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DETERMINATION OF THE OPTIMAL FERTILIZATION RATE BY A NEW BIOORGANIC FERTILIZER TO ENHANCE THE GROWTH PARAMETERS AND NUTRITIONAL QUALITY OF RED BEETROOT (*BETA VULGARIS L. SSP. VULGARIS*)

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ABSTRACT

The goal of this study was to investigate the effect of different rates of a new bioorganic fertilizer and an inorganic fertilizer on the parameters growth and the nutritional quality of red beetroot.
A field trial was conducted using the randomized complete block design with six treatments and six replications: (T1= inorganic fertilizer, NPK 20-45-25 ha^{-1}, T2= Bio-Organic Fertilizer "BOF" at 10%, T3= BOF at 20%, T4= BOF at 30%, T5= BOF at 60% and T6= unfertilized control). The total carbohydrate, protein and nitrate content of harvested red beetroot was measured analytically. Quantification of betanin was determined spectrophotometrically, while the antioxidant activity assessed free radical scavenging activities against DPPH.
Statistical analysis of soils treated with 20 and 30% BOF showed similar results for the total weight of red beetroot and the weight and diameter of their bulbs. However, these treatments significantly ($p < 0.05$) increased the betanin content of red beetroot by 16 % compared to the conventionally grown samples. However, the investigation of the effect of the different rates of BOF on the IC_{50} value showed a decrease of up to 52% in favor of the T5 treatment compared to the inorganic fertilizer. In contrast, organically and conventionally growing red beetroot had similar average nitrate levels.
In light of the crucial role of BOF rates, high levels of macro and micronutrients in the soil negatively affect all quality parameters of red beetroot.

Keywords: Bio-organic fertilizer, inorganic fertilizer, red beetroot, growth parameters, nutritional quality, antioxidant activity

INTRODUCTION

Due to increasing consumer demand for chemical-free and higher-quality agricultural products, farmers are looking to invest in environment-friendly fertilizers that can improve plant nutrition and ensure high yields simultaneously. Inorganic fertilizers cause serious environmental problems such as massive soil and groundwater pollution, energy depletion, imbalance of soil nutrients and negative impact on the population of beneficial microorganisms **(Farouk and Sharawy, 2016; Syed *et al*., 2021)**. Hence, using fertilizers that maintain and improve long-term soil fertility is a significant challenge for organic production. The primary purpose of organic fertilization is to increase the amount of macronutrients, especially C, N, and P, in the soil to provide nutrients necessary for crop growth **(Carter *et al*., 2012)**. Besides, it can improve soil quality by providing several types of microorganisms capable of producing many different enzymes that can hydrolyze the macromolecule and then facilitate its plant uptake **(Bamforth and Cook, 2019; Liu *et al*., 2021)**. Fertilizing the soil with efficient microbes and a suitable substrate is more effective than applying these agents directly to the soil **(Yousry, 2021)**. As a result, bioorganic fertilizers (BOF) have attracted the interest of many researchers, and their use is rapidly expanding. BOFs are the result of secondary solid-state fermentation of suitable substrates by selected microbial strains **(Wang *et al*., 2017)**. They have the particularity of having beneficial functions in the development of the microorganisms newly added for plant growth, in addition to their usual role with the organic fertilizers in improving the efficiency of plants to use nutrients **(Feng *et al*., 2020; Zhao *et al*., 2018)**. Many scientists have demonstrated that the application of a BOF can improve the yield and quality of crops and reduce the incidence of diseases in a large number of crops such as potatoes, bananas, watermelons, tomatoes, pepper, tobacco**...(Ma *et al*., 2018; Wu *et al*., 2015; Xue *et al*., 2019; Yanbin and Yazheng, 2009; Ye *et al*., 2020; Yuan *et al*., 2013)**. Moreover, BOF even showed efficacy in soils contaminated with phthalic acid esters by increasing the yield and quality of Chinese flowering cabbage **(Feng *et al*., 2020)**. In general, specific microbes known as plant growth-promoting microorganisms "PGPM" ensure BOF fermentation. The PGPM are agronomically beneficial soil microorganisms that can improve the decomposition and release nutrients and organic matter **(Peng *et al*., 2023)**. Applying bioorganic fertilizers containing PGPM promoted plant root growth, soil fertility, and soil-born pathogens **(Zhao *et al*., 2021).** Nevertheless, common rhizospheric and endophytic microorganisms are the most widely used PGPMs without considering the other potential groups of organisms, including LAB. **(Jaffar *et al*., 2023)** reported that particular strains of LAB can increase the availability of nutrients released by compost and other forms of organic or inorganic matter to plants. Also, LAB is widely used with other microorganisms to stimulate decomposition during the composting process and compost teas for soil fertilization and nutrient mineralization before and after seeding **(Murindangabo *et al*., 2023).** Despite the properties of the lactic acid bacteria/yeast combination, no study to date has demonstrated the efficacy of a BOF based on agricultural by-products and inoculated with selected strains of lactic acid bacteria of the species *"Lactiplantibacillus plantarum*" and "*Levilactobacillus brevis*" and yeasts of the species "*Candida famata*" on the growth parameters and the nutritional quality of red beetroot. Recently, most studies have focused on the influence of mineral, organic, biological and some BOF based on PGPM on the growth parameters of this plant **(Carrillo *et al*., 2019; Chung *et al*., 2011; Dlamini *et al*., 2020; Kale *et al*., 2018; Petek *et al*., 2019; Rubóczki *et al*., 2015; Sapkota *et al*., 2021a; Shanmugam *et al*., 2022).**
Red beetroot (*Beta vulgaris* L.), native to the Mediterranean region, is a widely consumed vegetable in many world countries **(SzéKely *et al*., 2019)**. It contains significant nutritional elements such as carbohydrates, fiber, protein, and essential and non-essential amino acids **(Hadipour *et al*., 2020)**. Moreover, its composition is rich in micronutrients, including vitamins (vitamin C, thiamine B1, vitamin B6, β-carotene, vitamin A, vitamin K and vitamin E), minerals (calcium, phosphorus, sodium, potassium, selenium and zinc), carotenoids **(Ceclu and Nistor, 2020; Fu *et al*., 2020; Stagnari *et al*., 2014; SzéKely *et al*., 2019)**. Over the past decades, *Beta vulgaris subsp. vulgaris* L has attracted increasing interest from researchers as a food with valuable therapeutic functions. It ranks among the top 10 vegetables for its potent antioxidant properties attributed to several bioactive compounds such as betalains, phenolic acids, flavonoids, saponins, sterols, triterpenes and ascorbic acids, which are naturally assimilated in the intestine after consumption

