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Effects of N, Fe and Zn Nutrition on Vegetative and Reproductive Growth and Fruit Quality of Grapevine (*Vitis viniferea* L.)

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Abstract

This Research aimed to study on effect of foliar spraying with zinc (as zinc sulphate), nitrogen (as urea) and iron (as sulphate and chelated Fe) on growth parameters, quality of fruit, flower characteristics, chemical constituents and nutrients content of leaves on grapevine. Applied nutrients were zinc sulphate (Zn) of 0.0 and 1.5gl^{-1} and urea (N) at 0.0 and 5 gl^{-1} and Fe (1) control (low Fe), (2) Fe (III)-chelate 3mgl^{-1}, (3) 20 g/ per tree soil application and (4) Fe sulfate ($FeSO_4$) 3mgl^{-1} which were applied both alone and in combinations with each other as foliar spraying. In a pot experiment, effects of N, Zn and Fe fertilizer were evaluated on concentrations of Fe, N and Zn in leaves, yield and the fruit quality of grape (*Vitis viniferea*). Results showed that application of urea and Zn and Fe increased vegetative growth and length of young branch. Highest size of berry achieved in N_2= 5 gl^{-1}× Zn= 1.5 mg l^{-1} × Fe= chelates Fe 3 mg l^{-1}. Nitrogen (N), iron (Fe) and zink (Zn) are essential element in grape vine causes increased contain N, Zn and Fe on leaves. Also results showed N Fe had significant difference ($p \leq 0.05$) on quality and quantity(?) characters in grape vine, Zn and Fe have no significant difference ($p \leq 0.05$).

Keywords: Antifungal, Plant extracts, Control, *Alternaria sesami*.

INTRODUCTION

Grapevine (*Vitis vinifera* L.), belongs to Vitaceae family, and is one of the most produced agricultural crops all over the world. The cultivated grapevine (*Vitis vinifera* L. subsp. vinifera) has economic importance (Ruel and Walker, 2006). Grapevine (*Vitis vinifera* L.) is a deciduous plant that of genetic varieties has expanded in the world. These varieties can produce such high value processed products as grape juice, jellies, table grapes, and dietary supplements (Zhang *et al*., 2009). The rare European– Middle Asian wild grapevine (V. vinifera subsp. sylvestris), which is believed to be the living ancestor of modern grapevine cultivars (This *et al*., 2006).

Nitrogen can be stored in the forms of organic and/or inorganic. The organic nitrogen approximately makes up 90% of soil's total nitrogen content, which is originated from living plants and animals, or as humus, i.e. decomposition product of plant and animal residues. Nitrate (NO^3) is the final form of N transformation from both organic and inorganic sources (Alva *et al*., 2006). In agricultural systems, overuse of N-fertilizer may result in NO^{3-} displacement to deeper soil layers that may eventually end up in the groundwater (De Paz, and Ramos, 2004). Researchers showed that when N concentration increased from 87 kg ha^{-1} to 393 kg ha^{-1}, the lack of N increased from 8% into 48%. (Dasberg *et al*., 1984).

Zn is an important essential micronutrient for plant. Zinc is an essential metal for normal plant growth and development since it's a structural element of proteins and enzymes in living organisms. High concentrations of Zn in many soils, indicated correct management methods including application of sewage sludge or animal manure. In rice production, yields are often reduced and Zn in the grains is low when Zn supply isn't sufficient (Gao, *et al*., 2006). Zn is the second microelement after iron (Fe) and is involved in various biological processes in organisms (Broadley *et al*., 2007).

Iron (Fe) deficiency induces chlorosis is a major nutritional disorder in in calcareous soils (Álvarez- Fernández *et al*., 2006). Iron deficiency in fruit trees causes chlorosis, decreases in vegetative growth, reduced fruit yield and quality. Iron deficiency (iron chlorosis) in fruit trees is results from impaired acquisition and use of the metal by plants, rather than from a low level of Fe in soils. Therefore, Fe fertilizers, either applied to the soil or delivered to the foliage, are used every year to control Fe deficiency (Abadía *et al*., 2011). Fe deficiency decrease in the amounts of chlorophylls (Abadı *et al*., 1989) with the appearance of leaf chlorosis, the visible symptom in Prunus (Pe´rez *et al*., 1995) and orange trees (Pestana *et al*., 2001).

In this study, we will a report about main characteristics of vegetative and reproductive growth of grape vine under application of N, Zn and Fe in both foliar spray and soil application.

