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Research Article

Shortage on (Magnesium and Calcium) Dry Weight, Fresh Weight, Root and Shoot Length, Leaf Relative Water Content (RWC), Chlorophyll Content and Malondialdehyde Activity in Fenugreek (*Trigonella Foenum Graceum*)

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ABSTRACT

Fenugreek (*Trigonella Foenum Graceum*) is one of the medicinal plants that have been used as a folk medicine in Iran and many countries. In this study, effects of macronutrient element shortage (Nitrogen, Phosphorous, Potassium, Magnesium and Calcium) on dry and fresh weight, root and shoot length, Leaf Relative Water and chlorophyll content in Fenugreek plant was investigated. In relation to Magnesium shortage which caused, increase of root and shoot dry weight, root and shoot fresh weight, root and shoot length and leaf relative water content, it was observed that this increment was meaningful in 0/05 of statistical level. In Calcium shortage, root and shoot length, root and shoot dry weight and shoot fresh weight decreased, which in statistical level 0/05 showed meaningful difference than blank level, while in relation to leaf relative water content, there was no meaningful difference to the control level. In Potassium shortage, shoot length, root and shoot fresh weight and leaf relative water content meaningful increased, while root and shoot dry weight, observed decrement in statistical level of 0/05 which was meaningful than blank level. Investigating the effect of Phosphorous shortage, shoot length meaningfully increased than in blank level, while in root dry weight, shoot fresh weight and leaf relative water content, meaningful decrement was observed than in the blank level ($P<0.05$). In Nitrogen shortage, root length and fresh weight and leaf relative water content meaningfully increased than blank, while shoot fresh weight, root and shoot dry weight were meaningfully decreased in statistical levels of 0/05 than blank. Macronutrient elements shortage caused decrement of chlorophyll in Fenugreek leaves with Nitrogen, Potassium and Phosphorous recording shortage than the blank level. In plants with Calcium and magnesium shortage, increase of chlorophyll was observed. Macronutrient elements shortage caused meaningful decrease in chlorophyll b and total chlorophyll concentration in Fenugreek leaves ($P<0.05$). Also, macronutrient elements shortage caused decrease in Malondialdehyde activity in root and shoot of Fenugreek plant.

