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CROP PRODUCTION AND MANAGEMENT - Article

Yield, berry quality and physiological response of grapevine to foliar humic acid application

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ABSTRACT: Environmentally friendly grape growing is an important objective of modern viticulture. The aim of the study was to examine the response of two grapevine *Vitis vinifera* L. cultivars to humic acids (HA) foliar application as biostimulant in order to provide sustainable viticultural practices for farmers. The experiment was carried out under field conditions and repeated in two consecutive years. In order to evaluate the effect of humic acids application, the grapevines were treated with three concentrations: 30 ml.L^{-1} (HA_{T1}), 40 ml·L^{-1} (HA_{T2}) and 50 ml·L^{-1} (HA_{T3}). The grapevines were represented by two grape varieties: cv. Feteasca Regala (cv. FR) and cv. Riesling Italian (cv. RI). Foliar sprays with humic acid derived from vermicompost at a concentration of 50 ml·L^{-1} induced a significant increase in the total leaf area, yield and total soluble solids. For several measurements performed in plants we found no significant difference between grapevines treated with HA_{T2} and those with HA_{T3}. The experimental results in the present study confirm that foliar application with humic acid may improve growth, yield, and berry quality attributes of grapevine.

Key words: biostimulants, humic acids, physiological response, sustainable viticulture, *Vitis vinifera*, yield and berry quality, vermicomposts.

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INTRODUCTION

Grapevine (*Vitis vinifera* L. ssp. *sativa*) is an important horticultural crop, one of the earliest domesticated non-climacteric fruit crops, and one of the most important perennial crops grown in Romania, used especially as table fruit, dried raisins, and for the vitivinicultural industry. In 2015, the total area under vines in Romania vineyards was 178 294 ha and total wine production was 3.627 mil hl.

In recent years, new strategies, methods, instruments, and technologies have been studied in order to improve the sustainability of agricultural ecosystems. A potential tool to improve the agro-enviromental perfomance in farms is the use of humic acids as plant biostimulants. These active natural compounds obtained from soil and compost organic matter can enhance yield and quality parameters of crops, nutrient efficiency, physiological performance of horticultural crops, and abiotic stress tolerance (Calvo et al. 2014). However, the potential of humic acids for grapevines has received little research attention. Canellas et al. (2015) reported that most humic substances used in agricultural systems are in the present produced from non-renewable resources. This is why new sustainable sources of humic commercial products need to be identified. Vermicomposts could be a potential source to develop new humic commercial products. Aguiar et al. (2013) noted that vermicomposts are an environmentally friendly substitute for peat and that it is enriched with highly bioactive humic acids substances.

Foliar applications of biostimulants have been widely recognized to improve plant growth, yield and physiological processes of horticultural crops. Foliar applications require fewer amounts of biostimulants and it allows for nutrients to be absorbed fast and directly by the leaf. According to Ferrara and Brunetti (2010), foliar sprays of humic compounds applied at different plant phenological growth stages increases berry weight and it enhanced grape fruit quality parameters such as titratable acidity and soluble solids.

Traditionally, vineyard fertilization is managed by adding different fertilizers directly to the soil, which may increase the risk of nutrient pollution and reduce the sustainability of agricultural systems (Garde-Cerdán et al. 2014). One solution is to use foliar applications because this technique is quicker and more precise (Lasa et al. 2012). In order to overcome nutrient deficiency in agro-ecosystems, foliar fertilization is an important practice increasingly applied in agriculture (Sabir et al. 2014). Foliar application with biostimulants may improve the uptake and the efficient use of nutrients. Humic acid applications represent a sustainable solution for agricultural systems to be integrated in new eco-friendly approaches for future agriculture. Biostimulants may have positive effects on plant growth due to an improvement of physiological processes and in plant nutrient acquisition.

Thus, the aim of this work was to evaluate the efficacy of the foliar application of humic substances derived from vermicompost, on the physiological activity, yield and berry quality attributes of grapevines. In the current study we present the results of a two-year field experiment with two local commercial wine grapevine cultivars.

MATERIAL AND METHODS

Vineyard description, growth conditions, and experimental design

Romanian viticultural areas are divided into 8 regions and 37 production areas. Stefanesti is one of the most important and suitable locations for viticulture in Romania. A two-year field experiment was conducted in a Stefanesti non-irrigated mature vineyard at 270 m altitude located between lat 44°42’ and 44°55’ N, long 24°54’ and 25°15’ E. The climate of this area is continental temperate. Long-term records show that the mean annual precipitation is 644 mm. The mean annual temperature is 9.6 °C – 10.1 °C and the insolation duration during the vegetative period is 1430 hours. The soil in the experimental vineyard was a luvisol (IUSS Working Group WRB 2006), slightly carbonated, medium levels in available P and K and low in available N. The pH of the soil is slightly acid. The vineyard was non-irrigated and had a trellis system. The vines had been pruned as Guyot double with mix of canes and spurs on the semi-stem sustained on draperies with 3 wires. The vines were planted at 2.4 m between rows and 1.2 m within rows (3472 vines per hectare) with a North–South row orientation. The vines were grafted on rootstock hybrid Kober 5 BB (*Vitis berlandieri* x *Vitis riparia*).