MATERIALS AND METHODS

This study was conducted on mature bearing vine trees in Lorestan province, Iran. The garden soil texture, was clay loam (34% clay and 39% sand), consisting of 7.76% $CaCO_3$, and has a pH of 7.3. The study was conducted based on a completely randomized block design with 16 treatment and four replications, with 64 10-year trees. At the end of the 15-days drought period soil water potential (Ψsoil) was calculated according to soil moisture/water potential curves previously assessed for the pot substrate (Lovisolo and Schubert, 1998).

1. Plant material and growth conditions

10-year-old plants of grapevine were used. Three replicate plants per genotype (were grown in 12 L containers filled with a substrate composed of sandy-loam soil/expanded clay/peat mixture (4:2:1 in volume), with a final pH of 7.3. Containers were placed in a Researchers Garden of Khoram Abad. This study was carried out on cultivar 'Asgari'. This obtained cultivar had high yielding capacity and high quality of fruits. 'Asgari' is a vigorous and early cultivar obtained from the University of Lorestan and well-adapted to the Khoram Abad.

2. Treatment

Treatments including (1) control (low Fe diet), (2) Fe (III)-chelates3mgl^{-1}, (3) 20 g/ per tree soli application Fe and (4) Fe sulfate ($FeSO_4$) 3mgl^{-1}, second factor is Zn sulfate 0 and 1.5 mgl^{-1} and end factor N treatments 0 and 5 mg l^{-1}.

3. Yield

Fruits of each tree were selected on the basis of weight average and yield per tree was calculated. At the end of the experiment, number of fruits per raceme was recorded. To obtain yield, the number of fruits is divided by the number of raceme. Berry length was measured using a calliper. At the end of the experiment, weights of racemes were recorded.

4. Total soluble solids

A sample of 20 grapevines was randomly selected and harvested for quality measurements from each replication. To characterize the maturity and quality of the fruit total soluble solids (TSS), titratable acidity (TA) were determined. TSS, expressed as °Brix, was measured with a portable refract meter. (Moraes Barros *et al*., 2012).

5. Plant-growth parameters:

At the end of experiment, three young branches were selected, and then some vegetative traits (branch's length and width) were determined.

6. Concentration of N, Zn and Fe in leaves

After sixty days spray of fertilizers, five leaves were randomly selected from each tree. The leaves was washed with tap water, followed by deionizer water, and then with 0.01 M HCl. The N concentration was determined according to the Kjeldahl Method. Subsamples were dry-ashed at 450 _C and digested in HNO3 and HCl following the A.O.A.C. procedure (A.O.A.C., 1990). The Zn, Fe concentration was determined by atomic absorption spectrophotometer (Pye Unicam, Cambridge, UK).

7. Statistical analysis

Data were analyze of variance based on the General Linear Models procedure in SAS (Statistical Analysis Systems, 19871), and then mean comparisons was performed using LSD test ($p \leq 0.05$). Pearson's correlation analysis was performed between the antioxidant activity and the total phenolic or ascorbic acid contents.

RESULTS AND DISSCUSION

1. Yield

Under moderate N, Fe and Zn levels, the yield fruit number and fruit length had significant genotypic variations (Table 2). N and Zn had a highly significant effect on the number of flowers per plant. For the total yield per tree, as shown in Table 2, N, Fe and Zn had the highest yield in this study in comparison to the control. Treatment with $N1Zn_2Fe_2$ had the greatest fruit weight average. With increasing of fertilizer concentration, increased yield, increased number of fruits per raceme and fruit weight in treatment $N_1Zn_2Fe_4$ and $N_1 \times Zn_2 \times Fe_2$ respectively. The greatest yield Achieved in $N_1 \times Zn_2 \times Fe_3$.