(Hadipour *et al*., 2020). It should be noted that betanin (one of the red betalains “betacyanins”) represents between 75 and 90% of the total pigments in red beetroot species **(Nouairi *et al*., 2021; Nowacki *et al*., 2015)**.
Beetroot is one of the vegetables that can be easily cultivated in the field. Thanks to its high productivity and short growing period, it is generally characterized by the absence of pest attacks and the appearance of diseases **(Sapkota *et al*., 2021)**.
The specific aim of this study is to evaluate the effect of this type of BOF on the growth parameters and chemical composition of red beetroot. We hypothesized that (1) the physicochemical properties of the soil could affect the growth parameters and nutritional quality of beetroot. (2) A single application of BOF may increase the weight and diameter of beetroot in the same way as an inorganic fertilizer. (3) The application rate of the BOF may affect beet growth parameters and nutritional quality.

MATERIAL & METHODS

Experimental site

The agricultural trial was conducted by the Faculty of Sciences, Ibn Tofail University of Kenitra. The geographical location of the experimental site is 6°35'18.27" W and 34° 14' 45.94" N. The cultivation occurred between May and August with a moderately warm temperature during the whole duration of the trial (24 °C to 29 °C, according to the history of the weather reports in Kenitra). The plot's surface to be cultivated is 18 m^2 (6m length and 3m width).

Plant material and cultivation

The cultivar used for the red beetroot is "Detroit improved," one of the main cultivars used in Morocco. The seed crops were cultivated 10/05/2021 in plastic honeycomb trays and transplanted to the plot after germination. Germinated seeds were thinned to keep only one plant per hole at each sub-block. The main plot was divided into 18 lines, with a spacing of 30 cm, and the distance between plants was 30 cm with 108 seedlings. The irrigation was ensured twice daily, maintaining the same amount of water throughout the trial.

BOF preparation

The bioorganic fertilizer used in the field trial is based on three wastes generated by the food industry: olive mill wastewater, molasses and rice hulls. Lactic acid bacteria (LAB) of the species “*Lactiplantibacillus plantarum”* and “*Levilactobacillus brevis*” and of the yeast of the species “*candida famata*” were inoculated to control the fermentation process. The process of the controlled fermentation is described by **(Atfaoui *et al*., 2021)**. Table 1 summarizes its physicochemical characteristics.

Table 1 Physicochemical characteristics of the bioorganic fertilizer applied.

	Unit	Bioorganic fertilizer (BOF)
pH	----	4.08 ± 0.08
Acidity	% Lactic acid	1.42 ± 0.04
OM[a]	%	80.20 ± 2.30
TOC[b]	%	48.2 ± 1.26
TN[c]	%	1.41 ± 0.08
C[ode]	g/l	43,2 ± 1.01
C/N	%	34.28 ± 4.01
TP[d]	%	0.11 ± 0.01
Potassium	%	0.91 ± 0.01
Calcium	%	0.49 ± 0.02
Zinc	mg/kg	14 ± 1.50

Except for pH value, all data are expressed on a dry basis. ± present the standard deviations (n = 3).
[An] Organic matter, [b] Total organic carbon, [c] Total nitrogen, [d] Total phosphorus, [e] Chemical oxygen demand.

Fertilization treatments

The experiment consists of the study of the effect of a single factor "type of fertilizer" on the growth of the same cultivar of red beetroot (*Vulgaris var.*). In total, there are six treatments related to the type of fertilizer: T1= inorganic fertilizer 80 kg N ha^{-1}, 180 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} (NPK 20-45-25), T2= Bio-Organic Fertilizer “BOF” at 10%, T3= BOF at 20%, T4= BOF at 30%, T5= BOF at 60% and T6 with 0% of BOF as a control culture.
The inorganic fertilizer was counted in the soil before the cultivation of the seeds and at the stage of the growth of the roots through irrigation. At the same time, the BOF was added to the soil 15 days before sowing by an auger to ensure compliance with the different percentages used.

Experimental design

The experiment was conducted in a randomized complete block design (or Fisher block design). The plot consisted of 6 blocks; within each block, there are three identical sub-blocks (Fig. 1). The different treatments are randomly assigned within each sub-block, where the treatment appears only once. The interior of a block is as homogeneous as possible. On the other hand, within the same field, the blocks are as different as possible.

