sandy loam	0.4	6.8	0.88	1.0	3.88	230	4.4	0.06	0.59	3	22	0.76	7.86

Table 2. Some chemical properties of irrigation water

$meq.l^{-1}$									SAR	EC_{sw} $dS.m^{-1}$	pH
Cations	K^+	Na^+	Mg^{2+}	Ca^{2+}	Anions	SO_4^{2-}	Cl^-	HCO_3^-			
3.89	0.02	0.55	0.8	2.5	3.9	2.1	0.6	2.1	0.42	0.38	7.2

Table 3. Some chemical properties of iron oxide powder

P_2O_5	Zn	Fe	EC	pH	Cd (total)
$mg.kg^{-1}$ (available)			$dS.m^{-1}$	1:20	$mg.kg^{-1}$
2	0.14	18.9	0.32	9	1

Effects of irrigation water acidification on shoot and root dry weight

In comparison of two irrigation waters, acidification of irrigation water significantly increased dry weight of two plants (in first harvesting), single plant (in second harvesting) and root, (Table 4).

It seems that complete elimination of bicarbonate in irrigation water due to acidification has led increase in dry weight with regard to the fact that in various scientific resources have been referred to the negative effects of bicarbonate irrigation water to absorb iron and other food elements (Shahabi and Malekuti, 2001; Wallace *et al.,* 1984).

Main effects of fertilizer treatments on shoot and root dry weights

The highest dry weight of two shoots, single shoot and root were achieved from T_6 (Table 5) and the lowest was achieved from T_2 which had significantly difference with T_6 and T_7 in first cutting and with all of treats in second cutting.

In the first cut, T_6 and T_7 had significantly difference with T_1 and T_2 but in second cut, T_6 had significant difference with T_1, T_2 and T_4, but T_7 had difference only with T_2 and it does not had significantly difference with blank (T_1). Adding of iron sulfate had no significant effect on dry weight of single shoot. Adding of iron sulfate with organic matter to blank increased the shoot dry weight not significantly. The lowest and highest root dry weight was belonging to T_3 and T_6, respectively. Some references have indicated that using of sulfur with *Thiobacillus* has improved soil chemical properties (such as pH) slightly in the rhizospher zone. In this condition, the availability of nutrient elements increases due to biological oxidation of sulfur with *Thiobacillus* bacteria and making of sulfuric acid (Besharati kelaye, 1998).These results is demonstrated by Bradiya *et al.* (1982). In Bradiya *et al.*, (1982) experiment, sulfur (0.5 soil weight percent) and *Thiobacillus* bacteria (10^6 cell.gr soil) reduced the pH of calcareous soil from 7.9 to 6.49 and increased the phosphorus and iron concentration from 4.8 to 7.53 and 2.13 to 4.2 $mg.kg^{-1}$, respectively.

Table 4. Effects of irrigation water acidification on corn shoot and root dry weight

irrigation	Double shoot after 80 days	Single shoot after 120 days	Root
		$gr.pot^{-1}$	
Ordinary water	8.57 b	26.79 b	5.54 a
Acidified water	9.27 a	28.78 a	6.18 a
% (CV)	14.48	7.17	9.02

* In each column means with the same letter, don’t have statistical significance with each other in five percent level

Effects of irrigation water acidification on Fe, Zn and P concentration

Acidification of irrigation water significantly increased Fe, Zn and P concentration in plant in first cutting and significantly increased Zn and P concentration in second cutting but has no significant effect on Fe concentration (Table 6). Acidification of irrigation water (improving water quality) may increase the solubility of these elements and the ability of plant roots to take up these elements readily. Swift and Haynes (1985) appointed the beneficial effects of acidification on solubility of Fe and Zn.

Main effects of Fe treatments on Fe, Zn and P concentration of corn

Based on the results of table 7 in first sampling, iron treatments had no significant effect on iron concentration in shoot. Different treatments of iron had significant effects on Zn and P concentration. Highest concentration of Zn was belonging to T_6 and the lowest was belonging to T_4 which had significantly difference with all other treatments in five percent level. In single shoot, different treatments of iron had no significant effect on Fe and Zn concentration but the highest effect on P concentration was belong to T_7 and the lowest was belong to T_2. It seems that different treatments which had been used in this experiment, instead of effects on Fe concentration had higher effects on Zn and especially on P concentration. Corn has strategy II for uptaking of micronutrients especially Fe. Perhaps it has used this strategy for increasing the availability of Fe and in this way it has averted needs for elemental micronutrients.

