

## **Post-harvest nutritional and antioxidant profile of *Beta vulgaris* L. grown in low emission soilless microgarden system with organic and inorganic nutriments**

### **Perfil nutricional y antioxidante post-cosecha de *Beta vulgaris* L. cultivada en microhuerto sin suelo de baja emisión con nutrientes orgánicos e inorgánicos**

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#### **ABSTRACT**

Beetroot was grown in an open soilless cultivation system with nutrition supplied by organic and inorganic sources. This low emission system was tested for microgardening high quality red beets with high water use efficiency and less pollutant emission in the environs. For this purpose, a pot experiment was planned according to completely randomized design. For inorganically grown red beets, peat moss was combined with 150, 200 and 250 ppm  $\text{NH}_4\text{NO}_3$ , whereas for organic red beets, peat moss was amended with compost having nitrogen equivalent to the mentioned  $\text{NH}_4\text{NO}_3$  concentrations. Rosette and roots were analysed for fresh and dry biomass. Nitrate content, total soluble solids, titratable acidity, ripening index, ascorbic acid, betacyanins, flavonols and antioxidant capacity were assessed as beetroot quality attributes. Combination of peat moss with  $\text{NH}_4\text{NO}_3$  showed comparatively lower fresh plant biomass, fresh and dry biomasses of rosette and root, and root to rosette ratio. However, enhanced antioxidant activity and bioaccumulation of ascorbic acid, total soluble solids, betacyanins, flavonols and reduced titratable acids, resulting in higher ripening index and good quality were observed in peat moss combined  $\text{NH}_4\text{NO}_3$  treated beetroots. Overall, combination of peat moss with  $\text{NH}_4\text{NO}_3$  led to higher nutritional and antioxidant quality of red beet plants.

#### **Keywords**

beetroot • *Beta vulgaris* • compost • organic growing medium • red beet • soilless culture • substrate • sphagnum peat moss • organic agriculture

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## RESUMEN

La remolacha se cultivó en un sistema de cultivo abierto sin suelo con nutrición suministrada por fuentes orgánicas e inorgánicas. Este sistema de baja emisión fue probado para la microhorticultura de remolachas rojas de alta calidad con una eficiencia alta de uso de agua y menor emisión de contaminantes a los alrededores. Con esta finalidad se planificó un experimento en macetas de acuerdo con un diseño completamente al azar. Para las remolachas rojas cultivadas inorgánicamente se combinó musgo de turbera con 150, 200 y 250 ppm de  $\text{NH}_4\text{NO}_3$ , mientras que para las orgánicas se adecuó el musgo de turbera con compost con un contenido de nitrógeno equivalente a las concentraciones de  $\text{NH}_4\text{NO}_3$  mencionadas. Se analizó el contenido de biomasa fresca y seca de la roseta y de la raíz. El contenido de nitratos, los sólidos solubles totales, la acidez titulable, el índice de maduración, el ácido ascórbico, las betacianinas, los flavonoles y la capacidad antioxidante se evaluaron como atributos de calidad de la remolacha. La combinación de musgo de turbera con  $\text{NH}_4\text{NO}_3$  mostró valores comparativamente más bajos de biomasa fresca de planta, de biomasa fresca y seca de roseta y raíz, y de relación raíz-roseta. Sin embargo, también se observaron valores más altos de actividad antioxidante y bioacumulación de ácido ascórbico, sólidos solubles totales, betacianinas, flavonoles y menos ácidos titulables, lo que resultó en un mayor índice de maduración y buena calidad. En general, la combinación de musgo de turbera con  $\text{NH}_4\text{NO}_3$  condujo a una mayor calidad nutricional y antioxidante de las plantas de remolacha roja.

### Palabras clave

remolacha • *Beta vulgaris* • compost • medio de cultivo orgánico • remolacha roja • cultivo sin suelo • sustrato • musgo de turbera sphagnum • agricultura orgánica

## INTRODUCTION

Owing to the extensive use of synthetic chemicals in crop production, soil and underground water can get contaminated to sub-standard levels that might not be suitable for livestock and human consumption (1, 15). For example, drinking water high in nitrate content may cause methaemoglobinaemia (impairment of oxygen delivery to tissue) in infants and could be a possible cause of stomach cancer (16). Therefore, strict usage regulations aiming at the reduction of emissions of nutrients and pesticides to soil and groundwater have been introduced in Europe and many developed countries (23).

To overcome pollution problem in agriculture, soilless cultivation not only provides control over water and nutrient use efficiency but also prevents chemicals and pathogen transmitting from growing system to soil or backward (22). It is an innovative production system that is extensively used in protected horticulture. For soilless production, interest is increasing worldwide generally for private growing and particularly in urban areas where people do not have access to agriculture land (26). In urban areas, exploitation of small areas such as balconies, roofs, backyards, public areas and small gardens to grow herbs,

vegetables and flowers allows self-supply to some extent and significantly improve the nutritional quality of food. Such cultivation is often performed at sub-optimal climatic conditions and requires a particularly specialized and optimized management of nutrients when no natural soil of sufficient quality is available. This requires carefully composed substrates or combinations of substrates with continuous provision of nutrient solutions (21). The aim is to provide simple, low-budget and easy to manage solutions for soilless culture technology adapted for the resources-poor urban families. This technology is known as "microgarden systems" (6).