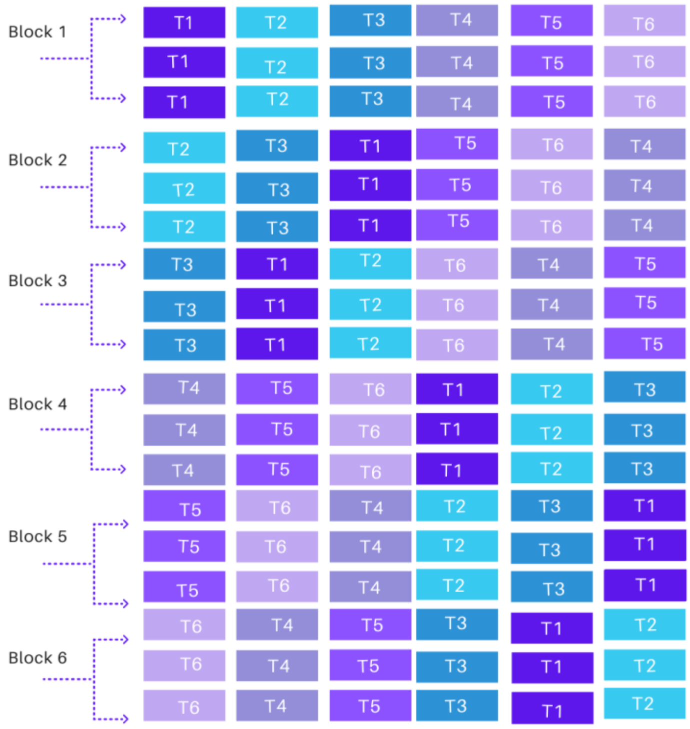

Figure 1 Theoretical distribution of the treatments within the plot according to the randomized complete block design

Physico-chemical parameters of soil

A sandy soil (95% sand) was collected from a plot dedicated to agricultural trials with no additional fertilization. The soil was sampled at a depth of 20 cm and 40 cm. The samples were mixed and immediately transported to the laboratory and stored at + 2°C. The analyses carried out concern the soil before and after its fertilization by the different doses of the BOF (Table 1).

Samples were analyzed according to the following methods:
pH was determined electrometrically using a digital pH meter (JP SELECTA, pH-2005), water suspension (1:5, w/v), Electrical conductivity was quantified in a 1:5 soil/water suspension and measured with a HANNA benchtop conductivity meter (ModelHI2300) at 25°C. Organic matter was obtained by evaluating the difference between the dry mass and the mass after calcination at 500°C for 24h (SELECT-HRN**) (Navarro *et al*., 1993)**. A TOC analyzer (Shimadzu, model TOC-V CPN, Japan) was used to measure the total organic carbon **(da Silva *et al*., 2008)**, and nitrogen by the Kjeldahl method using Pro Nitro Distiller, SELECTA **(Bremner, 1965).** Phosphorus (P) content was performed by reduction spectrophotometry with molybdenum vanadate according to ISO 6491 (1998) using a Jenway® 6850 double beam UV / visible spectrophotometer type spectrometer **(Straus *et al*., 2012)**.

K, Na, Ca, Mg, Fe, Mn, Cu, and Zn minerals were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES), Model Optima 8000 (Perkin Elmer, Waltham, Massachusetts, USA) according to the method described by **(Chevallier *et al*., 2015)**.

Table 2 Soil physicochemical properties regarding fertilization treatments

	Unity	Soil	T2	T3	T4	T5
pH		8.02± 0.41	7.29±0.24	7.03±0.18	6.48±0.13	5.82±0.12
Ec	µS/cm	140±3.10	1430±28.91	1960±39.28	3750±77.33	7690±158.87
Organic Matter	%	1.06± 0.02	3.19±0.07	4.72±0.11	6.22±0.17	13.81±0.31
Total Carbon	%	61.26±1.32	1.86±0.03	2. 74±0.06	3.61±0.08	8.01±0.16
C/N	----	10.21±0.26	10.88±0.28	11.39±0.34	11.28±0.31	12.32±0.37
N	mg/kg	600±12.62	1700±36.96	2400±52.68	3200±65.81	6500±163.21
P	mg/kg	28.35±0.60	93.52±1. 97	135.84±2.82	240.72±4.85	569.23±11.20
K	mg/kg	62.76±1.01	1337±26.65	2828±56. 58	4388.6±87.84	8207.21±168.15
Na	g/kg	0.02±0	0.69±0.01	1.38±0.02	2.63±0.06	4.78±0.12
Ca	g/kg	5.16±0.11	7.52±0.16	7.85±0.18	8.98±0.20	15.71±0.35
Mg	mg/kg	37.60±0. 74	134.63±2.62	251.7±.5.08	476.6±9.01	566.85±11.89
Cu	mg/kg	0.43±0.01	0.51±0.01	0.61±0.01	0.76±0.02	1.52±0.03
Zn	mg/kg	3.89±0.09	4.13±0.08	8.42±0.17	11.32±0.21	23.06±0.44
Mn	mg/kg	6.98±0.17	25.51±0.48	47.66±0. 96	57.28±1.16	107.58±2.26
F	mg/kg	15.54±0.31	17.61±0.36	36.8±0.69	56.7±1.13	159±3.02
Cl	mg/kg	0.35±0	342.6±7.19	812.4±16.24	1248±26.20	3047.1±60.94

The mean value standard deviation (n= 3). T2=10% of BOF, T3=20% of BOF, T4=30% of BOF, T5=60% of BOF.

