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Effect of nitrogen, iron, magnesium, manganese and molybdenum deficiencies on biochemical and ecophysiological characteristics of pistachio seedling *(Pistacia vera)*

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Abstract. The effects of nitrogen, iron, magnesium, manganese and molybdenum deficiencies on ecophysiologicy, biochemical indices characteristics of pistachio seedling (*Pistacia Vera*) were studied in sand culture. The following treatments were employed: (i) Complete Hoagland's nutrient solution; (ii) Nutrient solution lacking N; (iii) Nutrient solution lacking Fe; (iv) Nutrient solution lacking Mg; (v) Nutrient solution lacking Mn; (vi) Nutrient solution lacking Mo; (vii) Nutrient solution lacking Fe and N; (viii) Nutrient solution lacking Fe and Mn; (ix) Nutrient solution lacking Fe-Mo; and (x) 10-distilled water. The experimental design used in this study was complete randomized block design with three replications. Analysis of the some biochemical indices (chlorophyll content) and ecophysiologicy indices such as primary florescence chlorophyll (Fo), ratio of variable chlorophyll to maximum (Fv/Fm), temperature of leaf area, transpiration, stomata conduction and resistance of stomata showed that fractions of chlorophyll, transpiration, stomata conduction and resistant of stomata in treated seedlings particularly N-deficient seedlings declined significantly. Chlorophyll (Fo) and ratio of variable chlorophyll to maximum (Fv/Fm) were significant at 5% level.

Keywords. Transpiration – Florescence – Hoagland – Stomata conduction – Resistance of stomata.

Effet de la carence en azote, fer, magnésium, manganèse et molybdène sur les caractéristiques biochimiques et écophysiologiques de semis de pistachier (*Pistacia vera*)

Résumé. *Les effets de la carence en azote, fer, magnésium, manganèse et molybdène sur les caractéristiques biochimiques et écophysiologiques de semis de pistachiers (*Pistacia vera*) ont été étudiés pour une culture sur lit de sable. Les traitements suivants ont été utilisés: (i) Solution nutritive complète de Hoagland ; (ii) Solution avec carence en N ; (iii) Solution avec carence en Fe ; (iv) Solution avec carence en Mg ; (v) Solution avec carence en Mn ; (vi) Solution avec carence en Mo ; (vii) Solution avec carence en Fe et N ; (viii) Solution avec carence en Fe et Mn ; (ix) Solution avec carence en Fe et Mo ; et (x) Eau distillée. Le dispositif expérimental utilisé dans cette étude consistait en blocs totalement aléatoires à trois répétitions. L'analyse de certains indices biochimiques (chlorophylle) et d'indices écophysiologiques tels que la chlorophylle dans la floraison primaire (Fo), le ratio de la chlorophylle variable par rapport au maximum (Fv / Fm), la température de la surface foliaire, la transpiration, la conduction stomatique et la résistance des stomates, ont montré que les fractions de la chlorophylle, la transpiration, la conduction stomatique et la résistance des stomates, en particulier pour les jeunes plants traités, surtout ceux carencés en N, ont diminué. La chlorophylle (Fo) et le ratio de la chlorophylle variable par rapport au maximum (Fv / Fm) ont été significatifs à 5%.*

Mots-clés. *Transpiration – Floraison – Hoagland – Conduction stomatique – Résistance des stomates.*

I – Introduction

Pistachio (*Pistacia vera*) is the principal tree and one of the most important horticultural products of Iran and during the past 50 years, has been embraced as one of the main commercial products. The main habitat of pistachio is the Middle East, especially Iran. Rafsanjan (a town) is one of the most important areas of Pistachio production in the world. In

