# Visual deficiency and multi-deficiency symptoms of macro and micro nutrients element in pistachio seedling (Pistacia vera)

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*in*

**Zakynthinos G. (ed.).**
**XIV GREMPA Meeting on Pistachios and Almonds**

**Zaragoza : CIHEAM / FAO / AUA / TEI Kalamatas / NAGREF**
**Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 94**

**2010**
pages 37-52



# Visual deficiency and multi-deficiency symptoms of macro and micro nutrients element in pistachio seedling (*Pistacia vera*)

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**Abstract.** The effects of nitrogen, iron, magnesium, manganese and molybdenum deficiencies and multi-deficiencies on pistachio seedling (*Pistaia vera*) was studied in sand culture. The following treatments were employed: 1-Complete Hoagland's nutrient solution; 2-Nutrient solution lacking N; 3-Nutrient solution lacking Fe, 4-Nutrient solution lacking Mg; 5-Nutrient solution lacking Mn; 6-Nutrient solution lacking Mo; 7-distilled water; 8- Nutrient solution lacking Fe and N; 9- Nutrient solution lacking Fe and Mn; and 10- Nutrient solution lacking Fe and Mo. The experimental design used in this study was complete randomized block design with three replications. Deficiency symptoms observed were mainly leaf discoloration, necrosis, scorching, defoliation and growth stunting. Seedlings that received complete nutrient solution were healthy with dark green foliage. Reductions in height, leaf area and leaf number were noticed for various levels of deficiencies of nutrient elements. Shoot and root growth of the seedlings deficient in nutrients were also affected. Seven months after planting, shoot and root biomasses production were statistically significant at one percent level. Multi-Deficiency symptoms sound like single deficiency. Also shoot and root growth of the seedlings deficient of nutrients were affected. Visual symptoms of nitrogen- deficient seedlings also coincided with the reduction in foliar levels of the concerned element. The typical symptoms of deficiencies of various nutrient elements could be used as a guideline for diagnosing nutrient deficiencies of pistachio in commercial nurseries and plantations. The present study also showed the multi-deficiency of nutrients, new symptom appears in leaves, differs from single deficiencies. In these conditions, leaf symptoms don't agree with Minimum Law. This is a new incorporated sign that appears in leaves.

**Keywords.** Necrosis – Multi-Deficiency – Dry weight – Defoliation.

***Symptômes visuels de la carence ou de carences multiples en macro- et micro-éléments chez des plants de pistachier***

***Résumé.*** *L'effet de la carence ou de carences multiples en azote, fer, magnésium, manganèse et molybdène sur des plants de pistachier* (Pistacia vera*) a été étudié sur lit sableux. Les traitements suivants ont été utilisés: 1- Solution nutritive complète de Hoagland ; 2- Solution nutritive carencée en N ; 3- Solution nutritive carencée en Fe ; 4- Solution nutritive carencée en Mg ; 5- Solution nutritive carencée en Mn ; 6- Solution nutritive carencée en Mo ; 7- Eau distillée ; 8- Solution nutritive carencée en Fe et N ; 9- Solution nutritive carencée en Fe et Mn ; 10- Solution nutritive carencée en Fe et Mo. Le dispositif expérimental utilisé dans cette étude consistait en blocs totalement aléatoires à trois répétitions. Les symptômes de carences observés étaient principalement la décoloration des feuilles, la nécrose, le dessèchement, la défoliation et le nanisme. Les plants recevant une solution complète de nutriments étaient sains et avaient un feuillage vert sombre. Des réductions en hauteur, surface foliaire et nombre de feuilles ont été observées pour plusieurs niveaux de carence en éléments nutritifs. La croissance des pousses et des racines a également été affectée pour les plants carencés en nutriments. Plusieurs mois après la plantation, la production de biomasse pour les pousses et les racines était statistiquement significative à un niveau de un pour cent. Les symptômes de carences multiples étaient difficiles à différencier de ceux d'une seule carence. La croissance des pousses et racines des plants carencés en nutriments était également affectée. Les symptômes visuels des plants carencés en azote coïncidaient également avec la réduction des niveaux foliaires de l'élément en question. Les symptômes typiques de carence en plusieurs éléments nutritifs pouvaient être utilisés comme piste pour le diagnostic des carences nutritives chez les pistachiers dans les pépinières commerciales et les plantations. La présente étude a également montré que la carence multiple en nutriments, avec de nouveaux symptômes apparaissant sur les feuilles, diffère des carences simples. Dans ces conditions, les symptômes foliaires ne sont pas en concordance avec la Loi du Minimum. Ceci est un nouveau signal incorporé qui apparaît sur les feuilles.*

***Mots-clés.*** *Nécrose – Carences multiples – Poids sec – Défoliation.*

## I – Introduction

Pistachio (*Pistacia vera*) is the principle 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. 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. 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 (Razavi, 2005). 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 macronutrients (N, P, K, Ca, Mg and S) or micronutrients (Fe, Mn, Cl, B, Cu, Zn, Ni and 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 nutrient availability and the methods used to diagnose and correct deficiencies. This experiment will discuss basic, but important principles of plant nutrition to develop a specific set of recommendations for dealing with severe nutritional disorders that have been observed in pistachio seedlings grown in nurseries (Uriu and Pearson, 1984). The primary objective of this study was to induce symptoms of deficiency of various nutrient elements in pistachio seedlings grown in sand culture to establish a rough, practical nutrient guideline. By using these guidelines nursery managers and farmers may be able to diagnose pistachio nutrient deficiencies and apply the correct fertilizer to attempt to solve the exact nutrient deficiency. The present study was also aimed at investigating the effect of various nutrient elements on growth at the end of the seedling stage.

