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Research paper

Impact of different fertilizers on some phytochemical constituents and biological activities of *Beta vulgaris* L. leaves

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Abstract

Red beet leaves are used in some countries in salads, while others consider them as leftover. Our goal is to conduct field experiment, for two seasons, to evaluate the effect of various combinations of (organic, chemical and bio-/yeast) fertilizers and different harvest dates on some leaves growth parameters, biocostituents and biological activities. The applied fertilizers significantly increased most of the growth parameters compared to control and highest values were produced by 100%chemical fertilizer (2), followed by treatments of 75%biofertilizer (4) and 75%yeast (8). Highest chlorophyll (a&b) and carotenoids contents were recorded by treatments 2, 4 and 8, particularly those of the second stage. Most of the studied fertilizers significantly increased betanin and polyphenolic contents compared to control and highest values were attained by treatments 4 and 8. Maximum values of betanin and polyphenols were reached in the first and third stages, respectively. Additionally, highest antioxidant activity was afforded by leaves of treatments 4 and 8, whereas leaves of treatment 4 provided a broader antimicrobial activity than control. Moreover, the highest cytotoxic activity was exerted by leaves of treatment 4. Finally, it is recommended to harvest beet leaves at the appropriate stage to obtain high growth values and reasonable percentages of bioactive metabolites.

Keywords: *Antimicrobial; Antioxidant; Cytotoxic; Fertilizers; Red Beet Leaves.*

1. Introduction

Red beet leaves or commonly known as beet greens are edible organs of the plant *Beta vulgaris* L. ssp. *vulgaris*, family Chenopodiaceae. They are underexploited by many countries and could be treated in the Egyptian markets as a leftover. In the kitchen, beet greens can be enjoyed sautéed or as a salad. Besides supplying good amounts of proteins, minerals (magnesium, copper, calcium, sodium, potassium, iron, manganese, and phosphorus), vitamins (A, C, K and B-complex group) and carotenoids (ß-carotene, lutein and zea-xanthin), beet greens are also a great source of fibers. They possess more minerals, vitamins, and fibers than beetroot; meanwhile they contain less fats, sugar and no cholesterol [1]. Additionally, they are considered as an excellent source of omega-3, phenolic and antioxidant compounds [2]. On tracing the available literature, very few studies were concerned with the biological studies of beet greens [3].

Plants need essential nutrients, in the suitable proportion, to afford healthy growth and maximum yield. These necessities can be fulfilled by the use of fertilizers (biofertilizers, organic and chemical) singly or as a combination mixture. Food and Agriculture Organization of the United Nations (FAO) developed a strategy named Integrated Plant Nutrition System (IPNS) to promote crop and soil productivity through the use of a balanced combination of chemical, organic and biofertilizers. Moreover, the consumers, nowadays, are greatly cocerned with the harmful effects of plant chemical fertilizers on their health, as well as polluting the environment [4].

The impact of different fertilizers on production of secondary metabolites in plants is not widely studied, yet some authors suggested that organic fertilization can enhance the production of these metabolites versus inorganic fertilizers [4-7]. As no studies could be traced concerning the efficacy of various fertilizers on beet greens metabolites and in a continuation to our previous study regarding evaluation of the effect of different fertilizers on growth and bioconstituents of red beet bulbs [8], thus, the present work aimed at investigating the impact of fertilizers on some growth parameters, phytochemical constituents and biological activities of the beet leaves.

2. Materials and methods

2.1. Experimental location, plant materials and treatments

The authors conducted the experiments (two field) at the Experimental Farm of the Applied Research Center for Medicinal Plants, National Organization for drug Control and Research (NODCAR), Egypt, during 2012/2013 and 2013/2014 seasons to evaluate the impact of some combinations of fertilizers (organic, chemical, biofertilizers and yeast) on vegetative parameters and some chemical constituents of the leaves. The biofertilizer used in this work was a mixture of nitrobein, phosphorein and potassiumage that contained efficient strains of nitrogen fixing bacteria namely; *Azotobacter chroococcum* ($1x10^9$ cfu/ml), phosphate dissolving bacteria: *Bacillus megaterium* var *phosphaticum* ($1x10^{11}$ cfu/ml) and *Bacillus circulans* ($1x10^8$ cfu/ml), respectively. Moreover, the yeast fertilizer was a suspension of *Saccharomyces cervisiae* in a concentration of ~10^8 cfu/ml. The planting of the seeds was performed as reported by Farouk and Sharawy [8] and three replicates were established for each treatment.