2004, Iran had a share of 53% of the world's planted area. In 2003, the area of pistachio plantation in Kerman province was 45.5% and in Rafsanjan, about 20.6% of total world pistachio orchards. The total world pistachio production in 2003 was about 663.3 Kt, 16.6% originating from Rafsanjan. In 2003, Iran was the most important pistachio exporter with USA in second place, having a of 69% and 8.9% share, respectively of the world exports. In 2003, the five main pistachio importers were Hong Kong, Spain, Germany, Italy and China (Razavi, 2005). Kerman province, in particular the Rafsanjan region, has the largest number of different cultivars with recognizable characteristics in cultivation and use, compared with other areas of Iran, or with any other country. Despite its immense popularity and commercial importance, the nutritional aspects of pistachio have seldom been studied, especially at the nursery stage. Pistachio trees, as most plants, require 14 elements for normal growth and reproduction. These essential elements are classified as either macro- (N, P, K, Ca, Mg, S) or micronutrients (Fe, Mn, Cl, B, Cu, Zn, Ni, Mo) based on the concentration normally present in plants. Each is essential for particular functions in the plant (Uriu and Pearson, 1983). Plant nutrients are also important in disease resistance and fruit quality, and the balance between the various elements can affect pistachio plant health and productivity (Uriu and Pearson, 1984). Optimization of pistachio productivity and quality requires an understanding of the nutrient requirements of the tree, the factors that influence in ecophysiological and biochemical characteristics used to diagnose and correct deficiencies. The present study is aimed at investigating the effect of various nutrients elements on ecophysiological and biochemical characteristics to develop a specific set of recommendations for dealing with nutritional disorders that have been observed in pistachio seedlings grown in nurseries (Uriu and Pearson, 1984).

II – Material and methods

Seed of cv. 'Badami-e-zarand', the main pistachio rootstock in Iran's pistachio plantation area, were germinated in trays of sterilized sand and pre-treated for 24 h with 0.01% Captan solution. Perlite used in the experiments was thoroughly washed. Sterilized perlite (autoclave-sterilized: 121°C, 15 min, at 103 kPa) was used for the sand culture studies (Erowid, 2007). Hoagland's solution (Hoagland, 1948) for plant nutrition studies was divided into two stock solutions (described in detail below): with 4 macronutrients and 7 micronutrients. We used 1/2- or 1/4-strength Hoagland's solution for lower level nutrient conditions. These simple, but relative amounts and proportions are important because for deficiency studies, a micronutrient can be eliminated easily in this way. The studies were conducted inside polyethylene tubes (50 cm in height without restricting the growth of the main root), one plant/tube that was filled with sterilized perlite. Tubes were placed under a controlled environment glasshouse with day/night temperatures of 30/25°C, 30/35% relative humidity and a 16 h photoperiod. All the experimental seedlings received ½-strength Hoagland's nutrient solution (macro- and micronutrients) for a period of 10 days until they established well in perlite. The composition of the 1/2-strength nutrient solution, expressed in mmol l^{-1} was 30 KNO_3, 2.0 $Ca(NO_3)_2.4H_2O$, 1.0 $NH_4H_2PO_4$, 0.5 $MgSO_4.7H_2O$; and in µmol l^{-1}, 25 Cl, 13 B, 1.0 Mn, 1.0 Zn, 0.25 Cu, 0.25 Mo and 10 Fe (supplied as ferric-sodium ethylene diamin etetra acetate). Then the following treatments were employed: (i) Complete Hoagland's nutrient solution; (ii) Complete Hoagland's solution, but completely lacking one of the following: N, Fe, Mg, Mn, Mo and (iii) distilled water.

Biochemical indice (chlorophyll content) and ecophysiological indices (primary florescence chlorophyll (Fo), ratio of variable chlorophyll to maximum (Fv/Fm) (with Opti Sciences Inc ADC, UK), temperature of leaf area, transpiration, stomata Conduction and resistance of stomata (with LCA4, ADC, Bioscientific LTD, UK), photosynthesis (with the method of Starner &Hardley, 1967) were analysed.

1. Measuring of choloropyll content

Chlorophyll is vital for photosynthesis, which allows plants to obtain energy from light. Chlorophyll is a chlorin pigment, which is structurally similar to and produced through the same metabolic pathway as other porphyrin pigments such as heme. At the center of the chlorin ring there is a magnesium ion. Green plants have six closely-related photosynthetic pigments (Zare *et al.*, 1995) (in order of increasing polarity):

Carotene - an orange pigment
Xanthophyll - a yellow pigment
Chlorophyll a - a blue-green pigment
Chlorophyll b - a yellow-green pigment
Phaeophytin a - a gray-brown pigment
Phaeophytin b - a yellow-brown pigment (Cramer and Butler, -1968)

