

Visual Deficiency Symptoms of Nitrogen, Iron, Magnesium, Manganese and Molybdenum (Macro- and Micronutrients) on Pistachio Seedlings

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ABSTRACT

The effect of nitrogen, iron, magnesium, manganese and molybdenum deficiencies on the growth characteristics of pistachio seedlings (*Pistacia vera*) were studied in sand culture. Cv. 'Badami-e-Zarand' was used in this experiment as common rootstock. The following treatments were employed: 1) Complete Hoagland's nutrient solution; Nutrient solution lacking either: 2) N, 3) Fe, 4) Mg, 5) Mn, or 6) Mo; 7) distilled water. Seven months after planting, deficiency symptoms mainly included leaf discoloration, necrosis, scorching, defoliation and stunted growth. Seedlings that received complete nutrient solution were healthy with dark green foliage. A reduction in height, leaf area and leaf number was noticed for various levels of nutrient element deficiencies. These typical symptoms of deficiencies of various nutrient elements could serve as a practical guideline for diagnosing nutrient deficiencies of pistachio in commercial nurseries and plantations.

Keywords: discoloration, dry weight, Hoagland, *Pistacia*

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. 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 nutrient availability and the methods used to diagnose and correct defi-

ciencies.

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.

MATERIALS 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 ½- or ¼-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

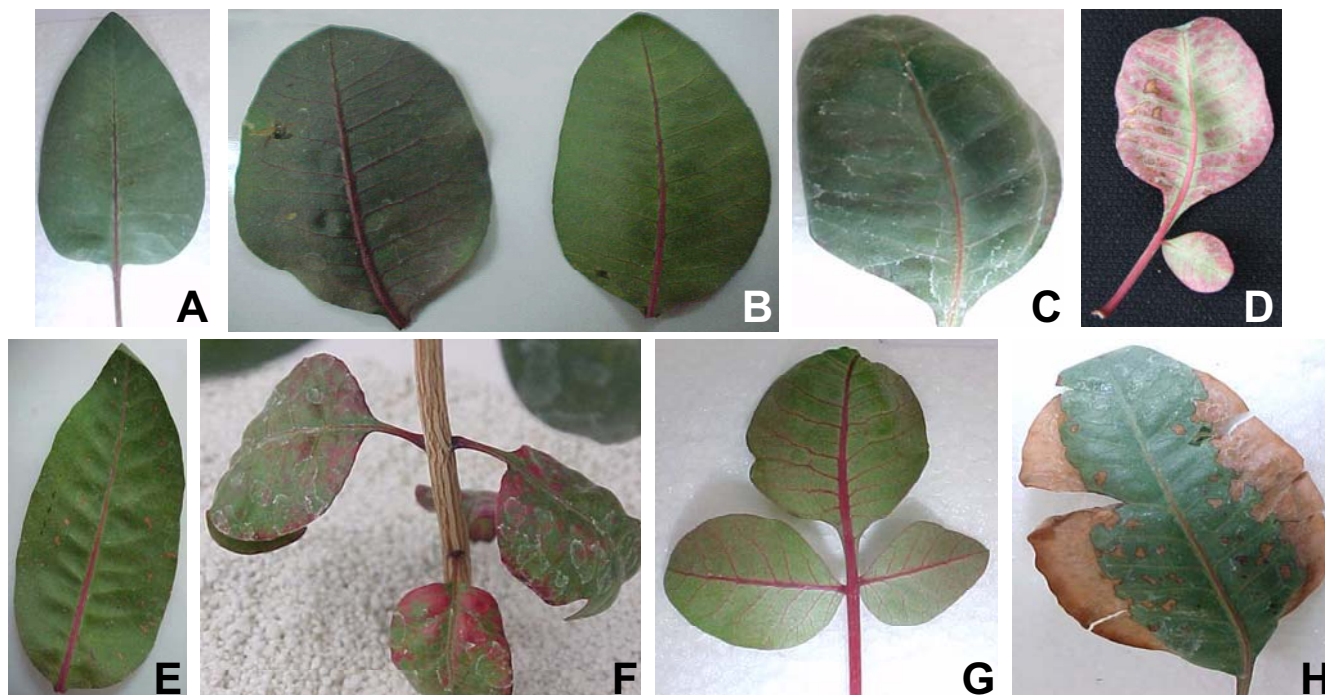


Fig. 1 Visual symptoms of macro- and micronutrient deficiencies in pistachio leaves. (A) Normal leaf; (B) Mo-deficiency; (C) Mg-deficiency; (D) N-deficiency; (E) Mn-deficiency; (F) N-deficiency; (G) Mn-deficiency; (H) Fe-deficiency.

10 days until they established well in perlite. The composition of the $\frac{1}{2}$ -strength nutrient solution, expressed in mmol l^{-1} was 30 KNO_3 , $2.0 \text{ Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $1.0 \text{ NH}_4\text{H}_2\text{PO}_4$, $0.5 \text{ MgSO}_4 \cdot 7\text{H}_2\text{O}$; and in $\mu\text{mol l}^{-1}$, 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: a) Complete Hoagland's nutrient solution; Complete Hoagland's solution, but completely lacking one of the following: b) N; c) Fe; d) Mg; e) Mn; f) Mo; f) distilled water.