The grapevines selected for this study included two varieties, *Vitis vinifera* cv. Feteasca Regala (henceforth RF) and cv. Riesling Italian (henceforth RI). These occupy large areas in Romanian viticulture and they are widely used by the winemaking industry. The cultivars have white berry colors. Grapes obtained from these cultivars are excellent to make white wine with PDO (protected designation of origin) labels in order to satisfy quality standards. Feteasca Regala is the most cultivated cultivar in Romania, with 13723 ha in 2015, and Riesling Italian, with 5853 ha, occupies the fourth place.

In this experiment we used commercial humic acids (BioHumusSol Company Ltd., Romania) at the pre-bloom and fruit set phenological stages according to the BBCH scale (Lorenz et al. 1995). BioHumusSol, available in liquid form, is a natural biostimulant product based on humic acids extracted from composts using earthworms. This product is compatible with organic production for use as a crop fertilizer, according to the guidelines of European Union laws. The nutrient content of BioHumusSol product is: 14.5 g humic substances L^{-1}, 19 ppm nitrate nitrogen (NO_3^--N), 104 ppm ammonium nitrate (NH_4^+-N), 22.5 ppm P, 132 ppm K^+, 39 ppm Ca^{2+}, 75 ppm Mg^{2+}, 75 ppm Na^+. During the two-year field experiment there were no other fertilizer applications. Pest control, soil management and other viticultural operations were conducted according to standard practices.

The field trial was established for two consecutive growing seasons: 2013-2014 and 2014-2015. In a factorial experimental design, three different concentrations of humic acids and a control were used as treatments. Treatment plots were separated from each other by an untreated row. Each treatment plot was 86.4 m^2 and had three rows of ten plants per row. In order to minimize possible contamination effects from nearby treatments, data was collected only from the inner rows of each plot. During each foliar humic acid application the foliage of each plant was fully wetted. The cultivars were manually treated with foliar humic acids at three different concentrations: 30 ml·L^{-1} (HA_{T1}), 40 ml·L^{-1} (HA_{T2}) and 50 ml·L^{-1} (HA_{T3}). The control plants were manually sprayed with tap water alone. Foliar spray treatments with commercial humic acids were performed twice: at the pre-bloom and fruit set phenological stages.

Data and Measurements

Effects of the foliar treatments with humic acids on leaf growth were expressed using the following parameters: total leaf area (m^2·$vine^{-1}$), leaf fresh weight (g), and leaf dry weight (g). These measurements were performed on mature leaves, before harvest time, when shoot growth had reached its peak. The collected leaf samples, preserved in the ice box at 4 °C, were transported to the laboratory for estimation of physiological parameters. In order to evaluate the physiological response of foliar application with humic acids, the following were determined: photosynthesis rate (µmol CO_2 m^{-2}·s^{-1}) in leaves, the content of chlorophyll a (Chl a, mg·g^{-1}), chlorophyll b (Chl b, mg·g^{-1}), and carotenoids (Car, mg·g^{-1}) in leaves. Photosynthesis rate was determined with a S151 Infrared CO_2 portable plant analyzer (IRGA from Qubit Biology Inc., Ontario, Canada). During testing, photosynthetic photon flux density was 1300 – 1500 µmol.m^{-2}·s^{-1}, air temperature was 25 °C ± 2 °C, and ambient relative humidity was between 55% and 60%. Photosynthetic pigments were determined using a spectrophotometer (BOECO S-20VIS from Boeckel & Co, Hamburg, Germany). Leaves were collected in the morning, and transported to the laboratory where photosynthetic pigments were extracted in 80% acetone. The amounts of chlorophyll a, chlorophyll b and carotenoid pigments were calculated according to the formula described by Holm (1954).

Yield of vines and some grape quality parameters were evaluated at harvest time in 2014 and 2015. The following parameters were measured: yield (kg·$vine^{-1}$), cluster weight (g), 100 berries weight (g), berry volume (ml·100 $berries^{-1}$), total soluble solids (°Brix), and titratable acidity (g H_2SO_4 L^{-1}). For each vine from the experimental unit grapes were manually collected at harvest time. The fresh weight and the volume of 100 berries from each sample were recorded. Total soluble solids (TSS) were determined using an Abbe refractometer. Titratable acidity (TA) was measured with sodium hydroxide titration. Titratable acidity was expressed as sulfuric acid (g·L^{-1}). Finally, two important indexes for evaluation of efficacy of leaf area to fruit yield and for information about the hastening or delay of the ripening process were calculated: the leaf area to yield (m^2·kg^{-1}) and the °Brix/TA ratios.