Chlorophyll a is the most common of the six, present in every plant that performs photosynthesis. The reason that there are so many pigments is that each absorbs light more efficiently in a different part of the spectrum. Chlorophyll a absorbs well at a wavelength of about 400-450 nm and at 650-700 nm; chlorophyll b at 450-500 nm and at 600-650 nm. Xanthophyll absorbs well at 400-530 nm. However, none of the pigments absorbs well in the green-yellow region, which is responsible for the abundant green we see in nature (Demmig *et al*, 1996). Measurement of the absorption of light is complicated by the solvent used to extract it from plant material, which affects the values obtained. In diethyl ether, chlorophyll a has approximate absorbance maxima of 430 nm and 662 nm, while chlorophyll b has approximate maxima of 453 nm and 642 nm (Hall and Rao, 1994). The absorption peaks of Chlorophyll a are at 665 nm and 465 nm. Chlorophyll a fluoresces at 673 nm. The peak molar absorption coefficient of chlorophyll a exceeds 105 M^{-1} cm^{-1}, which is among the highest for organic compounds.

The chlorophyll content of the leaf was estimated spectro-photometrically in a known aliquot 80% acetone extract. The absorbance was measured at 645 and 663 nm for the estimation of chlorophyll A, chlorophyll B and total chlorophyll. The following formulae suggested by Starner and Hardley (1967) were used for the estimation of different fractions of chlorophyll:

Chlorophyll A =12.7 (Abs. at 663 nm) - 2.69 (Abs. at 645 nm) x V/l000 x w
Chlorophyll B = 22.9 (Abs. at 645nm) -4.68 (Abs. at 663 nm) x VI 1000 x W
Total chlorophyll =20.2 (Abs. at 645nm) + 8.02 (Abs. at 663 nm) x V/1000 X W

where:

Abs = absorbance
V = final volume of chlorophyll extract (mg)
W =fresh weight of the leaf extract (g)

The experiments were laid out in a complete randomized block design with three replications and data was analyzed using MSTATC.

III – Results and discution

1. Chlorophyll content and photosynthesis

The plant shows deficiency help of biochemical parameters are also useful. In our study, the rates of chlorophyll were statistically significant at one percent level. The seedlings receiving complete nutrient solution had higher contents of all fractions of chlorophyll. The chlorophyll content of the leaves was significantly influenced by the deficiency of various nutrient elements

(Table 1). All the fractions of chlorophyll of treated seedlings, particularly N-deficient and Mn-deficient seedlings, reduced considerably (Fig. 1). This was because of inadequate supply of N for chloroplast protein synthesis (chlorosis of the older leaves). Therefore Nitrogen deficiency appeared as Yellow chlorotic patches in the older leaves and severe chlorosis of the entire seedling. Nitrogen deficiency was the obvious noticeable effect on the growth behavior of seedlings particularly with regard to shoot growth. This was because of decreasing of chlorophyll. In botany, chlorosis is a condition in which leaves produce insufficient chlorophyll.

Table1. Variance analysis results of the effect of nutrient deficiencies on biochemical parameters of seedlings

Mean of Square					
Source of varience	**Degree of freedom**	**Photosynthesis**	**Content of chlorophyll (mg/g in fresh leaf)**		
			Total chlorophyll	**Chlorophyll A**	**Chlorophyll B**
Treatment	9	5.626 *	0.001**	0.0004 **	0.0003**
Error	18	2.743	0.0002	0.0001	0.0001
CV %	–	5.49	4.58	19.3	4.3

ns = not significant; significant at *P = 0.05 or at **P = 0.001.

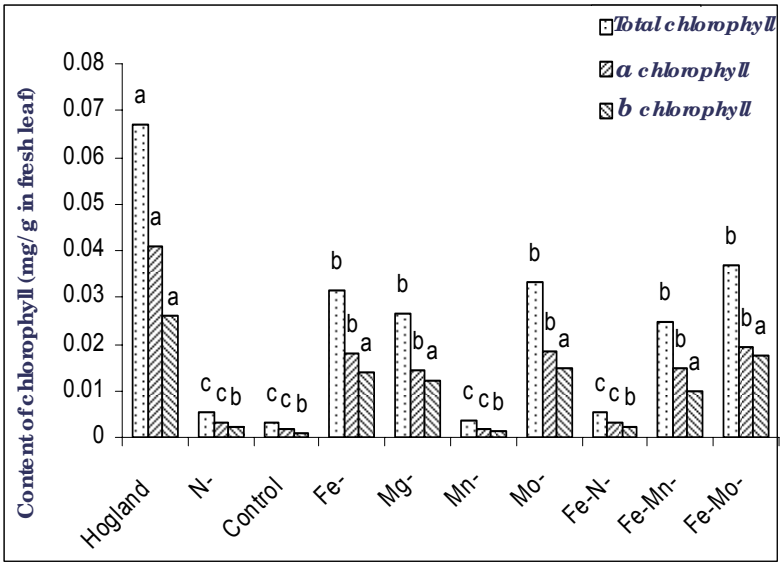

Fig. 1. The effect of nutrient deficiencies on biochemical parameters.

