

Effective Factors in Little Leaf Disease on Pistachio Trees

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Abstract:

In this survey, effective factors in little leaf disease on trees were studied in different pistachio orchards in Kerman province. Little leaf disease is the most serious disease of pistachio in Iran. Affected trees reduced growth rates. The first symptoms of little leaf disease are those of nutrient deficiency: a slight yellowing and shortening of the needles and reducing of shoot growth. New needles are discolored and shorter than normal, and the tree loses all but the new needles near the tips of the branches. This study examined the causes of the disease through several researches conducted in various places (Anar, Rafsanjan, Shahrbabak, Noogh, Koshkoeih), in Kerman, south of IRAN. The following treatments were employed: 1- healthy leaflet from healthy tree, 2- healthy leaflet from problem tree, 3- problem leaflet from affected trees. Little leaf disease was divided into two groups (trees with severe and low problem). External leaflet, total leaf and leaf stalk analysis were performed to determine nutrient element content. The results showed that concentration of iron in leaf stalk healthy leaflet from problem tree were statistically significant because of fixed and decreasing mobility of iron. Reductions in iron, manganese, cooper and zinc were noticed in healthy leaflet from problem tree (healthy leaflet from healthy tree). In places where the soil is characterized by low and poor iron, manganese, cooper and zinc, alone or together, the disease appears in leaflets. Therefore any foliar application can tackle the problem for the following year.

Keywords: little leaf disease, micro nutrient element, pistachio

Introduction

Pistachio (*Pistacia vera*) is the principal tree and one of the most important horticultural products of Iran. During the past 50 years, it has been embraced as one of the main commercial products. The main habitat of pistachio is the Middle East, especially Iran (Razavi, 2005). Pistachio is economically regarded as an important source of income for the region. It has been grown popular because of its adaptability to

environmental conditions including water and soil salinity, low water and drought resistance, and also due to its high nutritional value. Probably the most noticeable problem in the most of pistachio orchards in Kerman province associated with nutrient deficiency could be little leaf (Qermezo disorder). The reasons of this complication have not been yet established. The nutrition experts have different opinions and offer a variety of treatment methods, though none have been found effective so far. Plants under hot and dry climate

conditions with limestone soils and high PH absorb little amount of trace elements such as zinc, copper, manganese and iron. Based on the key role of zinc, Fe, Cu and Mn in pistachio, the following objectives can be set:

1-What simely factors have been found in affected orchards to use it as treatments for improving their problem.

2- The interaction between various nutrients identified and showed in issues. Interaction between various nutrients should be considered, until we can provide suitable conditions for nutrition plants.

Material and Methods

This experiment conducted as the following:

1- Selecting affected sites in different places in the region.

2- Completing form about some information for each orchard.

3-Preparation of photograph from the symptoms of the affected leaf.

4- Taking sample separately from affected and non- affected leaf of every each orchard in late July.

Leaves from healthy trees with symptoms, healthy leaf from the nearest tree without symptoms and leaves of the affected trees were prepared as treatments. Treatments were selected from the middle branches of trees, without fruit and leaves from the tip of the third and fourth branches. In this study, the little leaf was divided into three groups: severe, moderate and poor categories from leaf sampling.

5-taking sample of soil from depths of 80-120 cm, 40-80 cm, 0-40 cm for each orchard.

Soil samples can be proper from different layers in profiles separately.

6-taking sample of irrigation water for each orchard.

7-At the end of this study macro and micro elements in the leaves and soil should be determined. The factors CEC, PH, EC, texture (by the method of hydrometric), equivalent calcium carbonate, gypsum, organic carbon, SP and FC at different depths of soil were also determined. The analysis of water should be performed as well.

In this study, different areas of Pistachio orchards with little leaf disorder were selected. Information for each orchard was filled in little leaf disorder. Leaf sampling in late July with healthy leaves from the problem tree, healthy leaf from the nearest tree without problem and symptoms leaves of trees of the problem tree separately from middle branches of trees, without fruit and leaves the third and fourth from the tip of branches were selected. The little leaf disorder in the samples was of high and low types.

