Comparison effect of Nano-Iron Chelate and Iron Chelate on Growth Parameters and Antioxidant Enzymes activity of MUNG bean (Vigna Radiate L.)

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INTRODUCTION

Contribution of pulses to agriculture and daily life has been tremendous besides being one of the important constituents of our diet. The green gram, Vigna radiata (Fabaceae) is one of the important pulse crops in India. It is a protein rich staple food. It contains about 25 percent protein [36]. An important feature of the mung bean...
Iron plays many essential roles in plant growth and development, including chlorophyll synthesis, thylakoid synthesis, chloroplast development, contributes in RNA synthesis and improves the performance of photosystems [28,39]. Despite of being abundant, due to its inaccessibility, its shortage is common for plants. Most Shapes of iron is insoluble, and only a small amount of iron is soluble (Fe (OH)2, Fe (OH)+2, Fe+3, Fe+2). Amount of these soluble forms of iron depends on pH so that they are maximum in acidic pH and minimum in pH range between 6.5 and 7.5 [19]. Abbas et al. [1] applied 0, 4, 8, 12 and 12 kg ha-1 in the form of iron sulphate to the soil and showed that iron fertilization increased Fe and protein contents of the wheat grain. With application of 150 g ha-1 iron in the form of Fe2O3, Habib [13] reported that iron and protein contents of the wheat grain were enhanced. Zeidan et al. [50] applied foliar Fe fertilizer (1.0% FeSO4) and reported that Fe application increased protein and Fe contents of wheat grain. Liu et al. [24] reported that nano-Fe2O3 promoted the growth and photosynthesis of peanut. Sheykhbaghlo et al. [39] showed that application of nano-iron oxide particles increased soybean yield. Prasad et al. [34] reported that nano-scale zinc oxide particles increased stem and root growth and pod yield of peanut as compared with ZnSO4 application. Suitable and useful usage of different kind of fertilizers is the main way for reformation and maintain of soil fertility and increasing of crops yield [37]. Metal chelate fertilizers can include many different chemicals that are usually referred to by their abbreviated names and include EDTA (ethylene-diamine-tetra-acetic acid), DTPA (diethylene-triamine-pentaacetic acid), or EDDHA (ethylene-diaminedi-o-hydroxyphenylacetic acid). All of these materials are water soluble, but it is important to choose the right one since they have different abilities to form stable metal complexes depending on the soil pH. For example, EDTA prefers to chelate calcium rather than iron or zinc at neutral to alkaline pH (pH > 7.0). For iron fertilization, the best chemical is EDDHA (Sequestrene 138). This metal chelator is highly stable and can last for several years in the soil since it recycles many times to dissolve more iron in the soil and deliver it to the roots. The applied amount of a trace metal fertilizer depends on the type of fertilizer material as well as the severity of the deficiency [11]. Welch and Graham [44] and Cakmak (2008) suggested that Fe deficiency in wheat grain can be alleviated by breeding and selection of cultivars that could absorb more Fe from the soil and accumulate it in the grain, whereas Yip [48] proposed that Fe deficiency could be overcome by food fortification. However, plant breeding is time consuming and iron fertilizers applied to crops by these methods may reach to target site of crops much below the minimum effective concentration. In addition, the effectiveness of inorganic and chelated forms of Fe fertilizers (FeSO4, FeEDTA, FeDTPA, FeEDDHA, Fe-citrate) in overcoming Fe deficiency is highly variable depending on their solubility, stability, penetration ability through leaf cuticle, mobility and translocation following diffusion into the leaf tissues [38,12]. Iron chelates based on EDDHA is stable in soil and prevents from iron deposition for a reasonable period of time. Chelation agent EDDHA stors ferric iron with high power and prevents from its deposition in soil. Thus the iron concentration in the soil increases but these fertilizers have a problem that is they very high cost [14].

Iron compounds can use as foliar on leaves and as seed coating. With production of nano fertilizers, this nano compounds rapidly and completely absorbed by plants and fix it's nutrients shortages and needs [14].

