VALUATION OF VEGETAL RESIDUE OF GRAPE SEEDS RESULTING FROM THE EXTRACTIVE PROCESSES OF PHENOLIC COMPOUNDS

VALORIFICAREA REZIDUULUI VEGETAL DE SEMINȚE DE STRUGURI REZULTAT DIN PROCESELE EXTRACTIVE ALE COMPUŞILOR FENOLICI

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Abstract. *The stepwise extraction process of phenolic compounds from grape seeds ultimately leads to the production of a residue rich in protein, cellulose and substances without nitrogen which can be biodegraded in nature by microorganisms from the soil. The purpose of this research was to verify whether the vegetable residue of grape seeds, resulting from the extraction of polymerized proanthocyanidins can be used as an organic fertilizer in the soil. The soil biodegradation process was assessed by microbiological analysis and analysis of current and potential dehydrogenase activity. The results obtained showed that the vegetal residue led to the increase of the number of soil microorganisms involved in the nitrogen circuit and carbon, as a result of the triggering of the biodegradation process as well as its non-polluting effect supported by current and potential dehydrogenase activity determined in dynamics over a year in experimental plots.*

Key words: organic fertilizer, microbiological, dehydrogenase activity

Rezumat. *Procesul de extracție etapizată a compuşilor fenolici din semințele de struguri conduce în final la obținerea unui reziduu vegetal bogat în proteină, celuloză şi substanțe neazotate, ce pot fi biodegradate în natură, de către microrganismele din sol. Scopul cercetărilor efectuate a fost de a verifica dacă reziduul vegetal de semințe de struguri rezultat după extracția proantocianidinelor condensate polimerice poate fi utilizat ca îngrăşământ organic în sol. Procesul de biodegradare a reziduului administrat în sol a fost apreciat prin analize microbiologice şi analiza activității dehidrogenazice actuale şi potențiale. Rezultatele obținute au evidențiat activitatea favorizantă a reziduului vegetal asupra creşteri numărului de microorganisme din sol implicate în circuitul azotului şi carbonului, ca urmare a declanşării procesului de biodegradare, precum şi efectul nepoluant al acestuia susținut de activitatea dehidrogenazică actuală şi potențială determinată în dinamică pe parcursul unui an în parcelele experimentale*

Cuvinte cheie: îngrăşământ organic, activitate microbiologică, enzimatică

INTRODUCTION

Maintaining and increasing soil fertility and biological activity is an important objective in organic viticulture (Bernaz *et. al*, 1999). The solution of this

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problem can be achieved by using conventional (organic and mineral fertilizers) or unconventional products provided that they are applied at well-defined doses in order not to induce soil imbalances in natural microflora (Ulea *et al*, 2008).

In the technology of exploiting the bioactive components in the grape waste, elaborated by Viticulture and Oenology Research and Development Station in Iasi (SCDVV Iasi), the gradual extraction process of phenolic compounds from grape seeds ultimately lead to a residue rich in vegetable protein (9.610 wt%), cellulose (51.720 wt%) and neazotate substance (34.80 wt%) or carbohydrates, which are the substances necessary for the activity of soil microorganisms.

The purpose of these researches was to verify the fertilizer quality of the plant residue resulting from the extraction of polymer condensed proanthocyanidins. The process of biodegradation in the soil of the residue given was assessed by the determination of pH, microbiological analysis and analysis of current and potential dehydrogenase activity.

MATERIAL AND METHOD

The experience was placed in a vineyard plantation belonging to SCDVV Iasi with plan land, chernozem cambic soil and south-western exposure. The amount of vegetable residue administered the test plots was calculated based on the results obtained in preliminary tests. Thus, in the plot V1 administered an amount of 1.5 kg/m^2 of vegetal residue and in plot V2 3.0 kg/m^2.The vegetal waste was applied by spreading on the vineyards, at the surface of the soil and incorporating it into the ground with the large digging up to the depth of 18-20 cm.