The major ingredients in soilless production or container culture include compost, sphagnum peat moss, perlite, and vermiculite. Among these, compost and sphagnum peat moss are extensively used in organic and soilless farming equally. Rhizosphere ecology such as population shift among microorganism species is largely dependent on organic matter in the growing medium (19). Compost improves soil tilth, provides a blend of nutrients and affects growth and yield of vegetables (4, 8). It prevents some of the diseases by stimulating beneficial microbial growth (29). Similarly, sphagnum peat moss is a recognized growing medium for nursery plants and greenhouse cultivation with high water holding capacity, cation exchange capacity and capillary porosity. These characteristics hugely contribute to the plant growth and make it an excellent growing medium (29). Hence, soilless cultivation could be utilized in microgardening to grow nutritious and chemical free vegetables such as beetroot.

Beetroot (*Beta vulgaris* L.), commonly referred as red beet, is a good source of fibre, mineral components, folic acid,

phenolic compounds and betacyanin pigments. Dietary nitrate administered in the form of red beet juice decreases resting systolic blood pressure and O<sub>2</sub> consumption during walking and exercises (13). The red beet juice delivers a high amount of bio-accessible antioxidants and may be a cost effective and convenient method of increasing antioxidant status (27). However, beneficial effects of red beet could be compromised if grown with unmanaged irrigation and nutrients systems.

The objective of the study was to set a trial for soilless production in containers, to develop a nutrient system consisting of various combinations of organic and inorganic nutrients, which is independent from the continuous supply of nutrient solutions and to measure its efficiency in the form of production and quality of root vegetable red beet.

## MATERIALS AND METHODS

### Development of low emission microgarden system and experimental plan

The study was carried out at the University of Natural Resources and Life Sciences, Vienna, Austria. A low emission soilless microgarden system based on container cultivation was developed for growing red beets at polyhouse in Universität für Bodenkultur, Vienna. Seeds of red beet cv. Rocket purchased from Austrosaat Österreichische Samenzucht-und Handels AG, Austria were sown directly in each pot and after germination thinned out to three plants per pot. Pots with capacity of 7.5 litres and diameter of 30 cm were used for this purpose. The basic growing medium in pots was sphagnum peat

moss (SPM) obtained from Floragard Vertiebs GmbH, Germany. However, either ammonium nitrate (33% N) or compost was supplemented as organic and inorganic N nutrients, respectively. For this study, compost was prepared at the Vegetable Research Area of University of Natural Resources and Life Sciences, Vienna. Compost material consisted mainly of residues from the harvesting of different vegetable and its maturity was determined through seed germination test. The general characteristics of the peat moss and compost are presented in table 1. Completely randomized design was used with seven nutrient combinations that were replicated four times. Peat moss was used as the only growing medium in pots supplemented with  $\text{NH}_4\text{NO}_3$ . There were three levels of N including 150, 200 and 250 ppm. A 500 mL solution of each N level was fertigated twice a week to minimize N leaching and maintain nutrients availability in root zone of red beets. The application of

compost was proportionate to N equivalent of  $\text{NH}_4\text{NO}_3$  concentrations.

The combinations of peat moss, compost and  $\text{NH}_4\text{NO}_3$  and their labels (to be used hereafter) are presented in Table 2. All plants were fertigated with P and K after 21 days of sowing, whereas selected plants were fertigated with  $\text{NH}_4\text{NO}_3$ . Roots of red beet were harvested at edible physiological maturity and analysed for their consumable quality.

### Preparation of beetroot extracts

Beetroot extracts were derived from fresh roots by using a blender. The extract was differentially centrifuged for 5 minutes at 1000 x g and the supernatant was clarified by using 10-20  $\mu\text{m}$  cellulose filter papers (VWR, France). The extract was used for the determination of nitrate content, total soluble solids (TSS), titratable acidity, ripening index, potential acidity (pH), electrical conductivity (EC), redox potential (Eh) and ascorbic acid in the beetroots.

**Table 1.** Characteristics of Sphagnum peat moss and compost.

**Tabla 1.** Características del musgo de turbera Sphagnum y del compost.

	Sphagnum peat moss	Compost
pH	5.5 to 6.0	7.5 to 7.75
Electrical conductivity	0.2 to 0.5 $\text{dS m}^{-1}$	0.5 to 0.6 $\text{dS m}^{-1}$
Redox potential	260 to 265 mV	190 to 195 mV
Nutrients	NPK fertilizer (18-10-20) $0.8 \text{ kg m}^{-3}$	On dry weight basis: Carbon to nitrogen ratio 17:1; $\text{P}_2\text{O}_5$ 0.8%; $\text{K}_2\text{O}$ 0.7%

**Table 2.** Treatment combinations and their labels.

**Tabla 2.** Combinaciones de tratamiento y sus etiquetas.

Treatments	Combination	Labels
T1	Sphagnum peat moss (100%)	SPM
T2	Peat moss + $\text{NH}_4\text{NO}_3$ (1%)	AmN1
T3	Peat moss + $\text{NH}_4\text{NO}_3$ (2%)	AmN2
T4	Peat moss + $\text{NH}_4\text{NO}_3$ (3%)	AmN3
T5	Peat moss (75%) + compost (25%)	Comp25
T6	Peat moss (50%) + compost (50%)	Comp50
T7	Peat moss (25%) + compost (75%)	Comp75

### Physical quality attributes

Fresh biomasses (Fb) of plant, rosette and roots were recorded immediately after harvesting. Root to rosette ratio was determined on fresh weight basis. For dry biomass determination, rosette and roots were dried for 72 hours at 80 °C in an oven. Dry matter ( $\text{mg g}^{-1} \text{F}_b$ ) and moisture content of rosette and roots were also measured.

