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Effects of Foliar Seaweed and Humic Acid Treatments on Monoterpene Profile and Biochemical Properties of cv. Riesling Berry (*V. vinifera* L.) Throughout the Maturation Period

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This study has been conducted to find out the effects of foliar treatments of seaweed (SW) and humic acid (HA) on cv. Riesling wine grape throughout ripening period from veraison period to harvest period at 15 days of interval. Such as veraison period, 15 days after veraison period and 30 days after veraison period aimed to examine the biochemical variations in berry. In this research, the doses of 0, 1000, 2000 ppm of SW and HA treatments have been selected, and applied to grapevines three times at different phenological stages of grapevine. Among the berry sampling, in terms of measurement and analysis, the best quality characteristics were respectively obtained from the period of 30 days after veraison, 15 days after veraison and veraison, and the results showed that the foliar sprays of SW and HA could modify the biochemical characteristics of berries in cv. Riesling. In terms of free volatile terpenes (FVTs) and potentially volatile terpenes (PVTs) of berries, which are also very important for aromatic grape cultivars, 1000 ppm doses of both of SW and HA treatments respectively displayed the highest contents in FVTs (0.880 and 0.804 mg L⁻¹) and PVTs (2.153 and 2.084 mg L⁻¹). Consequently, 1000 ppm doses of SW and HA foliar treatments provided the best improvements in most quality characteristics of berry when they were harvested at 30 days after veraison period.

Keywords: *V. vinifera* L., berry quality, foliar fertilizer treatment, humic acid, seaweed

Yapraktan Uygulanan Deniz Yosunu ve Hümik Asit Uygulamalarının Olgunlaşma Dönemi Süresince Riesling (*V. vinifera* L.) Üzüm Çeşidinin Monoterpen Profili ve Biyokimyasal Özellikleri Üzerine Etkileri

Bu çalışma, yapraktan uygulanan deniz yosunu ve hümik asit uygulamalarının şaraplık Riesling üzüm çeşidinde ben düşme döneminden hasat dönemine kadar olan süreçte (ben düşme dönemi, ben düşmeden 15 gün sonra ve ben düşmeden 30 gün sonra olmak üzere) tanedeki biyokimyasal değişimleri incelemek için gerçekleştirilmiştir. Araştırmada, deniz yosunu ve hümik asit uygulamalarının 0, 1000 ve 2000 ppm dozlarından yararlanılmış ve bu dozlar asmalara 3 farklı fenolojik gelişme döneminde uygulanmıştır. Yapılacak ölçüm ve analizler için düşünülen örnek toplama dönemleri arasında en iyi kalite özellikleri sırasıyla ben düşme döneminden 30 gün sonra, ben düşme döneminden 15 gün sonra ve ben düşme dönemlerinden elde edilmiş ve yapraktan uygulanan deniz yosunu ve hümik asit uygulamalarının Riesling üzüm çeşidinin tane biyokimyasal özelliklerini değiştirdiği görülmüştür. Aromatik üzüm çeşitleri açısından da oldukça önemli olan serbest uçucu terpen bileşikler ile potansiyel uçucu terpen bileşikler yönüyle, her iki deniz yosunu ve hümik asit uygulamalarının 1000 ppm dozları sırasıyla üzümde en yüksek serbest uçucu terpen bileşikler (0.880 and 0.804 mg L⁻¹) ile potansiyel uçucu terpen bileşiklerin (2.153 and 2.084 mg L⁻¹) oluşmasına neden olmuştur. Sonuç olarak, deniz yosunu ve hümik asit uygulamalarından özellikle 1000 ppm dozları, üzümler ben düşme döneminden 30 gün sonra hasat edildiklerinde en iyi tane kalite özelliklerinin ortaya çıkmasına neden olmuştur.

Anahtar Kelimeler: *V. vinifera* L., üzüm kalitesi, yaprak gübresi uygulaması, hümik asit, deniz yosunu

Introduction

Numerous plant growth regulators have been found in seaweed and seaweed extract, including cytokinins, auxins, gibberellins, abscisic acid and betaines. Seaweed-based amendments can increase crop yield (Rathore et al., 2009;

Chouliaras et al., 2009) and quality characteristics (Jayaraj et al., 2008; Jayaraj et al., 2010; Kok et al., 2010) in plants. Strik et al. (2004) reported that the seaweed extracts are found effective fertilizers for many crops.