Alva *et al*. (2003) evaluated different combinations of irrigation and nitrogen management. Fruit yield of 36- year-old 'Valencia' orange trees was greater with the application of N as fustigation compared to that of the trees which received a similar rate of N as three soil applications of granular product. Previous studies showed a lack of response to fustigation in fruit yield. Koo (1980) also reported that dry nitrogen fertilizer (2 items) resulted in similar fruit yields as 10 fustigation applications. Arshad *et al*., (2006) reported foliage spraying with urea 1 and 1.5% increased berry set and the weight of berries. Alva *et al*. (1998) have showed similar results in 2 years of a 3-year study. Koo (1986) and Boman (1993) also found no difference in yield between two applications of dry soluble fertilizer with controlled release N sources. The objective of N management consists of supplying enough N to achieve maximum crop yields (Amanullah, 2010). Efficient N management in tomato production can be attained by using suitable evaluations of the plant's nutritional status. Adequate availability of N during the critical stages of fruit initiation and development is important to support optimal yield and fine quality in citrus fruits (Davies and Albrigo, 1994; Syvertsen and Smith, 1996). Therefore ZnSO4 is applied to increase fruit number, size and quality (Abdollahi *et al*., 2010). These researchers reported that application of Fe increased photosynthesis and increased yield of grapevine. Yamdagni *et al*., (1979) reported application of Zn caused increased yield and weight of raceme on grape vine. Dixi and Gamdagin (1978) reported that a foliage spraying with $ZnSO_4$ on March and April increased size of oranges. Their results had shown Fe fertilizer application improves yield and fruit quality in several crops and is a standard practice in regions of fruit production (Álvarez-Fernández, *et al*., 2006). Researchers showed that fruit yield and quality depends on different concentrations of N, Fe and Zn. Abdollahi *et al*., (2010) reported application of chelate Fe on soil increased quality and production of fruits of grapevine. The main aim of experiment was to increase yield and to enhance reproductive growth.

2. TSS

As shown in Table 3, effect of N, Zn and Fe on TSS have no significant difference ($p \leq 0.05$). The results showed that no significant difference were observed among treatment's impact on T.S.S. T.S.S was observed in maximum concentrations of $N_1 \times Zn_2 \times Fe_4$. Zn increased T.S.S content significantly; also it was found no significant difference among treatments on T.S.S of fruits as compared to the control. N application increased TSS content as compared to the control.

It was reported that a foliar spraying with $ZnSO_4$ at March and April increased TSS and juice of oranges (Dixi and Gamdagin , 1978). Dobroluybsikii *et al*., 1981, 1982 reported application of zinc sulfate can increase TSS in fruits of guava. PBZ used for quality, reduces fruit soluble solids content (Curry and Williams, 1986, 1990; Elfving *et al*., 1990). Singh (2002) reported micro nutrient had effect on fruit quality of grapes and T.S.S and quality of fruit increased. Mustafa *et al*. (1986) reported that an application of $ZnSO_4$ increased weight and number of raceme, T.S.S and the juice content of grapevine. Brar *et al*. (1992) reported using urea can increase T.S.S in grapevine for up to 3 years after use. These results are in accordance with the finding of Yamdagni *et al*. (1979) about the application of Zn sulphate on grapevine that increased T.S.S content. It can be concluded that fruit quality parameters like fruit's color, can be improved by external use of Fe, Zn & N fertilizers.

3. Plant-growth parameters

Plants of each genotype were sampled randomly before the treatments and 90 days after treatments. Effects of treatments on trees indicated that increased nutrient supply could increase growth. Analyzing the differences between treatments revealed that proper management of nitrogen had a significant effect on the shoots growth. The N fertilization significantly affected growth of branches (Table 2). The percentages of nitrogen in new vegetative growth were higher than the control. In the lower concentrations, there was no change in growth and the highest growth of branches belonged to $N_2 \times Zn_2 \times Fe_1$.

Fertilizers increased shoot weight as compared to the control. This result is consistent with observations that plants invest most heavily in the organs that capture the resource in shortest supply (Abdelhadi *et al*., 1986). The most evident effect of Fe deficiency is a decreased content of photosynthetic pigments, which results in the relative enrichment of carotenoids over chlorophylls

(Chl) and leads to the yellow colour that is characteristic of chlorotic leaves and decreased of vegetative growth (Abadía *et al*., 2011). It was reported that $ZnSO_4$ increased vegetative growth of strawberry cultivar 'Camarosa' and 'Armore' respectively (Nazarpur, 2005). Lolaei, (2011) reported that nitrogen application increased vegetative growth of strawberry; this result is supported by Sharma (2002). From this experiment, it is found that increasing the yield decreased vegetative growth.

4. Concentration of macroelement in leaves

The results showed that treatments have significant difference ($p \leq 0.05$). The Fe concentration in grapevine leaves decreased markedly under Fe deficiency, but recovered rapidly in response to the application of Fe, either to the leaves or the nutrient solution and caused increased Fe concentration in leaves. When Zn and N were added to the nutrient solution, total recovery from the symptoms of increase in Zn concentration was observed in leaves.

