Alfalfa (*Medicago sativa* L.) growth and fodder yield in response to iron and humic acid spraying

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Abstract

The experiment was carried out on loamy sand soil in the Al - Zubair district throughout the 2022–2023 winter growing season. The district is located in the southeast of the Basrah Governorate, 20 km west of the Basra Governorate Centre, at longitude 47.05° and latitude 30.28°. The purpose of the study was to determine how the application of iron and humic sprays, as well as their interaction, affected certain growth traits and the quantity of dry and green feed given of the local variety of Alfalfa crop (*Medicago sativa* L.). A completely randomized block design (R. C. B. D.) was used in a split - plot arrangement with 3 replications, Iron concentrations were put (Fe0, Fe1=400, F2=800 mg L^{-1}) in the main plots using Aqueous iron sulphate fertilizer H2SO4.7H2O (%20 Fe) as a source of iron, while Humic acid) concentrations (0, 3, 6 ,9 gm L^{-1}), which used the symbols HA0,HA1,HA2,HA3 were put in the sub-pilots. The administration of iron foliar had noteworthy influence on height of the plant and No. of branches m^{-2} at the 1st and 3rd cutting, whereas these traits were significantly affected by spraying of humic acid at all cuttings. In the case of Iron and humic acid, both Fe1 and HA3 showed the best result of green fodder yield at all cutting, total green fodder yield and protein. Interaction between Fe and HA showed a significantly effect on some of the studied characteristics, the combination (Fe1×HA3) has the highest values for overall production of dry fodder (1367.1 kg ha-1) and total yield of green fodder (2634.6 kg ha-1).

Key words: Alfalfa, Iron, Humic acid, Fodder yield, Protein

1. Introduction

Alfalfa, scientific name *Medicago sativa* L. is a vital herbaceous fodder legume in Iraq and around the globe. It performs a significant role in the agroecosystem. It is a perennial crop that remains in the soil for 3-4 years and produces a lot of green fodder that is high in protein and other nutrients that are beneficial to the animal's health and productivity [1]. It is produced as silage and hay. This crop is highly adaptable to many agricultural environments, making it excellent for production in all irrigated fields, particularly in the central and southern parts of Iraq. In addition to being palatable to animals, it can fix significant amounts of nitrogen in the soil [2]. In Iraq, alfalfa is mostly grown for its fodder, with the intention of producing seed only being of minor importance. Suitable fodder yield requires the availability of all nutrients in the right quantities [3]. One of the best strategies to avoid nutrient shortages in the soil is to apply foliar fertilization, particularly for micronutrients. One of the micronutrients that is commonly lacking is Iron (Fe). The availability of Fe depends on the pH level of the soil and is higher in acidic soils; in contrast, it is quite low in alkaline soils. Soils having a pH higher than 6.0 are more probable to have deficiencies. [4]. In contrast, the majority of Iraq's central and southern soils had a pH of greater than 7.5[5]. Consequently, it is essential to apply Iron directly to foliage rather than through the soil. Iron is essential for many aspects of plant growth and development., including respiration, the manufacture of chlorophyll, the development of chloroplasts, and enhanced photosystem function [6]. It is an essential component of several enzymes and It is essential for nucleic acid metabolism. [7]. [8] found that Alfalfa's photosynthetic capacity as well as yield increased at 0.6% ferrous sulphate concentration. Additionally, there are now challenges for reducing the risks of pollution from chemical fertilizers. Thus, the importance of humic acid and other organic fertilizers, which have a quick effect and are safe for people, animals, and plants, as well as the fact that they include minerals that promote plant development [9] [10] and [11] produced the most dry and green fodder on average during two

seasons was obtained with the increase of humic acid concentration. The goal of this investigation was to determine the impact of foliar iron and humic acid treatments on Alfalfa growth and fodder output.