Determination of ecophysiological groups of microorganisms was carried out according to the method authors Pochon S. and Tardeux J. (1954) cited by Dunca S. (2007). The ecophysiological groups were assessed by the size order of the soil suspension dilution at which the microorganisms developed, and their number was expressed in logarithm based on 2/g soil.

Dehydrogenase enzyme activity current and potential were determined according to the method Casida (Drăgan - Bularda, 2000).

RESULTS AND DISCUSSIONS

Microorganisms in the soil a remarkable capacity to adapt the biodegradation processes of natural organic compounds. Most of the soil microorganisms prefer a pH close to neutrality. This aspect was monitored during the fertilizer quality assay of the plant residue.

The data on the variation of pH values in the soil in the control plot and the two plots in the experimental variants V1 and V2 are presented in table 1.

Table 1

The evolution of the soil pH values in the time March to September, 2016

Variantion	Months						
	March	April	May	June	July	August	September
Control plot (M)	6.50	6.41	6.50	5.90	5.90	6.11	6.68
Plot V1 1.5 kg/m^2	6.61	6.26	6.35	5.91	6.02	6.13	6.71
Plot V2 3.0 kg/m^2	6.50	6.21	6.20	5.85	6.04	6.19	6.72

From analysis of pH variation data was found, between March and June 2016 a moderate decrease of the values in the soil in the control plot (from 6.5 to 5.9) and the plot V1 (from 6.61 to 5.91) and more pronounced in the soil in the plot V2 (from 6.5 to 5.85).

The decrease in the pH values of the product was due to the precipitation of this time. In the plot V2 the decrease was also accentuated due to the higher quantity of vegetal residuals. Starting of month July observed a progressive increase in the soil pH values V1 and V2 plots, values higher than those recorded in the control plot soil.

Considering the chemical composition of the vegetable residue, namely rich in proteins, cellulose and carbohydrates, microbiological analyzes in this study focused on the determination of the eco-physiological groups of microorganisms involved in the nitrogen and carbon circuit.

In the context of climatic factors in the spring of 2016 (the average temperature higher than the normal level of soil moisture to 69%), determinations made before administration of the vegetable residue in the soil for the evaluation of potential microorganisms in ecophysiological groups, showed that they were well represented (tab. 2).

Table 2

The number of ecophysiological microorganisms (logarithm in base 2)

Plots	Total number of microorganisms								
	Ammo-niacal microflora	nitrification microflora		Denitrifying microflora	Nitrogen fixation microflora		Proteolytic microflora	Cellulosylium microflora	
		nitric bacteria	nitroas bacteria		aerobic	anaerobic		aerobic	anaerobic
MARCH 2016									
M	63.044	9.551	9.551	22.839	8.644	15.458	16.811	23.304	18.610
V1	63.043	8.814	6.845	21.254	7.966	12.873	15.988	22.666	17.932
V 2	59.932	9.343	9.892	22.632	8.814	14.610	15.458	21.966	19.310
JUNE 2016									
M	60.380	15.986	7.644	15.458	15.456	8.229	28.746	15.458	11.773
V1	74.082	20.510	8.814	14.873	17.922	10.966	36.467	16.536	11.966
V 2	75.389	20.133	9.892	13.214	17.923	12.316	36.467	21.245	12.136
SEPTEMBER 2016									
M	48.677	13.873	1.100	0.900	0.900	0.900	12.873	0.900	0.900
V1	47.507	17.195	1.100	0.900	0.900	0.900	12.666	0.900	0.900
V 2	52.529	17.610	1.150	0.900	0.900	0.900	12.873	0.900	0.900
MARCH 2017									
M	59.996	7.644	7.229	18.780	7.956	7.644	13.214	11.966	14.288
V1	59.865	11.288	11.288	18.417	7.956	8.451	16.205	12.001	17.932
V 2	63.317	12.883	11.966	21.932	8.451	11.288	16.747	16.536	19.858

In order of the number of microorganisms determined in the nitrogen circuit, the most abundant were the ammonifier, denitrifying, proteolytic, anaerobic nitrogen fixators, nitrite bacteria, nitratbacteria and aerobic nitrogen fixators. In order of the number of microorganisms involved in the carbon chain, the most numerous were aerobic cellulosic bacteria, followed by anaerobic cellulosic.