Data analysis

The experimental design consisted of a randomized complete block with four treatments and three replicates. A statistical analysis was performed using the ANOVA in the SPSS 16.0 software. Data was analyzed using the SPSS 16.0 statistical software (IBM Corporation, Armonk, New York, USA). Means were compared using Duncan's multiple range tests at $p < 0.05$. The results of this study are expressed as means. Standard deviations of the means were calculated.

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RESULTS AND DISCUSSION

Physiological and growth response to foliar humic acid application

In the current experiment, the effects of foliar treatments with humic acids on leaf development were evaluated by determining the following parameters: total leaf area, leaf fresh weight, and leaf dry weight (Table 1). The results showed that the total leaf area and the leaf fresh and dry weight in both cultivars increased with some treatments, and that there were some significant differences between grapevines treated with HA_{T2} or HA_{T3} and control samples. Total leaf area of cv. Riesling Italian plants had the same statistical trend in 2015 as in 2014 to the foliar application of humic acids. Regarding leaf dry weight in cv. FR during both growing seasons we found no significant differences between treatments. The maximum leaf development of cv. FR and cv. RI plants occurred with humic acids at 40 ml·L^{-1} and 50 ml·L^{-1} (HA_{T2} and HA_{T3}). No significant differences regarding the leaf growth features of cv. FR and cv. RI during 2014 and 2015 seasons were found between the untreated grapevine and HA_{T1} – 30 ml·L^{-1}. In the case of cv. FR plants, the influence of HA_{T2} or HA_{T3} on leaf development did not show any significant difference during the two years of study, except in the case of leaf fresh weight and total leaf area observed in 2015.

Studies regarding the effect of foliar application of humic acids on grapevine are still limited. Only a few studies have been published mainly for table grapevine (Sánchez-Sánchez et al. 2006; Ferrara and Brunetti 2008; Ferrara and Brunetti 2010; Mohamadineia et al. 2015).

Ferrara and Brunetti (2010) observed that the foliar application of humic acids improves grapevine production. Generally, experiments related to humic acid application on plant growth and its development were conducted in controlled environments, and few open-field experiments have been reported.

Ahmad et al. (2013) showed that humic acids foliar application provides positive influence on growth, vase life, and corm characteristics of gladiolus. HA have been shown to have a positive effect, especially to stimulate plant growth and consequently yield for several horticultural crops: gerbera (Haghighi et al. 2014), gladiolus (Ahmad et al. 2013), and tomatoes (Olivares et al. 2015). The physiological mechanisms of the humic acid, applied as biostimulant on grapevine, were investigated in terms of photosynthesis and assimilatory pigments in leaves. Chlorophylls and carotenoids are the main pigments of leaf photosynthesis. Efficacy of foliar application with humic acids varies according to the physiological status of plants. In our study we found a positive effect of humic substance sprays on the photosynthesis rate. Photosynthesis in leaves of cv. Feteasca Regala and cv. Riesling Italian showed the same trend in response to the foliar application of humic acids (Table 2). The HA_{T2} and HA_{T3} treatments induced a statistically significant increase in the photosynthesis rate compared to the controls. The biostimulant effect of humic acids on photosynthesis was more pronounced, in both years of the study, in cv. FR treated with 40 ml·L^{-1} and 50 ml·L^{-1} humic acids than in the untreated vines or the plants treated with 30 ml·L^{-1}. In the case of the photosynthesis rate for cv. FR, we did not find significant differences for the plants treated with 40 ml·L^{-1} or 50 ml·L^{-1} humic acid. The photosynthetic response of cv. Riesling Italian plants to humic substances →

Table 1. Effect of humic acid foliar application on leaf development in grapevine during the 2014 and 2015 seasons (*Vitis vinifera* L. cv. Feteasca Regala and cv. Riesling Italian) grown in the Stefanesti region, Romania.