Separating from each group was sampled leaves. Using dry ash method, the mineral nutrition elements were determined in leaf. Parts of plant placed in electric furnace in the temperature 550°C. Then, 5cc HCl 6 N added to samples and the digestion was done. With filter paper flat inside the balloons 100ml and finally reached to the volume of 100ml. The amount of calcium and magnesium was determined by titration method whereas P by spectrophotometry with using the reaction of ammonium. Micro-elements such as iron, zinc, manganese and copper were measured by atomic absorption. Finally, research data were statistically analyzed by SPSS.

Results and Discussion

Zinc is an essential element for plants that absorbs in the form of bivalent cations. This element acts as cofactor regulators in many enzymes on the plant. Zn is used in the building at least four enzymes carbonic ahydraz, alcohol dehydrogenase, superoxide dismutase

and polymerase in plants. This element is essential for indole acetic acid Tryptophan. A small amount of auxin that results in reduced growth and dwarf plants and small leaves, and eventually leads to reduce long-term tree and product decreases in plants (Tandon and kimmo, 1993; Nijjar, 1990).

Deficiency of Zinc prevents protein synthesis and metabolism of carbohydrates as well. The plasma membrane permeability in plants with zinc leads to increase the exit of potassium, nitrate and organic compounds from the root cell. Zinc strongly relative to other parts of the plant. Zinc also affects on fruit and flowers in plant. Flower bud should always be formed in the growing season on the plant. Zinc is enough to ensure future product (Adames, 1996). On pistachio plant, zinc has key roles. Zinc is necessary for insemination and fertility of male pistachio trees by pollen and also has a direct role in pollen and the development of seed and grain. Shortage of Zinc causes osteoporosis (Uriu and pearson, 1986).

Zinc deficiency symptoms in Pistachio plants appeared in the early growing season. The first signs were the delay of growth in leaf buds and flowers flourish in the tree. Buds may open a few days to several weeks and in severe cases delayed for period of one month. When vegetative buds open terminal leaves were small, pale and complex mode (Tufts). Plant suffered from little leaf had limited leaf development. Fruit trees significantly were smaller and red fruits than normal Fruit. Due to the nature of inactive zinc, deficiency symptoms of terminal leaves may be limited to one branch of the tree (Brown, 1994). In the pistachio trees, fruits are small size and seed number may be low on the cluster. Red color seed and seed osteoporosis due to severe

deficiency (Ashworth *et al.*, 1985). Symptoms of zinc deficiency appear in amounts less than 7 PPM in pistachio leaves and the concentration of 2-3 PPM, plant is suffering from severe deficiency. In the deficiency of zinc, levels of nitrogen and phosphorus increase in the plant. Overall results in the pistachio plant showed that zinc element was not very dynamic (Durzan, 1995). Foliaration with zinc in the deficiency can be resolved. If only one side foliarate the tree, we will just disappear deficiency symptoms one side and the other side will remain the same. Analysis healthy pistachio indicated that zinc is a lot in early May, but then quickly can drop late June and till October remains constant (Uriu and Crane, 1977; Durzan, 1995). Uriu and pearson (1984) thinning Fruit and getting red spots scattered on leaves of pistachio were introduced as deficiency of zinc (Webb and longeragan, 1990). In a study in this field by Uriu and Pearson in pistachio orchards in America observed in the severe shortage, more than 80% of grains were thinning while trees without symptoms only 20% of the grain were thinning. In this study, symptoms of zinc deficiency in leaves were observed when the amounts of zinc were less than 7 Mgg^{-1} (Uriu and Pearson, 1986). According to research Karimian (1995) on the relationship between nutritional nitrogen, phosphorus and zinc in corn, found that the nitrogen treatments increased zinc and zinc nutritional status improved, but decreased concentrations of P and P/Zn plant. Many researchers reported that the use of phosphate fertilizer caused being slow on the transfer and decreased zinc concentration in shoots and deficiency symptoms appeared in trees (Morvedt *et al.*, 1972). In relation to the distribution of zinc between roots and shoots, Sharma *et al.*, (1968) according to a greenhouse research on leave corn and Tomato, studied interaction between phosphorus and zinc. They observed deficiency symptoms of zinc in both part of