Vegetative growth parameters

After 94 days of plant growth, all plants from each block were collected to measure the following characteristics: root and shoot length (cm), total weight (g), leaves numbers, bulb length (cm) and diameter (cm).

Nutritional quality of beetroot

Plant samples were blotted with filter paper after being carefully cleaned with tap and deionized water. The fresh plants were stored at 4 °C for quality measurements as soon as possible.

Determination of total carbohydrate content

The total carbohydrate content in fresh beetroot was determined spectrophotometrically according to the method given by **(Dubois *et al*., 1956)**. The results were measured with a Jenway® 6850 UV/visible double beam spectrophotometer at 490 nm, using a standard curve with concentrations of 0.3 g/l glucose and 0.3 g/l fructose.

Determination of Protein Content

The protein content is calculated by multiplying the total N (%) (Determined by the Kjeldhal method) by the coefficient 6.25 using Pro Nitro Distiller, SELECTA.

Betanin content

20 g of fresh beetroot was collected separately from each treatment and homogenized with a mortar and pestle. Samples were extracted with approximately 90 ml of 99% acidified water (with 1% HCl) by sonication for 10 minutes. The mixtures were filtered through Whatman No. 1 filter paper and then were made to 100 ml with acidified water "Betanin Extract." 1 ml solution was diluted to 10 ml with acidified water to obtain high-resolution spectra.
The absorbance of samples at 538 nm was analyzed in a UV-VIS spectrophotometer, and the betanin extracted from 20 g of red beet was calculated using the following equation **(Nouairi *et al*., 2021**), then converted to units of mg per 100 g of fresh weight "FW."

$$\mathbf{m_i = (A_i.D_f .M/\varepsilon.l)* (V_e/1000*m_s)}$$

Where:
mi: quantity of betanin extracted,
Ai: Sample absorption (L),
ABetanin= 1.095×(A_{538}−A_{600}) **(Skalicky *et al*., 2020)**
Betanin has its A_{max} at 538 nm. Measurements at 600 nm were used to correct for the presence of impurities.
M: average molecular mass (550 g.mol^{-1} for betanin),
ε: molar extinction coefficient (1120 L.cm^{-1}. mole^{-1} for betanin)
l: Cuvette length (1cm)
V_e: Volume of extract in ml (after extraction),
m_s: mass of red beet used for extraction,
D_f: Dilution factor (1/10).

Nitrate content

The content of nitrate (NO_3^-) ion was conducted following the method proposed by **(Bahadoran *et al*., 2016)** following ISO 6635,1975a, b, 1984, 2004.
In order to extract nitrate, portions of 1 to 10 g of fresh red beetroot samples were treated with sodium tetraborate, Carrez solutions containing potassium hexacyanoferrate (II) trihydrate (Carrez I) and zinc acetate solution (Carrez II). After extraction, samples were filtrated on the Whatman filter. Then, a reduction of nitrate to nitrite by metallic cadmium was performed. Afterward, sulfanilamide chloride and N-(1 -naphthyl) ethylene diamine dihydrochloride were added to the filtrate and the red complex obtained was measured spectrometrically at 538 nm.

DPPH Radical Scavenging Assay

The antioxidant activity was assessed by the radical scavenging DPPH method with slight modifications **(Shakeel *et al*., 2020).**
Extracts were prepared precisely as "betanin extract" described previously using methanol instead of acidified water. Then, aliquotes (0.05, 0.1, 0.15 ml) were added to 5ml of a freshly prepared methanolic solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH, 20mg/l) and completed to 10 ml by methanol. The mixtures remained at room temperature in the dark for 30 min. A calibration curve was prepared with Trolox as the reference standard. Absorbance was recorded at 517 nm against blank samples lacking scavenger.
Antioxidant activity was expressed as percent scavenging of DPPH radical, and the following formula was used:

$$\mathbf{SA_{DPPH}\ (\%) = (Abs_{control} - Abs_{sample}/Abs_{control})\ x\ 100}$$

The IC_{50}, the concentration at which the DPPH radicals were scavenged by 50%, was calculated for each case from the curve drawn for the I% of each sample versus the corresponding concentrations in mg/ml.

Statistical analysis

One-way analysis of variance (ANOVA) was performed at a significance level of 5%. Comparison of means followed Tukey test using IBM SPSS STATISTICS (25th edition, United States). The analysis of variance (ANOVA) was performed according to a randomized complete block design. The Graph Pad Prism7 software was used to draw the graphs.

RESULTS

Vegetative growth parameters

According to (Table 2), only the total weight of red beets, the weight and the diameter of the bulbs were significantly influenced ($p < 0.05$) by the type and rate of fertilizer added to the soil. At the same time, the other parameters were not significantly different at $P \leq 0.05$.
Results reported that almost all treatments significantly increased these three parameters, except T5, compared to control plants. Moreover, the highest values were scored by those who received only the inorganic fertilizer "T1" and the bio-organic fertilizer at 20% "T3" and 30% "T4". Cross sections of red beet bulbs showed visible differences between bulbs from these three treatments and those from treatments T2, T5 and the control T6 (Fig. 2).

Figure 2 Cross sections of red beet bulbs from different treatments, 94 days after sowing.

The results showed that amendment with 20% BOF and 30% BOF achieved statistically the same growth parameters of red beetroots as the inorganic fertilizer. The bulb's weight and diameter ranged from 97,94g, 5,889 cm (T3) to 108,22g, 5,972 cm (T3), respectively.