## 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: 121ºC, 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 30/25ºC, 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 ethylenediaminetetraacetate). Then the following treatments were employed: (i) Complete Hoagland's nutrient solution (Table 1); (ii) complete Hoagland's solution, but completely lacking one of the following: N, Fe, Mg, Mn, Mo; and (iii) distilled water.

**Table 1. Hoagland's solution (Hoagland, 1948)**

| Compounds | Concentration of elements (ppm) | |
|---|---|---|
| | Element | Concentration |
| $KNO_3$ | N | 101.1 |
| $Ca(NO_3)_2.4H_2O$ | Ca | 118.07 |
| $NH_4H_2PO_4$ | P | 115.08 |
| $MgSO_4.7H_2O$ | Mg | 123.09 |
| $H_3BO_3$ | B | 8.57 |
| $MnSO_4.H_2O$ | Mn | 6.153 |
| $ZnSO_4.7H_2O$ | Zn | 4.39 |
| $CuSO_4.5H_2O$ | Cu | 0.98 |
| $(NH_4)_6Mo_7O_{24}.4H_2O$ | Mo | 0.09 |
| Fe – EDTA | Fe | 10 |

**Concentration of microelements in nutrient solution**

| Microelements | Concentration (mg/l) | Fe-N | Fe-Mn | Fe-Mo |
|---|---|---|---|---|
| $(NH_4)_6MO_7O_{24},4H_2O$ | 0.05 | 0.05 | 0.05 | |
| $H_3BO_3$ | 1.5 | 1.5 | 1.5 | 1.5 |
| $MnSo_4,4H_2O$ | 2 | 2 | | 2 |
| $CuSo_4,5H_2O$ | 0.25 | 0.25 | 0.25 | 0.25 |
| $ZnSo_4,7H_2O$ | 1 | 1 | 1 | 1 |
| Sequesteren Fe 138 | 10 | | | |
| Total | 1 | 2 | 1 | 4 |

**Complete nutrient solution**

| | $NO_3$ | $PO_4$ | $SO_4$ | Cl | Total |
|---|---|---|---|---|---|
| K | 1 | | | | 1 |
| Na | | | | | |
| Ca | 1 | | | | 1 |
| Mg | | | 1 | | 1 |
| $NH_4$ | | 1 | | | 1 |
| H | | | | | |
| Total | 2 | 1 | 1 | | 4 |

**Complete nutrient solution lacking N**

| | $NO_3$ | $PO_4$ | $SO_4$ | Cl | Total |
|---|---|---|---|---|---|
| K | | 0.5 | | 0.5 | 1 |
| Na | | | | | |
| Ca | | 0.5 | 0.5 | 0.5 | 1.5 |
| Mg | | | 1.5 | | 1.5 |
| $NH_4$ | | | | | |
| H | | | | | |
| Total | | 1 | 2 | 1 | 4 |

The experiments were laid out in a 1-factor block design with three replications and data was analyzed using MSTATC. A total of nine plants were used for each treatment, and three plants per experimental unit. The nutrient solutions required for each treatment were carefully prepared in bulk by eliminating the desired nutrient element from the stock. On every alternate day, each plant received 300 ml of the nutrient solution and on days in between 300 ml of distilled water. The seedlings were observed daily for deficiency symptoms and height, leaf area, leaf number and leaf area were recorded for various levels of nutrient element deficiencies.

## 1. Shoot growth parameter

At the end of this experiment, seedlings that received complete nutrient solution had a maximum height growth of 41.03 cm while the N-deficiency seedlings recorded the lowest height growth of 18.73 cm. Among the various nutrients, Mn produced maximum height growth (36.3 cm). This was followed by Mg- and Mo. Seedlings that had two deficient elements were on a par with those deficient in others in terms of height at the end of the study period. Height increment was relatively less in all the treatments compared with the control (Table 2).

The seedlings receiving complete nutrient solution produced the highest number of leaves (44) at the end of the study period (Table 2). At the end of the study the N-deficiency seedlings had a leaf number of 26. This was followed by Mn-deficient (36), Mg-deficient (34) and Mo-deficient (32) seedlings. The Fe N-deficient, Fe Mn-deficient and Fe Mo-deficient seedlings produced a leaf number of 31, 30 and 24, respectively. With regard to secondary internodes (internodes after doing treatments), N-deficiency seedlings recorded 0.53 like the seedlings grown in complete nutrient solution (0.52), Fe Mn-deficiency (0.53) and Fe Mo-deficiency (0.58). The mean internodes for Fe-deficient seedlings were 0.9 cm at the end of the study. The Mn-deficient and Mg-deficient seedlings produced internodes of 0.88 and 0.84 respectively at the end of the study. Seedlings that were Mo-deficient were on a par with those deficient in Fe N- in terms of internodes at the end of the study period (Table 2).