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The soil of the layout was thoroughly mixed with organic fertilizer (compost constituted of plant sources and cattle manure at the rate of 5 m^3/fed) and that represented the control treatment in this work. The plants were fertilized with combinations of chemical and bio-/yeast fertilizers as recommended by Farouk and Sharawy [8]. All the plants were harvested after 2 (1st stage), 3 (2nd stage) and 4 (3rd stage) months from the planting time.
The developed plant was authenticated by Dr. Abd El Halim Abd El Mogali Mohamed, senior researcher, Flora and phytotaxonomy researches department, Horticultural research institute, Agricultural Research Center, Egypt. A voucher specimen was kept at the department of medicinal plants and natural products.

2.2. Determination of vegetative parameters

Three plants were selected randomly, from each treatment, for the purpose of recording observations. The growth parameters *viz.* weight, length and number of leaves were determined at the different harvest dates.

2.3. Solvents and chemicals

Methanol, absolute ethanol, hydrochloric acid (HCl), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), folin-ciocalteu reagent and gallic acid were purchased from Sigma chemicals, USA.
Double distilled water was used in the course of this work.

2.4. Preparation of extracts for phytochemical and biological evaluation

About 20 g of small sliced red veins of the leaves, of each treatment, were extracted by 90 ml of 99% acidified water (with 1% HCl) with the aid of sonication for 10 minutes. The samples were then filtered and the volume was adjusted to 100 ml with acidified water (stock solution). Such aqueous stock solution was used for determination of betanin and total polyphenolic contents.
Another similar weight of 20 g was treated in the same manner but using methanol as solvent to yield a methanolic stock solution to be used in the evaluation of antioxidant activity.
The samples, used in the study of antimicrobial and cytotoxic activities, were lyophilized juice resulting from blending of the leaves with minimal amount of water in a mixer then squeezed manually using a cloth of muslin.

2.5. Phytochemical evaluation

2.5.1. Determination of chlorophyll content

Chlorophyll content of the leaves was estimated by collecting the healthy fully matured leaves at different stages. Chlorophyll (a & b) and total carotenoids contents were colorimetrically determined in leaves according to the method described by Inskeep and Bloom [9] and is expressed in mg/100g fresh weight.

2.5.2. Determination of betanin content

One ml of the aqueous stock solution was diluted to 10 ml with acidified water. Betanin content was quantified by spectrophotometer (Unicam, UK) at 538 nm using absorptivity value (A_1%) which was 1120, according to Piattelli and Minale [10] and then calculated as mg/100g fresh weight.

2.5.3. Determination of total polyphenolic content

Total polyphenolic content was determined by using Folin-Ciocalteu's reagent, according to the method of Lachman et al. [11] with some modifications. An aliquot of 0.25 ml from the aqueous stock solution was introduced into test tubes, then 1.0 ml Folin-Ciocalteu's reagent and 1 ml sodium carbonate (4.0%) were added. The volume was adjusted to 5 ml by distilled water. The absorbance of all samples was measured at 765 nm using a spectrophotometer after incubating at ambient temperature for 30 minutes. Results were expressed as gallic acid equivalents (GAE) and calculated as mg/100g fresh weight.

2.6. Biological evaluation

The samples that recorded highest growth values and reasonable amounts of bioconstituents, together with the control and 100% chemical fertilizer, were selected to be subjected to further biological activities. *In vitro* biological studies were applied to these samples to evaluate their antioxidant, antimicrobial and cytotoxic activities.

2.6.1. Antioxidant activity using DPPH method

In vitro antioxidant activity was employed using DPPH according to Brandt-Williams method [12] with some modifications. Aliquots of 0.05, 0.01 and 0.15 ml of the methanolic stock solution were mixed with equal volumes (2.5 ml) of freshly prepared DPPH methanolic solution (20 mg/l) then the volume was adjusted with methanol to 5 ml. The absorbances of the samples and a control solution (containing 2.5 ml DPPH solution and 2.5 ml methanol) were read at 517 nm using a spectrophotometer. The inhibition percentage of DPPH (I%) was calculated, for each sample, according to the following equation: $I\% = [(A_C - A_S)/A_C] \times 100$
Where: A_C is the absorbance of control solution, and A_S is the absorbance of the sample solution. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted of I% versus concentration.

2.6.2. Antimicrobial activity

The antimicrobial activity was tested using agar well diffusion method [13]. A concentration of 100 mg/ml of each sample were separately prepared in DMSO and tested on four microorganisms; Gram-negative bacteria; *Escherichia coli* (NCTC-10416) and *Pseudomonas aeruginosa* (NCIB-9016), Gram-positive bacteria; *Bacillus subtilis* (NCIB-3610) and *Staphylococcus aureus* (NCTC-7447). The tested microorganisms were kindly supplied by the Fermentation Biotechnology and Applied Microbiology Center, Al-Azhar University, Cairo, Egypt. The well was loaded by 100 µl of the samples. Paper discs impregnated with 20 µl of a solution of 10 mg/ml of Ciprofloxacin were used as standard antibacterial.