The affected plant has little or no ability to manufacture carbohydrates through photosynthesis and may die unless the cause of its chlorophyll insufficiency is treated. Specific nutrient deficiencies produce chlorosis (Goedheer, 1964). Reduced considerably in all the fractions of chlorophyll of treated seedlings, particularly nitrogen-deficient seedlings had been reported by Gopikumar and Varghese (2004) in teak, Paliwal *et al.* (2004) in soybean, Ronaghi *et al.* (2002) in spinach, Koal *et al*. (1972) in teak and Anoop (1993) in *Ailanthus*. The functions of

manganese are regarded as being closely associated with those of iron and as being concerned with chlorophyll formation. Hence, when manganese is deficient, chlorosis is a common symptom (Govindjee, 2007). Chlorophyll content also declined in Mn-deficient seedlings at the end of the study. Deeksha (1999) reported reduction in chlorophyll content, stomatal conductance and photosynthetic rate in the seedlings under deficiency. Kamala and Angadi (1998) observed a reduction in leaf area, and a decrease in amount of chlorophyll and photosynthetic efficiency of the leaves suffering trace element deficiency.

In a process called photosynthesis, plants use energy from the sun to change carbon dioxide (CO_2 - carbon and oxygen) and water (H_2O- hydrogen and oxygen) into starches and sugars. These starches and sugars are the plant's food (Goedheer, 1964). In the case of chlorophyll, a spectral analysis shows the wavelengths of sunlight absorbed, which is actually the combined absorption of two different chlorophylls, a and b. The maximum absorbance of chlorophyll a is at 420 and 660 nm and the maximum absorbance of chlorophyll b is at 435 and 643 nm (Hall and Rao, 1994). In leaves, chlorophyll is bound to thylakoid membranes in the chloroplasts, and absorbed wavelengths of light are converted to chemical energy. When chlorophyll is extracted from leaves, light energy cannot be transferred to the chloroplasts. Chlorophyll a is the most common in every plant that performs photosynthesis (Robert *et al,* 1990). Dabi, 1997 represented deficiency of different nutrient elements in leaves presumably due to break of chloroplast, earlier old age in leaves, decreasing of chlorophyll and finally decrease of photosynthesis. Also limiting of nutrient elements due to starch synthesis and accumulation of sugars has been limiting effect in photosynthetic chemical reactions that are equivalent reactions. Same and flore (1983) reported reduction photosynthesis was to correlate reduction nucleic acid and protein in cherry. Loss of photosynthetic can result from several processes including the breakdown of proteins (especially of PSII and Rubisco), the destruction of membranes by lipid degradation and decreased acid nucleic (Thomas, 1982, 1987). In our study, the rates of photosynthesis were statistically significant at 5 percent level (Table 1). The rate of photosynthesis showed decreased in treated seedlings compared with complete nutrition solution. Multi-deficiency of Fe-N- has the most effective in photosynthesis (Fig. 2). Chlorophyll molecules are specifically arranged in and around pigment protein complexes called photosystems which are embedded in the thylakoid membranes of chloroplasts (Zare *et al*., 1995). Chlorophyll and thylakoid proteins represent about 25% of the total nitrogen in a mature leaf (Evans, 1988), so if a significant proportion of protein. Nitrogen is used by plants to synthesize amino acids and nucleic acids that are necessary for all functions of the plant. The lower total N content is partly due to accumulated carbohydrates in leaf. So the deficiency of N, the rates of photosynthesis reduced. Photosynthesis depends on: fully expanded leaves in darkness with maximum amounts of chlorophyll, sufficient turgar pressure utilization of carbohydrates and present adult leaves with sphagnum mesophyll cells (Schaffr *et al.*, 1994). So rate of photosynthesis in the treated seedlings was dropped down. This was because of reduction growth parameters such as number, size leaves and the color (related to chlorophyll). As chlorophyll is responsible for the green colour of leaves, chlorotic leaves are pale, yellow, or yellow-white (Pavia *et al.*, 1982). The reason iron deficiency causes chlorosis is that iron is used in the active site of glutamyl-tRNA reductase, an enzyme needed for the formation of 5-Aminolevulinic acid which is a precursor of heme and chlorophyll (Nicol,1938).