Maximum leaf area was recorded by seedlings grown in complete nutrient solution (12340 $cm^2$) (Table 2). The lowest leaf area (3556 $cm^2$) was recorded by seedlings lacking N. the second lowest in leaf area were seedlings which were deficient in Mn. In the case of Fe-deficient and Mn Fe-deficient plants the leaf areas were 4568 and 11710 respectively. The difference in leaf area between the others deficient seedlings was not significant.

## 2. Fresh and dry weight of shoot

Seedlings that received complete nutrient solution recorded 15.37 g. Among the nutrient elements, Mg-deficient and Mn-deficient seedlings recorded the highest shoot fresh weights of 16.97 and 16.61 g respectively. The shoot fresh weights of the rest of the treatments were in the order Fe Mo-< Fe Mn-< Fe N-<N-<Mo-<Fe-<Hogland. With regard to dry weight, the treatments were in the order Fe Mo-< Fe Mn-< Fe N-<N-<Mo-< Hogland< -Fe< Mg -<Mn- (Table 2).

## 3. Root growth parameters

The length of the main root did not show any significant difference due to the treatment (Table 2). At the end of the study, Fe N-deficient Seedlings recorded the lowest root length (43.3 cm). Fe Mn-deficient Seedlings recorded maximum root length (57.67 cm). N- and Fe Mo- deficient Seedlings recorded 51 and 50.33 at the end of the study. Fe N-, Fe Mn- and Fe Mo- had the second lowest number of secondary roots at the end of the study. With regard to root length, the treatments were in the order Fe N -< Fe -< Mo -< Hogland < Mg -< Mn -< Fe Mo -< Fe Mn -.

**Table 2. Comparison of ten treatments on pistachio seedling growth parameters**

| Treatment | No. of leaves | Plant height (cm) | Inter-nodes (cm) | No. of secondary leaves | Secondary plant height (cm) | Secondary internodes (cm) | Fresh root weight (g) | Fresh stem weight (g) | Fresh leaf weight (g) | Dry root weight (g) | Dry stem weight (g) | Dry leaf weight (g) | Root length (cm) | Leaf area ($cm^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hoagland | 49.89$^{a}$ | 41.03$^{a}$ | 0.77$^{bc}$ | 26.67$^{a}$ | 24.38$^{a}$ | 0.5167$^{c}$ | 9.187$^{b}$ | 8.077$^{a}$ | 12.293$^{a}$ | 7.1$^{b}$ | 4.51$^{a}$ | 7.913$^{a}$ | 53.33$^{a}$ | 12340$^{a}$ |
| –N | 26.67$^{cd}$ | 18.73$^{c}$ | 0.68$^{c}$ | 14.83$^{cd}$ | 9.120$^{bc}$ | 0.5367$^{c}$ | 10.39$^{b}$ | 3.657$^{c}$ | 5.233$^{bc}$ | 5.8$^{bc}$ | 2.11$^{bc}$ | 2.257$^{de}$ | 51$^{a}$ | 3961$^{c}$ |
| Distilled water | 19.78$^{d}$ | 15.47$^{c}$ | 0.7767$^{bc}$ | 9.22$^{d}$ | 4.057$^{c}$ | 0.4667$^{c}$ | 4.837$^{d}$ | 1.953$^{c}$ | 2.89$^{c}$ | 2.87$^{c}$ | 1.177$^{c}$ | 1.363$^{e}$ | 31.5$^{c}$ | 3556$^{c}$ |
| –Fe | 36.67$^{b}$ | 28.97$^{bc}$ | 0.94 0$^{a}$ | 23.89$^{b}$ | 14.78$^{bc}$ | 0.91$^{a}$ | 13.80$^{b}$ | 6.113$^{b}$ | 8.564$^{b}$ | 7.05$^{b}$ | 3.433 $^{b}$ | 4.1$^{b}$ | 44$^{b}$ | 4568 $^{c}$ |
| –Mg | 34.22$^{bc}$ | 33.27$^{bc}$ | 0.9467$^{a}$ | 19.78$^{bc}$ | 18.28$^{bc}$ | 0.8467$^{ab}$ | 18.3$^{a}$ | 6.65$^{b}$ | 10.32$^{ab}$ | 9.29$^{a}$ | 3.683 $^{b}$ | 4.497$^{b}$ | 49$^{ab}$ | 9871$^{ab}$ |
| –Mn | 36.89$^{b}$ | 36.37$^{b}$ | 0.9867$^{a}$ | 22.33$^{b}$ | 21.06$^{b}$ | 0.8833$^{ab}$ | 19.43$^{a}$ | 6.43$^{b}$ | 10.19$^{ab}$ | 9.6$^{a}$ | 3.737 $^{b}$ | 4.573$^{b}$ | 49.83$^{ab}$ | 11710$^{ab}$ |
| –Mo | 32.67$^{bc}$ | 30.10 $^{bc}$ | 0.8867$^{ab}$ | 18.22$^{bcd}$ | 16.18$^{bc}$ | 0.7200$^{bc}$ | 19.10$^{a}$ | 4.217$^{bc}$ | 8.86$^{b}$ | 7.98 $^{b}$ | 2.607$^{b}$ | 3.75$^{bcd}$ | 45$^{b}$ | 7305 $^{bc}$ |
| Fe-N- | 31.33$^{bcd}$ | 27.43$^{bc}$ | 0.88$^{ab}$ | 16.89$^{bcd}$ | 12.28$^{bc}$ | 0.7244$^{bc}$ | 6.967$^{cd}$ | 2.827$^{c}$ | 5.5$^{bc}$ | 3.27$^{c}$ | 1.583$^{bc}$ | 2.58$^{de}$ | 43.33$^{b}$ | 7135$^{bc}$ |
| Fe-Mn- | 30$^{bcd}$ | 22.97$^{bc}$ | 0.85$^{abc}$ | 14.67$^{bcd}$ | 7.244$^{c}$ | 0.5322$^{c}$ | 8.177$^{cd}$ | 2.617$^{c}$ | 4.72$^{bc}$ | 5.08$^{bc}$ | 1.567$^{bc}$ | 2.34$^{de}$ | 57.67$^{a}$ | 6843$^{bc}$ |
| Fe-Mo- | 24$^{cd}$ | 20.6$^{bc}$ | 0.91$^{ab}$ | 11.67$^{cd}$ | 7.2$^{c}$ | 0.5768$^{c}$ | 5 / 680 $^{cd}$ | 2.537$^{c}$ | 4.10$^{bc}$ | 3.96$^{bc}$ | 1.417$^{bc}$ | 1.813$^{e}$ | 50.33$^{a}$ | 6681$^{bc}$ |