These values were higher than those found by **(Dlamini *et al.*, 2020)** in soils amended with cattle manure (80 kg/ha). However **(El-Sherbeny and Da Silva, 2013)** reported values more outstanding than our results, which reached a weight and a diameter of 134.62 g 7.08 cm, respectively, after a foliar application of tyrosine.

Effect of different treatments on protein and total sugar content

Application of different types of fertilizers (BOF and IF) and progressive rates of BOF on soil caused a significant change in the protein and total sugar content of red beetroot. Results from (Fig. 3) indicated a significant difference in protein and total sugar content. T1 provided the highest protein content with a value of 1.767 g/100g, followed by T3, then T2 and T5 in the third position, and finally, T4 and the control crop with the lowest contents of about 1.38 and 1.453 g/100g, respectively. Such protein contents are higher than those reported by **(Souci *et al.*. 2000)**, which did not exceed 1.05 g/100g. Whereas the report of the United States Department of Agriculture USDA (2019) states that red beetroot contains a content of 1.61 mg/100g of fresh matter **(Akan *et al.*. 2021)**. **(Szopińska and Gawęda. 2013)** suggested that the high levels of readily available nitrogen in inorganic fertilizers lead to increased synthesis of amino acids and proteins, which explains the high protein content obtained by the treatment T1.

Table 3 Vegetative growth parameters of red beetroot regarding fertilization treatments

Treatments	Root length	Shoot length	Total weight*	Leaves number	Bulb weight*	Bulb diameter*
T1	11.74ᵃ ±0.34	33.47ᵃ ±1. 09	156.18ᵃ±4.14	13.83ᵃ ±0. 78	100.67ᵃ ±2.84	6.19ᵃ ±0.18
T2	11.7ᵃ ±0.31	31.45ᵃ ±0.68	99.36ᵃᵇ ± 2.21	12.33ᵃ ±0.26	56.72ᵃᶜ ±1. 70	4.86ᵃᵇ ±0.14
T3	12.32ᵃ ±0.84	33.37ᵃ ±1.02	166.42ᵃ ±5.11	13.89ᵃ ±0.28	97.94ᵃ ±2.04	5.89ᵃ ±0.18
T4	12.32ᵃ ±0.68	33.81ᵃ ±1.23	164.9ᵃ ±4.94	13.0ᵃ ±0.38	108.22ᵃ ±3.56	5.97ᵃ ±0.18
T5	12.04ᵃ ±0. 59	29.89ᵃ ±0. 96	75.81ᵇ ±2.23	11.54ᵃ ±0.39	40.78ᶜ ±1.20	4.34ᵇ ±0.11
T6	12.18ᵃ ±0.46	30.93ᵃ ±0.88	57.33ᵇ ±1.60	10.01ᵃ ±0.25	27.89ᶜ ±0.57	3.66ᵇ ±0.08

T1= inorganic fertilizer. T2= 10% of BOF. T3= 20% of BOF. T4= 30% of BOF. T5= 60% of BOF. Values are the mean of 18 replicates. mean± standard deviation.
* Statistically significant compared to control at P≤0.05. Numbers followed by the same letters in the same column are not significantly different at P ≤ 0.05 by the Student–Newman–Keuls test.

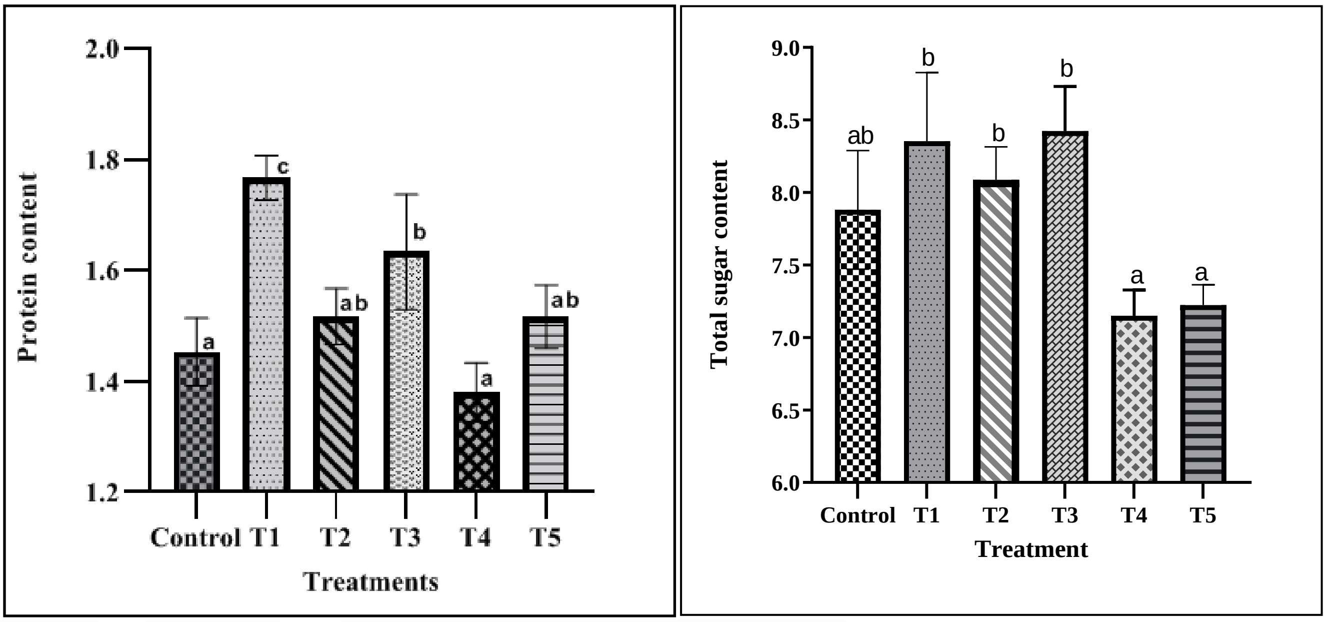

Figure 3 Protein (left) and total sugar (right) contents regarding fertilization treatments in red beetroot. T1= inorganic fertilizer. T2= 10% of BOF. T3= 20% of BOF. T4= 30% of BOF. T5= 60% of BOF. Graph bars marked with different letters at the top represent statistically significant results (P<0.05) based on Tukey's post hoc one-way ANOVA analysis.