Humic acid is the active constituent of organic humus, which can play a very important role in

soil conditioning and plant growth and they have different effects on plants. Chen et al. (2004) showed evidence of stimulation on plant growth by humic substances and consequently increased yield by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water, nutrient uptake and enzyme activities.

Grape is the most broadly consumed fruits in the world and aromatic cultivars among these grape cultivars have an important role for winemaking. Various monoterpene compounds are distinguished in *Vitis vinifera* L. grape cultivars and wine, the amplex of which are linalool, geraniol, nerol, citronellol and terpeniol (Gonzalez, 2003). Gholami et al. (2008) inform that monoterpenes are plant secondary metabolites, of which more than 50 compounds have been identified in grapes and wine.

Monoterpene compounds contribute significantly to the characteristic flavor of grapes and generally present only at low levels in the floral grape cultivars, and they are responsible for the distinctive flavor of grape cultivars like Riesling, Gewürztraminer and all muscat cultivars. These are present as odor-active FVT and as PVT, specifically glycosides and polyols capable of releasing FVTs via temperature, pH or enzyme-induced hydrolysis (Reynolds and Wardle, 1997).

Profiles of monoterpene compounds in the grape are chiefly dependent on the cultivar, environmental variables, various foliar chemical treatments (Kok and Bal, 2014) and different canopy management practices (Kok et al., 2013) such as cluster thinning treatment (Kok, 2011; Sun et al., 2012), leaf removal treatment (Roberts et al., 2007) and shoot thinning treatment (Sun et al., 2012).

Since veraison is brief physiological period of grape that represents the onset of berry ripening when sugars begin to accumulate, organic acid concentrations decline, anthocyanin pigments accumulate in color cultivars, aroma compounds occur in aromatic cultivars, and grapes soften (Rubio et al., 2009).

Researches on concentration changes of volatile compounds in the course of grape physiological

development have focused on the period from veraison to harvest (Coelho et al., 2007).

For this reason, this study is intended to compare the effects of variable doses of foliar SW and HA treatments on contents of FVT and PVT monoterpene and other biochemical characteristics of cv. Riesling berry throughout its maturity period in terms of three different timings such as veraison period, 15 days after veraison period and 30 days after veraison period at an interval of 15 days.

Material and Methods

Research Site and Plant Materials

This study has been conducted in a commercial vineyard located in Tekirdag province of Turkey (lat.41°00' N; long. 27°40' E; 60 m. a.s.l.) by using cv. Riesling berry in the course of 2011 growing season.

Grapevines used in the research were spur-pruned on a bilateral cordon with 12 nodes per grapevine and trained to a vertical shoot position trellis system grafted onto 5BB rootstock. They were given a space of 2.50 m inter-row and 1.25 m intra-row, and row orientation of grapevines was north-south. The vineyard has been designed and managed by following the local standard viticulture practices without applying a single irrigation throughout its whole growing season.

Climatic conditions of research area

In research area, climate is mild and in order to annual mean temperature, per day sunshine duration, relative humidity and total rainfall have been recorded as 13.91°C, 6.23 h, 78.01% and 578.76 mm, respectively as averages of long terms for the last 50 years.

Foliar extract types, treatment doses and treatment times

In this study, grapevines were treated with foliar liquid of SW namely, *Ascophyllum nodosum* and HA extract, and their contents are given in Table 1.

Table 1. Chemical characteristics of foliar SW and HA extracts applied to grapevines of cv. Riesling

Foliar extract	Organic matter (%)	pH
SW	15	9.0-11
HA	16	6.5-8.5

In order to prepare foliar SW and HA extracts, three different doses of SW and HA have been applied such as 0, 1000, 2000 ppm based on their organic matter contents. They have been approximately applied to grapevines using three replications in accordance to three different phenological growth stages of grapevines of cv. Riesling. They were: shoots with height of 15-20 cm, pre-bloom and berries pea-size at 15 days of interval.