INTRODUCTION

Fenugreek(*Trigonellafoenum* L.) is an angiosperm plant. This plant has dicotyledonous petals from the order of roses, peas groups, subgroups of groups and genus (Trigonela L.) from group trifoliate. The name of this plant is combined from the Greek word trigon and Foenum-graecum. Fenugreek is herbaceous and an annual plant that has its height at 50cm. Fenugreek is one of the medicinal plants that have been used in Iran and several nations traditional medicines and it has many health benefits. Fenugreek has a wide range of therapeutic effects such as analgesic, anti-atherosclerotic, anti-inflammatory, carminative, antispasmodic, anti-cancer, chelating blood sugar, increasing sexual desire, astringent, cardiac tonic, bladder, laxative, expectorant diuretic, reducing cholesterol, reducing blood lipids, reducing blood pressure, uterine tonic, anti-worm. Growth and yield of agricultural plants is function of environmental factors and their interactions. Lowit (1980)suggests that stress is a result of abnormal physiological processes obtained from the effects of a combination of environmental factors. Actually, the amount and severity of these adverse factors can cause problems in organisms and also direct and indirect damages in plant or its components. These limiting factors are called environmental stress and they are divided into two types: biological and non-biological (Meybodi,1381).Mature stage has significant effects on plant minerals and one of the most important influences is mature plants with high phosphorus reduction. Nitrogen, phosphorus and potassium in young plants and young tissue plants are high. On the other hand, calcium, manganese and iron are greater in older plants and more mature parts of the plant. Levels of mineral will change with increasing plant age. Calcium increases with increasing plant age while P reduces with increasing plant age. In different regions of world, concentration of minerals in plants, depend on the interaction of several factors such as soil, plant species, and stage of growth, climate, and production and interaction elements in the absorption time. Plants reduce the harmful effect of stress with increase in metabolism and sets osmotic potential by the accumulation of organic and inorganic materials in the cells.This method works by the absorption of inorganic ions by plant in the external environment such as increasing the concentration of potassium in the shoots and/or synthesis of compatible solutes by high concentrations of materials such as osmolyte In response to stress, changes created in the ionic current. Sodium including elements that will accumulate when applied to environmental stress in the plant and it can lead to a disrupted membrane and inhibition of ion transport of nutrients from roots to shoots. Magnesium is a mineral component of the chlorophyll molecule. Magnesium contributes in the construction of the plant oil and it sets phosphorus uptake in the plant and also it is effective in hydrocarbon and sugar production. One of the symptoms of magnesium deficiency in plants is the yellowing between the veins. First, the symptoms of deficiency are observed in old leaves and leaves fall by acute shortage. Calcium plays an important role in the stability of the cell wall, cell development and internal processes, membrane stability, balance of cations and anions, activator of some enzymes and osmotic pressure. Calcium deficiency causes analysis of cell wall and dried leaves. This element is necessary for synthesis and transport of nutrients in plants and eliminates some adverse effects of nutrient imbalances of plants in soils. Also this element is effective in the regulation of water uptake. Phosphorus becomes unusable because of their specific reactions such as soil adsorption and sediment formation for plant. In calcareous soils and calcareous – plaster (soils of Iran), one of the problems of plant nutrition in the soils is the lack of plants with available phosphorus because conversion of soluble phosphorus to less soluble compounds such as calcium phosphates. Therefore the entrance of P in different reactions with composition of soil and low phosphorus availability in soil, amounts of this element is necessary for appropriate concentrations of growth in soils (Govere, 2004).

MATERIALS AND METHODS

Fenugreek seeds were prepared from the research institute of forest and rangeland and study was performed in the plant biology laboratory in Islamic Azad University of Tonekabon in spring 1390.Produced seeds had the same size. For disinfection of seeds, we used 5% sodium hypochlorite (Distilled Water 5%) in 10 minutes and then seeds were washed with distilled water (Falahati, 2007). For germination, seeds were cultured in medium Petri dishes in germinator with temperature of 25° C. After four days of growth, seeds were planted in pots with 30 cm diameter and length with sand planted. Then, pots were placed in a growth room, with temperature of 1 ± 23 (1 ± 26 °c during the dark and light periods) and light intensity 25 kilo lux and 16-h photoperiod. In the first week after planting in pots, irrigation was performed with 40 ml of distilled water. After the first week order to supply of mineral, irrigation was performed by 40 ml of distilled water with Hoagland solution. After 13 days according to the deficiency, 40 ml prepared solution was given to each pot. Number of pots for 5 treatments with 4 replicates and a control group were included.

Treatment of Plant

After 7 days, Hoagland solution with PH of 5/7 was given to all pots shown in table 1. Also, the material requirements expressed in table 1 is to apply for any deficiency (Norikane, 2003). Order to evaluate dry weight,

seedlings were placed in an oven with temperature of 50 ° C and after 24 hours the dry weight was measured.

Statistical Analysis

Studies were conducted in a completely randomized design with four replicates and data analysis with analysis of variance and Duncan's mean comparison test reviewed by SPSS and chart drawn with Excel.