Cultivar	Treatment	Total leaf area (m²·vine⁻¹)		Leaf fresh weight (g)		Leaf dry weight (g)	
		2014	2015	2014	2015	2014	2015
cv. FR	Control	5.78 ± 0.08c	5.82 ± 0.19c	2.12 ± 0.06b	2.16 ± 0.03c	0.53 ± 0.13a	0.53 ± 0.11a
	HA_{T1}	5.96 ± 0.48bc	5.92 ± 0.16c	2.22 ± 0.09b	2.20 ± 0.11c	0.57 ± 0.11a	0.59 ± 0.20a
	HA_{T2}	6.37 ± 0.23ab	6.44 ± 0.21b	2.45 ± 0.15a	2.61 ± 0.17b	0.68 ± 0.10a	0.72 ± 0.19a
	HA_{T3}	6.50 ± 0.15a	6.76 ± 0.21a	2.51 ± 0.16a	2.81 ± 0.12a	0.71 ± 0.12a	0,79 ± 0.14a
cv. RI	Control	4.28 ± 0.22c	4.29 ± 0.09c	1.98 ± 0.17b	2.07 ± 0.11c	0.50 ± 0.10a	0.51 ± 0.10b
	HA_{T1}	4.51 ± 0.13c	4.52 ± 0.23c	2.08 ± 0.09ab	2.14 ± 0.08bc	0.53 ± 0.11a	0.53 ± 0.07ab
	HA_{T2}	4.88 ± 0.22b	4.90 ± 0.17b	2.14 ± 0.04ab	2.28 ± 0.12ab	0.62 ± 0.13a	0.65 ± 0.13ab
	HA_{T3}	5.21 ± 0.13a	5.23 ± 0.08a	2.24 ± 0.09a	2.35 ± 0.17a	0.67 ± 0.07a	0.69 ± 0.12a

Data presented as mean ± standard deviation. Mean values followed by the same letter within columns are not significantly different according to Duncan's multiple range test ($p < 0.05$). Control: untreated grapevine; HA_{T1} – 30 ml·L^{-1}; HA_{T2} – 40 ml·L^{-1}; HA_{T3} – 50 ml·L^{-1} humic acids.

application showed the same trend during both years of the experiment. Regarding the influence of humic acids on assimilatory pigments, these natural compounds increased the content of Chl a, Chl b, and Car in the leaves (Figure 1). A statistically significant increase for Chl a and for Car in cv. FR during the first growing season was found for HA_{T2} and HA_{T3} compared to HA_{T1} or untreated plants. However, the difference in Chl a, Chl b and Car between the untreated grapevines and the plants treated with HA_{T1} of cv. RI and cv. FR was not significant for both growing seasons. In 2015, the carotenoids content in leaves of grapevine treated with humic acids was significantly higher at the concentration of 50 $ml{\cdot}L^{-1}$ (HA_{T3}) than for HA_{T1} and the control samples. No statistical differences in carotenoids pigments were observed between the plants treated with HA_{T1} and untreated grapevines. In the case of Chl b for cv. RI in 2015, we did not find any statistically significant difference between all the humic acid concentrations and the control samples.

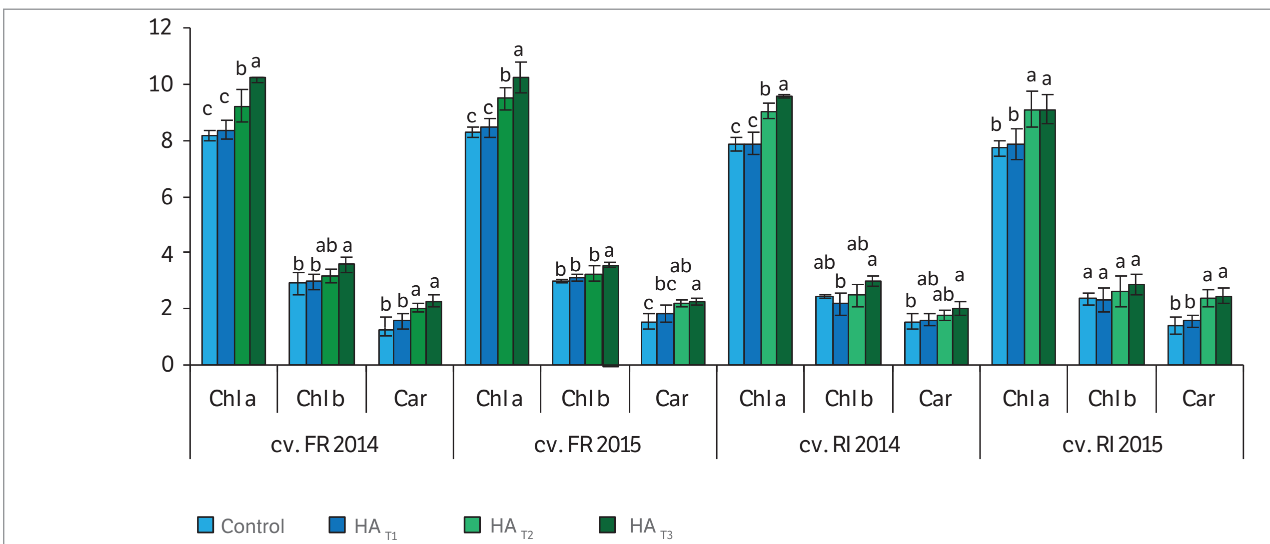
In the present study, humic acid foliar application also enhanced the leaf chlorophyll content, in accordance with the results of Sabir et al. (2014), who determined higher chlorophyll content in the leaves of vines treated with *Ascophyllum nodosum* L., a seaweed extract frequently used in agriculture. The seaweed extracts used as biostimulants in grapevine increased the chlorophyll content, yields and berry weight (Sabir et al. 2014).