Quality and yield parameters used in study

In this research, quality parameters such as total soluble solids (TSS, %), sugar concentration (SC, gL⁻¹), titratable acidity (TA, gL⁻¹), pH, monoterpenes as free volatile terpenes (FVTs, mgL⁻¹) and potentially volatile terpenes (PVTs, mgL⁻¹) have been analyzed along with yield parameters such as berry length (BL, mm), berry width (BWi, mm), berry weight (BWE, g), cluster length (CL, cm), cluster width (CWi, cm) and cluster weight (CWe, g) were determined for cv. Riesling berry.

Berry sampling and harvesting

Collecting of berry samplings from cv. Riesling have been conducted at three different berry development stages such as veraison; 15 days after veraison and 30 days after veraison. Berries were harvested early due to the excessive rainy weather during the final sampling period. As far as the laboratory analyses are concerned, samples of 250-berries have been collected from replicate of each treatment and eventually used to determine TSS, SC, TA, pH of berry. Moreover, samples consisted of 300 berries have also been collected aimed to determine the concentrations of FVT and PVT. For this purpose, berry samples were stored at -25°C till monoterpene analyses. Prior to

monoterpene analyses, berry samples were removed from -25°C, allowed to thaw overnight at 4°C and then homogenized in a commercial laboratory blender for 20 s.

Monoterpene analyses

Monoterpene analyses were performed on the berries sampled at three different stages by using the method of Dimitriadis and Williams (1984) as modified by Reynolds and Wardle (1989).

Canopy assessment of grapevines

Canopy point quadrat analyses in grapevines have been conducted aimed to characterize canopy density at veraison period (Smart and Robinson, 1992). Twenty insertions per replicate of treatment were performed at ca. 30° angles horizontally into the cluster zone with a thin 1 m-long probe at veraison period. The number of contacts per insertion and the nature of each contact have also been recorded. Calculations have been done from obtained data for leaf layer numbers (no.), percentage of canopy gap (%), percentage of sun exposed leaves (%) and percentage of sun exposed clusters (%), which are given in Table 2.

Statistical analyses

This study has been designed by using the Randomized Complete Block Design (RCBD) applying two factor-factorial with four replications and selected two grapevines from per replication. Analysis of variance (ANOVA) was performed by means of SPSS statistical software (18.0 for windows). Treatments were compared to separate means by using LSD (least significant difference) multiple range test at the level of 5%.

Table 2. Influences of different doses of foliar SW and HA treatments on canopy characteristics of cv. Riesling grapevine determined by point quadrat analysis

Doses of foliar extracts	Leaf layer number (No.)	Canopy gap (%)	Leaves	Clusters
			Sun exposed (%)	Sun exposed (%)
0 ppm	3.00 N.S.	0.00	62.72 N.S.	16.67 N.S.
1000 ppm SW	3.00 N.S.	0.00	71.89 N.S.	9.83 N.S.
2000 ppm SW	2.62 N.S.	0.00	66.80 N.S.	16.08 N.S.
1000 ppm HA	2.87 N.S.	0.00	63.99 N.S.	41.97 N.S.
2000 ppm HA	2.62 N.S.	0.00	76.00 N.S.	21.31 N.S.

Means followed by different letters in each column are for comparing of foliar SW and HA treatments and indicate significant difference by LSD test at 5% level. N.S.: Not significant

Results and Discussion

Grape ripening is normally associated with an increase in sugars, while decrease in acidity and development of characteristic color, texture and flavor (Hui, 2006). Cooke (2004) mentioned that TSS and SC values in grapes show similar variation trend and optimal values of TSS, TA and pH with the levels of 20.0-22.5%, 8-10 gL⁻¹ and 3.20-3.40 respectively in white wine grapes. In present study, berries of grapevines treated with 1000 ppm SW exhibited in higher mean values of TSS and SC (13.27%; 117.78 gL⁻¹) as compared to 2000 ppm SW (12.94%; 113.97 gL⁻¹), 1000 ppm HA (12.62%; 110.54 gL⁻¹), 2000 ppm HA (12.33%; 107.09 gL⁻¹) based on main effect of foliar treatment. Moreover, berry samplings that have been done at 30 days after veraison period showed the highest mean values of TSS and SC (19.02%; 181.41 gL⁻¹). On the other hand, berry samplings that have been taken at 15 days after veraison period (13.51%; 120.13 gL⁻¹) and veraison period (5.21%; 28.41 gL⁻¹) followed it ($p \leq 0.05$, Table 3).