**Results of ANOVA.** ***Mean of square***

| Source of variance (***deg. of freedom***) | No. of leaves | Plant height (cm) | Inter-nodes (cm) | No. of secondary leaves | Secondary plant height (cm) | Secondary internodes (cm) | Fresh root weight (g) | Fresh stem weight (g) | Fresh leaf weight (g) | Dry root weight (g) | Dry stem weight (g) | Dry leaf weight (g) | Root length (cm) | Leaf area ($cm^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment *(9)* | 145.7$^{*}$ | 197.7$^{*}$ | 0.02 $^{ns}$ | 90.43$^{*}$ | 132.8$^{**}$ | 0.83$^{*}$ | 95$^{**}$ | 10.2$^{**}$ | 22.7$^{**}$ | 17.007$^{**}$ | 3.21$^{**}$ | 4.14$^{**}$ | 67.7$^{*}$ | 5509$^{**}$ |
| Error *(18)* | 41.18 | 48.60 | 0.016 | 29.553 | 32.531 | 0.25 | 8.64 | 1.41 | 4.57 | 7.22 | 0.54 | 0.70 | 35.9 | 63364 |
| CV% | 4.5 | 5.03 | 3.8 | 1.5 | 4.9 | 4.9 | 5.03 | 5.25 | 5.45 | 5.2 | 5.2 | 5.2 | 3.5 | 3.9 |

Mean separation by Duncan's Multiple Range Test at P = 0.05. The same letters within a column are not significantly different.
ns = not significant; significant at *P = 0.05 or at **P = 0.001

### 4. Fresh and dry weight of roots

Root fresh and dry weights were influenced by the different treatments (Table 2). Seedlings grown in Mn-deficient recorded 9/18g. Among the nutrient elements, Mn-deficient and Mo-deficient Seedlings recorded the highest shoot fresh weights of 16.43 and 19.10 g respectively at this study. The shoot fresh weights of the rest of the treatments were in the order Fe Mo-< Fe N -< Fe Mn -< Hogland < N -<Fe-< Mg-.With regard to dry weight, the treatments were in the order Fe N -< Fe Mo -< Fe N-<N-~ Fe Mn-< Hogland <Fe -<Mo-< Mg -<Mn.

## III – Results and discussion

The present study was conducted with an objective of inducing and describing the symptoms of deficiency of various nutrient elements in pistachio seedlings. This also intended to provide information on understanding the importance of nutrient elements. Knowing the actual role, quantity required and uptake pattern of these nutrients will eventually benefit the managers and farmers for the production of healthy and vigorous seedlings for extensive planting programmers.

The seedlings that received all the nutrients through complete Hoagland's nutrient solution were found to be very vigorous, with healthy growth and produced dark green, normal-shaped foliage throughout the study period. These seedlings did not show any visual symptoms of deficiency (Fig. 1). Sand culture of teak Gopikumar and Varghese (2004) and aloe (Massiah and Pire, 1998) revealed similar results. In the former study, the objective was to induce symptoms of deficiency of various nutrient elements (N, P, K, Mg, S, Zn and Mo) in seedlings of teak grown in sand culture. The effects of nutrients on the growth were investigated. Seedlings that received complete nutrient solution were healthy and had dark-green leaves. Shoot and root growth of the seedlings deficient of nutrients were negatively affected (Gopikumar and Varghese, 2004). In the latter study, Massiah and Pire grew *Aloe barbadensis Mill*. In sand culture for 18 months supplied Hoagland's solution (complete or lacking N, P, K, Ca or Mg) or with demineralized Water. Leaf length, width, thickness and weight showed the highest values in plants receiving complete solution and the lowest values in plants receiving solution without N or water only. Plants supplied with solutions without N showed less luminosity (L* values) than those receiving water only, with plants from the other treatments having higher values. Chroma or colour purity was highest in plants receiving the complete solution or solution without N, thus reflecting the homogeneity of colour in those treatments.

