

# Electrochemical Properties and Biochemical Composition of Cv. Shiraz Wine Grape (*V. vinifera* L.) Depending on Various Dose and Application Time of Foliar Microbial Fertilizer Treatments

Demir Kok<sup>1</sup> · Erdinc Bal<sup>1</sup>

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**Abstract** Utilization from bio fertilization is well known a considerable tool to improve the yield and fruit quality of various crop fruits through the increasing emphasis on maintain of soil health, minimize environmental pollution and decrease the use of chemical fertilization. In this study, in order to improve wine grape quality features of cv. Shiraz, four different doses of foliar microbial fertilizer, including 0, 1000, 2000 and 3000 ppm were applied at two different terms as Term I (mostly; pre-bloom applications) and Term II (mostly; post-bloom applications). However, there were no influences of application terms of foliar microbial fertilizer treatments; treatment doses had considerable effects on yield and quality parameters. The lowest *p*-values, meaning the highest berry quality, from doses of foliar microbial fertilizer were obtained from 2000 ppm (105.08  $\mu$ W), 1000 ppm (110.40  $\mu$ W), 3000 ppm (112.97  $\mu$ W) and 0 ppm (119.58  $\mu$ W). Comparing the applications of foliar microbial fertilizer, it was observed that doses of 2000 ppm (3155.56 mg/kg), 1000 ppm (3000.92 mg/kg) and 3000 ppm (2530.19 mg/kg) exhibited higher total phenolic compounds content when compared with 0 ppm treatment (2206.97 mg/kg). Berries from grapevines applied with the doses of 2000, 1000 and 3000 ppm foliar microbial fertilizer respectively shown higher total anthocyanin content such as 1230.19, 1160.85 and 865.86 mg/kg compared to 0 ppm (637.37 mg/kg). As a result, research the findings indicated that 2000 and 1000 ppm doses of foliar microbial fertilizer were obviously effective on wine grape quality features of cv.

Shiraz in terms of electrochemical property, total phenolic compounds content and total anthocyanin content.

**Keywords** Wine grape · Foliar microbial fertilizer · *P*-value · Phenolic compounds · Anthocyanins

**Elektrochemische Eigenschaften und biochemische Zusammensetzung bei der Rebsorte ‘Shiraz’ (*V. vinifera* L.) in Abhängigkeit von Dosierung und Ausbringungszeitpunkt mikrobieller Blattdünger**

**Schlüsselwörter** Weintrauben · Mikrobielle Blattdünger · *P*-Wert · Phenolverbindungen · Anthocyane

## Introduction

The quality of wine grapes depends on biochemical constituents such as sugar, organic acids, phenolic compounds and anthocyanin. Therefore, it is important to know the factors, which regulate biosynthesis of these grape components in the course of ripening (Topalovic et al. 2011).

In grape growing, quality parameters can be different according to assessment forms of grape. While the total soluble solids, total acidity, skin color, flavor, presence of bloom and total soluble solids: total acid ratio are important for table grapes; total soluble solids, total acidity, juice pH, skin color, phenolic compounds, tannins and flavor gain importance for wine grape quality (Creasy and Creasy 2009).

According to the assessment situation of grape, offering of the best quality grape to consumers is considerable subject in grape growing. For this aim, it can be utilized from quantitative parameters and qualitative indicators for

✉ Demir Kok  
dkok@nku.edu.tr

<sup>1</sup> Agricultural Faculty, Department of Horticulture, Namik Kemal University, 59030 Tekirdağ, Turkey

evaluation of wine grape maturity. Quantitative parameters are sugar content, titratable acidity, ratio of malate to tartrate, juice pH, sugar: acid ratio, sugar  $\times$  pH, sugar  $\times$  pH<sup>2</sup>, total phenolic content, total anthocyanin content and total tannin content. However, qualitative indicators consist of soft berries, red fruit flavors, pulp ripeness, skin ripeness, brown seeds and clean pedicel. These physical and physico-chemical methods are used to determine quality of grapes (Goldammer 2015).