Organic acids of wine grape have contributions to the flavor and balance of wine and acidity and pH have must depended on several factors, but especially on grape ripeness and growing region (Moreno and Peinado, 2012). The effects of both foliar SW as well as HA treatments were found statistically significant on TA content of berries in reference to main effects of treatment and term, interaction effects of foliar treatment and term ($p \leq 0.05$); whereas the effect of these foliar treatments on pH of berry juice have not been found statistically significant except for main effect of term ($p \geq 0.05$). Mean values of TA ranked from

18.43 to 20.98 gL⁻¹ for main effect of foliar treatment; from 8.29 to 33.31 gL⁻¹ for main effect of term; from 7.62 to 34.87 gL⁻¹ for interaction effects of foliar treatment and term (Table 3 and 4). Concerning pH of berry juice, from high to low mean values were obtained from the stage of 30 days after veraison period (3.30), 15 days after veraison period (2.76) and veraison period (2.57) in terms of main effect of term ($p \leq 0.05$, Table 3).

Monoterpene compounds contribute significantly to the characteristic flavor of grapes and these aroma components, which are common constituents of many fruits, are present in free odor form and abundantly as non-volatile glycosides. Several authors have showed that

terpenes play a significant role in the varietal flavour of wines and they are located in skin and linked to sugars in the berries (Gunata et al. 1985; Wilson et al., 1986). Regarding contents of FVT and PVT, significant differences were statistically observed in terms of main effects of foliar treatment and term for FVT and main effect of term for PVT ($p \leq 0.05$; Table 3). Among the main effects of foliar treatments, grapevines treated with 1000 ppm SW showed the highest mean value of FVT content (0.48 mgL⁻¹) and the lowest mean of FVT content was noted as 0.33 mgL⁻¹ for 0 ppm. In relation to main effect of term, means of FVT content varied from 0.14 to 0.75 mgL⁻¹ for veraison period and 30 days after veraison period. However, it was also observed that forwarding term from 15 days after veraison period (1.13 mgL⁻¹) to 30 days after veraison period (2.00 mgL⁻¹) brought about a sharp increase in PVT content of berries as compared to veraison period (0.67 mgL⁻¹).

Both physical (berry size, shape and color, the nature of waxy cuticle; etc.) and biochemical properties (moisture, sugar, acidity contents; etc.) of grapes at harvesting stage may affect grape quality. Characteristics of berry sizes such as BL, BWi and BWe are important yield attribute for grapes (Hui, 2006). It was observed in this study that various doses of SW and HA treatments had significant effects on characteristics of BL given in Table 3 and 4 ($p \leq 0.05$). With respect to main effect of foliar treatment, BL was significantly affected by foliar treatments with increasing means of 0 ppm (11.67 mm), 2000 ppm HA (12.05 mm), 1000 ppm HA (12.54 mm), 2000 ppm SW (12.59 mm), 1000 ppm SW (13.05 mm). Concerning main effects of term, increasing mean values of BL for veraison period, 15 days after veraison period and 30 days after veraison period have been noted as 11.02 mm, 12.52 mm and 13.60 mm, respectively. In respect to interaction effects of foliar treatment and term, the lowest mean value has been obtained from 0 ppm (10.57 mm), while the highest one was 14.33 mm from 1000 ppm SW.