**Fig. 1. Complete Hoagland's nutrient: healthy growth and produced dark green, normal-shaped foliage.**

The macronutrient, nitrogen (N), is the most widely needed fertilizer element in Pistachios (Gonzales, 1985). Nitrogen is used by plants to synthesize amino acids and nucleic acids that

are necessary for all functions of the plant. Nitrogen deficiency symptoms will eventually appear in most orchards if N is withheld. Annual leaf tissue analysis can ensure that sufficient N is applied to meet the crop needs without wasting money on unnecessary application, without encouraging vegetative growth at the expense of reproductive growth, and without polluting surface and ground water supplies. Shoot growth is reduced in N-deficient pistachios. Shoots are thinner, shorter and in more severe deficiencies have reddish bark that related to produce anthosyanine pigments. Nitrogen is mobile and new leaf production is at the expense of older leaves if N is deficient. Young leaves pale as older leaves turn yellow and drop from the tree early. Excessive leaf drop results in a tree with sparse foliage. The petioles and midribs of N-deficient leaves become red (Gonzales, 1985). The characteristic deficiency symptom of N is the appearance of uniform yellowing of leaves including the veins, this being more pronounced on older leaves as expressed in rabbit-eye (*Vaccinium virgatum, syn*. V. ashei) and blueberries (section Cyanococcus of the genus *Vaccinium*) (Tamada, 1989), fescue (*Festuca arundinacea*) (Razmjoo *et al.,* 1997), *Ailanthus triphysa* (Anoop *et al*., 1998), chili (*Capsicum annum*) (Balakrishnan, 1999) and sugarcane (*Saccharum officinarum*) (Nautiyal *et al.,* 2000). The leaves become stiff and erect. In dicotyledonous crops the leaves detach easily under extreme deficiency. Cereal crops show characteristic 'V'-shaped yellowing at the tip of lower leaves. O'Sullivan *et al*. (1993) observed relatively small and pale green leaves with a dull appearance in sweet potato. If such nitrogen stress conditions persist, the result is a decrease in foliage and shoot growth, as occurs for black pepper (*Piper nigrum*) (Nybe and Nair, 1986), douglas fir (*Pseudotsuga menziesii*) (Friend *et al*., 1990) and sapota (*Manilkara achras*) (Nachegowda *et al.* 1992). In our study the symptoms of N deficiency appeared at the end of the first month. During the initial stages, yellow patches appeared towards the margins of older leaves. After seven months the entire lamina turned pale yellow. Stunting of the seedlings was also noticed at this stage. In the acute stage of deficiency, the entire seedling appeared severely chlorotic and these leaves gradual1y dried prematurely (Fig. 2).

**Fig. 2a. N -deficient in older leaves: severely chlorotic and these leaves gradual1y dried prematurely.**

**Fig. 2b. N -deficient: Young leaves pale and small.**

Massial and Pire (1998) also noted similar symptoms in aloe. Nitrogen deficiency has noticeable effect on the growth behavior of seedlings particularly with regard to shoot growth. This early senescence probably related to the effect of then supply on the synthesis and translocation of cytokinins. According to investigation of Wagner and Michael (1971) the synthesis of cytokinins is depressed when N-nutrition in adequate. Similar observations were also made by Koal *et al*. (1972) in teak and Anoop (1993) in Ailanthus. The reduction in vegetative growth may be due to the fact that N Largely controls the use of carbohydrates and hence determines whether the plant will make vegetative or reproductive growth (Jones & Embleton 1959). With regard to tissue concentration at the end of this experiment, N concentration fell to the least of the severe stage of deficiency when the entire seedling appeared chlorotic followed by premature drying and defoliation. In this study nitrogen element was the most effective in the leaves and stems

biomass. As Razzaque and Hanafi (2003) reported More than 80% of the dry weight in leaves was related to the effect of nitrogen in the dry matter leaves. Root growth is affected and in particular branching is going on. The root/shoot ratio is increased by N deficiency. In our study shoot / root ratio increased to 0.85. As Carver *et al.* 1992 reported N deficiency resulted increasing in specific hydrate carbon in root.