2.6.3. Cytotoxic activity

The cytotoxic activities of the samples in different concentrations (in the range of 0-50 µg/ml) were determined using sulforhodamine B method as reported by Skehan and Storeng [15], with slight modifications, against breast (MCF-7) cell lines. Doxorubicin (DOX), the standard cytotoxic drug, was used as a positive control. IC_{50} (the concentration of DOX / samples that produced 50% inhibition of cell growth) was calculated from the curve of surviving fraction versus concentration.

2.7. Statistical analysis

Analysis of variance (ANOVA) was used to test the difference between means, which were analyzed by the Dunette´s test at 95% ($p < 0.05$) level of significance using SPSS software version 20.

3. Results and discussion

3.1. Determination of vegetative parameters

Data in table (1) revealed that the application of the yeast and biofertilizers significantly increased almost all of the growth values *viz.* leaves weight, length and number, compared to the control treatment in both seasons and this is in agreement with Farouk and Sharawy [8]. These results ensure that the growth of the bulbs and leaves of the same plant responded to the different treatments in a

similar manner. The highest leaves growth parameters, throughout this experiment, were almost afforded at the 3-months harvest (2nd stage), after which the dryness of the leaves occurred and resulted in decreased values of weight and length at the 4-months harvest (3rd stage). This disagreed with our previous work (8) as maximum growth parameters of the bulbs were afforded at the third stage.

Moreover, the highest growth values were attained by the 100% chemical fertilizer (treatment 2), 75% biofertilizer (treatment 4) and 75% yeast (treatment 8). Our results regarding the effect of the combination of biofertilizers and chemical fertilizer in increasing the growth values of leaves, are in agreement with those reported by El Khawaga [16] on guava trees and Farahat et al. [17] on *Paulownia kawakamii*. Also, our results concerning the

Table 1: Effect of Different Fertilizers on Weight, Length and Number of *Beta vulgaris* L. Leaves, During Two Seasons, at Age of 2, 3 and 4 Months after Planting

Treatments (no.)	Leaves weight (g)						Leaves length (cm)						Number of leaves					
	First season			Second season			First season			Second season			First season			Second season		
	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage
Control (1)	57.75 ± 4.65	62.33 ± .441	53.2 ± 1.39	56.50 ± 0.88	106.75 ± 4.73	83.97 ± 1.54	27.77 ± 0.37	25.00 ± 1.32	21.00 ± 1.15	31.00 ± 2.08	33.00 ± 4.62	33.50 ± 2.75	9.00 ± 1.00	13.67 ± 1.45	12.00 ± 0.58	10.00 ± 0.58	15.00 ± 0.67	15.00 ± 0.58
100% Chemical fertilizer (2)	115.9 ± 3.29*	172.93 ± 2.92*	81.1 ± 2.71*	97.1 ± 1.50*	268.2 ± 1.59*	228.6 ± 3.09*	31.5 ± 3.01	32.00 ± 2.31	32.00 ± 2.08*	38.50 ± 0.29	40.00 ± 2.89	41.50 ± 4.91	12.67 ± 2.67	18.00 ± 1.15	16.33 ± 2.85	12.00 ± 1.00	17.00 ± 1.20	23.00 ± 1.15
100% Biofertilizer (3)	68.9 ± 4.04	135.97 ± 3.32*	34.27 ± 2.07*	63.45 ± 4.56	125.00 ± 3.05*	111.95 ± 2.28*	26.67 ± 2.60	28.50 ± 2.60	20.00 ± 1.53	30.25 ± 1.59	31.00 ± 0.58	34.33 ± 3.84	11.00 ± 0.58	14.00 ± 2.31	12.00 ± 1.00	10.00 ± 1.15	15.00 ± 1.73	17.33 ± 2.85
75% Bio+ 25% chemical fertilizer (4)	98.33 ± 4.65*	149.76 ± 3.01*	66.74 ± 1.66	110.47 ± 2.05*	251.00 ± 3.46*	207.80 ± 4.21*	30.00 ± 1.53	35.00 ± 1.44*	27.00 ± 1.26*	35.75 ± 2.45	41.00 ± 0.58	37.75 ± 1.30	12.33 ± 0.88	15.00 ± 2.65	15.00 ± 1.15	13.00 ± 1.73	17.00 ± 1.53	26.00 ± 1.00*
50% Bio+ 50% chemical fertilizer (5)	74.03 ± 4.16	125.89 ± 4.18*	63.30 ± 3.18	79.43 ± 2.09*	194.5 ± 4.27*	207.80 ± 4.21*	28.00 ± 3.51	30.50 ± 0.29	23.50 ± 0.29	33.67 ± 2.03	35.50 ± 0.29	36.00 ± 1.73	12.33 ± 0.88	14.00 ± 1.00	14.00 ± 2.00	10.00 ± 0.58	16.00 ± 1.15	19.00 ± 3.00
25% Bio+ 75% chemical fertilizer (6)	84.27 ± 4.46*	125.71 ± 4.33*	56.71 ± 4.24	86.60 ± 3.90*	175.00 ± 3.97*	192.45 ± 4.04*	28.00 ± 1.44	30.75 ± 1.30	23.00 ± 1.00	33.75 ± 0.14	38.00 ± 3.06	33.00. ± 1.73	10.67 ± 0.33	14.00 ± 2.52	11.00 ± 0.58	11.33 ± 1.20	16.00 ± 1.53	21.00 ± 2.89
100% Yeast fertilizer (7)	69.40 ± 5.38	89.00 ± 4.16*	38.93 ± 4.08*	59.77 ± 2.13	151.4 ± 0.60*	178.87 ± 2.29*	26.67 ± 3.18	31.00 ± 1.73	30.50 ± 0.29*	32.50 ± 4.91	33.33 ± 3.18	31.00 ± 0.58	11.00 ± 0.58	13.00 ± 1.53	13.00 ± 1.73	10.33 ± 0.88	16.00 ± 1.15	19.00 ± 0.58
75% yeast+ 25% chemical fertilizer (8)	88.63 ± 4.65*	203.9 ± 2.95*	85.24 ± 3.05*	99.33 ± 3.05*	192.33 ± 4.91*	96.2 ± 1.95	31 ± 3.06	35.50 ± 2.29*	32.00 ± 0.58*	36.50 ± 0.87	40.00 ± 3.46	38.00 ± 3.61	11.33 ± 0.58	16.00 ± 1.15	19.00 ± 0.58*	12.00 ± .200	22.00 ± 3.18	22.00 ± 3.46
50% yeast+ 50% chemical fertilizer (9)	70.1 ± 1.21	185.49 ± 4.59*	59.96 ± 4.47	69.07 ± 1.13*	135 ± 4.62*	121.2 ± 2.48*	26.17 ± 0.73	29.33 ± 2.19	24.67 ± 1.20	32.67 ± 2.96	38.00 ± 1.53	33.00 ± 4.62	10.00 ± 0.33	14.00 ± 2.31	16.00 ± 0.58	9.00 ± 0.58	14.00 ± 0.67	21.00 ± .231
25% yeast+ 75% chemical fertilizer (10)	71.43 ± 4.92	150.58 ± 2.96*	55.32 ± 4.68	76.33 ± 1.39*	180.5 ± 4.70*	120.53 ± 2.85*	27.5 ± 0.76	31.50 ± 0.29	26.00 ± 1.53	33.50 ± 1.76	36.67 ± 0.88	31.33 ± 3.93	10.00 ± 0.33	.1433 ± 2.85	12.00 ± 1.15	11.00 ± 1.15	15.00 ± 1.15	16.33 ± 1.20