plant and the zinc concentration in roots less affected by increased phosphorus. With increased phosphorus, phosphorus concentrations increased in shoots than roots and the presence of phosphorus treatments on the roots of corn has increased than the aerial (Sharma *et al.*, 1968). According to research Burleson in irrigated medium on cotton crop, which its roots had been divided into two parts and zinc fertilizers added separately from each other. They added at first phosphate fertilizer after that zinc fertilizer, and concluded that the total phosphorus increased on root and reduced on shoot. When they added fertilizer Zn at first, the total amount of phosphate fertilizer on roots increased and the amount of it reduced on shoots (Burleson, 1967). Parkd *et al.*, (1992) reported zinc deficiency interferences in the metabolism of phosphorus and its sorption increases from root and it passes to shoot. Under high phosphorus nutrition, phosphorus accumulation of toxic levels in leaves caused symptoms similar to zinc deficiency (Parker *et al.*, 1992). Iron was effective in construction and maintenance of chlorophyll, carbohydrate production, and cell respiration, chemical reduction of nitrate, sulfate and nitrogen uptake and had special role in nucleic acid metabolism and RNA (Tandon, 1995). The most famous duty of iron was in enzymatic systems in which the haem and haemin acts as a prostic group. The uptake of iron among the other micronutrient elements after chlorine had the highest amount in plants. Iron amount in the young seedlings could be very high and decreased with increasing age. If the values of iron were less than 50 Mgg⁻¹ would indicate deficiency in trees (Tandon, 1995). But this amount in the tissue of the meristem and developing parts was reached more and up to 200

Mgg⁻¹. Iron toxicity levels have been reported in 100 to Mgg⁻¹ 400 and even higher values (Tandon, 1995). Iron deficiency symptoms in the youngest in the pistachio, leaves were green and midribs remained yellowish and products would drop because of the reduction of photosynthetic activity (Adames, 1996). Singh and Stenberg experiments in barley plants found that zinc and its total amount increased with increasing iron soil, but the effect of zinc on iron concentration and total amount was unstable (Singh and Stenberg, 1975). Experiment the effect of iron ion on zinc absorption and its transport in rice were studied. It was found that high concentrations of iron in the culture medium decreased zinc absorption (Brar and Sekhon, 1976). Manganese had no effect as a main component of the enzyme, but acts as active of many enzymes. Manganese can also activate the enzyme transfers phosphate donor and replaced by Mg. Manganese activated Auxin oxidase enzyme and manganese toxicity may cause severe auxin due to degradation. Mn toxicity symptoms due to complexity of the leaf because of increasing in indole acetic Acid oxidase (Ohki, 1985; Nijjar, 1990). The important role of manganese was O₂ released during photosynthesis in the chloroplast. Therefore deficiencies of manganese first would affect photosynthetic O₂. Severe deficiencies of manganese have been decreased Destruction of chloroplast then decreased chloroplast concentrations (Tandon, 1995). Manganese deficiency symptoms would be determined first in young leaves as pale intercostals and burned bright spots. Gray areas appeared in the lower parts of younger leaves. This leaves changed from yellow to orange. Critical level deficiency varied mainly between 10 and 15 Mgg⁻¹ (Mordvedt *et al.*, 1991). In pistachio, leaf areas turned to light and burnt Mottling by weak chlorophyll production and adverse effects caused during photosynthesis and nitrogen metabolism and yield loss

by enzymatic processes (Adames, 1996). Singh and Steenberg studied the interaction between micro nutrition elements in barley plant that grew in soils with high zinc. They realized that total amount of zinc increased with increasing manganese in soil. Similarly the other hand, increased Mn concentration and total amount also increased with increasing soil Zn (Sing and Steenberg, 1974). Ming and Chungren were studied manganese and zinc application effects on nutritional balance. In this experiment reported zinc with adding NPK fertilizer significantly decreased manganese and iron in the grain, but increased the concentrations of copper (Ming and Chungren, 1995). Copper is a part of the building on a large number of plant enzymes and has a role in energy metabolism. Copper has role in cytochrome oxidase and protein components constituting chloroplast that involved the electron transport chain in photosynthesis, which is action, creates a linkage (Tandon, 1995). Copper deficiency reduces the formation of lignin common cause symptoms such as wilting and stem will bend. It is noteworthy that copper deficiency causes infertility vulva and reduced products. Copper have increased following the formation of vitamin A in the plant (Mortvedt *et al.*, 1997; Tandon, 1995). Copper deficiency in the pistachio is a relatively common deficiency symptoms in mid-summer it will appear near the branch tip of the burning leaves and started to progress from the leaves. New leaves in the tip end will burn and mid-to late summer necrosis and water was lost. Initially mild branches dieback and subsequently prune dieback occurred in late summer. Some terminals branches downward and bend in the bacilliform (Shepardos crook) and are often kernel is wrinkled (Brown,