Fan et al. (2014) described that the foliar application of humic acid improved the leaf area, net photosynthesis rate and the chlorophyll content in chrysanthemum compared with those of control plants. Regarding the results obtained

Table 2. Effect of humic acid foliar application on photosynthesis rate in leaves of grapevine during the 2014 and 2015 seasons (*Vitis vinifera* L. cv. Feteasca Regala and cv. Riesling Italian) grown in the Stefanesti region, Romania.

Treatment	Photosynthesis rate ($\mu mol\ CO_2{\cdot}m^{-2}\ s^{-1}$) cv. FR		Photosynthesis rate ($\mu mol\ CO_2{\cdot}m^{-2}\ s^{-1}$) cv. RI	
	2014	2015	2014	2015
Control	10.41 ± 0.50b	10.31 ± 0.43c	8.72 ± 0.35c	8.97 ± 0.32c
HA_{T1}	11.22 ± 0.75b	11.27 ± 0.24b	9.14 ± 0.42c	9.13 ± 0.23c
HA_{T2}	13.27 ± 0.76a	13.63 ± 0.31a	11.60 ± 0.38b	11.51 ± 0.61b
HA_{T3}	14.24 ± 0.61a	13.92 ± 0.41a	12.89 ± 0.35a	12.43 ± 0.88a

Data presented as mean ± standard deviation. Mean values followed by the same letter within columns are not significantly different according to Duncan's multiple range test ($p < 0.05$). Control: untreated grapevine; HA_{T1} – 30 $ml{\cdot}L^{-1}$; HA_{T2} – 40 $ml{\cdot}L^{-1}$; HA_{T3} – 50 $ml{\cdot}L^{-1}$ humic acids.



Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$).
Bars indicate standard deviation of mean.
Control: untreated grapevine; HA_{T1} – 30 $ml{\cdot}L^{-1}$; HA_{T2} – 40 $ml{\cdot}L^{-1}$; HA_{T3} – 50 $ml{\cdot}L^{-1}$ humic acids.
Chl a: Chlorophyll a in leaves ($mg{\cdot}g^{-1}$); Chl b: chlorophyll b in leaves ($mg{\cdot}g^{-1}$); Car: carotenoids in leaves ($mg{\cdot}g^{-1}$).

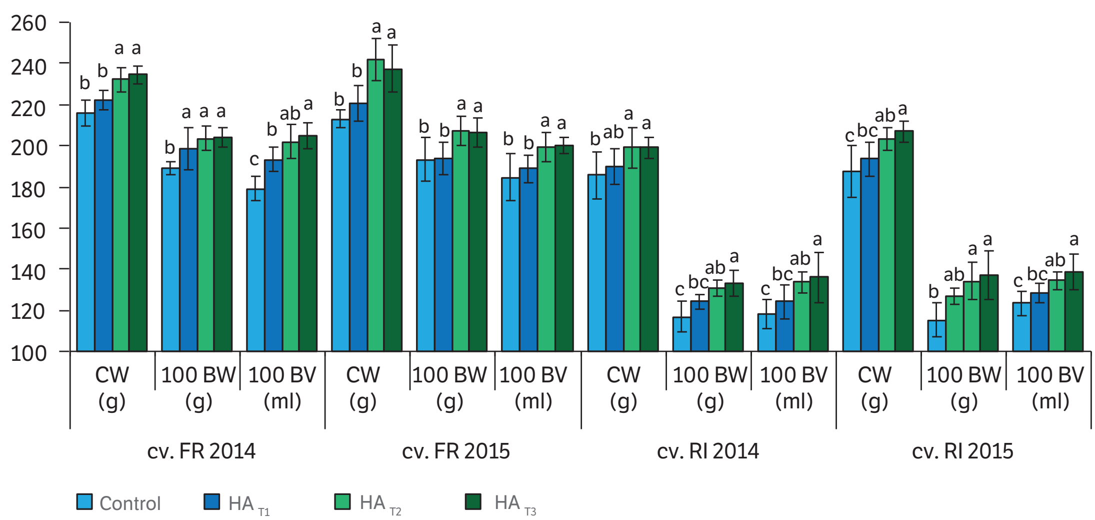
Figure 1. Effect of humic acids foliar application on assimilatory pigments in grapevine leaves during 2014 and 2015 seasons (*Vitis vinifera* L. cv. Feteasca Regala and cv. Riesling Italian).

for assimilatory pigments, our outcomes are in line with the findings of Haghighi et al. (2012), who demonstrated that the total leaf chlorophyll content was increased with the foliar application with humic acids.