Effects of irrigation water acidification on Fe, Zn and P uptake of corn

Acidification of irrigation water significantly increased Fe, Zn and P uptake in the double shoot, but in the single shoot its effect wasn't significant. It seems that acidification had better effect on uptake of Zn, Fe and P, perhaps in the first stages of growth due to incomplete extension of plant root system, it has not been capable to use strategy II, and therefore acidification of irrigation water had visible effect. In later stages of growth, root had extended and strategy II may increase solubility of nutrients and with reducing the role of acidified water, difference between two irrigation water wasn't significan

Table 5. Main effect of Iron treatments on shoot and root dry weight

Treatment	Double shoot after 80 days	Single shoot after 120 days	Root
		gr.pot^{-1}	
T_1	8.14 b	27.59 b	5.75 b
T_2	7.98 b	25.13 c	5.53 bc
T_3	8.99 ab	27.94 ab	5.03 c
T_4	8.57 ab	27.16 b	5.50 bc
T_5	9.19 ab	28.47 ab	6.54 a
T_6	9.83 a	30.01 a	7.01 a
T_7	9.76 a	28.20 ab	5.69 b

* In each column means with the same letter, don't have statistical significance with each other in five percent level
**T_1= control (without iron application), T_2= Iron sequesterin, T_3= Iron sulfate,
T_4= Iron oxide powder, T_5 = T_4 + powdered sulfur + *Thiobacillus* inoculants,
T_6= T_5 + organic matter, T_7 = T_3 + organic matter

Table 6. The Effect of acidification of irrigation water on Fe, Zn and P concentration

Irrigation	double shoot after 80 days			single shoot after 120 days		
	P	Zn	Fe	P	Zn	Fe
	%	mg.kg^{-1}		%	mg.kg^{-1}	
Ordinary	0.07 ab	26.18 b	53.32 b	0.137 b	25.50 b	41.02 a
Acidified	0.089 a	33.80 a	61.39 a	0.158 a	22.08 a	40.78
(%) CV	8.30	18.40	13.03	14.23	16.89	16.41

* In each column means with the same letter, don't have statistical significance with each other in five percent level

Table 7. Effects of Fe treatments on Fe, Zn and P concentration in plants

Treatment	double shoot after 80 days			single shoot after 120 days		
	P	Zn	Fe	P	Zn	Fe
	%	mg.kg^{-1}		%	mg.kg^{-1}	
T_1	0.071 d	25.25 d	54.57 a	0.136 b	24.81 a	39.88 a
T_2	0.065 e	31.13 c	61.13 a	0.132 b	26.00 a	38.63 a
T_3	0.080 cd	24.96 d	54.88 a	0.139 b	22.33 a	43.44 a
T_4	0.078 cd	18.33 e	54.88 a	0.148 b	24.38 a	39.38 a
T_5	0.089 b	28.13 cd	58.25 a	0.147 b	24.75 a	43.31 a
T_6	0.097 a	44.75 a	59.00 a	0.152 b	22.44 a	40.00 a
T_7	0.087 bc	37.38 ab	58.63 a	0.180 a	21.81	41.69 a

* In each column means with the same letter, don't have statistical significance with each other in five percent level
**T_1= control (without iron application), T_2= Iron sequesterin, T_3= Iron sulfate,
T_4= Iron oxide powder, T_5 = T_4 + powdered sulfur + *Thiobacillus* inoculants,
T_6= T_5 + organic matter, T_7 = T_3 + organic matter,
T_6= T_5 + organic matter, T_7 = T_3 + organic matter

Table 8. Effects of irrigation water acidification on Fe, Zn and P uptake

Irrigation	double shoot after 80 days			single shoot after 120 days		
	P	Zn	Fe	P	Zn	Fe
	mg.pot^{-1}					
Ordinary	6.42 b	0.22 b	0.44 b	38.1 a	0.68 a	1.1 a
Acidified	8.19 a	0.32 a	0.57 a	45.9 a	0.64 a	1.18 a
(%) CV	15.4	22	18	13.8	16	17

* In each column means with the same letter, don't have statistical significance with each other in five percent level

Effects of Fe treatments on Fe, Zn and P uptake

Effects of Iron treatments on Fe, Zn and P uptake in double shoots was significant in 5 percent level. Highest Fe uptake was belonging to T6 and T_7 and the lowest Fe uptake was belong to T_1 and T_2. Adding sulfur and *Thiobacillus* inoculant to iron oxide powder (T_5) had not significant effect on increase of Fe uptake, but adding organic matter to above treatment (T_6) induced Fe uptake was significant. Positive effect of organic matter was seen on treatment containing Iron sulfate (T_7) because of adding organic matter to iron sulfate, iron uptake increased but this increase wasn't significant in comparison to iron sulfate. Activities of *Thiobacillus* bacteria may reduce the pH of soil in rhizospher and then may increase the availability of Fe for plant.