Magnesium, also a macronutrient, is an activator for many enzymes; most of which are concerned with carbohydrate metabolism, phosphate transfer and decarboxilation and growth processes. (Dixon, 1949). It is a component of chlorophyll and thus is essential for photosynthesis (Bansal, 1989). Magnesium deficiency has not been widely reported in pistachio. Deficiencies are more likely to occur in sandy and acid soils. On the west side of the San Joaquin Valley pistachios are grown on alkaline, highly calcareous and boric soils. These soils may fail to provide sufficient available magnesium for optimal tree growth due to antagonistic competition for uptake by excessive calcium and other cations present on the soil colloids (Bolt *et al.,* 1991). In a similar manner, high rates of gypsum ($CaSO_4 \cdot 2H_2O$) may induce magnesium deficiency in some soils. Deficiency symptoms appear mid-season on the lower leaves of shoots as tip and lateral margin yellowing, or as interveinal yellowing. The leaf margins may later become scorched. The scorching progresses inward, leaving a green, inverted 'V' at the base of the leaf (Anoop, 1993). In severe deficiency, the interveinal yellowing may turn to scorching. Scorched leaves will then drop. Magnesium deficiency may be confused with potassium deficiency. Suspected Mg deficiency should be confirmed by leaf tissue analysis. Magnesium is also a catalyst for enzymes needed for carbohydrate and nitrogen metabolism. Magnesium is readily translocated within the plant, therefore deficiency symptoms occur first on the older plant tissues. In our study deficiency symptoms of Mg started appearing after the first month. The symptoms were first manifested on lower leaves. The older leaves were a green intending to yellowish (Fig. 3).


**Fig. 3a. Mg deficiency: younger leaves formed wavy areas that gradually spread through the midrib towards the leaf tip.**

**Fig. 3b. Mg deficiency: older leaves pale and yellowing may later become scorched.**

Because of the role of Magnesium in the proportion of protein N decreased and that of non-protein N increased (Mengel and Kirkby, 1982). From this it may be concluded that Mg deficiency inhibits protein synthesis. The basal leaves formed wavy areas that gradually spread through the midrib towards the leaf tip, thereafter progressing to the younger, higher leaves. The effects of Mg deficiency on ultra structural changes have been investigated by a number of workers. Marked differences occur in chloroplast structures and deformation of the lamellar structures might be expected. The mitochondria were also affected by Mg deficiency, the cristae being underdeveloped. This stage was noticed after seven months of the experiment. Mg deficiency primarily affects carbohydrate metabolism resulting in reduced plant growth and decreased transport rates of carbohydrates to sink organs, as reported by Gopikumar and Varghes (2004) in teak, Deotale (2005) in Nagpur orange (*Citrus eticulate* Blanco) and by Sun and Payn (1999) in radiata pine (*Pinus radiata*). The reduction of the photosynthesis due to starch accumulation in leaf (Antcliff, 1983), Magnesium element was the least effect in the leaves biomass compared of other nutrient elements. Similar observation has been reported by Gopikumar and vargese (2004).

Iron (Fe) is intimately associated with protein synthesis and its deficiency results in the accumulation of carbohydrates and soluble N compounds, thereby resulting in a breakdown and decrease in cambial tissues (Mengel and Kirkby, 2001). Fe deficiency symptoms are that the principal veins remain conspicuously green and the surrounding portion of the younger leaves turn yellow tending towards whiteness in chickpea (*Cicer arietinum)* (Mehrotra and Gupta 1990; Saxena *et al.,* 1990); groundnut (*Arachis hypogaea*) (Reddy *et al.,* 1993); radish (*Raphanus sativus*), cauliflower (also *Hematoma auris* or *Perichondrial hematoma*), cabbage (*Brassica campestris* sp. *Pekinensis olson*) and sorghum (*Sorghum bicolor*) (Preeti *et al*., 1994), lentil (*Lens culinaris*) (Zaiter and Ghalayini, 1994) and soybean (*Glycine max*) (Fonts and Cox, 1998). Under sever deficiency, most part of the leaf becomes white (Russelle and McGraw, 1986). Iron deficiency symptoms were noticed from two month onwards in our pistachio trials. The symptoms appeared on the lower leaves as chlorotic patches. Later the leaves developed necrotic patches and at severe stages the leaves had a burnt appearance. They also appeared as discoloration of the younger leaves from dark green to pale green (Fig. 4).

**Fig. 4a. Lower leaves as chlorotic patches. Later the leaves developed necrotic patches and at severe stages the leaves had a burnt appearance.**

**Fig. 4b. Fe deficiency in younger leaves turn yellow.**

Similar observations were also made by Deotale (2005) in Nagpur orange, Keil (1986) in

sunflower (*Helianthus annuus*) and Romheld and Marschner (1981) in potato (*Ipomoea batatas*). The iron element has been the highest mounts in root biomass. This might have resulted in the cluster appearance of the root seedlings. in pistachio plant the result had been shown that these physiological changes of roots under iron stress are accompanied by typical morphological changes such as thickening of root tips due an a largment of the cortex, additional division of rhizo- dermal cells and an intensified development of root hairs. Increasing in root growth is related to going on in charbohydrate , the role of iron in crebx cycle and phosphorilizathon oxidative.(Carver, 1992). Similar observations were also made by Romheld and Marschner (1979) in sun flower. A faierly well defined pattern of chemical composition is often shown in plant suffering from Fe chlorosis. In particular the P/Fe ratio is frequently higher than in comparative green tissues. In pistachio seedlings, this ratio has been going on from 0.0029 in complete nutrient solution to 0.0094 in Fe deficiency.