Recent studies have confirmed the hypothesis of a direct effect of humic acids on plant physiology (Haghighi et al. 2014). Humic substances seem to positively influence metabolic and signalling pathways involved in plant development, by acting directly on specific physiological mechanisms (Trevisan et al. 2010). According to Trevisan et al. (2010), humic acids influence plant development by interfering with the transcription of genes involved in meristem formation and organization, cell cycle, microtubule organization and cytokinesis. Humic acids play an important role for promoting the mechanism of cell division and differentiation. Canellas et al. (2015) noted that humic acids increased ATPase synthesis and activity in root cells. Some authors reported that humic acid bioactivity promotes plant growth by inducing hormone-like activities (Calvo et al. 2014; Canellas et al. 2015).

Yield and berry quality response to foliar humic acid application

In order to evaluate the effects of humic acid foliar sprays some quantitative parameters of grape production were analyzed: the recorded values are presented in Table 3 and Figure 2. The highest yield of grapes for both cultivars was obtained in plants treated with HA_{T3} and the lowest yield was observed in HA_{T1} and in the untreated vines (Table 3). For all the foliar treatments, humic acids in different concentration increased plant yield. However



Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$).
Bars indicate standard deviation of mean.
Control: untreated grapevine; HA_{T1} – 30 ml·L^{-1}; HA_{T2} – 40 ml·L^{-1}; HA_{T3} – 50 ml·L^{-1} humic acids.
CW: cluster weight; 100 BW: 100 berry weight; 100 BV: 100 berry volume.

Figure 2. Effect of foliar application of humic acid on cluster weight (g), 100 berry weight (g), berry volume (ml 100 berries^{-1}) in grapevine during 2014 and 2015 seasons (*Vitis vinifera* L. cv. Feteasca Regala and cv Riesling Italian).

Table 3. Effect of humic acids foliar application on the yield of grapevine during the 2014 and 2015 seasons (*Vitis vinifera* L. cv. Feteasca Regala and cv. Riesling Italian) grown in the Stefanesti region, Romania.

Treatment	Yield cv. FR (kg·vine^{-1})		Yield cv. RI (kg·vine^{-1})	
	2014	2015	2014	2015
Control	2.48 ± 0.10b	2.51 ± 0.28b	2.14 ± 0.19b	2.20 ± 0.15b
HA_{T1}	2.66 ± 0.14b	2.70 ± 0.17b	2.26 ± 0.23b	2.36 ± 0.11ab
HA_{T2}	3.20 ± 0.09a	3.15 ± 0.28a	2.80 ± 0.11a	2.71 ± 0.19a
HA_{T3}	3.24 ± 0.49a	3.31 ± 0.33a	2.92 ± 0.14a	2.90 ± 0.16a

Data presented as mean ± standard deviation. Mean values followed by the same letter within columns are not significantly different according to Duncan's multiple range test ($p < 0.05$). Control: untreated grapevine; HA_{T1} – 30 ml·L^{-1}; HA_{T2} – 40 ml·L^{-1}; HA_{T3} – 50 ml·L^{-1} humic acids.

there were no statistical differences between the plants treated with HA_{T2} and those treated with HA_{T3}. The yield of cv. FR plants was higher than those of cv. RI for both growing seasons.

The results for grape oenological parameters are shown in Table 4. The humic acid applied in concentration of 50 ml·L^{-1} (HA_{T3} 50 ml·L^{-1}) induced a significant increase of total soluble solids in grape and a significant improvement of the other quality parameters (titratable acidity) when compared to the controls. However, the difference in TSS of cv. RI was not significant between each of the samples treated with 30 ml·L^{-1} humic acid (HA_{T1}) and the control samples. In the second year of observations we found the same trend for TTS in cv. FR. Plants of cv. FR and cv. RI treated with humic acids at 40 ml·L^{-1} (HA_{T2}) and with 50 ml·L^{-1} (HA_{T3}) were statistically different in TTS (Table 4). In almost all the samples, the experimental results showed that the humic acids enhanced the TSS of plants of cv. FR. and cv. RI, but they generally caused a decrease of TA. There was no significant difference in TA between treatments with 40 ml·L^{-1} (HA_{T2}) and with 50 ml·L^{-1} humic acids (HA_{T3}), with the exception of cv. RI in 2014.