Table 1: Hoagland solution for supplying chemicals and imposed deficiencies

mM	Control	N	Ca	Mg	K	P
2	KNO3	K2SO4	KNO3	KNO3	NaNO3	KNO3
2	Ca(NO3)2	CaCl2	NaNO3	Ca(NO3)2	Ca(NO3)2	Ca(NO3)2
1	MgSO4.7H2O	MgSO4.7H2O	MgSO4.7H2O	Na2 SO4	MgSO4.7H2O	MgSO4.7H2O
0/67	NaH2PO4.2H2O	NaH2PO4.2H2O	NaH2PO4.2H2O	NaH2PO4.2H2O	NaH2PO4.2H2O	NaCL
0/05	Fe-EDTA	Fe-EDTA	Fe-EDTA	Fe-EDTA	Fe-EDTA	Fe-EDTA
5	Mn SO4.H2O	Mn SO4.H2O	Mn SO4.H2O	Mn SO4.H2O	Mn SO4.H2O	Mn SO4.H2O
0/05	Cu SO4.5 H2O	Cu SO4.5 H2O	Cu SO4.5 H2O	Cu SO4.5 H2O	Cu SO4.5 H2O	Cu SO4.5 H2O
0/5	Zn SO4.7H2O	Zn SO4.7H2O	Zn SO4.7H2O	Zn SO4.7H2O	Zn SO4.7H2O	Zn SO4.7H2O
16/5	H3BO3	H3BO3	H3BO3	H3BO3	H3BO3	H3BO3
0/1	Na MoO4.2H2O	Na MoO4.2H2O	Na MoO4.2H2O	Na MoO4.2H2O	Na MoO4.2H2O	Na MoO4.2H2O
0/05	CoSO4.7H2O	CoSO4.7H2O	CoSO4.7H2O	CoSO4.7H2O	CoSO4.7H2O	CoSO4.7H2O
0/05	Ni SO4.7H2O	Ni SO4.7H2O	Ni SO4.7H2O	Ni SO4.7H2O	Ni SO4.7H2O	Ni SO4.7H2O

After 42 days of planting the seeds in pots and 5 periods of treatment, plants were removed from pots and then the root length, length of shoot, root fresh weight, shoot fresh weight, relative water content, measurement of chlorophyll and measurement of malondialdehyde was calculated.

Relative Water of Shoot in Treated Plants and Control

First shoots were placed in two distilled water for 5 hours in Petri dish. After torgor, weight of torgor was measured with digital scales and the numbers were placed in following formula. It was calculated based on Smart and Bingham 1974.
DW =Dry weight
FW =Fresh weight
TW =Torgor weight

Measurement of MDA

Total size MDA was measured according to the method of Ohkawa 1979. According of this method, 01.0 g from fresh plant tissue (Root / Shoots) with Trichloroacetic acid 5% (TCA) was pulverized inside a mortar equal manner. Soluble moved the micro tube and separate operation was performed in rpm12000 centrifuge for 15 minutes. Thiobarbituric 5% was dissolved in TCA 20% and moved to test tubes for 25 minutes at a temperature of 100° C. Then,
these tubes were centrifuged at 10000 rpm for 5 minutes. Obtained solution uptake read at 532 nm and for review, data was read during the 600 nm wavelength. Extinction coefficient for MDA was 155 mM cm.

$$\hat{A} = \sum^{\wedge} \times B \times C$$

A =Absorption
$\sum^{\wedge}$ =Extinction Coefficient
B =Measured Cell Width
C=the Amount of Material

Measurement of Chlorophyll Content

The method of Arnon (1949) was used to measure chlorophyll. 1.0mg of fresh plant has been homogenized in the Chinese mortar by Stan. In order to prevent the withdrawal of magnesium in the chlorophyll building, calcium carbonate 5/0mM was added. The obtained solution was filtered with a Watman paper No. 2. Extract dilution was performed by 80% acetone. To calculate the amount of chlorophyll, absorbance was determined in the wavelengths of 645 and 663nm using 80% acetone control. Leaf chlorophyll content per mg per g wet tissue was calculated using the following formula:

Ca=(0/0127.A(663)-0/00269.A(645)mgr/mLit
Cb=(0/0229.A(645)-0/00486.A(663) mgr/mLit
CT=(0/0202.A(645)-0/0082.A(663) mgr/mLit