The use of nano fertilizer leads to an increased efficiency of the elements, reduce the toxicity of the soil, to at least reach the negative effects caused by the consumption of excessive consumption of fertilizers and reduce the frequency of application of fertilizers [14]. Harsini et al. [14] reported that Fe intake, increase yield and quantity of rapeseed and increase the height of the plant, the amount of nitrate reeducates activity and photosynthesis too. As well as studies showed that there was a significant linear relationship between Fe concentration and yield [14]. And similarly, Harsini et al. 2014 indicated that Strawberry fruit quality increased with foliar Fe fertilization. Harsini et al. [14] in an experiment comparison the effect of various Fe fertilizers on growth and propagation of Gladiolus and concluded that flowering Gladiolus occurs a few days earlier in Fe-enriched Peat and as well as cormel number per corm increase in this substrate. In another study, influence of Khazra iron nano fertilizer on rice yield Was examined and was shown that applied treatments have a significant effect to all Characteristics except grain Thousand weigh [14].

Iron chelate Nano fertilizer can be considered as a rich and reliable source of bivalentiron for plant because of its high stability and gradual release of iron in a wide pH range (3 to 11). One advantage of this Nano fertilizer is using no ethylene compounds in its structure. Ethylene enhances growth process and prevents appearing indications caused by chlorosisin leaves. Second advantage of iron chelate Nano fertilizer is increasing ratio of ferrous iron to ferric iron in chelate surface which results in increasing synthesis of chlorophyl in plant [16].
Base of iron nano fertilizer is natural quality and it made of organic and mineral material. This fertilizer is fully compatible with the environment and agricultural farms and organic materials with added to the soil to make it more organic material is to be [14], the results of the comparison effects of nano Fe chelate with Fe chelate on growth parameters of Ocimum basilicum showed that the replacement of iron fertilizer produced with nanotechnology in comparison with common Fe fertilizer can increase the growth of quantitative and qualitative plant in appropriate concentrations or less [33]. Regarding leaf Fe concentration, it was seen that the effect of foliar FeSO4 on leaf Fe concentrations was higher than of Fe-EDTA in Strawberry cultivars [14].

Nano-technology can present solution to increase the value of agricultural products and reducing environmental problems. By using of nano-particles and nano-powders, we can produce controlled or delayed releasing fertilizers. Nano-particles have high reactivity because of more specific surface area, more density of reactive areas, or increased reactivity of these areas on the particle surfaces. These features simplify the absorption of fertilizers and pesticides that produced in nano scale [5]. Studies showed that the effect of nanoparticles on plants can be beneficial (seedling growth and development) [51]. This trial was conducted to examine and determine the appropriate type of Fe fertilizer and method of its use to dispel the need for Fe in mung bean.

**MATERIAL AND METHODS**

In order to study, Comparison effects of nano-iron fertilizer chelate and Fe-EDDHA on growth parameters and antioxidant enzymes activity a pot experiment was with three replications in the research greenhouse of sciences faculty, Urmia University, Urmia, Iran in 2014. The physical-chemical characteristics of soil showed in Table 1.

<table>
<thead>
<tr>
<th>Field capacity (%)</th>
<th>pH EC (ms/cm) Clay (%)</th>
<th>Silt (%)</th>
<th>Sand (%)</th>
<th>Soil texture Fe</th>
<th></th>
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<tr>
<td>30.80</td>
<td>3.8</td>
<td>17</td>
<td>42</td>
<td>Loam</td>
<td>8.14</td>
</tr>
</tbody>
</table>

Treatments of experiment were consist of Soil application of Fe-EDDHA (S), Soil application of nano iron chelate (N), 10 ppm Fe-EDDHA (S1), 50 ppm Fe-EDDHA (S2), 100 ppm Fe-EDDHA (S3), 250 ppm Fe-EDDHA (S4), 10 ppm nano iron chelate (N1), 50 PPM nano iron chelate (N2), 100 PPM nano iron chelate (N3), 250 PPM nano iron chelate (N4), Control (C). The youngest fully expanded leaves were used for these analyses. Measure the length of stems and roots By a millimeter ruler and aerial parts fresh and dry weight was measured with a digital scale with an accuracy of 0.001. Pigments were extracted with 80% acetone. Method for measuring chlorophyll content (Lichtenthaler and Wellburn (1985) was used. Peroxidase enzyme activity measured according to Aebi (1984) (by spectrophotometer instrument) and Asada and Nakano (1981), respectively. Leaf protein using was extracted 3 ml of glycine Tris buffer. Extraction method took place.