The results obtained for total soluble solids and titratable acidity satisfy the quality standards for producing wine with PDO label in the viticultural region of Stefanesti. The influence of humic acids on the cluster weight of plants was significant in grapevine treated with HA_{T2} and HA_{T3} compared to plants treated with 30 m·L^{-1} humic acids or controls (Figure 2). The effect of humic acids on the weight and volume of berries was more pronounced in plants with HA_{T2} and HA_{T3} than with 30 ml·L^{-1} or in untreated plants. The quantitative parameters, expressed by cluster weight (g), 100 berries weight (g) and berry volume (ml 100 berries^{-1}), were higher in the plants of cv. FR than cv. RI for both growing seasons. The untreated vines had the lowest cluster weight, berry weight, and berry volume values for the two years. Among the humic acid treatments, HA_{T3} induced the highest average in cluster weight, berry weight, and berry volume of cv RI plants in 2014 or 2015 and was found to be significantly different from the others treatments.

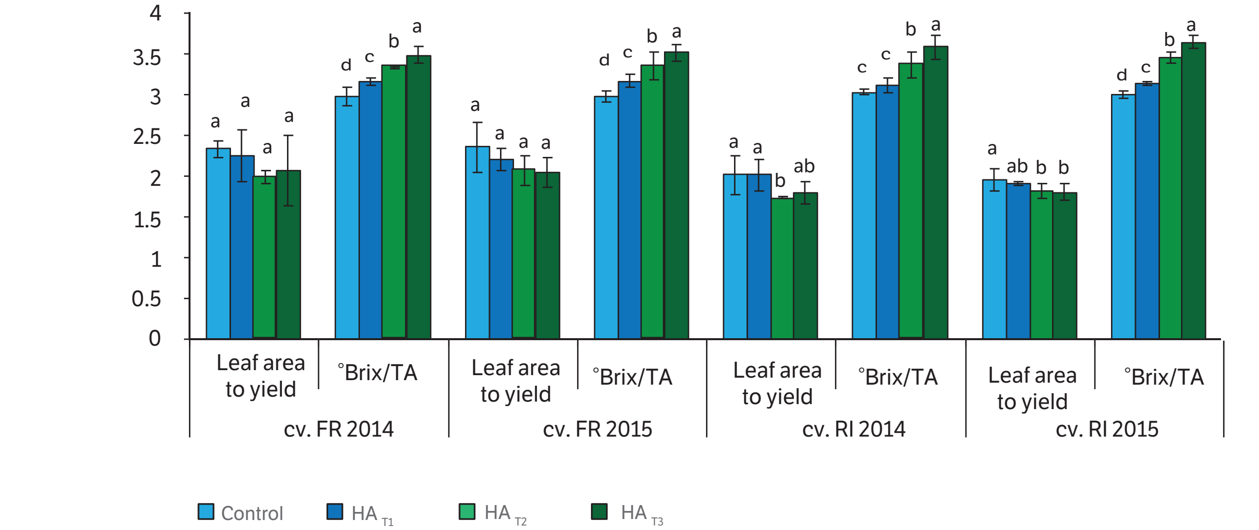
Two significant indicators, which reflect the relationship among the leaf area, yield and the oenological parameters in grapes, were analyzed under the influence of foliar application with humic acids (Figure 3). Humic acid application in cv FR showed no significant difference between treatments in the leaf area to yield ratio, while for cv. RI plants small but statistically significant differences between treatments were observed between untreated plants and the ones under HA_{T2} treatment. The greatest leaf area to yield ratio was observed in the treatment without humic acid, while the minimum values of this indicator was seen in the HA_{T3} treatment, except in the case of cvs. FR and RI in 2014 with a minimum ratio registered for the HA_{T2} treatment. The highest °Brix/TA ratio was observed in the plants from the HA_{T3} treatment. The lowest °Brix/TA ratio was found in the controls. By analyzing the °Brix/TA ratio, this study allowed us to observe significant differences between the HA_{T3}, HA_{T2}, and HA_{T1} treatments. The decrease of leaf area to yield ratio in Riesling Italian and Feteasca Regala cultivars likely increased the efficacy of leaf area to increase yield. The °Brix/TA ratio showed that treated vines had a faster ripening than control ones.

Some authors have shown humic acids extracted from vermicompost to be more efficient to enhance plant growth and yield than other products (Befrozfar et al. 2013). The

Table 4. Effect of humic acid foliar application on total soluble solids (°Brix) and titratable acidity (g H_2SO_4·L^{-1}) in grapevine during the 2014 and 2015 seasons (cv. FR; cv. RI).