RESULTS

Several studies have been performed on plants and the effects of nutrient stress on the growth factors at seedling stage that it shows a drop in growth of stress. In this study, results showed reduce stress effects on growth. Significant difference was observed among the treatments during radicle and the highest growth rate was observed in plants deficient in nitrogen and magnesium in comparison with control (Figure 1). Changes in length of shoot, the effect of nutrient deficiencies showed significant increase ($p < 0.05$) in plants with magnesium deficiency than in control. Whereas a significant decrease was observed in plants with calcium deficiency than in the control; but the difference was not significant in plants with phosphorus and potassium deficiency than the control (Figure 2). The root dry weight was significantly different ($p < 0.05$) among the different treatments and according to the observation on plants with magnesium deficiency than in the control showed significant increase, while the difference was not significant in calcium deficiency than in the control (Figure 3).Also, root fresh weight showed a significant increase than control in the effects of magnesium deficiency($P < 0.05$) whereas there was no significant difference in calcium deficiency than control (Figure 5).Shoot fresh weight showed the highest increase than control in magnesium deficiency plants and weight was equal in plants with nitrogen and calcium deficiency(Figure 6). According to numbers calculated in the above named formula, it was found that relative water content shoots had significant increase in plants potassium deficiency than control whereas it was equal in plants with calcium and magnesium deficiency and had a significant decrease in plants with phosphorus deficiency than control (Figure 7).