### Determination of nitrate content, total soluble solids, titratable acidity, ripening index, potential acidity, electrical conductivity, redox potential and ascorbic acid

Analysis of nitrate was performed using the remission photometry method (20) through digital reflectometer Reflec-toquant RQflex plus (Merck, Germany) and the results were expressed as  $\mu\text{g}$  nitrate per mL of beetroot extract. TSS content was analyzed with a digital refractometer PT-101 (Atago, Japan) and presented as °Brix value. The determination of titratable acidity was carried out as determined by Hortwitz (1960). All determinations were made in triplicate and results were expressed as mg citric acid per 100 mL of beetroot extract. Ripening index was determined by taking the ratio of TSS to titratable acidity. Ascorbic acid was determined as described by Ruck (1961) and expressed as mg ascorbic acid per 100 mL of beetroot extract. For this purpose, indophenol's titration technique was used. 0.4% oxalic acid solution was added to beet extract and filtered through Whatman No. 2 filter paper. Afterwards, aliquot from this filtrate was titrated against 2, 6- dichlorophenolindophenol dye. Titration dye was added until the light pink color appeared for at least 15 seconds. Electrical conductivity, pH and redox potential were simultaneously measured by a system of electrodes provided by WTW GmbH, Germany.

### Determination of total flavonols and betacyanins

0.5 g of homogenized beetroot material was transferred to a test tube containing 2% (v/v) HCl/methanol solution and stored for 48 hours at 4 °C. Subsequently, the extract was separated from tissue debris and centrifuged at 19,000 x g for 3 minutes. For betacyanin determination, 140  $\mu\text{L}$  of supernatant was added to 860  $\mu\text{L}$  HCl/methanol solution and absorbance was recorded at 538 nm by using spectrophotometer. For flavonols, semi-quantitative estimation was performed at 360 nm by using spectrophotometer. Results were expressed in as quercetin-equivalents for flavonols, whereas betacyanins content (BC) was calculated with a slight modification to the method determined by Cai *et al.* (1998).

$$\text{Betacyanins}_{(\text{mg}/100\text{g Fb})} = \frac{A_{538} (MW) V_a (DF) \times 100}{\epsilon L W_a}$$

$A_{520}$  is the absorption value at the absorption maximum of 538 nm for betacyanins, DF is the dilution factor,  $V_a$  is the total extract volume (mL),  $W_a$  is the fresh weight of extracting material (g), and L is the path-length (1 cm) of the cuvette. For quantification of betacyanins, the molecular weight (MW) and molar extinction coefficient ( $\epsilon$ ) of betanin [MW = 550 g/mol;  $\epsilon$  = 60000 L/mol.cm in  $\text{H}_2\text{O}$ ] were applied.

### Determination of trolox equivalent antioxidant capacity (TEAC)

0.25 g milled beetroot sample and 0.25 g quartz sand were homogenized in a mortar in 3 mL methanol and the homogenate was centrifuged for 10 min at 10,000 g and 4°C. The supernatant was used for 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

The TEAC of methanolic beetroot extracts was determined according to the DPPH method (2) with slight modifications. 0.1 mM DPPH solution in MeOH was

added to methanolic beetroot extracts and the absorbance was recorded against methanol as a blank at 517 nm after 30 min by using spectrophotometer. Trolox was used as reference compound and TEAC values were calculated by using the standard regression curve.

EC<sub>50</sub> values of Trolox and beetroot samples were calculated. EC<sub>50</sub> values of plant samples were compared with reference to Trolox and the results were expressed in µM TE/g root fresh biomass.

### Statistical analysis

Data analyses were carried out by using analysis of variance (ANOVA) technique after the data were tested for normality and the homogeneity of variance. General Linear Model (GLM) procedure of IBM SPSS (version 19) software was used for the test of main effects, whereas post hoc analysis was performed by Duncan's Multiple Range (DMR) test at  $\alpha = 5\%$ . Furthermore, the correlations between the various quality attributes were explored using Pearson's Coefficient of Correlation.

## RESULTS

Treatments significantly influenced fresh plant biomass, whereby Comp75 produced the highest fresh plant biomass, followed by Comp50, Comp25 and SPM, whereas AmN3 and AmN2 produced the lowest fresh plant biomass (table 3). Fresh plant biomass increased with the increase in the level of compost and decreased with the increase in the level of NH<sub>4</sub>NO<sub>3</sub>. Higher fresh and dry rosette biomasses were produced generally by compost nutrition and specifically by Comp75. SPM resulted in the least fresh and dry foliage biomass production. However, on dry matter (mg g<sup>-1</sup> rosette fresh biomass) basis, Comp75 produced the least and SPM produced the highest dry matter. Therefore, moisture content was highest in Comp75 and lowest in SPM grown plants.

Compost also produced more fresh and dry root biomass than other treatments (table 4, page 25). Among all nutritional variants, plants grown in Comp75 produced the maximum fresh and dry root biomass that was more than twice the minimum biomass produced by AmN3 fertilized plants.