2. Material and Methods:

In the winter growing season of 2020–2021, the experiment was carried out on loamy sand soil in the Al-Zubair district (southern of Iraq), To explore the impact of spraying with iron and humic and their interaction on growth, green and dry fodder yield of the Alfalfa. Soil samples have been taken from the field 30 cm from the surface, just before planting . The chemical and physical characteristics of the soil were assessed using the procedures mentioned in [12, 13] and listed in table (1). A completely randomized block design was used in a split-plot arrangement with three replications, Iron concentrations were put (Fe0 (0), 400, 800 mg L^{-1}) in the main plots using Aqueous iron sulphate fertilizer $H_2SO_4.7H_2O$ (%20 Fe) as a source of iron, while Humic acid (HA) concentrations (0, 3, 6 , 9 gm L^{-1}) which used the symbols HA0, HA1, HA2, HA3 were put in the sub-pilots . Hum plant fertilizer (80% HA), was used. Planting was done on 1st /10/2022 by hand sowing, with a 36 kg ha^{-1} sowing rate [14] , and the seeds were covered with a light layer of soil using the local variety, The experimental land was fertilized immediately after planting with urea fertilizer (46% N) at a level of 80 kg N ha^{-1} [15], in three doses, the 1st at planting and the 2nd and 3th after each cutting. Iron and humic acid were sprayed on the plants at the 9-leaf stage of growth, using cleaning solution at a concentration of 15.0 cm3 in order to increase efficiency absorption, reducing the surface tension of water, and causing complete wetness on the plant. The spraying was repeated at each cutting. Crop service operations were carried out, including irrigation and weeding, as needed. For each replication of each treatment, a sample area of alfalfa was selected at the early flowering stage, and a fresh sample of alfalfa was cut close to the ground. At every cutting, data was collected as well on the following parameters:

Height of the plant (cm)

No. of stems (m^{-2})

Fresh fodder yield (Kg ha^{-1}): It was calculated from the cutting of the harvested area ($1m^2$) of each experimental unit for three cutting. The sample is then weighed using an automatic weighing balance, and then the weight was converted to ton per hectare.

Dry fodder yield (Kg ha^{-1})

Leaf protein (%): it was determined by the AOAC Analytical Chemists [16].

Table -1- Field soil's physical and chemical characteristics.

Soil properties	Value
Clay (g kg^{-1})	205.3
Silt (g kg^{-1})	214.4
Sand (g kg^{-1})	580.3
Texture	Loamy Sand
Available N (mg kg^{-1})	41
Available P (mg kg^{-1})	2.59
Available K (mg kg^{-1})	22.56
Organic matter (g kg^{-1})	1.16
pH	7.78
Ec. (ds m^{-1})	24.10

Analysis of Statistics: To show the significant variations between the treatments, the LSD. test was used to statistically analyze the data using GenStat software (ver.12) at a method of acceptable level of 0.05

3. Results and Discussion

3.1. Height of the plant

Results presented in Table 2 indicate that iron fertilizer and humic in the first and third cuts had a significant (P <0.05) impact on plant height., However, results clearly indicated that spraying with iron increased plant height by 25.05 and 26.37cm at the1st and 3rd cut respectively. compared to the control which gave 18.65 and 29.88 cm respectively. Regarding the impact of humic acid, data showed (Table 2), spraying of the highest concentration of humic acid (9 gm L^{-1}) caused the plants to grow taller such as 28.54, 40.63, 39.30 cm at the 1st, 2nd, and 3rd cutting respectively. However, the results showed a noteworthy impact on the way iron spraying and humic acid interact. the maximum height of the plant was with application of Iron and the highest humic concentration (HA3) in the 1st and 2nd cuts such 31.00 and 42.50 cm respectively.

3.2. No. of branches (branch Plant^{-1})

Results show (Table 2) that the No. of branches was significantly (p≤ 0.05) impacted by the foliar application of iron, humic acid and their interaction. Foliar application of Fe resulted an increase in No. of branches by 61.26%, 10.18%, 11.16% when compare with control for three cuts respectively. No. of branches were increased when humic acid was applied at the higher rate (HA3) as compared with the control for the three cuts. Table 2 illustrates how the application of Fe enhanced the No. of branches when applied with higher rate of humic acid in Fe1 × HA3 interaction, were 1212.43,620.46 and 666.70 branch m^{-2} for three cutting respectively as compared to Fe0 ×HA0 (642.70, 457.81and 485.8 branch m^{-2} respectively)