The chloroplast is the only cellular organelle which shows marked structural changes in Mn-deficient leaves. These changes were primarily characterized by an increase in the number of thylakoids per grana stack and an almost complete loss of stroma lamellae. In Mn-deficient plants, most enzymic and structural components of photoreaction II are probably present in the membrane (Govindjee, 2007). The chloroplast is the most sensitive and the tissues have a small cell volume, cell walls dominate and the interepidermal tissue is shrunken. Mn deficiency cases interveinal chlorosis occurs in the leaves were significant after seven months. Older leaves produced small necrotic areas during the initial stages of deficiency and a characteristic chlorotic pattern between the veins was noticed. The acute stage of Mn deficiency results in red petioles and ribs because of accumulation of antocyanine pigments. Seedlings developed long internodes (Fig. 5). Metabolic functions such as enzyme cofactor, influence of valence changes, e.g. for nitrogenase, nitrate reductase, and sulfite reductases are strongly tied to N metabolism and Mo requirement depends on whether the plant is a $N_2$ fixer or not and the mode of N nutrition, i.e. the degree of dependence on nitrate vs. ammonium (Tandon, 1995). The leaf area of Mn -deficient seedlings was 11710 $cm^2$ less compared with the complete nutrient solution. The shoot fresh and dry weights also showed increased by 19.43 and 9.6 respectively compared with the complete nutrient solution.

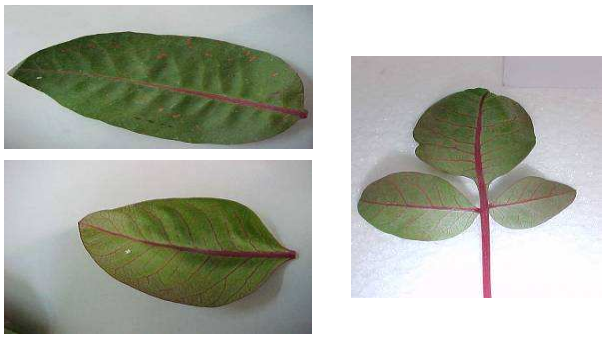

**Fig. 5. Mn- deficiency in leaf: chlorotic pattern between the veins and acute stage of Mn deficiency results in red petioles and ribs.**

Pistachio seedlings which lacked Mo developed deficiency symptoms after five months, first appearing on terminal leaves whose size reduced considerably. The leaves appeared to have narrowed. Mo deficiency in older leaves were small included marginal and interveinal then necrosis, scorching and downward curling of margins (Fig. 6) .The changes in isoenzyme pattern are the primary biochemical changes occurring at the cell level due to the deficiency of this element and on account of these physiological changes. The plant finally shows its effect on leaf anatomical and morphological characters further expressing as visible deficiency symptoms

as shown by Kamala and Angadi (1988) in sandalwood (*Santalum album*) and Gopikumar and Varghes (2004) in teak. A primary role of Mo in the plant is nitrate reduction. Due to its roles in plant nutrition, Mo deficiency often resembles N deficiency. Plants are pale yellow throughout showing N deficiency (Anchondo *et al.,* 2002 in chili peppers). The common symptoms of Mo deficiency in plants include a general yellowing, marginal and interveinal chlorosis, and marginal necrosis, rolling, scorching and downward curling of margins in poinsettia cultivars (Cox, 1992) and in various field, horticulture and forage crops (Gupta and Gupta, 1997). The deficiency of Mo in cauliflower causes a disorder described as 'whiptail' (Duval *et al.,* 1991). Mo-deficient seedlings, the roots were thin, multi- branch and brown intended to yellowish.

**Fig. 6a. Mo deficiency leaf pale yellow and purple narrowed.**

**Fig. 6b. Mo deficiency in young leaves include a general yellowing, marginal and interveinal chlorosis, marginal necrosis, rolling, scorching and downward curling of margins.**

Fe and N multi-deficiency symptoms first appeared as discoloration of the young leaves from dark green to pale green. The symptoms gradually advanced from the entire leaf. At the moderate stages of deficiency, the older leaves appeared red. Later, necrosis set in and at the acute stage the entire leaf developed chlorotic. The affected leaves were yellowish in color and sometimes off white. The youngest leaves may often be completely white and totally devoid of chlorophyll (Fig. 7). Seedlings were stunted in growth with short internodes and small clustered leaves. The length of the root was also affected significantly, and the secondary roots were the least compared with the complete nutrient solution (Table 2). The length of the root was thin, long and the brownish color. Multi-deficiency symptoms of Fe and Mn started appearing in the young leaves. The symptoms were first manifested on young leaves. New leaves were pale in colour, narrow in appearance and small clustered leaves. Older leaves gradually showed the bronze patches extended towards the entire leaf resulting in premature defoliation. These chlorotic areas gradually spread through the margin upwards. Necrosis progressed from the lower part of chlorotic leaves (Fig. 8). Leaf area has been shown minimum size considerably. The fresh and dry weights of shoot and root, the length of the shoot, the number of leaf and also leaf area showed decreased compared with the complete nutrient solution. The length of the

root was affected significantly, and the secondary of roots were the least compared with the complete nutrient solution (Table 2). Like Fe and N multi-deficiency the length of the root was thin, long and the brownish color. Fe and Mo multi-deficiency symptoms first appeared as discoloration of the young leaves from dark green to pale green. The affected leaves were yellowish in colour. The symptoms appeared on the lower leaves as chlorotic patches. Later the leaves developed necrotic patches and at the severe stages the leaves had a burnt appearance. The leaves were narrow in appearance (Fig. 9). The fresh and dry weights of shoot and root, the length of the shoot, the number of leaf and also leaf area showed decreased compared with the complete nutrient solution. The length of the root was affected significantly, and the secondary of roots were the least compared with the complete nutrient solution (Table 2).