2. Ecophysiological parameters

In our study Analysis of the ecophysiological indices such as primary florescence chlorophyll (Fo), ratio of variable chlorophyll to maximum (Fv/Fm), temperature of leaf area, transpiration, stomata conduction and resistance of stomata showed that fractions of chlorophyll, transpiration, stomata conduction and resistant of stomata in treated seedlings particularly N-deficient seedlings declined significantly (Table 1).

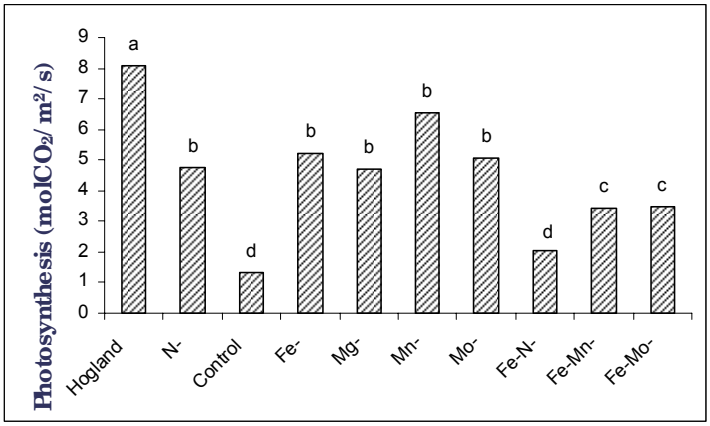

Fig. 2. The effect of nutrient deficiencies on photosynthesis.

Transpiration is the evaporation of water from the aerial parts of plants, especially leaves but also stems, flowers and roots. Leaf transpiration occurs through stomata which are found on the undersides of leaves, and can be thought of as a necessary "cost" associated with the opening of stomata to allow the diffusion of carbon dioxide gas from the air for photosynthesis. Transpiration also cools plants and enables mass flow of mineral nutrients from roots to shoots (Bilger *et al*, 1984). Mass flow is caused by the decrease in hydrostatic (water) pressure in the upper parts of the plants due to the diffusion of water out of stomata into the atmosphere. Water is absorbed at the roots by osmosis, and any dissolved mineral nutrients travel with it through the xylem. The rate of transpiration is directly related to the degree of stomatal opening, and to the evaporative demand of the atmosphere surrounding the leaf. Deficiency of elements can influence stomatal opening, and thus transpiration rate could be reduce (Fracheboud, 1999). The amount of transpiration by a plant depends on number, size leaf, leaf areas, plant's roots is used for this process. Transpiration rate of plants can be measured by a number of techniques, including potometers, lysimeters, porometers, and heat balance sap flow gauges (Demmig *et al.*, 1996). In the case of seedlings grown with nutrient solution lacking N and iron, showed a decreased in the transpiration (Fig. 3). Complete nutrient solution treatment recorded the highest value of transpiration. This was because of increasing photosynthesis and stomata conduction. There is linear correlation between photosynthesis and transpiration has been reported by David (2002) and Flexas *et al*. (2001). Transpiration rates go up as the temperature goes up, especially in deficiency of elements (Fig. 4). Transpiration rates go up especially during the growing season, when the number, size leaf, leaf areas, plant's roots are developing. Higher temperatures of leaf area cause the activity enzyme reduction and destroyed structure enzyme which control the gases transition and cause photosynthesis, Transpiration rate and stomata conduction decreased in treatments (Faust, 1989).

Fe deficiency has noticeable effect on the stomata conduction of seedlings because of importance in many enzyme systems and for energy transfer during photosynthesis. The seedlings received complete nutrient solution, had higher contents of all fractions of chlorophyll and photosynthesis, and showed an increasing in stomata conduction (Fig. 5). Similar observations were also made by Flaxes *et al*., 2001; Proctor, 1981. The deficiency of elements affected distribution of root, leaf area, physiological and growth factors and due to decreasing of ecological indices and increasing of leaf temperate.