The data of leaf ion concentrations in relation to N, Zn and Fe levels are presented in Table 4.

Therios and Sakellardis, (1988) reported that application of N increased N supply. Also, N application on strawberry, increased N content in leaves (Arshad *et al*., 2006). The increase in nitrogen levels is due to high nitrogen in leaves. Similar observation was reported by Sunitha (2006) in marigold and Singh (2000) in tuberose. Zn is an important structural nutrition in leaves and is necessary for vegetative growth (Xiao *et al*., 2010; Bonnet *et al*., 2000). Hao *et al*. (2007) showed that increased N content in nutrition could increase Zn content in leaves and also with increasing Zn levels, N levels in leaves was increased. Reducing N levels increased Fe levels which could be due to antagonistic effect of these two elements. Adding N fertilizer into micronutrient contents in leaves have shown that the levels of Fe, Zn, Cu and Mn increased in leaf under increased N application as compared to the control (Chandel *et al*., 2010; Rombolà and Tagliavini, 2006).

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Tables

Table 1. Anova of effects of N, Zn and Fe on grapevine.

(S.O.V)	df	Fruit number	Average fruit weight	Yield	Length of berry	T.S.S (% Brix)	Length of branch	Weight of raceme
N	1	0.25n.s	1.96n.s	8.86**	.007n.s	0.37n.s	0.13**	3.86**
Zn	1	1.33n.s	0.26n.s	1.93n.s	0.3n.s	0.02n.s	0.53n.s	2.57n.s
N*Zn	1	.007n.s	1.04n.s	0.02n.s	0.53n.s	0.18n.s	1.11n.s	1.11n.s
Fe	3	0.11n.s	1.24n.s	2.38n.s	0.22n.s	0.49n.s	0.31n.s	1.22n.s
N*Fe	3	0.22n.s	1.56n.s	0.48n.s	1.45n.s	1.43n.s	0.66n.s	0.46n.s
Zn*Fe	3	1.05n.s	0.08n.s	1.88n.s	0.75n.s	0.18n.s	0.23n.s	1.73n.s
N*Zn*Fe	3	0.46n.s	1.2n.s	0.01n.s	0.39n.s	1.16n.s	2.77n.s	5.13**
Error	48	787.6	0.38	49.06	0.86	3.32	124.4	27.9
cv	-	22.27	8.80	35.50	5.11	11.7	24.37	21.52

ns= Non-significant, .** Significance at P≤0.01 probability level.

Table 2. Effects of interaction within N, Zn and Fe on reproductive growth of grapevine

Treatments	Fruit number in raceme	Average fruit weight (g)	Yield (kg)	T.S.S (% Brix)	Length of berry (mm)	Length of branch (cm)	Weight of raceme (g)
$N_1 \times Zn_1 \times Fe_1$	114.3a	2.28ab	20.23abc	14.63a	18.1a	145.1a	238.8bc
$N_1 \times Zn_1 \times Fe_2$	134.3a	2.22ab	32.3abc	16.02a	18.1a	126.9a	270abc
$N_1 \times Zn_1 \times Fe_3$	131.6a	2.37ab	24.30ab	15.90a	18.2a	132.5a	255bc
$N_1 \times Zn_1 \times Fe_4$	113.8a	2.12b	16.90bc	14.75a	17.47a	136.4a	187.5c
$N_1 \times Zn_2 \times Fe_1$	124.7a	2.1b	25.45ab	16.65a	17.98a	126.3a	240bc
$N_1 \times Zn_2 \times Fe_2$	128.4a	2.47a	17.35bc	15a	18.60a	168.6a	225bc
$N_1 \times Zn_2 \times Fe_3$	128.8a	2.27ab	29.33a	15.88a	18.30a	150a	277.5ab
$N_1 \times Zn_2 \times Fe_4$	146.9a	2.25ab	22.67abc	16.85a	18.42a	159a	247.5a
$N_2 \times Zn_1 \times Fe_1$	110.1a	2.15ab	15.02bc	16.17a	18.35a	135a	203.6bc
$N_2 \times Zn_1 \times Fe_2$	125.8a	2.32ab	15.08bc	14.73a	17.33a	136.5a	200bc
$N_2 \times Zn_1 \times Fe_3$	124.7a	2.22ab	17.75abc	15.65a	18.48a	152.8a	233.5bc
$N_2 \times Zn_1 Fe_4$	121.7a	2.17ab	15.63bc	16.5a	18.33a	125.6a	265bc
$N_2 \times Zn_2 \times Fe_1$	141.1a	2.05b	19.38abc	16.7a	1852a	172.5a	265bc
$N_2 \times Zn_2 \times Fe_2$	117.8a	2.25ab	11.32c	14.88a	18.68a	133.6a	202.5bc
$N_2 \times Zn_2 \times Fe_3$	124.3a	2.32ab	21.48abc	15.65a	18.33a	136.3a	237.5bc
$N_2 \times Zn_2 \times Fe_4$	128.9a	2.15ab	20.63abc	15.40a	17.88a	137.5a	4.67a