**Experimental Procedures:**

Experiment was performed to evaluate the effect of different concentrations of Fe-EDDHA and nano-iron chelate on green gram with three replications. The treatments in the experiment were four concentrations of Fe-EDDHA (10, 50, 100 and 250 ppm) and four concentrations of nano-iron chelate (10, 50, 100 and 250 ppm) and an untreated control. Modified plant seeds of mung bean (Vigna radiate) of Agricultural Research Center dezfull Safiabad were prepared and in cultured Room located in the Faculty of Sciences, Urmia University, were killed. Seeds with similar size were randomly selected and sterilized in a 10% sodium hypochlorite solution for 3 min then rinsed thoroughly several times with distilled water. Of each concentration to size of 30 mm was added to the soil, And then every 14 days for 4 times the pots were treated. There is no iron fertilization in control treatment, experiment with three replications. In this way experiments with 9 treatments in 3 replications (27 pots) were performed. And the number of 8 seed in pots prepared with sand and soil a ratio of 3: 1 was killed. Two months after planting growth indices, the pigments content chlorophyll, activity enzymes ascorbate peroxidase Katalazv leaves were investigated Data were analyzed by analysis of variance (ANOVA) and Excel software used to draw graphs. The differences between the means were compared by least significant difference test (LSD) (p<0.05).

**RESULTS AND DISCUSSIONS**

In general, nano-iron chelate (N50ppm) application produced the highest chl b contents followed by respectively control and Fe-EDDHA (S10ppm), (Figure 13). Iron application increased chl a contents as compared with the control, but there was no marked differences between iron rates on these traits (Figure 12). The activities of ascorbate peroxidase (APX) were affected by Fe-EDDHA (100 ppm) and nano-iron chelate (10 ppm), but the activity of catalase (CAT) was highest by application of nano-iron chelate but was No significant difference in compared by s10, s50, s100 Fe-EDDHA and control, (Figure 9 and 10). Fe-EDDHA and nano-iron
chelate just in shoot fresh weight and shoot dry weight and protein in compared with each other were significant differences, but in other growth parameters was no significant difference (Figure 1, 6 and 11). Results showed that growth parameters increased in plants treated with Fe-EDDHA (50 ppm) and nano Fe chelate with 10 ppm; and exposure of mung bean to 50 ppm Fe-EDDHA indicated the greatest shoot fresh weight, shoot dry weight (by 71.67 % and 71.97 % more than control group) related to other treatments. Increasing nanoparticles concentration above 10 ppm reduced shoot fresh weight, shoot dry weight. It has not found any significant effects by Fe-EDDHA and nano iron chelate on elongation of shoot and root of mung bean (Figure 4 and 5). Application of 50 ppm concentration of nano iron chelate increased shoot length by 18.43% in comparison to the control, also 50 ppm concentration of Fe-EDDHA increased shoot length by 30.82% in comparison to the control; while 50 ppm nanoparticles iron in comparison to the 50 ppm iron chelate Not significant. The highest root biomass was achieved from concentration of 50 ppm Fe-EDDHA, but an increased concentrations of nano iron chelate significantly reduced root weight, results showed Differences in shoot dry weight, shoot fresh weight, leaf fresh weight, leaf dry weight, root fresh weight, root dry weight mung bean in the nano-iron chelate fertilizer in chelated fertilizer treatments (at 0.05) is significant. The highest mean shoot fresh weight (1.09 g), root fresh weight (2.08 g), leaf fresh weight (1.87 g), shoot dry weight (0.27 g), root dry weight (0.37 g), leaf dry weight (0.31 g) and shoot length (33 cm) was observed in treated S50 ppm. The highest mean root length was observed in the treated S250, (Figure 5). Most of the growth parameters except root length in treatment Fe-EDDHA (550ppm) was observed.

Shoot fresh weight concentrations of N10 to N250 reduced, N10 the highest fresh weight of shoot (0.878g) and the increase in shoot fresh weight of treated nano-iron chelate (N10) was significant compared to other treatments and control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in shoot fresh weight were respectively (0.561g and 1.097g), but it increased with increasing concentrations of 50 to 250 ppm caused a significant reduction shoot fresh weight that it was decrease, respectively, (1.097g, 0.663g and 0.648g).

