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# Vinegar and Iron Chelate Spray Affected Vegetative Growth and Yield of Grape cv. Thompson Seedless

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**Abstract**

In soils with high pH, iron is inactivated and bounded to the leaf apoplast developing leaf chlorosis. The inactive iron can be reduced artificially by lowering leaf apoplastic pH with spraying weak acid solutions, providing the symplast absorption of activated iron. The objective of this study was to determine the effect of vinegar spray, as an acidifying substance and iron chelate (Fe-EDTA) on iron nutrition and vegetative growth of grape vines (cv. Thompson Seedless) grown in high pH soils. Four years old grape vines of cv. Thompson Seedless which were grown in high pH soil (pH=8) were selected. Experimental design was a Randomized Complete Block Design (RCBD) with four spray treatments: 1) vinegar (0.8%), 2) iron chelate (0.2%), 3) combination of vinegar and iron chelate (0.8%, 0.2% respectively) and 4) distilled water (control). Vinegar treated plants had higher chlorophyll, active iron ($Fe^{2+}$) and yield. Vinegar is a by-product of grapes that can be easily produced by growers with very little cost and its application has effectively improved plant vegetative growth, yield and mitigated iron deficiency of "Thompson Seedless" grapes. Vinegar is very cheap compared to chelated iron. The vinegar spray has the potential to increases yield without increasing the cost for the growers, ensuring both profitability and sustainability of the production system.

**Keywords:** Ferrous & Ferric ions; Chlorophyll; Apoplastic pH; Grape; Iron deficiency chlorosis

## Introduction

In many areas around the world, fruit crops are grown in soils with high pH with abundant bicarbonates. In these high pH soils, iron, an essential element for plant growth and development, is inaccessible for plant metabolism. Most fruit crops in these soils exhibit high pH-induced iron deficiency, known as iron chlorosis, although there are enough iron in the soil and plant leaves [1].

Roots uptake iron by reducing $Fe^{3+}$ to $Fe^{2+}$ at the cell membrane of epidermal root cells [2]. After iron is reduced in the roots, it is re-oxidized back to $Fe^{3+}$ in the apoplast where $Fe^{3+}$ binds with citric acid (Schmidt 1999) and then transported to the leaves as $Fe^{3+}$-citrate. Iron is re-reduced in the leaf apoplast to the $Fe^{2+}$ and is actively transported across the plasma membrane into the symplast to be metabolized by the plant [2-5]. However, in calcareous soils, the reduction of $Fe^{3+}$ to $Fe^{2+}$ in the leaves is inhibited by the high pH environment of the apoplast [2,6,7].

Although iron is absorbed by roots at high pH soils, reports suggest that the chlorosis of vine rootstocks is caused by an inhibition of the absorption and translocation of iron due to inhibition of $Fe^{3+}$ reduction by root cells [8]. The high bicarbonate concentration in limestone soils buffers $H^+$ and increases the pH of the root apoplast inhibiting $Fe^{3+}$ reductase [9]. High bicarbonate concentration in a nutrient solution strongly inhibited iron uptake by roots and its translocation to shoots during a short-term absorption observed in many plant species [10,11].

A standard method to prevent iron chlorosis is to add chelated iron to the soil each year. Chelated iron is a very expensive fertilizer, which increases the cost of the fruit production on high pH soils. In United States, the cost of chelated iron is estimated at 80% of the total cost of fertilizers 50% of the total agricultural chemical [12]. The cost may be even higher in developing countries (personal communication with fertilizer suppliers).

Grape is a major horticultural product in many parts of the world with 70 million tonnes produced worldwide and 2.2 million tonnes in Iran [13] (FAO STAT). Grape is sensitive to iron deficiency induced by pH. The "Thompson Seedless" cultivar is still the main cultivar of grape and table grapes in the world [14]. Therefore, this study evaluates foliar application of a weak acid (vinegar) as a low cost alternative to the application of expensive chelated iron to the soil to prevent iron deficiency and increase the yield of grapes grown in high pH soils.