After long year studies about assessment of fruit quality, researchers were found out new method as known “Bio-electric Vincent Method”, which is better and faster than the other methods. This method was based on bioelectrical theory to derive electrical energy value of food from measurements of pH, redox potential and electrical resistance and *p*-value is an electrochemical parameter of quality in present method (Akay and Kara 2006; Paulauskiene et al. 2006; Ergun and Jezik 2011; Ergun 2012; Kok 2016; Kok and Bal 2016). Wolf and Rey (1997) inform that better commodity quality is obtained from a low redox potential and *p* values.

At the present time, excessive agricultural practices, which warrant high yield and quality, require intensive use of chemical fertilizers that are costly (Arikan et al. 2013). The continuous use of chemical fertilization also brings about the deterioration of soil attributes and fertility and may cause the accumulation of heavy metals in plant tissues, affecting the fruit nutritional value and edibility (Shimbo et al. 2001). However, biological fertilization is based on the use of natural inputs, including fertilizers, decaying remains of organic matter, excess crops, domestic sewage, animal manure and microorganisms such as fungi and bacteria (Chirinos et al. 2006).

Nowadays, there has been an increased interest in different types of foliar fertilization as an alternative or supplementary technique to traditional soil fertilization (Portu et al. 2015). Bio fertilizers, the organic preparations, containing microorganisms are useful for agricultural and also viticulture productions in terms of nutrient supply especially with respect to *N* and *P* (Wani et al. 2013).

One of the most important microorganism is bacteria and they are called plant growth promoting rhizobacteria (PGPR), containing the strains in genera *Azotobacter*, *Acinetobacter alcaligenes*, *Arthrobacter*, *Azospirillum*,

*Bacillus*, *Enterobacter*, *Rhizobium* and *Serratia* (Rodriguez and Fraga 1999; Sturz and Nowak 2000; Sudhakar et al. 2000).

The purpose of present research was to determine influences of foliar microbial fertilizer application doses and application times on wine grape quality features of *cv.* Shiraz.

## Materials and Methods

### Characteristics of Plant Materials and Research Area

The study was carried out in a *V. vinifera* L. *cv.* Shiraz vineyard in Tekirdağ, Turkey (lat. 40°55' N; long. 27°25' E; 140 m. a. s. l.) in the course of 2014 growing season. The grapevines, grafted on 5BB rootstock, were planted in 1995, trained on a vertical trellis system, pruned utilizing bilateral cordon system and organized in north-south row spaced 2.5 m apart, with 1.5 m between grapevines in the row. The vineyard was managed according to local standard viticulture practices for cultivar and region. A standard disease control program was also employed for fungal diseases.

The climatic attributes in study area is generally mild and averages of annual temperature, sunshine duration per day, relative humidity, total precipitation were successively 15.5 °C, 5.0 h, 89.3% and 815.2 mm for 2014 year. However, features of vineyard soil in current region was clay-loam with a pH of 7.64.

### Application Doses and Times of Foliar Microbial Fertilizer

In present study, it was utilized from a liquid microbial fertilizer – biological preparation prepared from the mixed populations of *Azotobacter chroococum* and *Azotobacter vinelandii*. In order to determine suitable application dose and application time, foliar microbial fertilizer was prepared at four different doses, consisting of 0, 1000, 2000 and 3000 ppm and applied at three times with 15-day interval in two distinct terms. Application times of foliar microbial fertilizer were respectively term I and term II according to E-L system in terms of growth stages of grapevine modified by Dry and Coombe (2004) (Table 1).