**Table 3.** The effects of organic and inorganic source of nutrients on physical attributes of red beet.

**Tabla 3.** Efectos de la fuente orgánica e inorgánica de nutrientes sobre los atributos físicos de la remolacha roja.

Treatments	Fresh plant biomass (g)	Fresh rosette biomass (g)	Dry rosette biomass (g)	Rosette dry matter (mg g <sup>-1</sup> plant Fb <sup>†</sup> )	Rosette moisture content (%)
SPM	232.1 ± 1.4 <sup>d</sup>	72.8 ± 1.6 <sup>c</sup>	6.9 ± 0.2 <sup>d</sup>	94.4 ± 1.2 <sup>a</sup>	90.6 ± 0.1 <sup>d</sup>
AmN1	217.4 ± 2.3 <sup>e</sup>	81.4 ± 1.9 <sup>c</sup>	7.3 ± 0.3 <sup>cd</sup>	88.9 ± 1.6 <sup>bc</sup>	91.1 ± 0.2 <sup>bc</sup>
AmN2	205.4 ± 1.6 <sup>f</sup>	90.9 ± 2.0 <sup>b</sup>	7.7 ± 0.2 <sup>bc</sup>	85.3 ± 0.6 <sup>cd</sup>	91.5 ± 0.1 <sup>ab</sup>
AmN3	203.7 ± 1.6 <sup>f</sup>	90.6 ± 2.6 <sup>b</sup>	8.1 ± 0.2 <sup>b</sup>	89.4 ± 0.9 <sup>abc</sup>	91.1 ± 0.1 <sup>bcd</sup>
Comp25	260.5 ± 4.2 <sup>c</sup>	92.7 ± 4.0 <sup>b</sup>	8.4 ± 0.2 <sup>b</sup>	90.9 ± 1.5 <sup>ab</sup>	90.9 ± 0.1 <sup>cd</sup>
Comp50	292.9 ± 3.4 <sup>b</sup>	95.1 ± 4.8 <sup>b</sup>	8.3 ± 0.1 <sup>b</sup>	87.8 ± 3.4 <sup>bc</sup>	91.2 ± 0.3 <sup>bc</sup>
Comp75	359.1 ± 1.4 <sup>a</sup>	113.6 ± 2.6 <sup>a</sup>	9.1 ± 0.2 <sup>a</sup>	80.3 ± 1.2 <sup>d</sup>	92.0 ± 0.1 <sup>a</sup>
Probability	***	***	***	***	***

<sup>†</sup>= Fresh biomass; values connected by the same letter within a column are statistically similar by DMR test at  $\alpha = 0.05$ ; \*\*\* =  $p < 0.001$ ; (Mean ± SE, n = 4).

<sup>†</sup> = Biomasa fresca; los valores conectados por la misma letra dentro de una columna son estadísticamente similares por la prueba de DMR en  $\alpha = 0,05$  - \*\*\* =  $p < 0,001$ ; (Media ± EE, n = 4).



**Table 4.** The effects of organic and inorganic source of nutrients on yield attributes of red beet.

**Tabla 4.** Efectos de la fuente orgánica e inorgánica de nutrientes sobre los atributos de rendimiento de la remolacha roja

Treatments	Fresh root biomass (g)	Dry root biomass (g)	Root dry matter (mg g <sup>-1</sup> plant Fb <sup>†</sup> )	Root moisture content (%)	Root to rosette ratio
SPM	159.4 ± 3.1 <sup>c</sup>	18.0 ± 1.3 <sup>bc</sup>	113.0 ± 8.5	88.7 ± 0.8	2.2 ± 0.1 <sup>a</sup>
AmN1	135.3 ± 2.7 <sup>d</sup>	15.3 ± 1.3 <sup>cd</sup>	113.3 ± 10.2	88.7 ± 1.0	1.7 ± 0.1 <sup>b</sup>
AmN2	114.2 ± 3.3 <sup>e</sup>	13.5 ± 0.9 <sup>d</sup>	118.7 ± 10.9	88.1 ± 1.1	1.3 ± 0.1 <sup>c</sup>
AmN3	112.5 ± 3.1 <sup>e</sup>	13.0 ± 0.7 <sup>d</sup>	116.4 ± 8.5	88.4 ± 0.8	1.3 ± 0.1 <sup>c</sup>
Comp25	167.4 ± 4.3 <sup>c</sup>	18.8 ± 1.5 <sup>bc</sup>	112.6 ± 9.0	88.7 ± 0.9	1.8 ± 0.1 <sup>b</sup>
Comp50	197.9 ± 3.4 <sup>b</sup>	21.0 ± 1.3 <sup>ab</sup>	106.1 ± 6.0	89.4 ± 0.6	2.2 ± 0.1 <sup>a</sup>
Comp75	244.9 ± 2.8 <sup>a</sup>	23.6 ± 1.2 <sup>a</sup>	96.2 ± 4.6	90.4 ± 0.5	2.2 ± 0.1 <sup>a</sup>
Probability	***	***	NS	NS	***

<sup>†</sup> = Fresh biomass; values connected by the same letter within a column are statistically similar by DMR test at  $\alpha=0.05$ ; \*\*\* =  $p<0.001$ ; NS = non-significant at  $\alpha=0.05$ ; (Mean ± SE,  $n= 4$ ).

<sup>†</sup> = Biomasa fresca; los valores conectados por la misma letra dentro de una columna son estadísticamente similares por la prueba de DMR en  $\alpha = 0,05$  - \*\*\* =  $p < 0,001$ ; NS = no significativo en  $\alpha = 0,05$ ; (Media ± EE,  $n = 4$ ).