3.3. Leaf protein (%)

The data's statistical analysis found that Alfalfa's protein content was greatly impacted (p≤ 0.05) by foliar application of Iron at the 2nd and 3rd cuts while it is affected by humic acid at three cuts (Table 2). Values of the data revealed that application of Fe1 = 400 mg L^{-1} produced maximum protein content were 18.24% and 16.84% for 2nd and 3rd cuts respectively, while the control gave 14.65% and 13.71% respectively. Foliar application of humic acid with HA3 (9 g L^{-1}) increased protein content for the three cuts (18.96% ,17.97%, 16.49% respectively) although There was not a difference between foliar applications with control, HA1 and HA3 concentration (Table2). Interaction of Iron and humic acid was significant. The highest protein content was recorded under (Fe0 × 9 g L^{-1} HA) at the 1st cut with values of 20.68 %, whereas the interaction Fe1 × HA2 and Fe1 × HA1 gave the highest protein at the 2nd and 3rd cut of 18.67% and 17.65%, respectively.

Table2. Iron and humic concentrations' effects on growth and Leaf protein.

Treatment's	Height of the plant (cm)			Number of Tillers Plant-^{1}			% Protein		
Fe conc. mg L^{-1}	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Fe0(Control)	18.65	35.21	29.88	642.70	457.81	485.8	15.74	14.65	13.71
Fe1	25.05	35.96	37.26	1036.43	504.44	540.0	16.47	18.24	16.84
Fe2	20.44	34.99	35.84	980.55	490.55	505.4	16.77	18.00	16.60
L.S.D. (p≤ 0.05)	2.765	N.S	1.533	33.34	12.54	31.48	N. S	1.320	0.673
Humic conc. (g L^{-1})									
HA0(Control)	10.23	24.05	26.40	524.43	383.96	400.00	14.33	15.25	13.57
HA1	24.13	38.09	34.15	788.43	448.05	528.90	15.18	15.52	15.20
HA2	24.50	39.55	34.42	899.56	528.03	504.00	15.94	17.04	15.85
HA3	28.54	40.63	39.30	1129.76	564.45	618.70	18.96	17.97	16.49
L.S.D. (p≤ 0.05)	1.762	4.079	1.216	19.94	23.17	37.83	1.824	1.778	1.062
Fe0HA0	9.00	26.31	25.20	524.43	375.11	437.3	12.93	12.72	11.60
Fe0HA1	20.14	35.64	27.20	396.43	433.88	471.1	14.13	12.80	12.74
Fe0HA2	19.41	40.30	31.00	602.67	513.80	464.0	15.21	15.41	14.15
Fe0HA3	26.07	38.58	36.10	1047.10	508.43	570.70	20.68	17.67	16.34
Fe1HA0	11.47	21.79	27.60	556.43	392.80	362.70	15.73	17.79	15.54
Fe1HA1	28.13	40.55	41.10	1180.43	462.22	586.70	16.23	18.24	17.65
Fe1HA2	29.60	38.80	37.83	1196.43	542.26	544.00	16.68	18.67	17.54
Fe1HA3	31.00	42.69	42.50	1212.43	620.46	666.70	17.25	18.27	16.64
Fe2HA0	12.31	22.41	25.46	648.14	415.55	391.00	17.65	16.78	14.16
Fe2HA1	28.46	38.25	30.55	987.21	550.78	458.21	18.00	17.16	15.66
Fe2HA2	29.11	40.56	33.44	1016.34	551.04	546.23	18.12	17.45	16.10
Fe2HA3	30.24	43.00	35.97	1143.22	580.56	609.44	19.45	17.46	16.15