Moghadam et al.,2012 reported Average wet weight of spinach aerial organs increased by different concentrations of iron chelate Nano fertilizer and using 4 kg/ha Nano fertilizer caused wet weight to increase 58% comparing to witness (Lack of fertilizer or control). These results are consistent with the results of the experiment that nano-iron chelate fertilizer (N10ppm) showed a significant increase compared to control group.

![Fig. 1: Means comparison of shoot fresh weight mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano -iron chelate at different concentration. The similar letters show non significant difference at P≤0.05.](image)

Root fresh weight concentrations of N10 to N250 reduced, N10 the highest fresh weight of root (2.01g) and the increase in root fresh weight of treated Nano-iron chelate (N10) was significant compared to other treatments and control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in root fresh weight were respectively (1.17g and 2.08g), but it increased with increasing concentrations of 50 to 250 ppm caused a significant reduction root fresh weight that it was decrease respectively, (2.08g, 1.30g and 1.12g ). Peyvandi et al. [33] Reported the highest mean dry and fresh weight of stems, roots, leaves at the lowest concentration of nano-iron chelated. Mohamadipoor et al. [30] reported the highest amounts of fresh weight root with 27.78 g was recorded from control treatment. However, no significant statistical difference was observed in this treatment with EDDHA and nano iron fertilizer treatments with 23.45and 22.85 g respectively. Also, data mean comparison shows that the best treatment for increasing the was EDDHA treatment that didn't show significant statistical difference with control and nano iron fertilizer treatments, This results are quite different from the results of this experiment may be due to differences in plant species.
Fig. 2: Means comparison of root fresh weight mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano-iron chelate at different concentration. The similar letters show non significant difference at P≤0.05

Appropriate use of different fertilizers creates more leaf area by producing more leafy texture and consequently leads to higher performance [29]. According to an increase in photosynthesis and plant dry weight, wet weight increased due to increase of maximum leaf area in treatments by iron chelate Nano fertilizer. Also Burger et al. [10] studied effect of iron chelate Nano fertilizer on qualitative and quantitative properties of various cut flowers and found that treatments by 1 and 1.5 gr/L iron chelate Nano fertilizer with possibility of 95% have a positive and significant effect on increasing this index [29].

Leaf fresh weight concentrations of N10 to N250 reduced, N10 the highest leaf fresh weight (1.53g) and the increase in leaf fresh weight of treated Nano-iron chelate (N10) was not significant compared to control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in leaf fresh weight were respectively (1.40g and 1.87g), but it increased with increasing concentrations of 50 to 250 ppm caused a significant reduction leaf fresh weight that it was decrease, respectively, (1.87g, 1.19g and 0.89g).

Fig. 3: Means comparison of leaf fresh weight mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano-iron chelate at different concentration. The similar letters show non significant difference at P≤0.05

Shoot lengh, concentrations of N50 to N250 decreed, N50 the highest Shoot lengh (30 cm) and the increase in Shoot lengh of treated Nano-iron chelate (N50 ppm) was significant compared to N100, N250 and control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in Shoot lengh were respectively (28 cm and 33 cm), but it increased with increasing concentrations of 50 to 250 ppm caused a significant reduction Shoot lengh that it was decrease respectively (33 cm, 26.66 cm and 25 cm). Yarnia et al. [48] reported that Fe intake, increase yield and quantity of rapeseed and increase the height of the plant, the amount of nitrate reductase activity and photosynthesis too. Nazaran et al 2009 Effect of foliar application of nano-organic iron chelated fertilizer on quantitative and qualitative characteristics of the dry land wheat results reached Nano iron chelate foliar application fertilizers at stem elongation stage best performance
with an increase of 99%. And an increase of 32.4% amount of iron seeds and increase the quantity and quality reported relative to control. Rajab beig et al., 2007 reported Basil plants grown treated with iron fell sharply compared with the control group. Before heavy metals can be defined and the term used in Life Sciences, it is necessary to mention two well known facts: first of all, heavy metals are not toxic per se but only when a certain threshold of internal concentrations exceeded. Secondly, some elements, called micronutrients or trace elements, have essential functions in plant cells. This has been shown for Co, Cu, Fe, Mn, Mo, Ni and Zn. Only when the internal concentration exceeds a certain threshold do they exert toxic effects and then they are commonly named heavy metals. Micronutrients are essential for biosynthesis and function of nucleic acids, growth substances, chlorophyll and secondary metabolites, carbohydrates as well as for growth and stress resistance. [21].