The results obtained three months after soil vegetal application (June 2016) revealed a numerical increase of the microorganisms in experimental plots V1 and V2 compared to their numerical representation in the control soil, a positive aspect supporting the quality of organic fertilizer natural vegetable residue.

The analysis of September results shows that the ecophysiological groups of microorganisms were affected by the evolution of climatic factors during the summer months (absence of precipitation, accentuation of the water deficit in the first layer of soil 0-20 cm from 47% in June to 77% and 86% in July and August). The number of ammonifying microorganisms in the M and V 1 plots was lower compared to the one determined in June. Also, the number of nitrate bacteria increased by 19% in V1 and 21% in V2.

Microorganisms in eco-physiological groups: nitritbacteria, denitrifying, aerobic and anaerobic nitrogen fixators, as well as aerobic and anaerobic celluloses were identified in the first soil dilution but were not identified as a number by the Mc Cray method in logarithm on base 2.

One year after the administration of the vegetal residues into the soil (March 2017), the number of ammonifying microorganisms in the soil was almost equal to the control plot V1 and higher by 5.2% in the soil in the plot V2. The number of nitrate bacteria and nitrite bacteria increased in value in the soil in plots V1 and V2 compared to the number determined in plot M. The number of denitrifying microorganisms determined was almost to the soil in the plots M and V1, and by 14% higher in the plot V2. The number of aerobic nitrogen fixation microorganisms / g of soil was equal in the plots M and V1 and higher by 5.8% in the experimental plot V2, and the number of anaerobic nitrogen fixation microorganisms / g of soil increased by 9% in experimental plot V1 and by 32% in experimental plot V2.

Compared to the number of microorganisms determined by the plot M, V1 and V2 in the plots, the number of the proteolytic microorganisms grown in plots V1 and V2 with 18% to 21%. The number of aerobic cellulosic microorganisms determined in this group was almost equal to the number determined in the control plots and V1 and higher by 27% in the experimental plot V2. Regarding the number of anaerobic cellulosic microorganisms, compared to the control plot their number was higher in plot V1 by 20% and in the experimental plot V2 by 28%.

The results of microbiological analyzes are supported by current and potential dehydrogenase enzymatic analyzes performed monthly. The enzyme activity in the soil is one of the first parameters that change under the influence of fertilization products, representing an early indicator of soil quality changes (Lee *et al.,* 2002; Garcia-Ruiz *et al.,* 2008).

The results of dehydrogenase enzymatic assays are presented graphically in figures 1 and 2.