Cultivar	Treatment	Total soluble solids (°Brix)		Titratable acidity (g H_2SO_4·L^{-1})	
		2014	2015	2014	2015
cv. FR	Control	17.13 ± 0.29d	17.17 ± 0.25d	5.74 ± 0.17b	5.75 ± 0.10b
	HA_{T1}	17.92 ± 0.31c	18.00 ± 0.19c	5.66 ± 0.11ab	5.68 ± 0.09ab
	HA_{T2}	18.48 ± 0.23b	18.58 ± 0.15b	5.54 ± 0.05ab	5.55 ± 0.25ab
	HA_{T3}	19.16 ± 0.30a	19.12 ± 0.12a	5.49 ± 0.11a	5.44 ± 0.10a
cv. RI	Control	19.02 ± 0.14c	18.88 ± 0.27d	6.26 ± 0.10c	6.28 ± 0.08b
	HA_{T1}	19.21 ± 0.17c	19.25 ± 0.06c	6.16 ± 0.17bc	6.14 ± 0.04b
	HA_{T2}	20.32 ± 0.52b	20.25 ± 0.17b	6.05 ± 0.13b	5.86 ± 0.05a
	HA_{T3}	21.05 ± 0.77a	20.89 ± 0.34a	5.88 ± 0.07a	5.74 ± 0.14a

Data presented as mean ± standard deviation. Mean values followed by the same letter within columns are not significantly different according to Duncan's multiple range test ($p < 0.05$). Control: untreated grapevine; HA_{T1} – 30 ml·L^{-1}; HA_{T2} – 40 ml·L^{-1}; HA_{T3} – 50 ml·L^{-1} humic acids.



Mean values followed by the same letter are not significantly different according to Duncan's multiple range test ($p < 0.05$).
Bars indicate standard deviation of mean.
Control: untreated grapevine; HA_{T1} – 30 $ml·L^{-1}$; HA_{T2} – 40 $ml·L^{-1}$; HA_{T3} – 50 $ml·L^{-1}$ humic acids.
°Brix: total soluble solids; TA: titratable acidity ($g\ H_2SO_4·L^{-1}$).

Figure 3. Effect of foliar application of humic acid on leaf area to yield ($m^2·kg^{-1}$) ratio and °Brix/TA ratio in grapevine during 2014 and 2015 seasons (*Vitis vinifera* L. cv. Feteasca Regala and cv Riesling Italian)

application of humic acids at 40 $ml·L^{-1}$ (HA_{T3}) and 50 $ml·L^{-1}$ (HA_{T3}) induced a significant increase in weight and volume of grape berries in cv. Feteasca Regala grapevine. These results are in agreement with data obtained by Ferrara and Brunetti (2010).

In this paper we found that high chlorophyll content in grapevine leaves can be partially responsible for the increase in net photosynthesis. The high leaf area and net photosynthesis likely contributed to hasten grape ripening, thus leading to a higher TSS content at maturity. Mohamadineia et al. (2015) evaluated the impact of foliar application at concentrations of 2.5 $mg·L^{-1}$ to 7.5 $mg·L^{-1}$ on yield and berry attributes in grapevine. In line with our findings, they reported that the influence of humic acids on yield, berry weight and volume, and TSS of grape was enhanced compared to the untreated vines. The leaf area to yield ratio is an indicator that influences TSS (Kliewer and Dokoozlian 2005; Filippetti et al. 2015).

In the current study, the leaf area to yield ratio in Riesling Italian and Feteasca Regala cultivars were influenced by the foliar application of humic substances derived from vermicompost, resulting in well-balanced grape production with a sufficient level of soluble solids.

Humic acids play a significant role for plant nutrition and physiological status as a source of nutrients and bio-stimulant. Our experiments demonstrate that BioHumusSol may provide such a dual role, especially when no fertilizer or other amendments were applied.

CONCLUSION

The humic acids sprayed at different concentrations in this study influenced leaf development, physiological activity, yield, and berry quality attributes of grapevine. Within the experimental conditions used in this study, we showed that the humic acids had a positive effect on plant growth and fruit quality when applied at the pre-bloom and fruit set phenological stages. In our study, the most responsive variety to the HA treatment was cv. Riesling Italian. In this regard, varieties responded in different ways to the HA treatments, but there is a similar likely positive effect for any grapevine variety. In order to improve the plant nutritional and physiological status, farmers may use commercial products based on humic substances.

For most of the treated samples, humic acids extracted from vermicompost significantly stimulated yield and quality parameters of the plants of Feteasca Regala and Riesling Italian grape cultivars. Results obtained in 2015 confirmed the trend observed in 2014. The experimental results in the present study indicate that foliar application with humic acid at a concentration of 40 mL^{-1} could be integrated into a sustainable technology package for viticulture management. A concentration of 40 mL^{-1} could represent the optimal humic acid application rate for grapevines, however, multi-location trials would be required to confirm this.

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