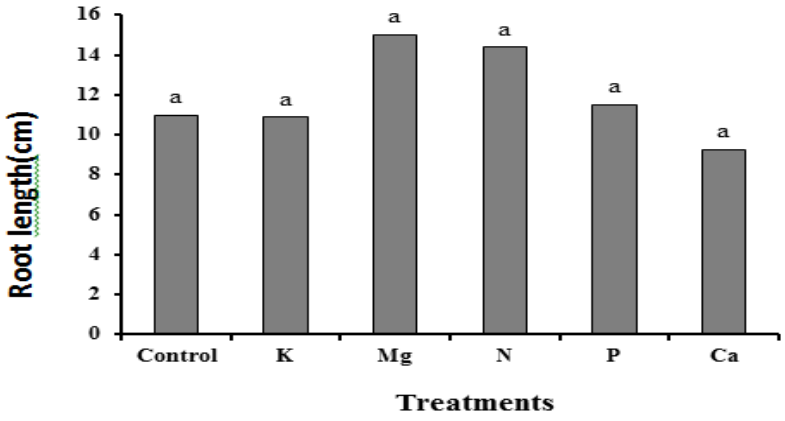

Figure 1: Effect of high-nutrient deficiency on root of Fenugreek

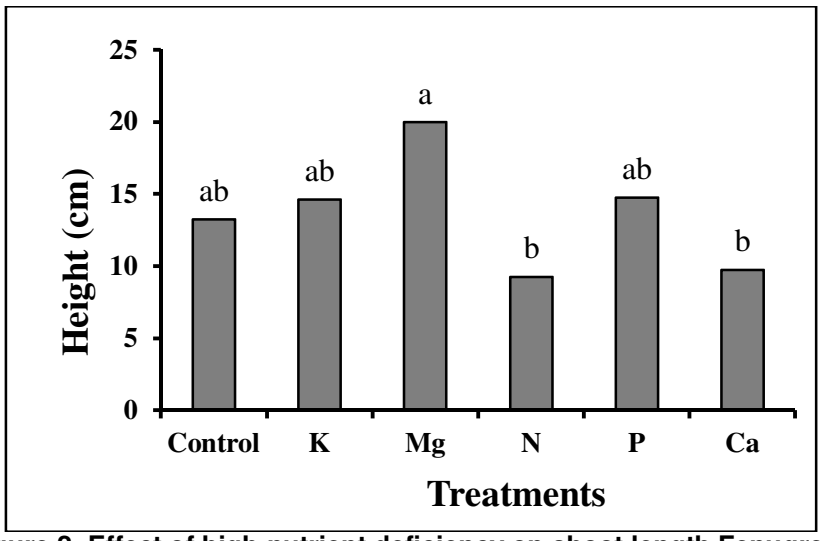

Figure 2: Effect of high-nutrient deficiency on shoot length Fenugreek

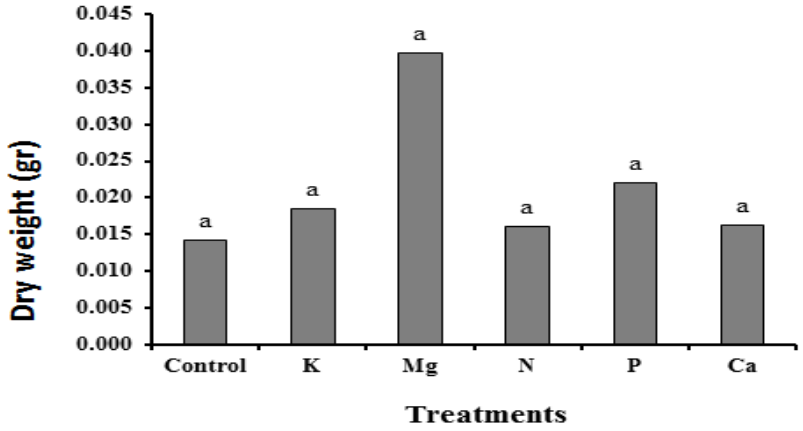

Figure 3: Effect of high-nutrient deficiency on dry weight of Fenugreek's root

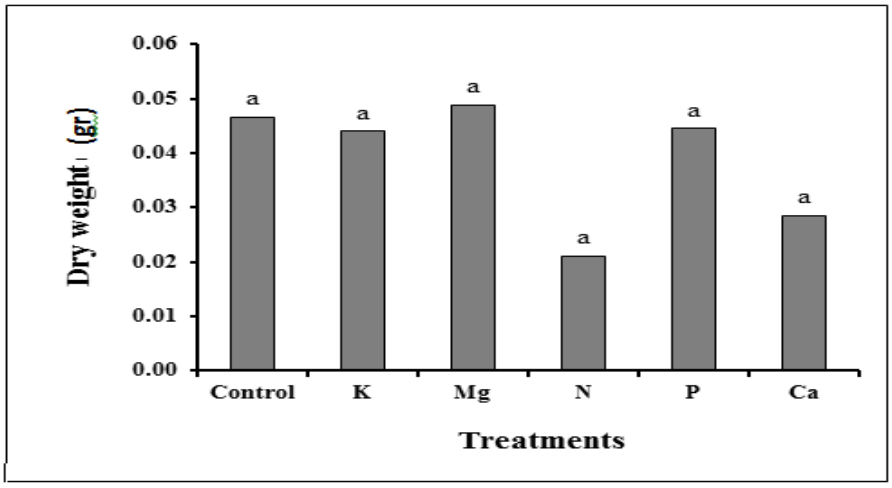