1994). trees may be only a few branches are affected and in severe case 100-90% of branches have signs of necrosis and death takes at the end points of growth. This deficiency in fruit trees is known as a prune dieback disease with decreased distance between nodes and eventually plant remains dwarf. The critical level of copper deficiencies has been reported 4 Mgg^{-1} in the pistachio trees and the toxicity level was more than 15-30 Mgg^{-1} (Uriu and pearson, 1986). Many researchers observed interaction between copper and zinc. In all have seen numerous reported that copper strongly prevent of zinc absorbed, on the other hand, zinc high concentration in soil solution effected on copper absorption by plant roots and deficiency copper may be intensify. It is not Because of this the dilution effect on the transfer or reduction of root to shoot. It seems that of zinc and copper are absorbed with a same mechanism. Thus, they compete with each other and prevent the absorption of another carrier to occupy the same positions in each other compete. Physiology experts are interested in proteins that a separate transmitter for each element, but an important distinction to be possible for some bivalent cations such as copper and zinc cation in Single transmission path in order provide for the two cations cell (Mortvedt *et al.*, 1991; Nijjar 1990; Tisdale *et al.*, 1984). Durzan *et al.* (1995) used amino acid as an indicator of zinc deficiency in plants in pistachio reported. zinc deficiency occurs in Low levels of zinc deficiency and also high levels of nitrogen, total soluble nitrogen and phosphorus. zinc deficiency caused accumulation of nitrogen in the form of arginines released and generally soluble nitrogen increases. In the contrast, dissoluble nitrogen decreased in the late of the season and phosphorus deficiency also leads to accumulation of arginine nitrogen was released. Thus, nitrogen in the form of arginine can be an indicator of nitrogen and zinc status in fruit trees should be used. While

considerable information existed on the band by amino acids and other micro elements, Amino acids and its relation to the physiological maintenance parts are still unknown. Using of amino acids as leaf sprayed was new method can be overcome zinc deficiency (Durzan *et al.*, 1995). Heidarinejad (1999) in relation to little leaf symptoms (Qermezo) in pistachio orchards reported that the incidence of this complication, mainly in orchards that have had nutrition poverty, despite annoying elements including sodium and magnesium and Nutritional balance has been foul. Antagonistic effects and soil problems in this complication, nutrition elements is not easily absorbed by plants. Positive reaction towards sprayed Fe and Cu were performed, leaves and fruits found their primary health care. Also reported that the little leaf rate in pistachio orchards relationship existed to weed type, soil texture, amount dieback with being black and red branches, plant age and plantation spacing. Being with more weeds, increased density in soil, plant age, and little leaf condition had been exacerbated. Tissue destruction, subsequent plant Vesicular, being red branches and dieback in the orchards were there. In orchards with heavy texture and inadequate irrigation water can be seen more passion this complication. Tadayyonnejad (1998) examined interaction with zinc, phosphate zinc and iron on seedlings pistachio (*Pistacia vera*). The concentration, total absorption zinc and iron levels decreased in the high level of phosphorus. Phosphorus concentrations decreased significantly on zinc treatments was much more tangible than iron treatments. They reported decreased of zinc and iron concentrations because of dilution effect with increasing growth due to fertilizer phosphorus and