## Materials and Methods

The field experiment was conducted at a grower's vineyard in Khandab, Arak, Iran in 2008-2009 with 4 years old vines on a trellis system. Irrigation was based on the recommendations from the Ministry of agriculture for the local area. The water and soil were analyzed and pH of the soil was in average 8.2 at three measured soil levels and bicarbonate ion at 189.1 $mgL^{-1}$.

Each experimental block had 6 grape vines. There were two vines between each experimental unit and a row between treatments to minimize the marginal effects. The experiment was conducted in a randomized complete block design in four replicates with four treatments: i) grape vinegar at 0.8%, ii) iron chelate at 0.2%, iii) mixture of grape vinegar and iron chelate (0.8% & 0.2% respectively) and iv) distilled water as control. Iron chelate (Fe-EDTA, Tradecorp) had 13.2% active ingredients and grape vinegar had 4.3% acidity. Vines

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were sprayed in full coverage until run off was observed. The vines were sprayed at six stages: 1) before bud break on April 1st; 2) after petal fall on June 4th; 3) small berries on June 12th; 4) two weeks after on June 26th; 5) 4 weeks after on July 10th; and 6) 6 weeks after which coincided with the beginning of veraison on July 24th.

The total chlorophyll concentration (a and b) was measured by Anon method [15]. The random leaf samples were washed with distilled water and 3 g of leaves were ground in a mortar and pestle and 20 ml of 80% acetone was added to the mixture and ground completely in a final solution of 100 ml. The solution was filtered by Whatman 42 paper and the chlorophyll content was measured at 645 and 663 nm absorbance with a spectrophotometer (Pharmacia Biotech Ultrospec 3000 UV/Visible Spectrophotometer, Cambridge, England). Acetone 80% was used as blank and total concentration of chlorophyll a and b was calculated as followed:

$$(\text{Chl a+ Chl b})\ \text{mg ml}^{-1} = (\text{A645 } 0.0202) + (\text{A663 } 0.00802)$$

Cell sap pH and stomatal conductance was measured one day after the 5th and 6th sprays. Cell sap was measured by the method of Barate et al. [16] with some modifications. Leaves were washed with distilled water, dried and frozen in liquid nitrogen and ground. Fifty ml of distilled water was added to two grams of the powdered leaves and incubated at 50°C on a water bath for 1 min and centrifuged at 3000 g for 15 min and pH of supernatant was measured. The stomatal conductance was measured with AP4-UM-3 Porometer between 1-2 pm one day after leaf sprays on fifth and sixth sprays.

Leaf total Fe, and $Fe^{2+}$ was measured one week after the 3rd spray. To measure leaf total iron, mature leaves were quickly washed with distilled water, dried in an oven at 70°C for 48 h and ground. One gram of the leaf powder was acid digested with HCl and total iron was measured by atomic absorption (Thermo Elemental, Solar). $Fe^{2+}$ was measured with ortophenanetroline method, by producing an orange complex and measuring the absorbance at 510 nm by spectrophotometer. The difference between total iron and $Fe^{2+}$ is the concentration of $Fe^{3+}$ iron [17]. Yield (Kg/plant) was recorded after harvest and all the data were analyzed using analysis of variance [18] (ANOVA, SAS Institute Inc., version 9.2). Means were compared using Duncan multiple range test at $p \leq 0.05\%$.

## Results and Discussion

### Leaf chlorophyll content

Leaf chlorophyll content (Figure 1A) at both fifth and sixth sprays were significantly higher in vinegar treated leaves (0.012 & 0.011 $mgml^{-1}$ respectively) compared to other treatments. There were no significant differences between control and Fe-EDTA spray, and mixture with vinegar. While other reports have shown that chelated iron spray increases chlorophyll index (SPAD unit) in Lychee and pear [12,19], it did not have such an effect in our experiment, may be because they used a different chelated iron (Fe-EDDHA & Fe-DTPA respectively). Krohling et al. [6] have also reported that Fe-EDTA leaf absorption is the least compared to $FeCl_3$ and Fe-citrate.