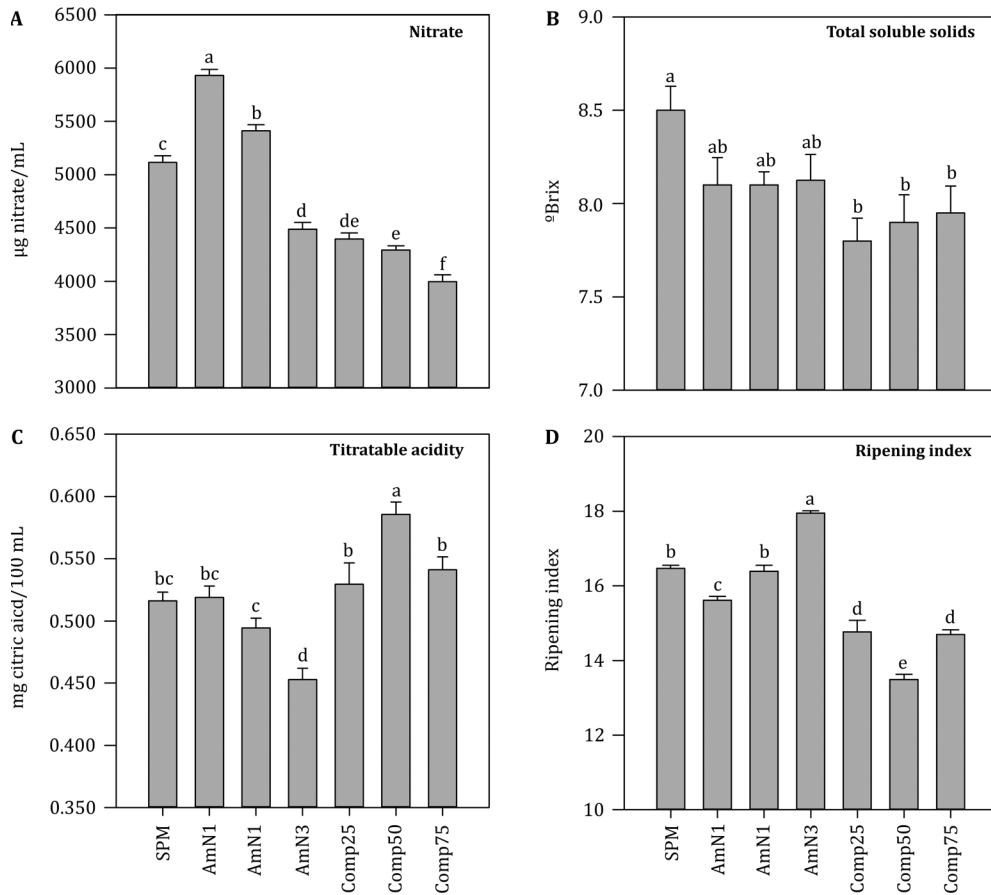
As a whole, the NH<sub>4</sub>NO<sub>3</sub> fertigated plants produced the lowest fresh and dry root biomasses. Furthermore, either increasing compost or decreasing NH<sub>4</sub>NO<sub>3</sub> both led to increase in fresh and dry root biomass. The maximum root dry matter observed in AmN2 was 23% more than the minimum dry matter produced by Comp75, but the difference was not statistically significant. Therefore, roots moisture content was highest in Comp75 and lowest in AmN2 grown plants. SPM, Comp50 and Comp75 resulted in highest root to rosette ratio (2.2), whereas NH<sub>4</sub>NO<sub>3</sub> fertigation produced the lowest ratios (1.3-1.7).

The highest nitrate content (5931.3 µg per mL) was observed in AmN1, followed by AmN2 (5412.0 µg per mL) and SPM (5114.8 µg per mL). However, increasing compost level resulted in lowering nitrate content: 4397.3 µg per mL in Comp25, 4294.8 in Comp50 and 3997.5 (the lowest) in Comp75. Generally, fertigation with NH<sub>4</sub>NO<sub>3</sub> resulted in higher nitrate contents, whereas compost amendments in lower contents. The increase in

the levels of both compost and NH<sub>4</sub>NO<sub>3</sub> decreased the nitrate content gradually (Figure 1a, page 26).

SPM grown red beet roots accumulated highest (8.50 °Brix) TSS content (Figure 1b, page 26), followed by AmN3 (8.13 °Brix), AmN2 (8.10 °Brix) and AmN1 (8.10 °Brix) grown plants. However, compost grown plants showed the lowest TSS content (7.80-7.95 °Brix). Significant effect of the nutrient sources was evident for titratable acidity (Figure 1c, page 26). Comp50 grown plants showed the highest titratable acidity (0.586%), followed by Comp75 and Comp25 (0.541 and 0.530%), whereas AmN3 produced the least acidity (0.453%). Generally, NH<sub>4</sub>NO<sub>3</sub> fertigation resulted in lower titratable acidity. Furthermore, increasing the concentration of NH<sub>4</sub>NO<sub>3</sub> led to decrease in titratable acidity.

Similarly, the ripening index was significantly affected by the nutritional variants (Figure 1d, page 26). AmN3 showed the highest index (17.95) that gradually decreased across AmN2 (16.39)



**Figure 1.** The effect of organic and inorganic source of nutrients.

**Figura 1.** Efecto de la fuente orgánica e inorgánica de nutrientes.

(A) nitrate content (B) total soluble solids, (C) titratable acidity and (D) ripening index. Different letters within a graph indicate significant differences ( $P < 0.05$ ) and error bars on the columns represent standard error.  $n=4$ .

(A) contenido de nitrato, (B) sólidos solubles totales, (C) acidez titulable y (D) índice de maduración. Letras diferentes dentro de un gráfico indican diferencias significativas ( $P < 0,05$ ) y las barras de error en las columnas representan el error estándar.  $N = 4$ .

and AmN1 (15.61), whereas the compost amendment resulted in red beets with the lowest ripening indices (13.49-14.76).  $\text{NH}_4\text{NO}_3$  fertilized plants resulted in the highest pH values (6.41-6.46) of their root extracts, followed by those treated

with SPM (6.39), whereas compost amendment resulted in the lowest pH values (6.34-6.35). Besides, EC and Eh values of various beetroot extracts were statistically similar and not influenced by the fertilization (table 5, page 27).