Foliar application of microelements such as Fe, Cu and Zn at the lowest amount in sugarcane caused increasing of plant weight, internode number, internode length and subsequently increasing of stem length and plant height [14]. Kumar et al., [22] reported that using of copper (1.5 mg.kg), increased in about 23% wheat height in compare with control treatment. El-Kassas [25] reported that application of iron to the soil or the foliage as chelate or sulphates improved the vegetative growth, gross yield and fruit quality of balady lime. Application of chelate iron Fe-EDDHA to the soil gave the highest response [30]. Liu et al. [24] reported that nano-Fe2O3 promoted the growth and photosynthesis of peanut. Sheykhabaglo et al. (2010) showed that application of nano-iron oxide particles increased soybean yield. Prasad et al. [34] reported that nano-scale zinc oxide particles increased stem and root growth and pod yield of peanut as compared with ZnSO4 application. The effects of heavy metals on plants are different in different growth stages of plants. In the early stage, Cd inhibits the photosynthesis and growth of rice, then inhibits the reproductive organs' differentiation, and finally distributes the nutrients transport and mobilization (Wang 1996), but a low concentration of Hg (10 -s mol/L) stimulated the growth of wheat seedlings. The reason for this may be that low concentrations of Hg increased the activities of amylase, proteinase and lipase, speed up the decomposition of endosperm and the respiration rate, so that the germination was more rapid (Ma and Hong 1998). According to the effects of mentioned heavy metals can be said iron as a heavy metal at low concentrations increased stem length. Concentration 50 ppm is Optimum concentrations in both fertilization for plants.

![Fig. 4: Means comparison of shoot length mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano-iron chelate at different concentration. The similar letters show non significant difference at P≤0.05](image)

N250 ppm the highest root length (21 cm) and the increase in root length of treated Nano-iron chelate (N250 ppm) was not significant compared to other treatments and control group. Application Fe-EDDHA, S250 ppm Maximum root length was (23.50 cm),and was significant (21.57%) compared to control group. Prasad et al. [34] reported that nano-scale zinc oxide particles increased stem and root growth and pod yield of peanut as compared with ZnSO4 application. Peyvandi et al [33] reported Average length of roots at the lowest concentration of iron chelated nanoparticles showed no significant difference with other treatments. and showed the highest growth parameters were observed at the highest concentration of iron chelates. The roots were one of the target organs of Cd pollution, so that the root growth of crops such as wheat, maize, pumpkin [24], cucumber (Chen 1990), and garlic (Allium sativum L) (Liu et al. 2000) were inhibited. In contrast to the above, maybe concentration of 250 ppm was not toxic to plants whose roots length increased compared to controls. Mohamadipoor et al. [30] reported Highest root length (25.01 cm) obtained from the control treatment that shows significant difference to other treatments. Nano iron fertilizer, EDDHA and FeSO4 treatments with 21.48, 21.73 and 23.01 cm respectively, didn’t show significant difference with each other. But they had significant difference with Fe-EDTA (the lowest root length).
Shoot dry weight concentrations of N10 to N250 reduced, N10 the highest Shoot dry weight (0.193g) and the increase in shoot dry weight of treated nano-iron chelate (N10) was significant compared to other treatments but was no significant compared to control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in shoot dry weight were respectively (0.184g and 0.270g), but it increased with increasing concentrations of 50 to 250 ppm caused a significant reduction shoot dry weight that it was decrease respectively, (0.270g, 0.151g and 0.144g).