### Total iron, $Fe^{3+}$ and $Fe^{2+}$

The leaf iron concentration measured one week after the third stage spraying (June 26th) showed that total iron did not differ statistically between treatments. However, in the vinegar-treated vines more than half of the iron is in soluble and active form ($Fe^{2+}$), while in other treatments the inactive iron ($Fe^{3+}$) is the common form. Chelated iron alone or mixed with vinegar increased $Fe^{3+}$ and did not affect active iron ($Fe^{2+}$) concentration. In the control plants, the active iron is very negligible (Figure 1B). The high concentration of inactive $Fe^{3+}$ in all treatments, with the exception of vinegar treated vines, indicates that iron is bound to the cell membranes in all treatments. Spraying chelated iron, alone or mixed with vinegar, did not reduce $Fe^{3+}$ to $Fe^{2+}$. However, the vinegar sprays alone reduced $Fe^{3+}$ membrane-bound to active soluble $Fe^{2+}$. As a result, the higher concentration of $Fe^{2+}$ increased the ratio of active ($Fe^{2+}$) to inactive iron ($Fe^{3+}$). This is not consistent with what Crane et al. [12] observed in Lychee where Fe-EDDHA mixed with acid increased the concentration of $Fe^{2+}$. However, both studies confirmed that chelated iron alone does not increase $Fe^{2+}$ and that an acid jet is needed. In orange trees, only total iron was measured and similarly, acid, chelated iron or a mixture could increase total iron concentration [1].

Plants sprayed with vinegar also had the highest chlorophyll content compared to other treatments. Previous reports have also shown that reduction of $Fe^{3+}$ is higher in photosynthetically active greener leaves [3,6,20]. This shows that iron reduction is directly dependent on photosynthetic electron donors such as NAD(P)H, which are more abundant in leaves with higher chlorophyll content. Photo-reduction of iron citrate in apoplast is critical for iron absorption in leaf laminas. The iron reduction diminishes in low chlorophyll content leaves and results to lower photosynthesis and higher membrane bound $Fe^{3+}$ as was seen in this experiment Brüraugman et al. [8,21,22].

### pH of the cell sap

Cell sap pH was the highest at chelated iron spray after fifth spray and at control sprayed vines after sixth spray. The vinegar treated leaves had the lowest pH and control and chelated iron treatments had the highest cell sap pH (Figure 1C). The vinegar spray effectively decreased cell sap pH and created a better condition for reduction of membrane bound $Fe^{3+}$-citrate. The reduced iron then became soluble and transported through symplast as active iron. Active iron $Fe^{2+}$ has improved the iron deficiency symptoms and increased crop yield and performance.

Based on the Kosegarten et al. [4], iron reduction and iron absorption increases in symplast under artificial low pH (such as acid spray). Therefore, re-greening of leaves after spraying with weak acids [23,24] can be due to the effect of acids in reducing the membrane bound iron and increasing soluble iron in leaf apoplast. Therefore, any treatment that can decrease leaf apoplastic pH can provide suitable conditions to reduce $Fe^{3+}$ and increase iron absorption in symplast. This condition was met in vinegar sprayed grape vines in our experiment.

### Stomatal conductance

Leaf stomatal conductance was higher in vinegar treated vines after fifth spray. However, no significance differences were observed after sixth spray (Figure 1D). The open stomata in vinegar sprayed vines may have increased the leaf gas exchange with the environment. This ensures higher $CO_2$ absorption and increased photosynthesis in synergy with increased chlorophyll content, which will lead to the improved photosynthesis. The results were reflected in the increased $Fe^{2+}$ and reduced cell sap pH.