L.S.D. (p≤ 0.05)	**2.578**	**N.S**	**1.659**	**29.97**	**28.83**	**48.28**	**2.430**	**2.247**	**1.330**

3.4. Fresh fodder yield (kg ha-1)

Table 3 presents information on the yield of green fodder, The data's analysis revealed that foliar application of Iron had significantly impacted on the Fresh fodder yield at 1st and 3rd cuttings and the total yield of fresh fodder The results of table 3 showed that foliar application of Iron lead to increased fresh fodder yield, Fe1gave the highest yield of fresh fodder by -1093.50- and 2162-Kg ha^{-1} at 1st and 3rd cuttings respectively. While, Fe0 gave lowest by 993.40- and 1517-Kg ha^{-1}. It was observed that spraying of humic acid had significant effect on fresh fodder yield of Alfalfa at all the three cuttings and the total yield. Higher fresh fodder yield observed in HA3 in all the 1st, 2nd and 3rd cuttings as 1243.70 Kg ha^{-1} in 1st cutting, 1760.3 Kg ha^{-1} in 2nd cutting and 2290 Kg ha^{-1} in 3rd cutting (Table3). Similar trend was also noted in total fresh yield of Alfalfa fodder as 5294 Kg ha-1. The interaction of Iron and humic acid has significant effect on fresh fodder yield. Foliar application of Fe1 with HA3, gave the highest fresh fodder yield by 1340.50 and 1789.3Kg ha^{-1} at the 1st and 2nd cuttings respectively. Whereas, at the 3rd cutting the spraying of Iron with HA1 and HA3 gave the highest fresh yield (2773Kg ha^{-1}).

3.5. Dry fodder yield (Kg ha^{-1})

Table (3) indicates that only the first cut showed a significant influence from iron, (p≤ 0.05). However, there were no significant influence on the other cutting and the total Yield of dry fodder. Foliar application of iron (Fe1) increased Yield of dry fodder (604.90 Kg ha^{-1}). Plants which did not received Fe (Fe0) had fewer dry fodder (532.1 Kg ha^{-1}). As seen in Table 3, Yield of dry fodder at 1st and 3rd and total Yield of dry fodder were significantly affected (p≤ 0.05) by humic concentrations, Yield of dry fodder increases with increasing humic acid concentrations. Maximum dry yield of 710.40, 564- and 1855-Kg ha^{-1}for the 1st, 3rd cuts, and total Yield of dry fodder respectively. The dry fodder yield was significantly impacted by the interaction between iron and humic acid. As the concentration of humic acid at the combination (Fe1 × 9 g L-1 HA) increased, so did the dry fodder yield. The highest fresh dry fodder yields were 727.20, 595.2, 576.0, and 1898.4 kg ha-1 for the first, second, third, and total dry fodder, respectively.

Table (3). Iron and humic concentrations' effects on green and dry fodder yield of Alfalfa

Treatment's	**Green fodder yield (Kg ha^{-1})**			**Total Green fodder yield (Kg ha^{-1})**	**Dry fodder yield (Kg/ha^{-1})**			**Total Dry fodder yield (Kg ha^{-1})**
(Fe conc. mg L $^{-1}$)	**1st cut**	**2nd cut**	**3rd cut**		**1st cut**	**2nd cut**	**3rd cut**	
Fe0	**993.40**	**1262.7**	**1517.4**	**3773.1**	**540.60**	**497.5**	**508.9**	**1547**
Fe1	**1093.50**	**1323.6**	**2162.3**	**4579.1**	**604.90**	**532.1**	**516.9**	**1653**
Fe2	**1009.41**	**1334.5**	**1805.2**	**4149.1**	**580.46**	**541.9**	**529.7**	**1652**
L.S.D. (p≤ 0.05)	**38.82**	**N. S**	**180.2**	**236.8**	**21.49**	**N. S**	**N. S**	**N. S**
Humic conc. (g L $^{-1}$)								
H0	**876.00**	**872.5**	**867**	**2615.5**	**421.2**	**440.8**	**450.3**	**1312.3**
H1	**1007.70**	**993.1**	**2080**	**4080.8**	**504.7**	**504.8**	**498.4**	**1507.9**
H2	**1046.40**	**1546.7**	**2121**	**4714.1**	**654.8**	**533.1**	**538.9**	**1727.4**
H3	**1243.70**	**1760.3**	**2290**	**5294**	**710.40**	**580.6**	**564**	**1855**
L.S.D. (p≤ 0.05)	**39.02**	**57.98**	**139.6**	**176.7**	**43.91**	**N. S**	**49.18**	**94.80**
Fe0H0	**850.40**	**833.1**	**912**	**2595.5**	**340.80**	**431.5**	**484.3**	**1257.6**