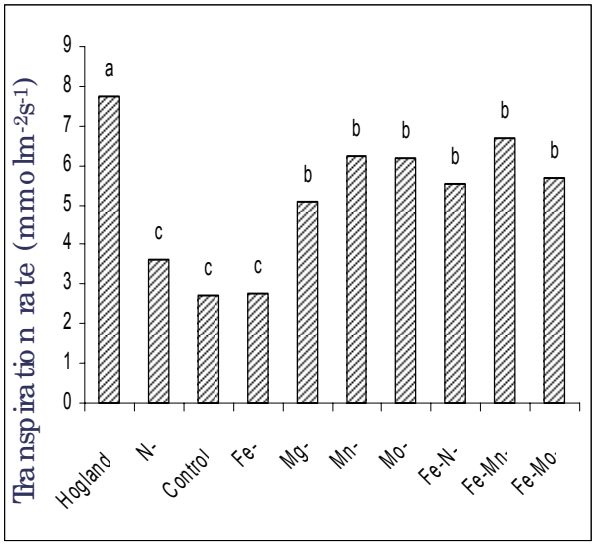

Fig. 3. The effect of nutrient deficiencies on transpiration.

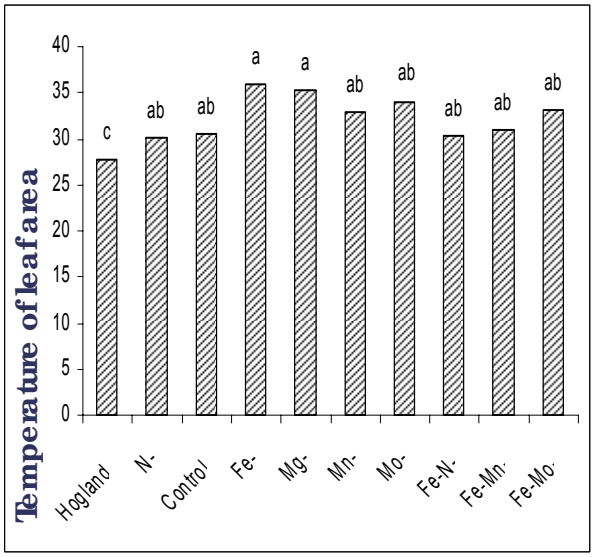

Fig. 4. The effect of nutrient deficiencies on temperature.

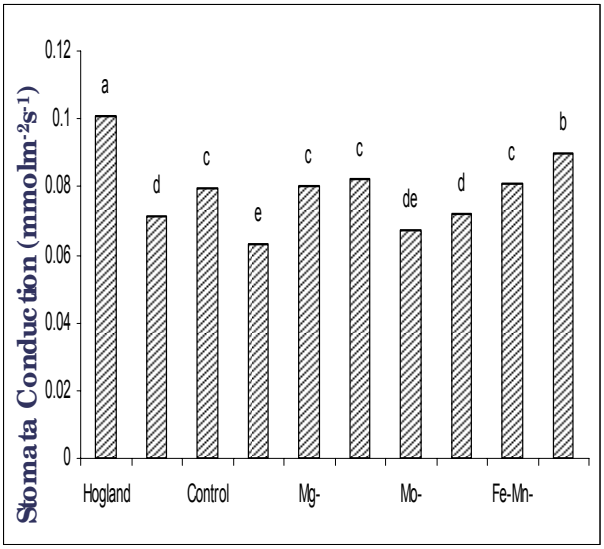

Fig. 5. The effect of nutrient deficiencies on stomata conduction.