affect of High phosphorus levels in uptake and transition in the plant. Interaction effects of phosphorus zinc and iron in high levels of these elements is more severe. SarcheshmehPour (1997) studied interaction between zinc elements iron, copper and manganese in pistachio plant (*Pistacia vera*). they reported zinc disrupt transfer other elements to aerial in the plant. While the copper elements decreased manganese and zinc absorption by plants, unlike copper and manganese, iron increased transferring of zinc to aerial plant. Afrousheh *et al.*, (2007) studied the effects of nutrient deficiency in seedlings of pistachio (*Pistacia vera*) in sand culture medium and reported that symptoms of manganese deficiency in the pistachio unlike most reports for other plants to create pale green in young leaves and advanced stage red color in the veins leaves. Visible sign of iron deficiency decreased chlorophyll content in young leaf and tended to create green to yellow. The browning leaves lower margins and subsequent deciduous leaves were observed. 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 at after seven months of the study. 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. Analysis of the plant in both intensity high and low severity of symptoms was observed that concentrations of boron, iron and zinc concentrations in areas with high intensity, has been less than critical level. Analysis of variance in both the intensity high

and low intensity condition on zinc, iron and on the petiole and leaflets containing the tree healthy condition, in Table 1 is shown. The degree of concentration on the 5% level of probability was significant. The results mean zinc concentration in leaflets showed that high intensity of a significant amount is reduced. ANOVA significant at 1% in connection with the boron and Mn concentration showed. Severity of symptoms in areas with high concentrations of manganese and the lowest concentration showed the highest rate. The petiole analyses at different intensity are shown in Table 1. High levels of iron in the petiole in areas with high intensity condition can be due to stabilization of this element in the petiole and its mobility is reduced. Analysis of variance in both the intensity

(low and high intensity) on phosphorus concentration and on the petiole, whole leaf and petiole of normal healthy tree is shown in Table 1. The results mean compare zinc concentration end of the petiole, leaf, petiole and total levels of 5% was significant. The highest concentration of high intensity is there. The results compare concentration P terminal leaflets, leaf petioles and total levels of 5% were significant. The lowest concentration of phosphorus was high in intensity. Analysis of variance in both the intensity (low and high intensity) on phosphorus concentration and on the petiole, leaf and petiole of the whole complicated tree is shown in Table 1. Manganese, copper, zinc, magnesium, phosphorus, the highest concentration was the intensity high.

Figures:

Fig1, Little leaf deficiency symptoms of terminal leaves may be limited to one branch of the tree and fruit trees with small size and seed number may

below on the cluster. Red seed color and seed osteoporosis a major problem due to severe deficiency



Table 1: concentration of nutrients with significant differences in different parts of the leaf in different degree condition

| healthy leaflet from healthy tree | | | | healthy leaflet from problem tree | | | | | |
|-----------------------------------|-----------|-------|--------|-----------------------------------|------|------|-------|-------|------|
| | Intensity | B | P | Intensity | Fe | Zn | Mn | B | |
| total | High | 144a | 0.27a | total | High | 59 a | 4/8 a | 29 a | 67 a |
| leaf | Low | 60b | 0.25b | leaf | Low | 89 a | 6/5 a | 55 a | 64 a |
| leaflet | High | 129a | 0.3 a | leaflet | High | 65 a | 4b | 34b | 167a |
| | Low | 56b | 0.29 a | | Low | 75 a | 6a | 57/5a | 64b |
| leaf | High | 15/6a | 0.12 a | leaf | High | 64a | 6/1 a | 48 a | 15 a |
| stalk | Low | 12/5b | 0.15 a | stalk | Low | 45b | 7/8 a | 51 a | 12 a |

| problem leaflet from problem tree | | | | | | | | | |
|-----------------------------------|-----------|-------|-------|-----|-------|-------|--------|-------|-------|
| | Intensity | Fe | Zn | Mn | Cu | B | P | Ca | Mg |
| total | High | 79 a | 59/6a | 47a | 6/6a | 150a | 0.32 a | 2/5 a | 1 a |
| leaf | Low | 82 a | 9/3b | 50a | 4/35b | 65b | 0.24 a | 3/2 a | 0.4 a |
| leaflet | High | 218a | 6 a | 47a | 7/3a | 157a | 0.3a | 4a | 0.7 a |
| | Low | 57b | 8 a | 46b | 4/6b | 51b | 0.2b | 3/1b | 0.4 a |
| leaf | High | 103 a | 5 a | 62a | 5/2 a | 18/6a | 0.12 a | 0/6a | 1/5a |
| stalk | Low | 65 a | 7 a | 55a | 5 a | 9/8b | 0.13 a | 1 a | 0.4b |

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