**Fig. 7a. Fe-N- deficiency: necrosis set in and at the acute stage the entire leaf developed chlorotic.**

**Fig. 7b. Fe-N- deficiency: discoloration of the young leaves from dark green to pale green.**

**Fig. 8a. Fe-Mn-deficinecy in young leaves: pale in colour, narrow in appearance and small clustered leaves.**

**Fig. 8b. Fe-Mn-deficiency in older leaves: the bronze patches extended towards the entire leaf resulting in premature defoliation. These chlorotic areas gradually spread through the margin upwards.**


**Fig. 9. Fe-Mo- deficiency the young leaves: yellowish in colour, chlorotic patches, and at the severe stages the leaves had a burnt appearance. The leaves were narrow in appearance and cup form.**

## IV – Concluding remarks and practical solutions

Reductions in height, leaf area and leaf number were noticed for various levels of deficiencies of nutrient elements, whose typical symptoms included leaf discoloration, necrosis, scorching, defoliation and growth stunting that could be used as guidelines for diagnosing nutrient deficiencies of pistachio in commercial nurseries and plantations. The root growth of treated seedlings particularly Mn- and N-deficient seedlings declined considerably. Also our research showed that deficiency symptoms of mineral nutrition in pistachio seedlings differed from those in other trees, especially Mn deficiency which showed a new symptom of Mn deficiency that has never been reported in other fruit trees: the older leaves produced small necrotic areas during the initial stages of deficiency and a characteristic chlorotic pattern between the veins was noticed. The acute stage of Mn deficiency results in red petioles and ribs. In other fruit trees, Mn deficiency is expressed in the principal veins as well as in smaller veins, becoming green, while the interveinal portion becomes chlorotic in *Ailanthus triphysa* (Anoop *et al.,* 1998), necrotic with browning of interveinal tissue in melons (*Cucumis melo*) (Simon *et al*., 1986) while the affected young leaves of bird's foot trefoil remain small and abscise before older leaves (Russelle and McGraw, 1986). In addition, Mg deficiency causes yellowing, but differs from nitrogen deficiency in that yellowing takes place in between veins of older leaves in *Picea abies* (Makkanen 1995) and veins remain green; to a greater extent necrosis of tissues occurs in birdsfoot trefoil (Russelle and McGraw, 1986), melons (Simon *et al.*, 1986), black pepper (*Piper nigrum* L.) (Nybe and Nair, 1987) and blueberry (*V. darrowi*) (Tamada, 1989). Mg deficiency may be induced in tomato (*Solanum lycopersicum*) by high levels of ammonium in the nutrient solution (Kafkafi *et al.*, 1971). This differs from our results in pistachio. In order to correct nutrient deficiencies in pistachio seedlings, nurserymen and growers should check symptom of deficiency and correct each deficiency by supplying the element in question and when the element is directly involved in the metabolism of the plant (Arnon, 1954). Reductions in height, internodes, leaf area, leaf number, shoot and root biomasses were noticed for various levels of deficiencies of nutrient elements. The present study also showed the multi-deficiency of nutrients, new symptom appears in leaves, which differs from single deficiencies. In these conditions, leaf symptoms don't agree with Minimum Low. As a result of which new guidelines will be set, through using these guidelines nursery managers and farmers may be able to diagnose pistachio nutrient deficiencies and apply the correct fertilizer to attempt to solve the exact nutrient deficiency.

For each element deficiency, we recommend the following: (i) Nitrogen: add organic matter to soil; apply N fertilizer, including legumes in crop rotation; use a 0.25-0.5% solution of urea applied as a foliar spray; (ii) Magnesium: apply dolomite limestone; foliar application of magnesium sulfate or magnesium nitrate solutions; (iii) Iron: foliar spray of 2% iron sulfate or 0.02-0.05% solution of iron chelate; use efficient cultivars, fertigation with chelated iron; (iv)

Molybdenum: lime acid soils; soil application of sodium ammonium molybdate; foliar spray of 0.07-0.1% solution of ammonium molybdate; and (v) Manganese: foliar application of 0.1% solution of manganese sulfate.

If the nutrient deficiency has been confirmed in a standing crop, the foliar application of selected nutrients by means of a spray is a quick way to get rid of stress symptoms and avoid yield loss. Foliar fertilization of macro- and micronutrients is the best practice whenever nutrient uptake through the roots is restricted due to adverse growing conditions (El-Fouly and El-Sayed, 1997), when topsoil is dry, particularly in semiarid regions (Grundon, 1980), under saline soils (El-Fouly and El-Sayed, 1997), and when root activity decreases during the reproductive stage. In calcareous soils where iron availability is generally very low and chlorosis is quite common, foliar spraying under these conditions is very beneficial (Horesh and Levy, 1981).

## Acknowledgments

Thanks of Dr. Hokmabadi and Dr. Ardalan for their comments and proofreading the manuscript, and Pistachio Research Institute and University of Tehran for financial support.

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