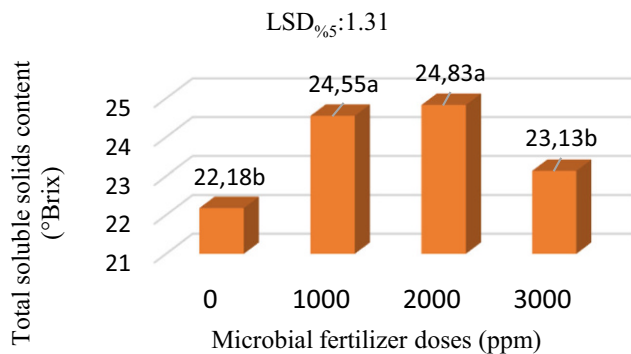
**Table 1** Application times of foliar microbial fertilizer doses according to grapevine's phenological growth stages

	1 <sup>st</sup> foliar application	2 <sup>nd</sup> foliar application	3 <sup>rd</sup> foliar application	
Term I	Shoots with 15–20 cm height (E-L No: 14)	Shoots with 16 leaves, onset of blooming (E-L No: 19)	After berry set and berries with 2 mm (E-L No: 27)	–
Term II	–	Shoots with 16 leaves, onset of blooming (E-L No: 19)	After berry set and berries with 2 mm (E-L No: 27)	–
	<i>Pre-bloom period</i>	<i>Bloom period</i>	<i>Post-bloom period</i>	<i>Post-bloom period</i>
	–	Shoots with 16 leaves, onset of blooming (E-L No: 19)	After berry set and berries with 2 mm (E-L No: 27)	Berries pea size with 7 mm (E-L No: 31)
		1 <sup>st</sup> foliar application	2 <sup>nd</sup> foliar application	3 <sup>rd</sup> foliar application

**Table 2** Dose effects of foliar microbial fertilizer on yield components of cv. Shiraz

Treatments	Berry length (mm)	Berry width (mm)	Berry weight (g)	Cluster length (cm)	Cluster width (cm)	Cluster weight (g)
0 ppm	14.35 <sup>b</sup>	12.85 <sup>c</sup>	1.77	14.36 <sup>b</sup>	6.81 <sup>c</sup>	222.04 <sup>c</sup>
1000 ppm	14.95 <sup>a</sup>	13.40 <sup>ab</sup>	1.91	17.12 <sup>a</sup>	10.28 <sup>a</sup>	285.78 <sup>ab</sup>
2000 ppm	15.21 <sup>a</sup>	13.71 <sup>a</sup>	1.99	17.49 <sup>a</sup>	10.79 <sup>a</sup>	313.20 <sup>a</sup>
3000 ppm	14.87 <sup>a</sup>	13.04 <sup>bc</sup>	1.89	15.02 <sup>b</sup>	8.55 <sup>b</sup>	252.22 <sup>bc</sup>
LSD <sub>5%</sub>	0.44	0.51	NS	1.35	1.11	36.44

Means values within columns designed by different letters are significantly different by LSD multiple range test at 5% level



**Fig. 1** Effects of foliar microbial fertilizer doses on total soluble solids content

### Yield and Quality Components Assessed in Research

In this research, berry length (cm), berry width (cm), berry weight (g) and cluster length (cm), cluster width (cm), cluster weight (g) were measured as yield parameters. Moreover, total soluble solids content (°Brix), titratable acidity (g/L), juice pH, *p*-value ( $\mu$ W), total phenolic compounds content (mg GAE/kg fw), total anthocyanin content (mg GAE/kg fw) were determined as quality parameters.

### Harvest Time and Berry Sampling

Wine grapes of cv. Shiraz were harvested at the end of August, 2014 and samples of 250-berry were collected from each treatment and finally used to determine total soluble solids content, total acid content, juice pH, *p*-value. However, 300-berry samples were used to find out total phenolic compounds content and total anthocyanin content. All berry samples were stored at  $-25^{\circ}\text{C}$  until analyses of total phenolic compounds content and total anthocyanin content; prior to these analyses, berry samples were removed from  $-25^{\circ}\text{C}$ , allowed to thaw overnight at  $4^{\circ}\text{C}$  and then homogenized in a commercial laboratory blender for 20 s.