**Table 5.** The effects of organic and inorganic source of nutrients on electrochemical properties of red beet.

**Tabla 5.** Efectos de la fuente orgánica e inorgánica de nutrientes sobre las propiedades electroquímicas de la remolacha roja.

Treatments	Potential acidity (pH)	Electrical conductivity (mS)	Redox potential (mV)
SPM	6.39 ± 0.018 <sup>bc</sup>	12.49 ± 0.10	309.30 ± 4.57
AmN1	6.42 ± 0.018 <sup>ab</sup>	12.42 ± 0.04	318.97 ± 13.68
AmN2	6.41 ± 0.018 <sup>ab</sup>	12.31 ± 0.30	323.24 ± 6.02
AmN3	6.46 ± 0.015 <sup>a</sup>	12.61 ± 0.03	334.24 ± 9.20
Comp25	6.34 ± 0.024 <sup>c</sup>	12.95 ± 0.12	321.32 ± 9.13
Comp50	6.34 ± 0.019 <sup>c</sup>	12.56 ± 0.22	324.85 ± 3.14
Comp75	6.35 ± 0.016 <sup>c</sup>	12.25 ± 0.11	339.98 ± 7.42
Probability	***	NS	NS

Values connected by the same letter within a column are statistically similar by DMR test at  $\alpha=0.05$ ; NS = non-significant at  $\alpha=0.05$ ; (Mean ± SE,  $n=4$ ).

Los valores conectados por la misma letra dentro de una columna son estadísticamente similares por la prueba de DMR en  $\alpha = 0,05$ ; NS = no significativo en  $\alpha = 0,05$ ; (Media ± EE,  $n = 4$ ).

Significant variation was observed for ascorbic acid in beetroot extracts in response to the nutrients (Figure 2a, page 28). SPM grown plants accumulated the highest ascorbic acid content (148.635 mg AA per 100 mL) in their roots, followed by AmN1, AmN2 and AmN3 (130.2, 123.0 and 121.0 mg AA per 100 mL, respectively), whereas Comp50 and Comp75 grown beetroot accumulated the lowest content (103.53 and 101.48 mg AA per 100 mL, respectively).

Moreover, a decreasing trend for ascorbic acid with the increase in the levels of both compost and  $\text{NH}_4\text{NO}_3$  was observed.

AmN1 produced the highest betacyanin content (51.84 mg/100 g  $F_b$ ) in beetroots, followed by AmN2 (49.31 mg/100 g  $F_b$ ), SPM (45.88 mg/100 g  $F_b$ ) and AmN3 (41.25 mg/100 g  $F_b$ ) treatments, respectively (Figure 2B, page 28).

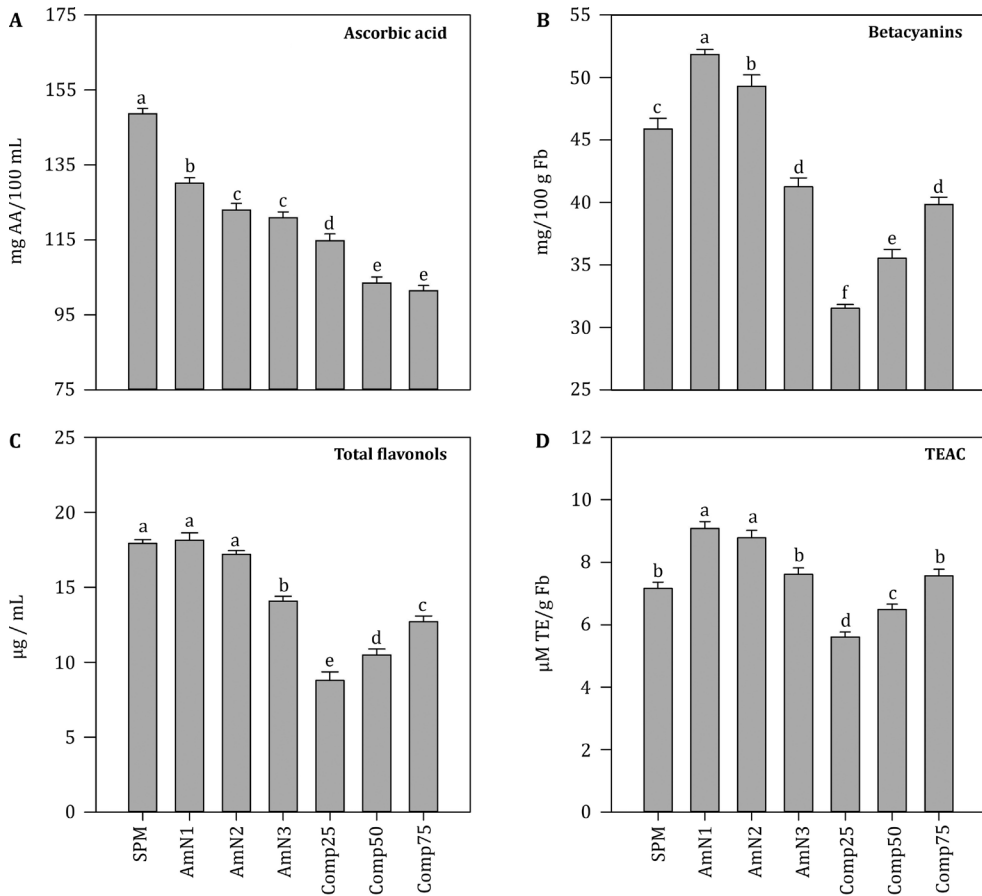
Plants grown in compost amendment accumulated the lowest betacyanin content (31.54-39.84 mg/100 g  $F_b$ ), the lowest being in Comp25. Moreover, the plants grown with the lowest level of  $\text{NH}_4\text{NO}_3$  (AmN1) had 65% higher betacyanins than those grown with the lowest level of compost (Comp25).