It seems that additions of organic matter (composted cow manure) has improved the condition for better activities of *Thiobacillus* bacteria or prepared some nutrient elements for plant.

The highest Zn uptake in double shoot was belong to T_6 (which had iron oxide powder + sulfur + *Thiobacillus* + organic matter), but this treatment had significant difference with T_3 (which had lonely iron sulfate). The highest P uptake in double shoots was belong to T_6 and its difference wasn't significant only with T_7 (Iron sulfate + organic matter) but with other treatments had significantly difference in five percent level. Lowest P uptake was belong to T_2 (Iron sequestrine). Based on table 1, the soil which was used for this experiment had low phosphorus and it seems that corn can positively responded to higher levels of phosphorus which was used in this experiment.

In single shoot, iron uptake was increased in different treatments, only in T_2 (Iron sequestrine) it was lower than blank (T_1) but differences with blank for other treatments wasn't significant in this growth stage. In later stages of growth, it had taken up more Fe than in earlier stage. Differences between treatments hadn't been significant because of sufficient Fe uptake in blank. It hadn't been significant difference between treatments on Zn uptake. T_7 (iron sulfate + organic matter) had highest phosphorus uptake and its difference with all treatments was significant. Adding organic matter had might prepared available phosphorus and performed as a plant feeder or prepared better root environmental condition for suitable growth and activity. Uptake of three nutrients was further in second step than in first step (Table 9).

Table 9. Effect of treatments on Fe, Zn and P uptake ($\alpha = 0.05$)

Treatment	double shoot after 80 days			single shoot after 120 days		
	P	Zn	Fe	P	Zn	Fe
	mg.pot^{-1}					
T_1	5.94 ef	0.21 bc	0.45 b	37 cd	0.68 a	1.1 ab
T_2	5.23 f	0.25 b	0.45 b	34.3 d	0.64 a	0.97b
T_3	7.23 cd	0.22 b	0.49 ab	43 bc	0.62 a	1.21 a
T_4	6.69 dc	0.16 c	0.47 b	40.4 bcd	0.66 a	1.08 ab
T_5	8.14 bc	0.25 b	0.53 ab	41.5 bc	0.71 a	1.22 a
T_6	9.34 a	0.44 a	0.59 a	45.6 b	0.67 a	1.19 a
T_7	8.56 ab	0.38 a	0.5 a	52.2 a	0.62 a	1.21 a

* In each column means with the same letter, don't have statistical significance with each other in five percent level
**T_1= control (without iron application), T_2= Iron sequesterin, T_3= Iron sulfate,
T_4= Iron oxide powder, T_5 = T_4 + powdered sulfur + *Thiobacillus* inoculants,
T_6= T_5 + organic matter, T_7 = T_3 + organic matter

Effects of acidified water on F_0, F_m, F_v, $T_{1/2}$, F_V/F_m and chlorophyll index

Acidification of irrigation water hadn't significant effect on F_0, $T_{1/2}$, F_V/F_m and leaf chlorophyll index, but it's effective on F_V and F_m. This two factor values had higher level in ordinary water than in acidified water (table 10). In studying of the plant photochemistry efficiency, the ratio of F_V/F_m is more important than separate factors (Hansatech Institutes Ltd, 1993). Based on changes in this ratio, it can be discussed the efficiency of treatments. It seems that in this study the acidification of irrigation water hasn't improved leaf photochemical efficiency.

Effects of iron treatments on F_0, F_m, F_v, $T_{1/2}$, F_V/F_m and chlorophyll index

In contrast with F_0, F_V/F_m and chlorophyll index, the effects of iron treatments on F_m, F_v, and $T_{1/2}$ were significant on five percent level (table 11). The lowest value of F_m was belonging to T_7 and its significantly differences with T_4, but differences between other treatments weren't significant. This trend was seen on F_V, too. The highest amount of chlorophyll index was seen in T_6 so that its significantly difference with T_1 but differences of other treatments with T_1 (blank) wasn't seen.