Figure 4: Effect of high-nutrient deficiency on dry weight of Fenugreek's shoot

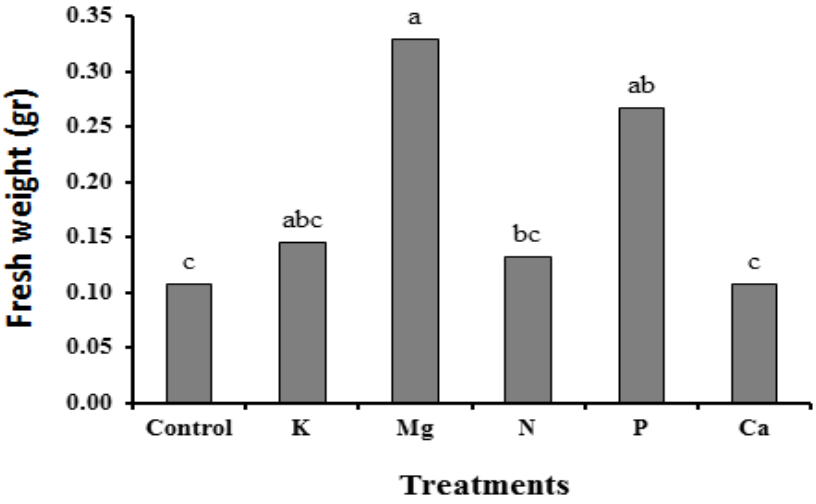

Figure 5: Effect of high-nutrient deficiency on fresh weight of Fenugreek's root

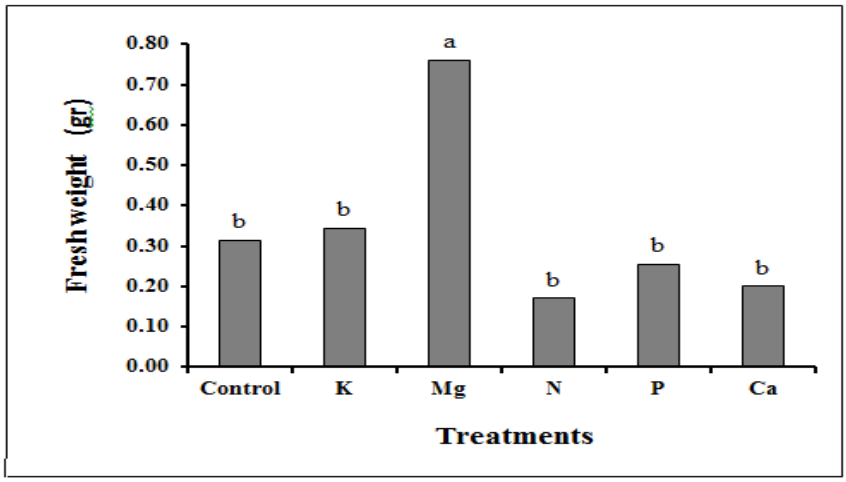

Figure 6: Effect of high-nutrient deficiency on fresh weight of Fenugreek's shoot

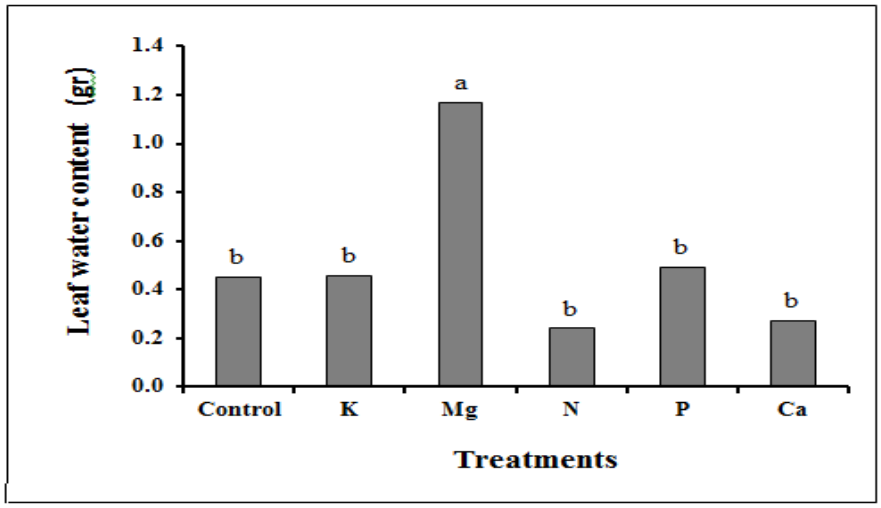

Figure 7: Effect of high-nutrient deficiency on relative water of Fenugreek's leaf