### Calculation of *P*-Value in Berries

*P*-value, consisting redox potential (mV), pH, *P*-value ( $\mu$ W) and resistivity (ohm) were formulated with an equation no-

ticed by Hoffmann (1991). In the study, *p*-values in samples of berry juices from different doses of foliar microbial fertilizer applied two distinct terms were calculated according to equation reported by Hoffmann (1991).

### Determination of Total Phenolic Compounds Content and Total Anthocyanin Content

Spectrophotometric methods notified by Singleton et al. (1978) and Di Stefano and Cravero (1991) were consecutively used to find out total phenolic compounds content and total anthocyanin content of cv. Shiraz.

### Statistical Analysis

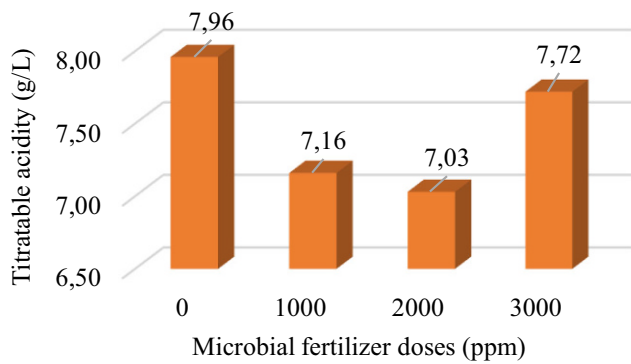
The research design was based on two factor-factorial randomized parcels with four replicates and all data were analyzed by TARIST statistical package program. When the differences at analysis of variance (ANOVA) were significant ( $P < 0.05$ ), means were separated by using Fisher's Least Significance Difference (LSD) multiple range test.

### Results and Discussion

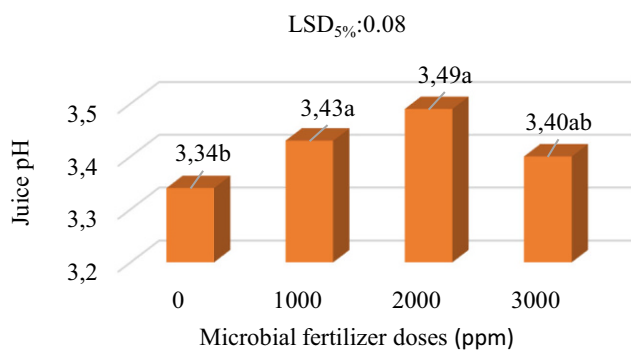
In present study, results of foliar microbial fertilizer applied at two different terms demonstrated that there were no statistically differences between two application times. However, different doses of foliar microbial fertilizer application significantly affected yield and quality components of cv. Shiraz (Table 2).

Berry size is broadly recognized as a prominent factor, determining wine grape quality (Matthews and Nuzzo 2007). In present research, grapevines treated with various doses of foliar microbial fertilizer with ascending order, 0 ppm (14.35 mm), 1000 ppm (14.95 mm), 2000 ppm (15.21 mm) and 3000 ppm (14.87 mm) increased berry length (Table 2).

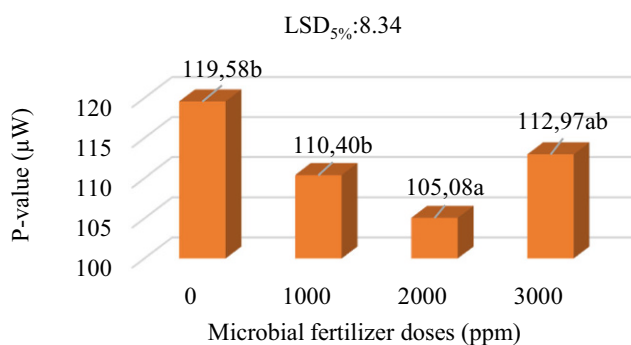
The results of obtained from berry width means represented that foliar microbial fertilizer applications contributed to increase in berry width and means were respec-



**Fig. 2** Effects of foliar microbial fertilizer doses on titratable acidity



**Fig. 3** Effects of foliar microbial fertilizer doses on juice pH



**Fig. 4** Effects of foliar microbial fertilizer doses on *p*-value

tively 12.85 mm (0 ppm), 13.40 mm (1000 ppm), 13.71 mm (2000 ppm) and 13.04 mm (3000 ppm) (Table 2).