The application of treatments T1, T2, T3 and T6 significantly increased the total sugar content of red beetroot compared to T4 and T5 values, which did not exceed 7.15 and 7.223 g/100g, respectively. The highest total sugar content was obtained after fertilization with BOF with a percentage of 20%, which reached about 8.423 g/100g. **(Szopińska and Gawęda, 2013)** reported the same findings and showed that fertilization with organic fertilizer resulted in higher total sugar levels in red beetroot compared to those produced by integrated and conventional cultivation. However, this level did not exceed 8.13 g/100g.
(Rembia\lkowska *et al.*, 2012) confirmed a higher content of total sugars in organic fruits and vegetables, mainly carrots, beets, potatoes, spinach, cherries, blackcurrants and apples, which contributed to increased technological and sensory (taste) quality of organic products. These high sugar levels are explained by the slow decomposition of organic and bio-organic fertilizers by microorganisms that progressively increase their availability and improve their absorption by the plants.

Effect of different rates of fertilizers on nitrate and betanin contents in red beetroot

Based on statistical analysis (Fig. 4), nitrate content was not significantly influenced by the type or rate of inorganic and bio-organic fertilizers (p <0.005). However, these contents, up to 242.3 mg/100g for T1, are higher than the mean nitrate values reported by **(Chung *et al*,. 2011; Rubóczki *et al*,. 2015; Siomos and Dogras. 2000)**, which did not exceed 137.9, 70.0-85.0, and 44.3-98.1 mg/100g respectively. The high nitrate levels can be explained mainly by the high nitrogen concentrations in the soil after fertilization. Despite this, organic red beets contain significantly less nitrate than conventional ones.

High concentrations of betanin content in red beetroot were registered with treatments T3, T4, T5 and T6, reaching 19.15, 19.08, 19.43 and 18.85 mg/100g of fresh matter "FM," respectively. In comparison, low levels were obtained in red beetroot from treatments T1 and T2 with values not exceeding 16.1 and 16.22 mg/100g of FM, respectively.

These results are close to the conclusions drawn by **(Farouk and Sharawy, 2016)** on the betanin content of peeled red beetroot, which varied between 23.60 and 25.37 mg/ 100g FM after the second stage of maturity for biofertilizer and yeast fertilizer, respectively. Nevertheless, the high betanin content observed in 8 red beet genotypes reported by (Lee *et al*., 2014) ranged from 50 to 65 mg/ 100g FM, which is higher than the values reported in this study.

The difference between all these values can be explained by the total betalains content, which varies considerably among the cultivars and parts of the beet analyzed**. (Sawicki *et al*., 2016; Slatnar *et al*., 2015)** proved that the outer layer of the beet had the highest betalains level compared to the inner layers.