As it is showed in Table 3 and 4, main effect of foliar treatments, BWi means were statistically different from each other and were recorded as 11.39 mm for 0 ppm, 11.63 mm for 2000 ppm HA, 11.76 mm for 1000 ppm HA, 11.96 mm for 2000 ppm SW and 12.48 mm for 1000 ppm SW. On the other hand, with respect to main effect of term, BWi means showed increase with forwarding term like 10.42 mm for veraison period, 11.86 mm for

15 days after veraison period and 13.25 mm for 30 days after veraison period. In terms of interaction effects of foliar treatment and term, means of BWi varied by depending on foliar treatment and treatment term, while the lowest mean was obtained from 0 ppm (10.06 mm) and the highest one was 13.79 mm from 1000 ppm SW ($p \leq 0.05$).

Concerning BWe means, despite the fact that main effects of foliar treatment and term were found statistically significant but interaction effects of foliar treatment and term did not show any significant effect ($p \leq 0.05$). According to BWe means obtained from main effect of foliar treatment given in Table 3, while 0 ppm treatment was showing lowest mean (1.27 g); 1000 ppm SW led to highest mean (1.51 g). In reference to main effect of term in BWe given in Table 3, it was observed that 30 days after veraison period caused the highest mean of BWe (1.76 g) as compared to the veraison period (0.88 g) and 15 days after veraison period (1.40 g).

Regarding CL characteristics, foliar SW and HA treatments showed statistically significant differences in terms of main effects of foliar treatments and term except for interaction effects ($p \leq 0.05$, Table 3). In relation to main effect of foliar treatment, the highest mean CL was obtained from those grapevines which treated with 1000 ppm SW (11.47 cm) and other

treatments consisted, in descending order, 2000 ppm SW (11.37 cm), 1000 ppm HA (10.91 cm), 2000 ppm HA (10.59 cm) and 0 ppm (10.07 cm). In terms of main effects of terms, 30 days after veraison period led to the highest mean of CL (11.71 cm) as compared to the veraison period (10.01 cm) and 15 days after veraison period (10.93 cm).

As far as the CWi characteristics are concerned, statistically significant differences have been found in terms of main effects of foliar treatment and term, given in Table 3 ($p \leq 0.05$). In descending order, treatments of 1000 ppm SW (9.16 cm), 1000 ppm HA (8.83 cm), 2000 ppm SW (8.68 cm) resulted higher CWi means. In relation to main effect of term, obtained mean values of CWi were 7.98 cm, 8.68 cm and 9.14 cm for veraison period, 15 days after veraison period and 30 days after veraison period, respectively.

As showed in Table 3 about CWe means that the significant differences were statistically determined in reference to main effects of foliar treatments and terms ($p \leq 0.05$). Mean values from low to high were 116.75, 128.70, 131.26, 141.27 and 158.86 g for 0 ppm, 2000 ppm SW, 2000 ppm HA, 1000 ppm HA and 1000 ppm SW in respect to main effect of foliar treatment. However, mean values of CWe increased from veraison period (86.45 g) to 30 days after veraison period (189.94 g).

Table 3. Influence of different doses of foliar SW and HA treatments on main effects of foliar treatments and term in cv. Riesling be

Main effect of foliar treatment							Main e		
Parameters	SW (ppm)			HA (ppm)		LSD _{%5}	Parameters	Veraison	Berry s 15 d ve
	0	1000	2000	1000	2000				
TSS (%)	11.74d	13.27a	12.94ab	12.62bc	12.33c	0.58	TSS (%)	5.21c	1
SC (g L ⁻¹)	100.53e	117.78a	113.97b	110.54c	107.09d	0.87	SC (g L ⁻¹)	28.41c	12
TA (g L ⁻¹)	20.98a	18.43c	18.85bc	19.91ab	19.79ab	1.33	TA (g L ⁻¹)	33.31a	1
pH	2.80	2.87	2.89	2.90	2.91	N.S.	pH	2.57c	2
FVT (mg L ⁻¹)	0.33b	0.48a	0.40ab	0.43a	0.39ab	0.093	FVT (mg L ⁻¹)	0.14c	0
PVT (mg L ⁻¹)	1.03	1.39	1.31	1.35	1.27	N.S.	PVT (mg L ⁻¹)	0.67c	1
BL (mm)	11.67d	13.05a	12.59b	12.54b	12.05c	0.37	BL (mm)	11.02c	1
BWi (mm)	11.39c	12.48a	11.96b	11.76b	11.63bc	0.33	BWi (mm)	10.42c	1
BWe (g)	1.27b	1.51a	1.35b	1.31b	1.31b	0.09	BWe (g)	0.88c	1
CL (cm)	10.07c	11.47a	11.37a	10.91ab	10.59bc	0.75	CL (cm)	10.01c	1
CWi (cm)	7.82c	9.16a	8.68ab	8.83ab	8.51b	0.63	CWi (cm)	7.98b	8
CWe (g)	116.75c	158.86a	128.70bc	141.27b	131.26bc	16.77	CWe (g)	86.45c	12