A significant increase in betacyanins was observed if either the level of compost increased or the concentration of  $\text{NH}_4\text{NO}_3$  decreased.

Figure 2c (page 28) shows that the highest content of total flavonols (18.14  $\mu\text{g}$  per mL) was observed in the AmN1 grown plants, which was statistically equal to the amount in beetroots grown in SPM (17.92  $\mu\text{g}$  per mL) and AmN2 (17.19  $\mu\text{g}$  per mL) nutrients. Composted plants contained the least concentration of flavonols in their root extracts that was up to 106% less than that of  $\text{NH}_4\text{NO}_3$  fertigated plants and up to 104% less than SPM grown beetroots.

Similar to betacyanins, a significantly increasing trend for flavonols with the increasing compost or decreasing  $\text{NH}_4\text{NO}_3$  was observed.

As a whole,  $\text{NH}_4\text{NO}_3$  fertigated plants showed higher TEAC values compared to SPM or compost grown plants (Figure 2d, page 28). AmN1 and AmN2 resulted in the highest TEAC (9.08 and 8.78  $\mu\text{M TE/g F}_b$ , respectively), whereas Comp25 the minimum TEAC (5.60  $\mu\text{M TE/g F}_b$ ).



**Figure 2.** The effect of organic and inorganic source of nutrients.

**Figura 2.** Efecto de la fuente orgánica e inorgánica de nutrientes.

(A) ascorbic acid, (B) betacyanins, (C) total flavonols and (D) antioxidant activity. Different letters within a graph indicate significant differences ( $P < 0.05$ ) and error bars on the columns represent standard error:  $n=4$ .

(A) ácido ascórbico, (B) betacianinas, (C) flavonoles totales y (D) actividad antioxidante. Letras diferentes dentro de un gráfico indican diferencias significativas ( $P < 0,05$ ) y las barras de error en las columnas representan el error estándar.  $N = 4$ .

Owing to the betacyanins and flavonols, significant increase in TEAC was found with the increase in compost or decrease in  $\text{NH}_4\text{NO}_3$  levels.

The statistical correlations between physical and biochemical quality attributes revealed that various quality parameters have linkage with each

other (table 6, page XXX). Fresh plant biomass had negative correlations that were medium in strength with nitrate, ascorbic acid, ripening index and pH, whereas weak in strength with betacyanins, flavonols and TEAC. A positive and medium correlation of fresh plant biomass with titratable acidity was also observed.

**Table 6.** Pearson's Coefficient of correlations between various yield and physiological quality attributes of red beets in response to organic and inorganic sources of nutrients.

**Tabla 6.** Coeficiente de Pearson de correlaciones entre el rendimiento y los atributos de calidad fisiológica de la remolacha roja en respuesta a fuentes orgánicas e inorgánicas de nutrientes.

	Fresh plant biomass	Fresh rosette biomass	Dry rosette biomass	Fresh root biomass	Dry root biomass	Root to rosette ratio	Nitrate content	Total soluble solids	Titrateable acidity	pH	Ascorbic acid	Total flavonols	Beta-cyanins
Nitrate content	-.70**	-.59**	-.66**	-.67**	-.56**	-.39*	1						
Total soluble solids	NS	-.66**	-.75**	NS	NS	NS	NS	1					
Titrateable acidity	.61**	NS	NS	.70**	.69**	.77**	NS	NS	1				
Ripening index	-.70**	NS	NS	-.73**	-.70**	-.61**	NS	.39*	-.91**				
pH	-.63**	NS	NS	-.67**	-.60**	-.64**	.43*	NS	-.69**	1			
Ascorbic acid	-.68**	-.77**	-.76**	-.58**	-.45*	NS	.70**	.52**	-.43*	.45*	1		
Total flavonols	-.52**	-.44*	-.61**	-.48**	-.46*	NS	.83**	.44*	NS	.52**	.58**	1	
Betacyanins	-.55**	-.53**	-.66**	-.49**	-.51**	NS	.79**	.55**	-.39*	.50**	.73**	.93**	1
TEAC	-.38*	NS	NS	-.39*	-.47*	-.39*	.67**	NS	NS	.47*	NS	.89**	.78**

\* = p<0.05; \*\* = p<0.01; NS = non-significant at  $\alpha=0.05$

\* = P & lt; 0,05; \*\* = p & lt; 0,01; NS = no significativo en  $\alpha = 0,05$

Further, negative correlations of fresh and dry rosette biomass with nitrate, TSS, ascorbic acid, betacyanins and flavonols were also evident.

However, fresh and dry root biomass were found to be negatively correlated with nitrate, titratable acidity, ripening index, pH, ascorbic acid, betacyanins, flavonols and TEAC, and positively correlated with titratable acidity. Although nitrate was found to be negatively correlated with the fresh and dry biomass parameters, it was positively linked with pH, ascorbic acid, betacyanins, flavonols and TEAC.

Similarly, TSS had a positive correlation with ascorbic acid, betacyanins and flavonols, whereas titratable acidity had a negative correlation with ascorbic acid and betacyanins. Also, pH was positively related to ascorbic acid, betacyanins, flavonols and TEAC. Positive correlations were observed between ascorbic acid, betacyanins and flavonols. Consequently, betacyanins and flavonols were positively and strongly linked to TEAC (table 6, page 29).