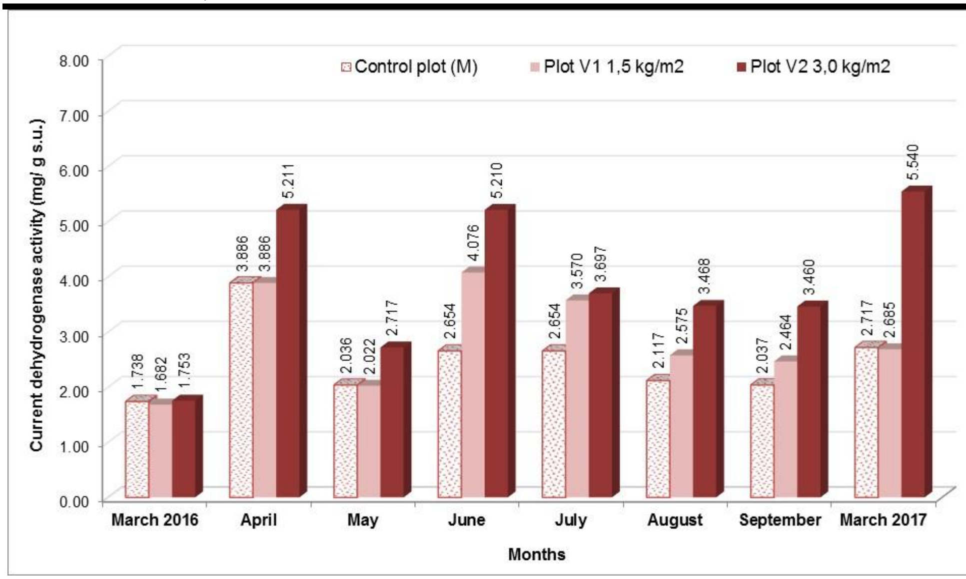

Fig. 1 The current dehydrogenase activity (mg/g s.u.)

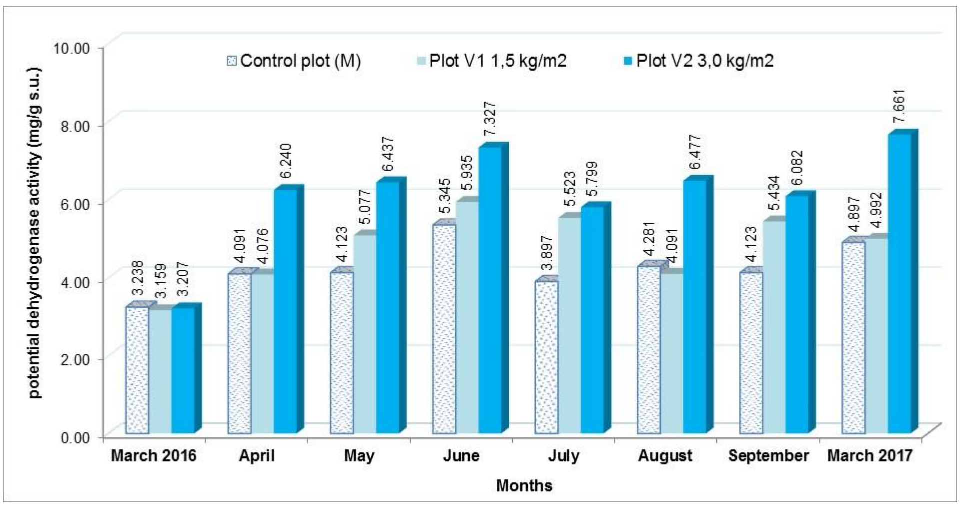

Fig. 2 The potential dehydrogenase activity (mg/g s.u.)

Determination of enzymatic activity, made in dynamics during the experiment, reveals that before application of the vegetal residue to the soil, the current and potential dehydrogenase activity values were close in the first case, between 1.682 – 1.753 mg formazan/g s.u. and 3.159 – 3.238 mg formazan/g s.u.

After administration of the residues in the soil, the values of dehydrogenase activity in the soil increased the plots V1 and V2, appearance which was maintained between April and September 2016.

One year after the administration of the vegetal residue into the soil, the actual and potential dehydrogenase activity values in plot V2 were higher compared to the mean values determined in parcels M and V1 by 50% for current dehydrogenase activity and by 35% for potential dehydrogenase activity.

CONCLUSIONS

1. The test vegetal residue has favored the development of microorganism populations, an effect mainly observed in the soil in experimental plot V2 where the highest amount of vegetal residue (3.0 kg / m2) was administered, which led to a larger number of ecophysiological microorganisms compared to the soil in the plot of V1.

2. Dehydrogenase activity has been shown to be a sensitive indicator of global microbiological activity. Higher values of current and potential dehydrogenic activity in V1 and V2 plots support the non-polluting effect of the test vegetal residue, which did not inhibit the development of soil eco-physiological groups involved in the nitrogen and carbon circuit.

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