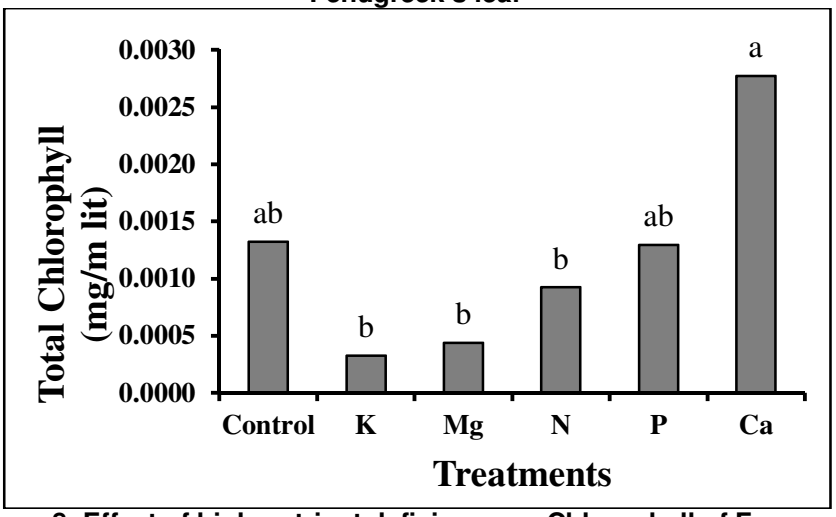

Figure 8: Effect of high-nutrient deficiency on Chlorophyll of Fenugreek

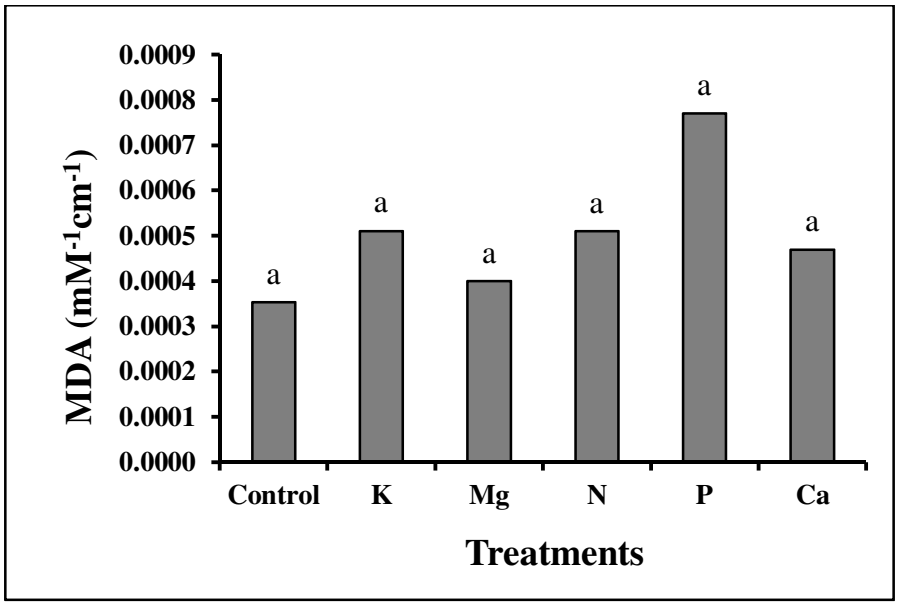

Figure 9: Effect of high-nutrient deficiency on Malondialdehyde of Fenugreek's shoot

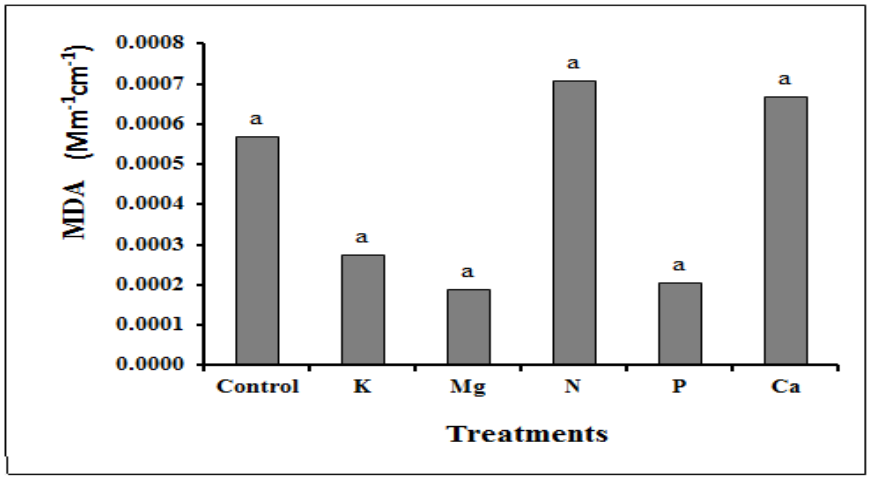

Figure 10: Effect of high-nutrient deficiency on Malondialdehyde of Fenugreek's root