Fe0H1	**1075.50**	**918.9**	**1387**	**3381.4**	**450.40**	**432.5**	**496.5**	**1378.4**
Fe0H2	**900.80**	**1567.5**	**1963**	**4431.3**	**677.60**	**560.0**	**502.9**	**1740.5**
Fe0H3	**1146.90**	**1731.2**	**1806**	**4684.1**	**693.60**	**565.9**	**552.0**	**1811.5**
Fe1H0	**901.60**	**912.0**	**821**	**2634.6**	**501.60**	**449.1**	**416.4**	**1367.1**
Fe1H1	**940.00**	**1067.2**	**2773**	**4780.2**	**559.00**	**578.1**	**500.3**	**1637.4**
Fe1H2	**1192.00**	**1525.9**	**2279**	**4996.9**	**632.00**	**506.1**	**574.9**	**1713**
Fe1H3	**1340.50**	**1789.3**	**2773**	**5902.8**	**727.20**	**595.2**	**576.0**	**1898.4**
Fe2H0	**911.60**	**920.0**	**850.4**	**2682.0**	**515.60**	**460.1**	**425.8**	**1401.5**
Fe2H1	**964.09**	**1117.2**	**2299**	**4380.1**	**560.5**	**569.7**	**510.3**	**1640.5**
Fe2H2	**1192.00**	**1525.9**	**2541**	**5258.9**	**620.49**	**511.8**	**559.2**	**1691.5**
Fe2H3	**1240.50**	**1750.3**	**2607**	**5597.8**	**700.57**	**584.9**	**545.1**	**1794.6**
L.S.D. ($p \leq 0.05$)	**50.87**	**78.02**	**191.7**	**245.1**	**54.46**	**48.73**	**66.26**	**117.2**

4. Discussion

Foliar application of 400 ppm Fe increased height of the plant, No. of branches, yield of green fodder and dry fodder yield contrasted with untreated Alfalfa plants, This is because iron affects critical functions by affecting the activity of many enzymes, having a role in the division of meristematic cells, and raising the height of the shoots, which led to an increase in the height of the plant. [17]. Since iron is necessary for the manufacture of cytochrome, ferredoxin, and chlorophyll in the chloroplasts that carry out photosynthesis, it also plays a part in cell division and elongation., [18,19,20], this is reflected in encouraging the growth characteristics and fodder yield of the crop. According to the results of table 2, foliar humic acid application enhanced plant height and number of branches m^{-2}. The concentration of HA3(9g L-1) produced higher, plant height and number of branches m^{-2} by 34.00%, 12.45% and 42.89% respectively as compared to the reason may be due to the effect of humic acid in increasing the vital activities of the plant, which leads to an increase in the growth rate of the plant. Or the reason may be due to the fact that humic acid has a hormonal effect, as it affects the cell protoplasm and the cell wall, which leads to rapid cell division and growth, thus increasing the height of the plant [20]. An increase in growth characteristics, yield of green and dry fodder and protein content of leaves and all cuttings was achieved when spraying the combination of iron (400 mg L^{-1} × 9 g L^{-1} humic acid). Accordingly, the action or role of iron and humic mentioned above in increasing growth characteristics pushed for an increase in fodder yield, and their action in explaining the reasons leading to an increase in plant height and the number of branches pushed towards an increase in green and dry fodder yield.

5. Conclusion:

With the greatest values for the components of the fodder output, green and dry fodder, and the percentage of protein in the alfalfa crop's leaves and for all cuts, the study concludes that there is a high reaction to iron element spraying at a level of 400 mg L^{-1}. Along with having the best yield and component values, humic acid sprayed at a quantity of 9 mg L-1 also had the highest percentage of protein in the leaves, indicating a considerable advantage for all attributes under study. The interaction between the level of Fe1 and H3 recorded a significant superiority in all studied traits. The results of this study clearly demonstrate that alfalfa has the potential to achieve high green and dry fodder yields under the challenging conditions of the desert environment in Al-Zubair district. This indicates that alfalfa is not only adaptable to arid soils and limited water resources, but also capable of contributing significantly to sustainable livestock feeding systems in such regions. Therefore, alfalfa can be considered a promising strategic fodder crop for desert agriculture, offering a reliable source of

nutrition for animals and supporting agricultural productivity in areas previously thought unsuitable for fodder cultivation.

6. References

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