In recent years, the technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. No investigation into the photosynthetic performance of plants under field conditions seems complete without some fluorescence data. Chlorophyll fluorescence is also very useful to study the effects of environmental stresses on photosynthesis in plants (Bron, 2004). Chlorophyll fluorescence allows us to study the different functional levels of photosynthesis indirectly (processes at the pigment level, primary light reactions, thylakoid electron transport reactions, dark-enzymatic stroma reactions and slow regulatory processes) (Govindjee, 1995). The electrons produced by the photochemical process are not necessarily used for carbon fixation. In conditions where carbon fixation is limited (e.g. low temperature of leaf area, shortage of CO_2 due to stomatal closure, etc.). Since photosynthesis is often reduced in plants experiencing adverse conditions, such as temperature, nutrient deficiency and attack by pathogens (Godedheer, 1964). A typical measurement on intact leaf by the saturation pulse method is that the plant was dark adapted for 20 min prior to the measurement. Upon the application of a saturating flash (8000 mmol m^{-2} s^{-1} for 1 s), fluorescence raises from the ground state value (Fo) to its maximum value, Fm. In this condition, QA, the first electron acceptor of PSII, is fully reduced. This allows the determination of the maximum quantum efficiency of photo system II (PSII) primary photochemistry, given by Fv/Fm = (Fm-Fo)/Fm. In healthy leaves, this value is always close to 0.8, independently of the plant species studied. A lower value indicates that a proportion of PSII reaction centers are damaged, a phenomenon called photo inhibition, often observed in plants under stress conditions (Bilger *et al.*, 1984, Maxwell and Johnson, 2000). Changes in the dawn Fv/Fm may, however, give important information concerning the effect on the plant of environmental stress. In present study, Chlorophyll (Fo) and ratio of variable chlorophyll to maximum (Fv/Fm) were significant at 5% level (Table 2). Maxwell and Johnson (2000) reported a direct correlation between photosynthesis and the ratio of variable chlorophyll to maximum. Nesterenko *et al.,* 2001, Neves *et al*., 2005 reported that in healthy leaves with high Chlorophyll had a maximum (Fv/Fm). Complete nutrition Seedlings recorded maximum Fv/Fm. The similar results have been reported by Faust, 1989 in apple. In the present study Fe- and Mn-deficient had maximum tens of seedlings (Fig. 6).

Table 2. Variance analysis results of the effect of nutrient deficiencies on ecophysiological parameters of seedlings

Square means								
Source of variance	**Degree of freedom**	**Transpiration (mmol m^{-2} s^{-1})**	**Stomata resistance (1/mmol m^{-2} s^{-1})**	**Stomata conduction (mmol m^{-2} s^{-1})**	**Temp. surface leaf (ºC)**	**Fo**	**Fv**	**Fv/Fm**
Treatment	9	7.16*	0.0003*	8.894*	20.4 *	16.985*	6668.44*	0.001*
Error	18	1.18	0.0001	7.354	4.4	6.916	1925.28	0.0001
C.V. %	–	4.6	4.49	4.59	2.5	1.6	1.85	1.75

ns = not significant; Significant at *P = 0.05 or at **P = 0.001.

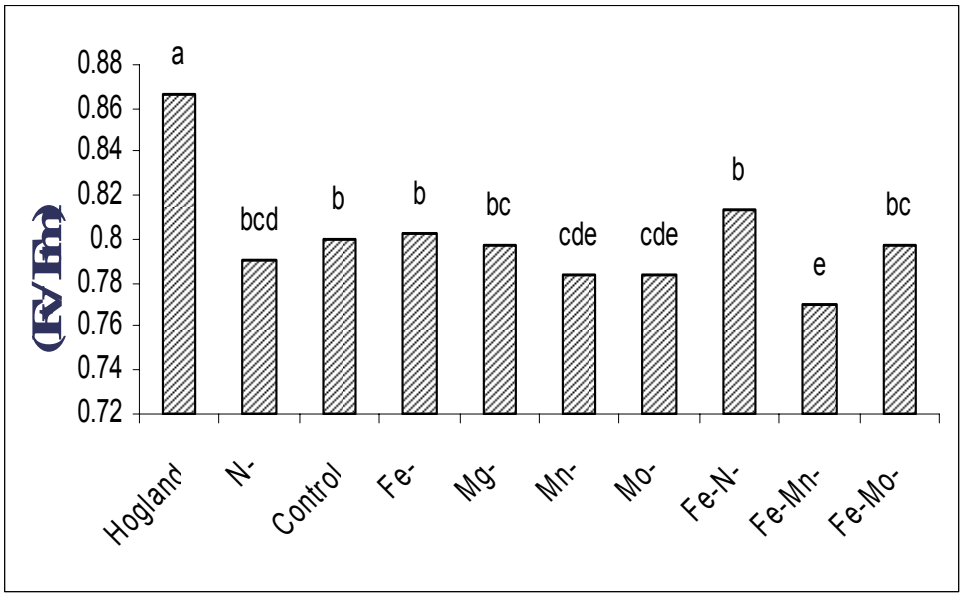

Fig. 6. The effect of nutrient deficiencies on Fv/Fm.

Acknowledgments

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