DISCUSSION

In this study, root and shoot increased in plants with magnesium deficiency. Also dry and fresh weight increased in roots and shoots. Relative water content in magnesium deficiency plants had no significant difference compared to control ($P < 0.05$). Jones and et al (1996) found that nitrogen and magnesium are necessary in formation of chlorophyll. Magnesium transfers phosphorus to seed of plants and also enabled dehydrogenase and decarboxylase enzymes and it is also important in cellular respiration. This element enabled enzymes in oxidation and reduction in plants and it separates oxygen molecules in photosynthesis. In this study, in plants with nitrogen deficiency, dry weight of roots and shoots had significant decrease compared with controls. Root fresh weight increased in plants with nitrogen deficiency but shoot fresh weight reduced compared with controls. Relative water content increased compared to control in nitrogen deficit. Gayne G (1979), found that with development deficiency on citrus stem elongation almost stopped. As a result, nitrogen deficiency led to decrease dry in all seedlings and all parts maker (branches and leaves, stems, roots).With nitrogen deficiency, growth was stopped in 76% of all the seedlings and small leaves appeared in complete nutrition in the control group. In this study, nitrogen deficiency led to reducing growth factors. Alizadeh et al (2008) found that increasing nitrogen fertilizer leads to increased dry matter in shoot and root length in corn whereas with consume more nitrogen root dry matter decreased. Jeyhooni (2009) expressed that nitrogen deficiency led to the stoppage of growth in tomato shoots. Nitrogen excused 2 to 5 percent of dry and because nitrogen interferes in molecular structure of chlorophyll, there is a significant positive relationship between nitrogen content of leaves and the

amount of chlorophyll (Cassman et al, 1994).Range of chloric in treatments of nitrogen and magnesium deficiency is less than chloric in treatments of phosphorus, potassium and calcium deficiency (Marschner, 1995).After nitrogen, phosphorus is the most important element of plant and its availability, rises as a limiting factor in the production of agriculture products worldwide.(Afif et al, 1993). An important sign of phosphorus deficiency is draft and short plants. Often, phosphorus deficient plants are confused with young plants and in vein are lattice and brown in older leaves in deficiency conditions (Salim, 2007). In this study, it was observed that plant growth reduced throughout the shoots and roots. Also plant height was higher in control than nitrogen deficient plants in greenhouse. One reason for the low high in plants under phosphorus deficiency could be due to the phosphorus effect in grain growth period. High in all micronutrient deficiency plants was lower compared to control. Also in nitrogen, high was reduced greatly. The largest decrease was in growth and dry weight of nitrogen treatment. Phosphorus is one of the key elements which play an important task in plants. This element has a role in energy transfer in plant metabolic processes, cell division, phospholipids building walls of plant cells, reproductive parts of plants, growth and development of lateral roots and hair and in formation and transport like sugars and starches in plant (Biswas and Mukherjee,1991; Bennett,1996. Havlin et al.,1999Marschner,2002).In this study, we also obtained similar results. In this study, relative water content increased in plants deficient in potassium than controls. Potassium is necessary for the synthesis and transport of nutrients in plants and the removal of some plant nutrient imbalance in soil and regulation of water uptake.(Wang, 2000).Potassium is important as an active enzyme in many important metabolic processes in plants, like transport in phloem, osmotic balance and photosynthesis (Jeyanny et al, 2009).As a result, potassium deficiency, slows growth which leads to dramatic reduction in root diameter, high, number of branches leaf and citrus plant biomass.

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