Berry weight was dependent on the cultivar and was closely related to proportions between its components such as berry skin flesh and seeds (Ferrer et al. 2014). As displayed in Table 2, feature of berry weight was not statistically influenced by doses of foliar microbial fertilizer. However, the highest berry weight was obtained from treatment of 2000 ppm (1.99 g) followed by treatments of 1000 ppm (1.91 g), 3000 ppm (1.89 g) and 0 ppm (1.77 g).

May (1972) declare that yield parameters of grapevine consist of several components such as grapevines per hectare, shoots per meter, cluster per shoot, cluster per

grapevine, cluster weight, berries per cluster and berry weight. Results of cluster length from study demonstrated that foliar microbial fertilizer applications significantly increased cluster length compared to 0 ppm treatment (Table 2). Application dose of 2000 ppm led to the highest cluster length (17.49 cm), followed by treatments of 1000 ppm (17.12 cm), 3000 ppm (15.02 cm) and 0 ppm (14.36 cm).

Features of cluster width were significantly affected by various doses of foliar microbial fertilizer. While the highest mean of cluster width was measuring in treatment of 2000 ppm (10.79 cm); treatments of 1000, 3000 and 0 ppm successively resulted in increase, including 10.28, 8.55 and 6.81 cm (Table 2).

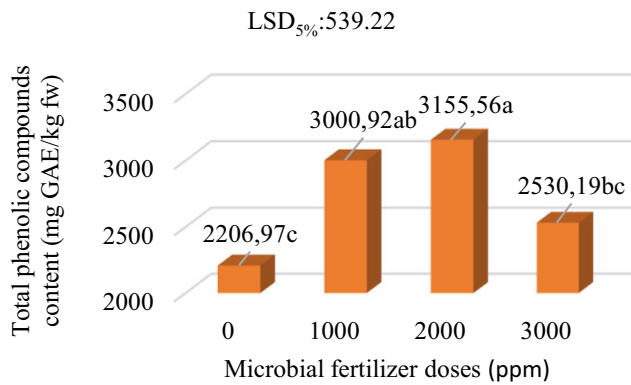
The significant variations in cluster weight means were recorded in grapevines applied with doses of 2000 ppm (313.20 g), 1000 ppm (285.78 g) and 3000 ppm (252.22 g) compared with 0 ppm treatment (222.04 g) (Table 2).

Although total soluble solids content varies according to assessment of grapes, the typical maturation level of total soluble solids content should lie between 22.5–24.5 °Brix (Rees et al. 2012; Shikhamany and Srinivasulu 2014). In this study, total soluble solids contents of berries were statistically influenced by doses of foliar microbial fertilizer (Fig. 1). Treatment of 2000 ppm caused the highest amount of total soluble solids content (24.83 °Brix) compared to doses of 0 ppm (22.18 °Brix), 1000 ppm (24.55 °Brix) and 3000 ppm (23.13 °Brix) and doses of foliar microbial fertilizer had varying effect in total soluble solids content of *cv.* Shiraz.