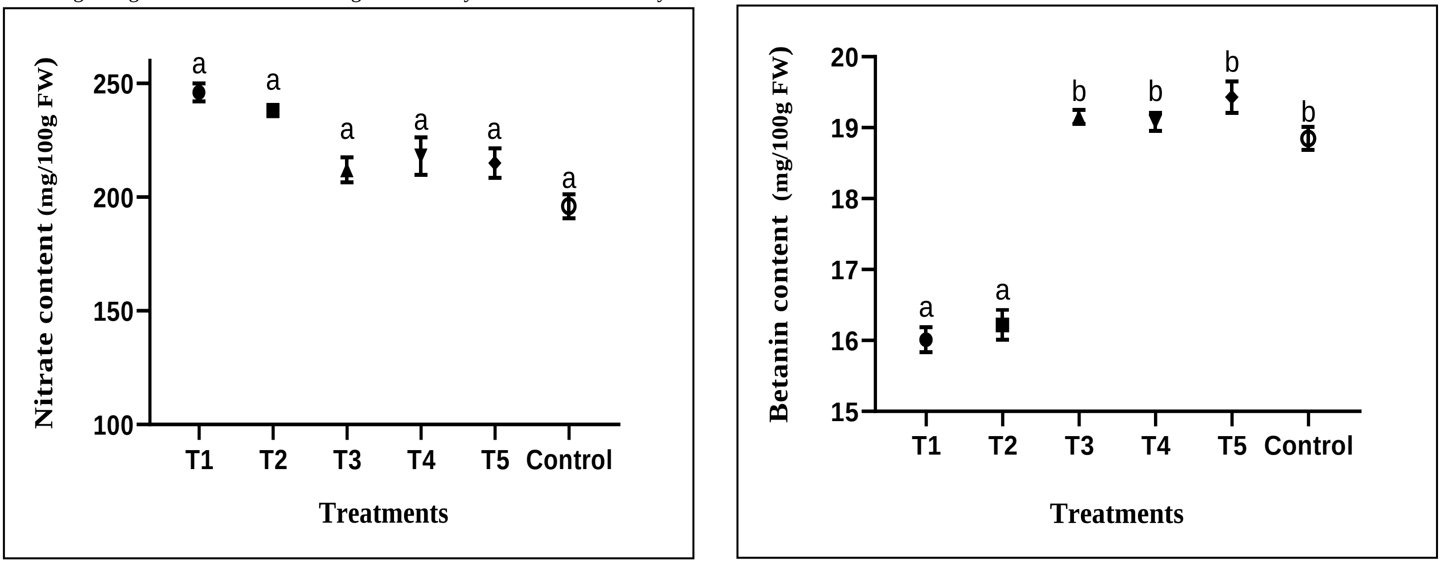

Figure 4 Nitrate (left) and betanin (right) contents regarding fertilization treatments in red beetroot. T1= inorganic fertilizer. T2= 10% of BOF. T3= 20% of BOF. T4= 30% of BOF. T5= 60% of BOF. Values are the mean of 3 replicates. Means mentioned by the same letters are not significantly different at P ≤ 0.05 based on the Tukey test.

Effect of different rates of fertilizers on antioxidant activity in red beetroot

The inhibition concentration, at which the DPPH radicals were scavenged by 50%, of red beetroot extract at different rates of BOF is shown in (Fig. 5). Thus, the lower the IC_{50} value, the more effective the antioxidant activity.

Only a tiny amount of bioorganic red beetroot was necessary to inhibit 50% of the free radical (IC_{50}). Therefore, the samples of T5 (16.23mg eq. trolox /ml) and T4 (17.61 mg eq. trolox / ml) showed lower concentrations to decolorate 50% of DPPH (IC_{50}), which means a higher free radical scavenging activity. In general, the IC_{50} value decreased by up to 40, 48 and 52% in favor of T3, T4 and T5 treatments compared to the inorganic fertilizer. Hence, applying different levels of BOF as a soil amendment caused a significant increase in the antioxidant activity of red beets compared to the inorganic fertilizer treatment.

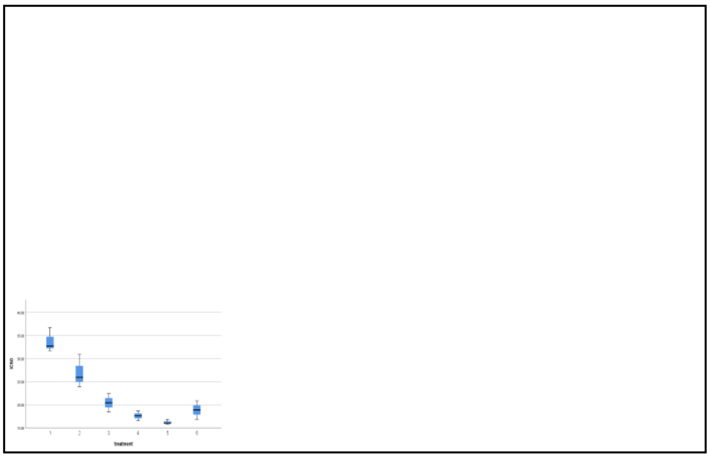

Figure 5 Free Radical Scavenging Activity by DPPH (IC50) of red beetroots from different soil treatments. IC50 = (mg eq. trolox/ml). Values are expressed as means in triplicate (n = 3).

It is difficult to compare our results with other data because of different red beetroot cultivars or parts (stems, roots, peeled or not) and various standards during the analysis. Otherwise**, (Carrillo *et al*., 2019)** analyzed the antioxidant capacity of red beetroot with three different methods and proved that the organic red beetroot significantly increased the total antioxidant capacities compared to the conventional samples.

DISCUSSION

The optimal soil pH for red beetroot cultivation ranges from 6.0 to 7.0, and it can tolerate alkaline soils with a pH between 9.0 and 10.0 **(Kumar *et al*.,. 2014)**. While **(Akan *et al*.. 2021)** confirmed that red beetroot does not tolerate pH values greater than or equal to 7.6.

Based on the results of this experiment, we confirm these statements by recording the best growth parameters and nutritional quality of red beetroot in the soil with a pH of 7.03, corresponding to the soil treated with 20% BOF (Table 1). In addition, we can conclude that acidic soils (60% of BOF) are unfavorable for the growth of red beetroot due to their high ability for cation exchange and components leaching phenomenon **(Soti *et al*.. 2015).**

Soil amendment with progressive percentages of BOF revealed no significant variation in C/N ratios, which are all under the recommended ratios **(Gell *et al*.. 2011; Walsh *et al*.. 2012)**. Thus, the significant differences observed in the growth parameters and nutritional quality of red beet after applying different treatments are not influenced by this parameter.

In general, the application of increasing rates of BOF (less than 60%) increased the red beetroot bulbs' diameter and weight. This could be explained by the large amounts of macronutrients available in soil (Table 1). In particular, nitrogen (ranged from 1700 to 3200 mg/kg for soils amended with 10 to 40% of BOF) is known by its role in the division and elongation of cells, which is therefore reflected in root diameter and weight **(Sinta and Garo. 2021)**. Also, **(Sapkota *et al*,. 2021)** confirmed that phosphorus (ranging from 93.52 to 240.72 mg/kg for soils with 10 to 40% of BOF) stimulates root growth, allowing for better absorption and translocation of nutrients.