Results of [2]. Hodgsin et al. [15] show that application of iron in soil before cultivation increases amount of soybean aerial organs dry weight by 46% in early vegetative season. Kumusu et al. and Kuhura et al. showed that iron chelate fertilizer increases significantly performance of tomato comparing to other iron fertilizers [18,20]. Peyvandi et al. [32] Reported the highest mean dry and fresh weight of stems, roots, leaves at the lowest concentration of nano-iron chelated, iron chelate showed the highest growth parameters were observed at the highest concentration of iron chelates.

Mohamadipoor et al. 2013 reported highest of dry weight of root with 5.53 g was recorded from EDDHA in soil method and lowest of this trait was recorded from Fe-EDTA in soil and foliar application with 0.9 and 0.63 g respectively. Root dry weight concentrations of N10 to N250 reduced, N10 the highest root dry weight (0.344g) and the increase in root dry weight of treated nano-iron chelate (N10) was significant compared to control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in root dry weight were respectively (0.175g and 0.375g), but it increased with increasing concentrations of 50 to 250 ppm caused a significant reduction root dry weight that it was decrease respectively, (0.375g, 0.223g and 0.172g).
Leaf dry weight concentrations of N50 to N250 reduced, N50 the highest leaf dry weight (0.323g) and the increase in leaf dry weight of treated nano-iron chelate (N50) was significant compared to other treatments control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in leaf dry weight were respectively (0.202g and 0.315g), but it increased with increasing concentrations of 50 to 250 ppm caused a significant reduction leaf dry weight that it was decrease respectively, (0.315g, 0.188g and 0.185g). It seems that the use of iron nano-particles causes increasing in pod and dry leaf weight and finally will increase total yield [39]. Iron is necessary for chlorophyll production and increasing of chlorophyll cause to increasing of leaf area and leaf weight. Iron deficit reduce leaf weight, leaf area, iron concentration and chlorophyll [26]. Thereby, iron treatment because of its effect on leaf area index and chlorophyll and subsequently increasing of photosynthesis had effective impact on dry matter accumulation. At higher concentrations, the dry matter content is low probably due to the higher concentration of heavy metals such as iron, are toxic for plants.

**Changes activity of antioxidant enzymes:**

Activities of ascorbate peroxidase (APX) and catalase activity (CAT) was affected by the nano-iron, but in compare to Fe-EDDHA and controls was not observed significant differences. the activities of these enzymes increased with decreasing iron rates. Increased in the enzymes activities might be due to triggering induction of CAT, POX and APX genes expression by iron application as reported in Brassica napus by Vansuyt et al. (1997). Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are important enzymes for plants protective enzymatic system. The harmonious interactions of the three enzymes make the balance of free radical production and elimination, and keep the level of free radicals in plants low to prevent the injury of cells by free
radical. The activities of POD, SOD and CAT are usually enhanced by heavy metal stress such as Cd, Cr^{6+}, Hg, Ni, Pb and Fe in low concentrations [23,31,43,46,47]. But, as the concentration of heavy metals increases, the activities decrease.

Ascorbate peroxidase, concentrations of N10 to N250 reduced, N10 the highest Ascorbate peroxidase (0.074g) and the increase in Ascorbate peroxidase of treated nano-iron chelate (N10) was significant compared to other treatments and control group. Application Fe-EDDHA, concentration of 10 to 100 ppm caused a significant increase in Ascorbate peroxidase were respectively (0.015g, 0.027 and 0.071g), but it increased with increasing concentrations of 100 to 250 ppm caused a significant reduction Ascorbate peroxidase that it was decrease respectively, (0.071g and 0.011g).

**Fig. 9:** Means comparison of Ascorbate peroxidase mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano -iron chelate at different concentration. The similar letters show non significant difference at P≤0.05

Catalase, concentrations of N10 to N250 reduced, N10 the highest Catalase (1.307g) and the increase in Catalase of treated nano-iron chelate (N10) was significant compared to other treatments and control group. Application Fe-EDDHA, concentration of 10 to 250 ppm caused Reduction in Catalase were respectively (1.006g, 0.986, 0.871 and 0.779g) and was no significent compared to control group.

