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BIOCHEMICAL CHANGES IN RED RASPBERRY *(Rubus idaeus* L.*)* DUE TO FOLIAR FERTILIZATION USING BIOFERTILIZER OBTAINED FROM HUMIC ACIDS AND FROM POLYPHENOLIC EXTRACT OF *Vitis vinifera*

DUMITRU Mihaela Gabriela, GANESCU Anca
University of Craiova, Faculty of Science, Department of Chemistry, România

Abstract. *Raspberry fruit can be considered as a potential product rich in bioactive compounds. This study examines the effects of the foliar fertilizer based on humic acids extracted from lignite and polyphenolic extract from the seeds of Vitis vinifera on some biochemical parameters of raspberry fruit (Rubus idaeus L.). Foliar treatment with biofertilizer was applied at the beginning of blooming process and every 10 days thereafter until ripening. Fertilizer concentration was about 50 mL L^{-1}. We determined biochemical parameters of the fruit (total polyphenols, ascorbic acid, and antioxidant activity). Fertilized samples recorded significantly higher values than the unfertilized samples.*
Key word: *Rubus idaeus* L, humic acids, polyphenols *Vitis vinifera*.

MODIFICĂRI BIOCHIMICE DATORATE FERTILIZĂRII FOLIARE CU BIOFERTILIZANT OBŢINUT DIN ACIZI HUMICI ŞI EXTRACT POLIFENOLIC DIN SEMINŢE DE *Vitis vinifera* LA ZMEURA ROŞIE (*Rubus idaeus* L.)

Rezumat. *Zmeura poate fi considerată un produs potențial bogat în compuși bioactivi. Acest studiu examinează efectele îngrășământului foliar bazat pe acizii humici extrași din lignit și extractul polifenolic din semințele de Vitis vinifera asupra unor parametri biochimici și fiziologici ai zmeurii (Rubus idaeus L.). Tratamentul foliar cu biofertilizator a fost aplicat la începutul procesului de înflorire și la fiecare 10 zile după aceea până la coacere. Concentrația fertilizantului a fost de 50 mL L^{-1}. S-au determinat parametrii biochimici ai fructului (polifenolii totali, acidul ascorbic și activitate antioxidantă). Probele fertilizate au înregistrat valori net superioare față de probele nefertilizate.*
Cuvinte cheie: *Rubus idaeus L, acizi humici, polifenoli Vitis vinífera.*

Introduction

Getting the fertilizer based on humic acids extracted from lignite and polyphenolic extract from the seeds of Vitis vinifera presents in premiere the use of these components in soil fertilization. It can be applied to the soil and extraroot when it is needed a rapid correction of nutritional deficiency especially in the reproductive organs formation stage, between flowering and fruiting, when there is a high consumption of nutrients and the plants can not feed from the soil. The combination of the principles of the classes of polyphenolic substances (gallic acid, monomers of flavan 3-ol:

catechin, epicatechin, gallocatechin, epicatechin 3-O gallate, dimers, trimers and polymers of proanthocyanidins have an antioxidant activity 20 times higher than vitamin C and by 50 times stronger than vitamin E [1; 2] with humic acids extracted from lignite provides a well-defined multitargeted effect of the biofertilizer (increasing the antioxidant activity of the plants, adjusting the water content, temperature, controls the enzyme activity, reduce the activity of heavy metals in plant etc.) [3-6].

Experimental part

To make the experimental part were used fruits of raspberry (*Rubus idaeus* L.). Samples of raspberry were harvested in Dolj county at the commercial maturity stage. After harvest, the samples were immediately stored at -20^{o}C until analysis.

For determinations were made two experimental variants:

- A variant consists of unfertilized red raspberries;
- A variant consists of fertilized red raspberries.

Foliar treatment with biofertilizer was applied at the beginning of blooming process and every 10 days thereafter until ripening. Fertilizer concentration was about 50 mL L^{-1}.

Determinations on the fruits

Extraction

Five grams of pooled raspberry samples were homogenized with 5 mL of a mixture of ethanol, distilled water, and HCl (70:30:1, v/v/v). The homogenate was then centrifuged at 14 000 rpm for 20 min at 4°C. The extraction was repeated twice with three replications. The supernatants were combined, and the final volume was increased to 20 mL with extraction solution [7].