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 50 ml of the nutrient solution and on days in between 50 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.

RESULTS AND DISCUSSION

Visual symptoms

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. 1A). 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 and 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.

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. 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 gradually dried prematurely (**Fig. 1D, 1E**). Massial and Pire (1998) also noted similar symptoms in aloe.

Magnesium, also a macronutrient, is an activator for many enzymes involved in energy transfer and growth processes. 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 ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) 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.

In our study deficiency symptoms of Mg started appearing after the first month. The symptoms were first manifested on lower leaves. The basal leaves formed wavy areas that gradually spread through the midrib towards the leaf tip, thereafter progressing to the younger, higher leaves. 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 (**Fig. 1C**), 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*).

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 severe 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. 1H**). 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 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). Mn deficiency symptoms were significant after seven months and older leaves produced small necrotic areas during the initial stages of deficiency and a characteristic chlorotic pattern between the veins was noticed (**Fig. 1E, 1G**). The acute stage of Mn deficiency results in red petioles and ribs.

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). Pistachio seedlings which lacked Mo developed deficiency symptoms after five months, first appearing on terminal leaves whose size reduced considerably (**Fig. 1D, 1F**). The leaves appeared to have narrowed. 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 chilli peppers).

The common symptoms of Mo deficiency in plants include a general yellowing, marginal and interveinal chlorosis, 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).

SHOOT GROWTH PARAMETERS

At the end of this experiment the seedlings that received complete nutrient solution (i.e. the controls) had a maximum height of 41.03 cm while the N-deficiency seedlings recorded the lowest height growth (18.73 cm). Among the various nutrients, Mn produced maximum height growth (36.3 cm). This was followed by Mg- and Mo-deficient plants. Height increment was relatively less in all the treatments compared with the complete nutrient solution (**Table 1**).

The seedlings receiving complete nutrient solution produced the most leaves (44) at the end of the study period (**Table 2**). At the end of the study the N-deficient seedlings had 26 leaves, followed by Mn-deficient (36), Mg-deficient (34) and Mo-deficient (32) seedlings.

Internodes of N-deficient seedlings were 0.53 cm long, insignificantly similar to those of seedlings grown in complete nutrient solution (0.52 cm). The mean internode length

Table 1 The effect of nutrient deficiencies on growth parameters of pistachio seedlings: results of ANOVA.

Source of variance	Degree of freedom	Mean of square							
		№ of leaves	Height of plant (cm)	Internodes (cm)	№ of Secondary leaves	Secondary height of plant (cm)	Secondary internodes (cm)	Length of root (cm)	Leaf area (cm ²)
Treatment	9	145.7*	197.7*	0.02 ns	90.43*	132.8**	0.83*	67.7*	5509**
Error	18	41.18	48.60	0.016	29.553	32.531	0.25	35.9	63364
C.V. %	-	4.5	5.03	3.8	1.5	4.9	4.9	3.5	3.9

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

Table 2 Comparison of several treatments on pistachio seedling growth parameters.

Treatment	No of leaves	Height of plant (cm)	Internodes (cm)	No of secondary leaves	Secondary height of plant (cm)	Secondary internodes (cm)	Root length (cm)	Leaf area (cm ²)
Hoagland	49.89 a	41.03 a	0.7700 bc	26.67 a	24.380 a	0.5167 c	53.33 a	12340 a
-N	26.67 cd	18.73 c	0.6800 c	14.83 cd	9.120 bc	0.5367 c	51.00 a	3961 c
Distilled water	19.78 d	15.47 c	0.7767 bc	9.22 d	4.057 c	0.4667 c	31.50 c	3556 c
-Fe	36.67 b	28.97 bc	0.9400 a	23.89 b	14.780 bc	0.9100 a	44.00 b	4568 c
-Mg	34.22 bc	33.27 bc	0.9467 a	19.78 bc	18.280 bc	0.8467 ab	49.00 ab	9871 ab
-Mn	36.89 b	36.37 b	0.9867 a	22.33 b	21.060 b	0.8833 ab	49.83 ab	11710 ab
-Mo	32.67 bc	30.10 bc	0.8867 ab	18.22 bcd	16.180 bc	0.7200 bc	45.00 b	7305 bc

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

for Fe-deficient seedlings was 0.9 cm at the end of the study while that for Mn-deficient and Mg-deficient seedlings was 0.88 and 0.84 cm, respectively at the end of the study.

Maximum leaf area was recorded by seedlings grown in complete nutrient solution (12,340 cm²) (Table 2). The lowest leaf area (3556 cm²) was recorded by seedlings lacking N. The second lowest leaf area was observed in seedlings which were deficient in Mn. In the case of Fe-deficient plants the leaf area was 4568 cm². The difference in leaf area between the other nutrient-deficient seedlings was not significant.

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. 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 (Fig. 1E, 1G). 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). For each element deficiency, we recommend the following:

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;

Magnesium: Apply dolomite limestone; foliar application of magnesium sulfate or magnesium nitrate solutions;

Iron: Foliar spray of 2% iron sulfate or 0.02-0.05% solution of iron chelate; use efficient cultivars, fertigation with chelated iron;

Molybdenum: Lime acid soils; soil application of sodium ammonium molybdate; foliar spray of 0.07-0.1% solution of ammonium molybdate

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).

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