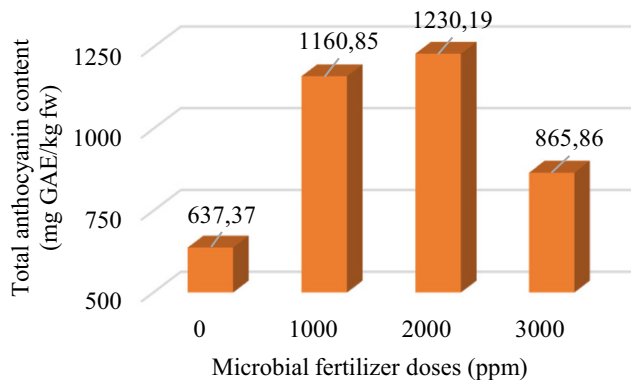
The acidity level of the grapes at harvest is very important to the structure of the wine and composition of the finished wine (Goldammer 2015) and value of titratable acidity should be between 6 and 8 g/L in red wine grapes (Shikhamany and Srinivasulu 2014). There was no effect of different doses of foliar microbial fertilizer on titratable acidity of berries in *cv.* Shiraz. Mean values of titratable acidity accumulated in berries of grapevines treated different doses of foliar microbial fertilizer ranged from 7.03 g/L (2000 ppm) to 7.96 g/L (0 ppm). As presented in Fig. 2, all doses of microbial fertilizer decreased content of titratable acid compared to 0 ppm treatment.

The pH of the grape juice at harvest is another important variable (Goldammer 2015). Red wine grapes have 3.3–3.5 of pH values (Shikhamany and Srinivasulu 2014). The mean values of juice pH at different doses of foliar microbial fertilizer applied to grapevines are presented in Fig. 3. It appeared that graded doses of microbial fertilizer could significantly increase the pH values of juice in 0 ppm (3.34), 3000 ppm (3.40), 1000 ppm (3.43) and 2000 ppm (3.49).

Calculation of *p*-value is a method representing of product quality (Keppel 2001; Krautgartner 2001; Kara et al. 2008, 2012) and Kara et al. (2008) declare that grapes with



**Fig. 5** Effects of foliar microbial fertilizer doses on total phenolic compounds content



**Fig. 6** Effects of foliar microbial fertilizer doses on total anthocyanin content

low  $p$ -value represent health promoting. Based on the findings of  $p$ -values in berries from applications, there were statistically significant relationships among the foliar microbial fertilizer doses (Fig. 4). While 2000 ppm application was decreasing  $p$ -value ( $105.08 \mu\text{W}$ ); doses of 1000, 3000 and 0 ppm decreased  $p$ -values of berries such as 110.40, 112.97 and  $119.58 \mu\text{W}$ . As shown, doses of microbial fertilizer reduced the mean of  $p$ -values in berries, leading to wine grapes with high quality.

The phenolic compounds have been reported to have several biological activities and have marked influence on the mouth-feel and taste sensations of products derived from grape (Ivanova et al. 2011). Concerning total phenolic compounds content of berry samples in *cv. Shiraz* illustrated in Fig. 5, 2000 ppm treatment had higher total phenolic compounds (3155.56 mg/kg) than treatments of 0 ppm (2206.97 mg GAE/kg fw), 1000 ppm (3000.92 mg GAE/kg fw) and 3000 ppm (2530.19 mg GAE/kg fw).

Anthocyanin and phenolic compounds contents in grapes are influenced by multi factors, including grape cultivar, soil composition and viticulture practices like different fertilizer treatments and canopy management practices and climate (Alvarez et al. 2007; Kok 2011, 2013). There were

no statistically significant differences among the foliar microbial fertilizer doses in terms of total anthocyanin content (Fig. 6). While effect of microbial fertilizer treatments on total anthocyanin content of berries was the lowest level (637.37 mg GAE/kg fw) in treatment of 0 ppm; the higher values were recorded in treatments of 2000 ppm (1230.19 mg GAE/kg fw), 1000 ppm (1160.85 mg GAE/kg fw) and 3000 ppm (865.86 mg GAE/kg fw) for same parameter.

## Conclusion

$P$ -value, an electrochemical measurement of commodity, would be beneficial together with other standard quality features for grape growers. In present study, increasing doses of foliar microbial fertilizer applications had favorable impacts on quality and yield components of *cv. Shiraz* grapes.

Consequently, doses of 2000, 1000 and 3000 ppm foliar microbial fertilizer successively caused improvements in wine grape quality of *cv. Shiraz* grape.

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