Table 10. Effects of acidification of irrigation water on F0, Fm, Fv, $T_{1/2}$, FV /Fm and chlorophyll index

Water	F_0	F_m	F_v	$T_{1/2}$	F_V/F_m	Chlorophyll index (SPAD)
Ordinary	0.691 a	3.151 a	2.45 a	70.82 a	0.780 a	27.719 a
Acidified	0.680 a	2.909 b	2.23 b	71.64 a	0.764 a	28.33 a
% CV	9.19	10.25	11.6	11.52	2.13	14.25

* In each column means with the same letter, don't have statistical significance with each other in five percent level

Table 11. Effects of treatments on F_0, F_m, F_v, $T_{1/2}$, F_V/F_m and chlorophyll index

Treatment	F_0	F_m	F_v	$T_{1/2}$	F_V/F_m	Chlorophyll index (SPAD)
T_1	0.692 a	3.118 ab	2.468 ab	72.06 ab	0.775 a	26.83 b
T_2	0.673 a	2.979 ab	2.278 ab	70.40 ab	0.769 a	28.51 ab
T_3	0.684 a	3.073 ab	2.388 ab	70.79 ab	0.777 a	27.87 ab
T_4	0.739 a	3.306 a	2.558 a	67.58 b	0.774 a	27.67 ab
T_5	0.674 a	3.043 ab	2.370 ab	72.75 ab	0.777 a	27.88 ab
T_6	0.669 a	2.927 ab	2.257 ab	74.44 a	0.769 a	29.15 a
T_7	0.667 a	2.763 b	2.085 b	73.58 ab	0.764 a	28.57 ab

* In each column means with the same letter, don't have statistical significance with each other in five percent level

**T_1= control (without iron application), T_2= Iron sequesterin, T_3= Iron sulfate,

T_4= Iron oxide powder, T_5 = T_4 + powdered sulfur + *Thiobacillus* inoculants,

T_6= T_5 + organic matter, T_7 = T_3 + organic matter

Table 12. Relation between variables of experiment

Variables	Equation	R^2	Pr> F*	Variables	Equation	R^2	Pr> F
Shoot dry weight and P uptake	y = 0.242 x + 24.7	0.7158	0.0001	F_V/F_m and Zn uptake	y= -6.731x + 6.126	0.2709	0.075
Shoot dry weight and Fe uptake	y = 12.72x + 15.7	0.7518	0.0001	F_V/F_m and chlorophyll index	y = -47.6x + 64.77	0.3502	0.026
Shoot dry weight and Zn uptake	y = 12.72x + 15	0.2542	0.066	P uptake and Fe uptake	y= 0.015x + 0.878	0.6404	0.0006
Shoot dry weight and F_V/F_m uptake	y = 9.051x + 28.2	0.1402	0.187	P uptake and Zn uptake	y= 0.006x + 0.612	0.169	0.154
F_V/F_m and P uptake	y = 468.3x +410	0.2691	0.044	Fe uptake and Zn uptake	y= 4390x + 0.203	0.2886	0.0478
F_V/F_m and Fe uptake	y = -9.028 x + 8.60	0.2896	0.047				

* Pr > F: probability of significantly difference

Correlation between variables

For determining the relation equation between variables and measuring correlation index (R^2), Excel software (2003) and SAS (9.1) was used. The highest correlation index was set to shoot dry weight with iron and phosphorus uptake (R^2= 0.75 and 0.72, respectively) and lowest was determined between shoot dry weight and F_V/F_m ratio (Table 12).

The highest F_V/F_m ratio correlation index achieved with chlorophyll index (SPAD) and relation between two variables was negative. Uptake elements, relation of phosphorus and iron uptake had highest correlation index (R^2= 0.64) so that by increasing in phosphorus uptake, iron uptake was increased. Lowest correlation index was between phosphorus and Zn uptake (R^2= 0.17).

CONCLUSION

Our society needs to improve the yield and quality of agricultural products and at the same time saving of the environment. This matter persuades us to pay attention to uptake nutrients from different fertilizer sources and soil nature.

Due to having different properties such as degree of purity, concentration, solubility and having other materials such as sulfur, different fertilizer sources may have prefer to use. Organic matter and sulfur combined with *Thiobacillus* inoculant and other materials which were examined in this research had attended results. Comparing fertilizer compounds which contains Fe (treatments T_3, T_4, T_7) showed that in some conditions (especially same as in this experiment), non-ordinary fertilizer compounds can be effective as ordinary fertilizers.

Therefore it is necessary to pay attention to other sources of this element which may be unknown. Results shows that plant roots and some of fertilizers may improve slightly soil chemical properties (such as pH and TNV) then increase solubility of natural elements in soil or nutrients in ordinary fertilizers. In this experiment acidified irrigation water has improved and increased the solubility of nutrients in soil and fertilizer compounds with some chemical reactions (such as lowering pH).

If so in this experiment factors of fluorescence had low correlation with nutrient uptake and yield, further studies need to be done for plant nutrient situation determining, besides of current indexes.

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