Furthermore, soils amended with 10 to 40% of BOF manifested substantial amounts of sodium that ranged from 0.69 to 2.63 g/kg. Many studies reported that sodium is as crucial as potassium and is necessary for maximum yield and healthy plant development **(Wakeel, 2013).** In contrast, **(Nieves-Cordones *et al*., 2016)** confirmed that high sodium levels contributed to soil salinity, which limits the growth of many plants due to reduced water availability and ion toxicity specific to high Na levels. Our findings are consistent with these studies and prove that soils with sodium concentrations greater than or equal to 4.78 g/kg had a negative influence on the growth parameters of plants.

Soil nutrients can provide essential substances and energy for the survival of various microorganisms in the soil and the growth of crops **(Liu *et al*.. 2021)**. However, excessive fertilization reduces the overall quality of the soil **(Shasha *et***

al., 2020). This is due to the release of large amounts of nutrients into the soil and the increased uptake of these nutrients by plants **(Chontal *et al*., 2019)**.
The study on the toxicity of micronutrients **(Havlin *et al*., 2016)** and analyses of the soil after amendments by our bio-organic fertilizer at different doses (Table 1), allows us to conclude that no micronutrient exceeds the expected value that should be contained in the soil.
The study carried out by **(Mengel. 1997)** on the influence of Potassium content on crop yield, taking into consideration that potassium is the only limiting factor, showed that the yield increases with the increase of K supply until the saturation of the soil, according to the "Mitscherlich curve" phenomenon. After this value, the crop yield starts to decline. This means high K levels in the FBO favor red beet growth up to 4388.6 mg/kg soil.
Our results confirmed that BOFs are essential for maintaining soil health and contributing to improving the nutritional properties of plants. This type of fertilizer provides necessary nutrients to crops and to the beneficial microorganisms resident in the soil, as well as stimulating their activities.
These conclusions are consistent with the results of other researchers. For example **(Ye *et al*.. 2020)** found in their recent study that organic tomatoes issued from a bio-organic fertilizer inoculated with Trichoderma have higher soluble sugar and vitamin C contents with values up to +24% and +57%, respectively, compared to those from treatments with 100% of inorganic fertilizer. In another study, **(Feng *et al*.. 2020)** confirmed a significant increase in vitamin C, vitamin B1, total protein, and starch content using a *Bacillus megaterium*-inoculated BOF compared to the control and mineral fertilizer.
Also, several researches have shown that BOFs inoculated with an efficient consortium of microorganisms produced better yields and increased nutrient uptake for areca nut **(Liu *et al*., 2023)** wheat **(Hu and Qi. 2010)**, soybean **(Moretti *et al*., 2020)**, rice **(Javaid. 2011)** and cotton **(Khaliq *et al*., 2006)** than plants grown with non-inoculated fertilizers. (**Kang *et al*., 2015)** studied the effect of an ECM consisting of *Rhodobacter sphaeroides*, *Lactobacillus plantarum* and *Saccharomyces cerevisiae* on the growth and development of cucumber and concluded that the combination of these three microorganisms increased cucumber growth, nutrient uptake and amino acid content. The increase in productivity and quality of crops treated with ECM-inoculated BOFs compared to untreated composts is likely due to the accelerated decomposition of organic compounds into plant-available nutrients. **(Giassi *et al*., 2016)** demonstrated that LABs could solubilize phosphate by producing organic acids and that three LAB strains isolated from sugarcane ferment can fix atmospheric nitrogen. Furthermore, research on the growth-stimulating effect of lactic acid bacteria on pepper showed that indole-3-acetic acid produced by LAB is responsible for promoting plant growth **(Shrestha *et al*., 2014).** Recently, the high phytohormone activity of Lactobacillus bacteria was confirmed by detecting Gibberellic acid, which stimulates plant growth and development, in wheat coleoptiles **(Kutlieva, 2021)**.
In comparison, yeasts stimulated plant growth by inhibiting pathogens, producing phytohormones and solubilizing phosphate **(Nassar *et al*., 2005).** For example, *S. cerevisiae* promoted plant growth under adverse environmental stress conditions **(Gao *et al*., 2014)**.
Also, **(Maroušek *et al*.. 2017)** reported that high nitrogen content in the soil has a negative influence on betanin content. Therefore, the low values of betanin content might be due to the high nitrogen supply by the different treatments, especially in the soil with 60% FBO (Table 1).

The correlation between betanin content and IC_{50} of red beetroot from different treatments was noted. Indeed, the decrease of IC_{50} was associated with increased concentrations of betanin in samples (r = –0.936). Also, **(Acharya *et al*., 2021; Belhadj Slimen *et al*., 2017; Kusznierewicz *et al*., 2021)** have observed the antioxidant activity of the betalains. **(Sawicki *et al*., 2016)** found a correlation between the red beetroot betalain and betacyanins and the antioxidant activity of red beetroot analyzed by the DPPH (r = 0.740 and r = 0.802, respectively).
Few studies have assessed the influence of bioorganic fertilizers on the antioxidant capacity of vegetables **(Abdelbaky *et al*., 2023; Bakhtiari *et al*., 2020; Sánchez-García *et al*., 2022)**. However, to the best of our knowledge, this is the first study to have evaluated the impact of a BOF on the total antioxidant capacity of red beetroot.

CONCLUSION

Our present study can confirm the positive effect of bioorganic fertilizer on red beetroot by considerably improving its nutritional quality compared to crops applied with only inorganic fertilizers. However, the amendment rate by BOF should not exceed 30% of the total weight of the soil; otherwise, it will show a toxic effect on the development of this type of microorganisms.

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