Determination of total polyphenol (TP)

The total polyphenol content was measured using the Folin Ciocalteu colorimetric method. The results were expressed as mg Gallic acid equivalents (GAE) g^{-1} of raspberry extracts using a standard curve generated with 50 µg, 100 µg, 300 µg and 500 µg Gallic acid per 100 mL. [8]

Determination of ascorbic acid

Ascorbic acid was quantitatively determined according to 2,6-dichlorophenol-indophenol dye method Results were expressed as mg 100 g^{-1} on fresh weight (fw) basis [9; 10].

DPPH radical scavenging activity

The DPPH radical scavenging activity (SA) of berry fruits was determined spectrophotometrically using the DPPH method. The capability to scavenge the DPPH radicals (DPPH radical scavenging activity) was calculated using the following equation [11]:

$$SA\ (\%) = 100 \times (A_{blank} - A_{sample})/A_{blank}$$

Where: A_{blank} is the absorbance of the blank, and A_{sample} is the absorbance of the sample.

Results and discussion

The results for total polyphenols, ascorbic acid and antioxidant activity obtained in this research are shown in Table 1.

Table 1. Total polyphenols, ascorbic acid and antioxidant activity of red raspberry

Parameter	Samples	
	Unfertilized red raspberries	Fertilized red raspberries
Total polyphenols, mg GAE $100g^{-1}$ FW	369.4	1022.5
Ascorbic acid, mg $100g^{-1}$ FW	26.1	60.0
Antioxidant *activity (DPPH), %*	55.2	91.2

From the results shown in Table 1 it can seen fertilizer role on the main composition parameters of raspberry fruits.

Total polyphenols

Polyphenols are phytochemicals found in plants which provide beneficial functions when consumed by humans and animals also. They are classified into several different categories depending upon their structures and vary from simple phenolic acids (hydroxybenzoic and hydroxycinnamic acids) to complex polyphenols (hydrolysable and condensed tannins) [12-14]. The color and flavor of fruits and vegetables are partly attributed to their phytochemical/polyphenolic components [14; 15].

By fertilization with humic acids we obtain an increase in polyphenols concentration; from of 364.9 mg GAE 100 g^{-1} FW in the sample nonfertilized to 1022.5 mg GAE $100g^{-1}$ FW in the sample fertilized.

This can be explained by the role of polyphenols from the fertiliser obtained from the seeds of Vitis vinifera that increase concentration of polyphenols in the plant cell.

Similar results have been reported by others researchers [16-19].

Ascorbic acid

L-Ascorbic acid is the major antioxidant found in many plants and fruits. Ascorbic acid is an important nutrient used as an antioxidant in beverages, food products and in pharmaceutics products with a role in human metabolic reactions [20]. Treatment contained humic acids caused greater levels of ascorbic acid (60.0 mg 100 g^{-1} FW comparing to unfertilized samples (26.1 mg 100 g^{-1} FW).

Results reveal that treatment had significant effect on ascorbic acid content and that raspberries are a good source of vitamin C (100 g of raspberries may provide up to 50% of the recommended daily allowance of vitamin C).

Similar results have been reported by other researchers to plants fertilized [21; 22].

Antioxidant activity (DPPH)

Evaluation of the antioxidant activity of any compound can be carried by in model DPPH. DPPH is a free stable radical being able to accept a hydrogen or an electron radical to become a stable diamagnetic molecule. The potential antioxidant activity of raspberry might be described to its high total polyphenols and ascorbic acid. The strongest radical scavenging activity is displayed by fruit of raspbery fertilized (91.2%) compared to unfertilized raspberry fruits (52%). Similar results have been reported by Kostecka-Gugała; Radovanovic [19; 23].

Conclusions

Foliar spraying treatments using from humic acid and extract from seed *Vitis vinifera* were associated significant effect on biochemical characters in raspberry and induced the highest values of total polyphenols, ascorbic acid and antioxidant activity (DPPH) (%) compared to blank.

It can be concluded from the study that the foliar treatment used in experiment (humic acids and polyphenolic extract of *Vitis vinifera* seeds), gave superior quality of raspberry fruit nutrient. The extract obtained from the seeds of *Vitis vinifera*, contain polyphenols which are responsable to protect chemical compounds against oxidation by increasing the antioxidant activity. In view of the better nutrition, raspberry fruit quality and improved nutrient use efficiency imparted by the humic acids and

polyphenolic extract of *Vitis vinifera* seeds based on fertilizer, might be considered for promotion among raspberry crop.

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