**Fig. 10:** Means comparision of Catalase mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano -iron chelate at different concentration. The similar letters show non significant difference at P≤0.05

Abbas *et al.* [1] applied 0, 4, 8, 12 and 12 kg ha^{-1} in the form of iron sulphate to the soil and showed that iron fertilization increased Fe and protein contents of the wheat grain. Habib [13] reported that iron and protein contents of the wheat grain were enhanced. Zeidan *et al.* [50] applied foliar Fe fertilizer (1.0% FeSO4) and reported that Fe application increased protein and Fe contents of wheat grain. Zeidan *et al.* [50] applied foliar Fe fertilizer (1.0% FeSO4) and reported that Fe application increased Fe and protein contents of wheat grain. Hong *et al.* (1991) and Li *et al.* [23] reported that the dissolubility of proteins increased under Cd stress, which might be a detoxifying mechanism (e.g. Cd-protein produced to reduce the toxicity of Cd). On the other hand, the results of Qin *et al.* [35] showed that the dissolubility of proteins of B. chinensis seedlings decreased with an increase in Cd concentration higher than 0.1 mg/L.
The protein, concentrations of N10 to N250 reduced, N10 the highest protein (1.66g) and the increase in protein of treated nano-iron chelate (N10) was no significant compared to other treatments and control group. Application Fe-EDDHA, concentration of 10 to 50 ppm caused a significant increase in protein were respectively (1.86g, and 3.18g) , but it increased with increasing concentrations of 50 to 250 ppm caused reduction protein that it was decrease respectively, (3.18g,3.12 and 2.57g) but was no significant.

Changes pigment composition of leaves:

Effects of heavy metals on the content of chlorophyll and photosynthesis yield depend on the concentration of heavy metals. Exposed to Cr 6+, the content of chlorophyll of H. dubia leaves rose in the solution of 4 and 8 mg/kg in the first few days, although it was reduced in 16 and 32 mg/kg treatments [47]. The same results were observed for B. schreberi, where the content of chlorophyll decreased and the chlorophyll a/b ratio was kept at 2 under Cr 6§ stress (10-40 mg/L) [46]. Sun and Wang, [43], however, reported that the chlorophyll a/b ratio of macrophytes decreased as the concentration of Cd increased and that Fe 3§ could abate the increasing trend [23]. Heavy metals affected the function of PSI and PS II, and it was stronger with the latter [45]. The chlorophyll proteins, which took protons for photosynthesis in PS II, were decomposed and decreased under Cd stress. The sub-microstructure of chloroplast was changed and the membrane system was destroyed. Therefore, the capacity of taking protons declined and the photosynthesis function was influenced [31]. The photosynthesis of woody plants decreased under pollution with Cd from the atmosphere before the observed symptoms had occurred [17]. Thus, the photosynthetic yield would be one of the indicators for air pollution.

In general, nano-iron chelate (N50ppm) application produced the highest chl b, The application of Fe-EDDHA (S10ppm) The maximum amount of chlorophyll a and total chlorophyll (a + b) compared to nano-iron chelate and Control was not significant. Iron application increased chl a, b and total chlorophyll contents as compared with the control, but there was no marked differences between iron rates on these traits. That was perhaps due to the association of Fe with chlorophyll formation [27]. In line with our results, Liu et al. [24] reported that nano-Fe2O3 application increased chlorophyll content of peanut and Amanullah et al. (2012) showed that application of iron sulphate in soil and foliar spray increased chlorophyll content of maize leaf. Borowski and Michalek [7] reported that foliar application of iron salt increased chlorophyll a, b and carotenoid contents of French bean. Increased in chlorophyll b content of mung bean in our experiment could be due to promotion of the absorption and utilization of nutrients such as nitrogen by nano-Fe compound as concluded by Liu et al. [24].