Different letters within a column indicate significant differences at 5% level using LSD multiple range test

Table 4. Influence of different doses of foliar SW and HA treatments on interaction effects of foliar treatments and term in cv. Riesling

Berry sampling term	Treatment	TSS (%)	SC (g L ⁻¹)	TA (g L ⁻¹)	pH	FVT (mg L ⁻¹)	PVT (mg L ⁻¹)	BL (mm)	BWi (mm)	BV (g)
Veraison	0 ppm	4.83	24.17	34.87a	2.53	0.125	0.411	10.57e	10.06c	0.8
	1000 ppm SW	5.49	31.60	31.25b	2.59	0.151	0.807	11.27cd	10.72ef	0.9
	2000 ppm SW	5.45	31.05	33.12ab	2.62	0.136	0.744	11.22cde	10.47fg	0.9
	1000 ppm HA	5.10	27.20	32.93ab	2.57	0.149	0.770	11.00de	10.56fg	0.8
	2000 ppm HA	5.19	28.02	34.37a	2.55	0.140	0.666	11.03cde	10.30fg	0.8
Berry sampling term	Treatment	TSS (%)	SC (g L ⁻¹)	TA (g L ⁻¹)	pH	FVT (mg L ⁻¹)	PVT (mg L ⁻¹)	BL (mm)	BWi (mm)	BV (g)
15 days after veraison	0 ppm	12.52	108.87	19.06c	2.69	0.283	0.934	11.34cd	11.19de	1.3
	1000 ppm SW	14.25	128.37	16.43de	2.71	0.408	1.232	13.56b	12.93bc	1.6
	2000 ppm SW	14.32	128.90	14.56e	2.76	0.324	1.180	12.98b	12.39c	1.4
	1000 ppm HA	13.45	119.60	18.87c	2.81	0.352	1.202	13.10b	11.52d	1.3
	2000 ppm HA	13.02	114.92	16.93cd	2.82	0.305	1.146	11.65c	11.27de	1.3
Berry sampling term	Treatment	TSS (%)	SC (g L ⁻¹)	TA (g L ⁻¹)	pH	FVT (mg L ⁻¹)	PVT (mg L ⁻¹)	BL (mm)	BWi (mm)	BV (g)
30 days after veraison	0 ppm	17.87	168.55	9.00f	3.20	0.581	1.746	13.09b	12.93bc	1.6
	1000 ppm SW	20.07	193.37	7.62f	3.33	0.880	2.153	14.33a	13.79a	1.9
	2000 ppm SW	19.05	181.97	8.87f	3.28	0.758	2.024	13.56b	13.01b	1.7
	1000 ppm HA	19.32	184.82	7.92f	3.31	0.804	2.084	13.51b	13.20b	1.6
	2000 ppm HA	18.80	178.32	8.06f	3.38	0.742	1.998	13.49b	13.33ab	1.7
	LSD ₅	N.S.	N.S.	2.30	N.S.	N.S.	N.S.	0.64	0.58	N.

Different letters within a column indicate significant differences at 5% level using LSD multiple range test

Conclusion

Results of this study showed that foliar treatments of SW and HA significantly influenced quality and yield parameters of cv. Riesling berry. Based on this research findings, 1000 ppm doses of SW and HA are advised for the best wine berry quality of cv. Riesling when the berries were harvested at 30 days after veraison period.

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