*In each column, means with the same letters are not significantly different.

Table 3. Effects of N, Zn and Fe on reproductive growth and quality of fruit of grapevine.

Treatments	Yield (kg)	Length of berry (mm)	Weight of raceme (g)	Length of branch (cm)	Fruit number in raceme	Average fruit weight (g)	T.S.S (% Brix)
N_1	16.05b	18.13a	229.5b	143b	127.8a	229.5b	15.46a
N_2	22.34a	18.15a	225.2a	146a	124.3a	225.2a	15.75a
Zn_1	18.20a	18.04a	231.9a	141a	122a	231.9a	15.55a
Zn_2	18.51a	18.24a	232.1a	148a	130.1a	232.1a	15.63a
Fe_1	20.40ab	18.24a	237.5a	144.7a	122.6a	237.5a	15.54a
Fe_2	26.71b	17.99a	224.4a	151.5a	126.5a	224.4a	15.16a
Fe_3	23.21a	18.19a	250.6a	142.1a	127.3a	250.6a	15.77a
Fe_4	18.96ab	18.15a	259.9a	139.7a	127.8a	259.9a	15.89a
$N_1 \times Zn_1$	20.98a	17.97a	237.8a	135.2a	123.5a	237.8a	15.32a
$N_1 \times Zn_2$	23.70a	8.34a	272.5a	151a	133.2a	272.5a	15.59a
$N_2 \times Zn_1$	16.05a	18.12a	225.9a	147.8a	120.5a	225.9a	15.78a
$N_2 \times Zn_2$	18.20a	18.15a	233.1a	145a	128a	233.1a	15.66a

*In each column, means with the same letters are not significantly different.

Table 4. Effects of N, Zn and Fe on Fe, Zn and N concentrations in leaves of grapevine.

treatment	Fe(ppm)	Zn(ppm)	N(%)
$N_1*Zn_1*Fe_1$	157.70	27.82	2.03
$N_1* Zn_1*Fe_2$	327.78	31.69	2.04
$N_1* Zn_1*Fe3$	210.92	25.12	1.93
$N_1*Zn_1*Fe_4$	417.46	29.16	2.54
$N_1*Zn_2*Fe_1$	166.08	208.34	2.85
$N_1*Zn_2*Fe_2$	428.42	193.26	2.83
$N_1*Zn_2*Fe_3$	189.01	211.05	2.21
$N_1*Zn_2*Fe_4$	580.46	188.63	2.37
$N_2*Zn_1*Fe_1$	177.67	40.45	2.86
$N_2*Zn_1*Fe_2$	320.84	43.07	2.44
$N_2*Zn_1*Fe_3$	183.73	36.46	2.63
$N_2*Zn_1*Fe_4$	554.3	32.33	2.64
$N_2*Zn_2*Fe_1$	197.39	222.9	2.58
$N_2*Zn_2*Fe_2$	458.13	183.6	2.64
$N_2*Zn_2*Fe_3$	194.30	237.85	2.73
$N_2*Zn_2*Fe_4$	553.92	175.87	2.26

*In each column, means with the same letters are not significantly different.

Table 5. Correlation between some characters growth, yield, and quality of grapevine.

Length of branch	T.S.S	Length of berry	Average fruit weight	Fruit number in raceme	Weight of raceme	Yield	Treatment
0.77 n.s	0.72 n.s	2.45*	2.019*	2.47**	5.17**	1	Yield
1.45 n.s	2.95**	3.27**	0.72n.s	6.12**	1		Weight of raceme
0.1 n.s	2.01*	0.07n.s	0.7n.s	1			Fruit number in raceme
1.96 n.s	0.004 n.s	3.63**	1				fruit Weight
1.5 n.s	0.8 n.s	1					Length of berry
0.39 n.s	1						T.S.S
1							Length of branch