Chlorophyll a, concentrations of N50 to N250 reduced, N50 the highest Chlorophyll a (14.69g) and the increase in Chlorophyll a of treated nano-iron chelate (N50) was significant compared to other treatments and but was no significant control group. Application Fe-EDDA, concentration of 10 to 100 ppm caused a significant decreased in Chlorophyll a were respectively (16.01g, 14.57 and 14.55g) , S10 ppm was the highest Chlorophyll a (16.01g) that in compare to S50 and S100 was significant but was no significant compare to control group. In accordance with the above, we can conclude that in the case of heavy metals in low concentrations of nano-iron chelate and chelate iron, the amount of photosynthesis and chlorophyll content is higher. Mohamadipoor et al. [30] reported The effect of iron fertilizer was so that the highest chlorophyll content (20.7) was obtained from EDDHA treatment. After EDDHA treatment, highest amounts of this index with the amounts of 14.86 and 12.43 was recorded from FeSo4 and nano iron fertilizer respectively, that were on a higher level compared to the control treatment with the amount 6.61 and Fe-EDTA treatment with the
amount 5.6, consistent with these results, the highest chlorophyll a content of the iron chelate fertilizer (S10 ppm) were found that, compared with nano-chelated fertilizer (N50 ppm) and control group was not significant. also Mohamadipoor et al. [30] reported In this index (chlorophyll), the amount of chlorophyll of the nano iron fertilizer was on higher level from control treatment but no superior have than EDDHA treatment These results are disagreement with the results of the amount of chlorophyll in application of nano iron fertilizer on Ocimum Basilicum plant by peyvendi et al. [33], this results are consistent with the results of this experiments that nano-iron chelate fertilizer (N50 ppm) was lower compare to the control group and showed no significant differences in comparison to control group and chelated fertilizer (S10 ppm).

![Fig. 12: Means comparison of Chlorophyll a mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano -iron chelate at different concentration. The similar letters show non significant difference at P≤0.05](image)

Fig. 12: Means comparison of Chlorophyll a mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano -iron chelate at different concentration. The similar letters show non significant difference at P≤0.05

Chlorophyll b, concentrations of N50 to N250 reduced, N50 the highest Chlorophyll b (5.30g) and the increase in Chlorophyll b of treated nano-iron chelate (N50) was significant compared to other treatments and but was no significant control group. Application Fe-EDDHA, concentration of 10 to 100 ppm caused a significant decreased in Chlorophyll b were respectively (4.70g, 4.04and 4.06g) , S10 ppm was the highest Chlorophyll b (4.70g) that in compare to S50 and S100 was significant but was no significant compare to control group.

![Fig. 13: Means comparison of Chlorophyll b mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano -iron chelate at different concentration. The similar letters show non significant difference at P≤0.05](image)

Fig. 13: Means comparison of Chlorophyll b mung bean (vigna radiata) plants affected by Soil application Fe-EDDHA and nano -iron chelate at different concentration. The similar letters show non significant difference at P≤0.05

Chlorophyll(b+a), concentrations of N50 to N250 reduced, N50 the highest Chlorophyll (b+a) (20g) and the increase in Chlorophyll (b+a) of treated nano-iron chelate (N50) was significant compared to other treatments and but was no significant control group. Application Fe-EDDHA, concentration of 10 to 100 ppm caused a significant decreased in Chlorophyll (b+a) were respectively (20.72g, 18.62 and 18.61g) , S10 ppm was the highest Chlorophyll (b+a) (20.72g) that in compare to S50 and S100 was significant but was no significant compare to control group.
Conclusion:

Application of Fe-EDDHA increased shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, leaf fresh weight, leaf dry leaf, shoot length, root length, chlorophyll a, total chlorophyll (a+b) and protein. But application of nano-iron chelate increased enzyme activities ascorbate peroxidase and catalase. Among two fertilizers treatment nano iron fertilizer and EDDHA that in most growth characteristics were on the similar surface, but nano iron fertilizer was significant superiority than other fertilizers particularly EDDHA, in the concentration of the plant nutrients. So use of nano iron fertilizer in addition to cost-effectiveness and cost much less than the imported fertilizer EDDHA, of terms reducing the harmful effects of chemical fertilizers such as FeSO₄ on the environment also has advantage. By employing nano iron fertilizer as alternative to common fertilizers, nutrients of the fertilizer are released in soil gradually and to be controlled and caused reduce the toxicity of the soil and to at least reach the negative effects due to excessive consumption of fertilizers. Application of nano iron fertilizer in foliar method recommended and EDDHA to both methods 'foliar and soil application' can be useful.

Abbreviations:

Fe-EDDHA: ethylene-diaminedi-o-hydroxyphenylacetic acid; spss: Statistical Package for the Social Sciences; ANOVA: Analysis of Variation; S: sequestrin iron fertilizer; N: nano-iron chelate.

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REFERENCES