### Yield

Yield is significantly lower in control (62 kg/plant) compared to other treatments (in average 77 kg/plant). There are no significant differences between vinegar and iron treatments (data not shown).

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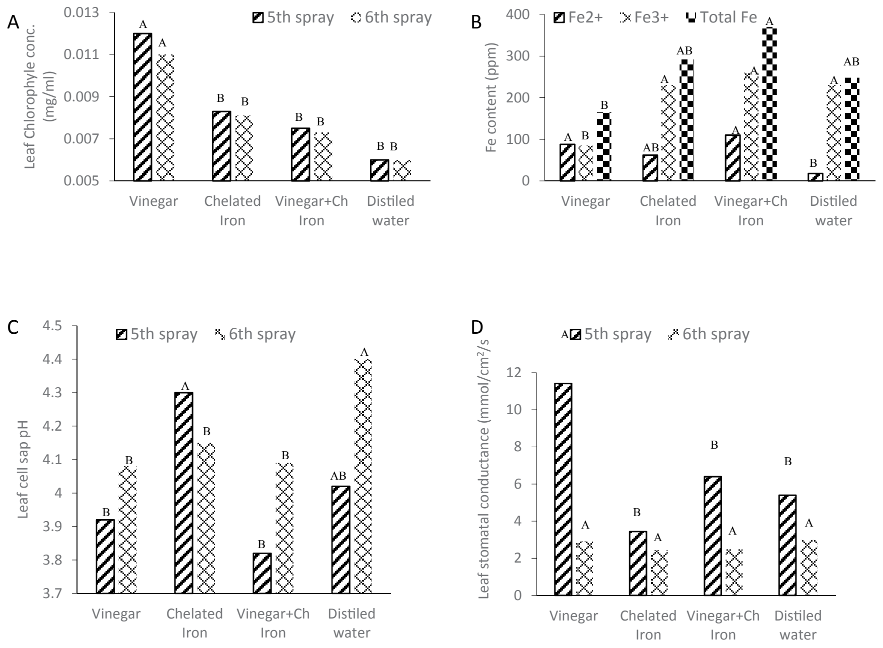


**Figure 1:** Charcteristics measured on grape vines treated with vinegar (8 ppm),chelated iron (Ch Iron, 2 ppm), mixture of vinegar and chelted iron (8, 2 ppm repectively) and water (control) in "Thompson Seedless" grape grown in a high pH soil. All characteristics measured one day after fifth spary (10 July) and sixth spray (24 July) except iron which was measured one week after the third spray. A) Leaf chlorophyll content, B) $Fe^{3+}$, $Fe^{2+}$ and total iron, C) Leaf cell sap pH, D) Leaf stomatal conductance. Bars with the same letters are not significantly different at $p \leq 0.05$.

## Conclusion

In vinegar treated grape vines, increased chlorophyll content increased reduction of iron ($Fe^{3+}$) and absorption of $Fe^{2+}$ through symplast and re-greening of cholorotic leaves mainly due to the reduced leaf cell sap pH. Only vinegar spray was able to reduce the membrane bound $Fe^{3+}$ to metabolically active $Fe^{2+}$ in the cell sap. The high concentration of inactive iron ($Fe^{3+}$) in control and chelated iron sprayed vines reveals the possibility of high iron deposits on the cell membrane. The vinegar treated leaves had the lowest pH, while control and chelated iron treatments had the highest cell sap pH. Any treatment that can decrease leaf apoplastic pH has the potential to provide suitable conditions to reduce $Fe^{3+}$ and diminishes the iron deficiency symptoms.

The reduced cell sap pH and high $Fe^{2+}$ and chlorophyll concentrations in vinegar treated vines in synergy with increased stomatal conductance are all reflected in re-greening of the vines and higher yield. Further research is needed to compare the different type of chelated-iron products to find, if there is any difference between Fe-EDTA, Fe-EDDHA and Fe-DTPA, as the results of this experiment do not comply with other reports where different chelated iron was used.

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