## DISCUSSION

Fresh and dry biomasses of rosette and root were maximum in compost treated plants because roots contribute most of the plant fresh biomass in beetroots. On the other hand, N application increases vegetative growth, that could lead to a higher foliage growth at the expense of root growth and, therefore, to a less total plant biomass. This also caused root to rosette ratio to increase with increasing compost level and decreasing  $\text{NH}_4\text{NO}_3$  level. Generally, crops grown from organic nutrient sources have higher fresh and dry weight contents (28).

Increase in the yield or plant biomass could be in response to improvement in soil physical, chemical and biological properties due to the amendment of peat moss and compost (5, 17).

Biomass partitioning (as measured by root:rosette ratio) indicated that not only above ground parts but also roots of compost treated beets grew better than those of  $\text{NH}_4\text{NO}_3$  fertilized beets.

The same was also obvious in fresh and dry rosette and root biomasses. Generally, besides providing nutrients, compost provides additional benefits such as soil tilth improvement, a balanced blend of other nutrients needed by plants (8) and organic compounds that act as bio-stimulant agent, all these creating conducive conditions for plant growth.

Different parts of a plant accumulate nitrate differently and generally, the parts in decreasing order by nitrate content are petiole, leaf, stem, root, inflorescence, tuber, bulb, fruit and seed (18). Low nitrate accumulation in the roots of compost treated plants is the result of organically applied nutrients. Applying compost not only provides nutrients to the plant but also improves soil structure and adds organic matter necessary for the growth of microorganisms.

However, this reduces the availability of nutrient over time. Low nitrate concentration was accompanied with high fresh and dry root biomass and vice versa. A possible explanation is the reduction of nitrate and its utilization in plant physiological processes. This result is in line with the findings of Ugrinović *et al.* (2012) where decrease of dry biomass in beetroot was observed with the increase of N application. Similar trends in carrots were reported by John *et al.* (2003). Further, increase in soil EC with increasing the  $\text{NH}_4\text{NO}_3$  level could have lowered the nitrate accumulation in roots.

However, contrary to fresh and dry biomass of roots, TSS in roots of compost treated plants was low. Normally, protein production of plant is accelerated with the continuous supply of nitrogen that results in exceeded growth of plant and, hence, production of more biomass, whereas accumulation of carbohydrates is less (28). Similarly, ascorbic acid was also low in the roots of compost treated plants, mainly owing to the scarcity of carbohydrates in these plants. Increased protein production is coupled with reduction in carbohydrate production which in turn, being precursor of ascorbic acid production, reduces vitamin C synthesis (28). Furthermore, massive growth of these plants might have a dilution effect on the concentration of TSS and ascorbic acid.

Compost grown beetroots showed less TSS content and higher titratable acidity that led to low ripening index for these plants. As in fruits and vegetables, the concentration and type of sugars and organic acids, and the balance between them essentially contribute to the product flavor, the higher ripening index in SPM and  $\text{NH}_4\text{NO}_3$  additions indicated a good flavouring quality. Therefore, as was obvious, a negative correlation was noticed between pH and titratable acidity.

In response to different treatments, similar trend for accumulation of betacyanins and flavonols was observed in red beets. In compost treated plants, the decrease in the concentrations of betacyanins and flavonols was correlated with lower TSS and nitrate, and higher fresh plant biomass and fresh and dry biomass of rosettes and roots.

Consequently, similar to ascorbic acid, betacyanins and flavonols, TEAC was also higher in AmN1 and AmN2 applications

than in compost amendments. In addition, a decreasing trend for ascorbic acid, betacyanins, flavonols and TEAC with the increasing levels of  $\text{NH}_4\text{NO}_3$  and an increasing trend for betacyanins, flavonols and TEAC with the increasing level of compost were also evident. Under limited supply of nutrients, biomass production is negatively correlated with phenolic component (14) and betacyanins (7), and therefore, although the fresh and dry biomasses were the highest in compost grown beetroots, the ascorbic acid, betacyanins, flavonols and TEAC were the lowest in these beetroots.

Furthermore, less nitrate accumulation in composted beetroots also indicates the deficiency of free N for other metabolic activities. Consequently, flavonol content increase significantly in N deficient plants, primarily, to prevent oxidative damage (24).

Moreover, according to the carbon nutrient balance, if there are certain nutrient levels, excess carbon leads to the synthesis of carbon based secondary metabolites and their precursors (10). Increasing the carbon increased the accumulation of carbohydrates over growth demands, which was evident from higher TSS in SPM grown beetroots, and therefore, plant used extra carbon for carbon based secondary metabolites.

However, contrary to high flavonols and betacyanins contents, plants grown in SPM and  $\text{NH}_4\text{NO}_3$  produced less fresh and dry biomasses. It could be due to the fact that secondary metabolites are negatively correlated to the biomass production (14) due to the competition between protein synthesis and secondary metabolite production (11).

## CONCLUSION

In red beet plants, although peat moss and  $\text{NH}_4\text{NO}_3$  nutriment showed lower yield attributes like less plant biomass, fresh and dry rosette and root biomasses, and root to rosette ratio but, on the other hand, they also showed good internal quality pre-requisites including higher bioaccumulation of ascorbic acid, betacyanins and flavonol content, total soluble solids, ripening index and anti-

oxidant activity and less titratable acids. In terms of production, beets grown in only sphagnum peat moss were better than  $\text{NH}_4\text{NO}_3$  fertigated beets; and also regarding internal quality, peat moss was the best substrate. It is further suggested to combine peat moss with  $\text{NH}_4\text{NO}_3$  application for higher biomass and overall better growth of red beet plants.

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