Values are mean of three results ± SE; * statistically significant from control at P≤0.05

combination of yeast and chemical fertilizer are in agreement with results of Nakayan et al. [18] on lettuce weight and Asal [19] who reported that yeast fertilizer has good efficiency on growth parameters of wheat plants.

3.2. Chlorophyll content

Regarding the results of chlorophyll contents in the leaves (table 2), it is clear that all of the applied treatments significantly increased chlorophyll (a) and (b) as compared to the control group (untreated plants) in the two seasons. However, the highest chlorophyll content was scored by the treatments 2, 4 and 8 in the two seasons. Moreover, the second stage harvest showed the highest values of chlorophyll (a) and (b) contents, followed by the first stage harvest, while the third stage harvest revealed the lowest chlorophyll content in the two seasons of this study. In addition all interactions between the studied fertilizers and harvest time statistically increased leaves chlorophyll (a & b) content when compared with control in the two seasons. Thus, treatments 2, 4 and 8 were the most effective in affording the richest chlorophyll (a) and (b) content especially those harvested at the second stage in the two seasons.

The out lined data of the carotenoids content in table (2), clarified that all of the studied fertilizers significantly increased the carotenoids content in the leaves, especially at the second stage in the two seasons. The highest values of carotenoids were recorded by treatments 2, 4 and 8, particularly those harvested at the second stage in the two seasons.

Our results emphasized the effect of the combinations of bio-/ yeast fertilizers and chemical fertilizer in increasing the photosynthetic pigments (chlorophyll a & b) and carotenoids, and these are in accordance with previous reports [17, 20-21].

3.3. Determination of betanin and polyphenolic contents

From results in table (3), it was observed that almost all results of betanin content were significantly higher than the control. The betanin content in all treatments was highest in the first stage then decreased in the second and the third stages, in both seasons. The betanin content significantly reached its maximum in leaves of treatment 4 among different concentrations of biofertilizers and 8 between the different yeast fertilizer treatments and even higher than treatment 2 in the first season. A similar trend was observed in the second season. Such results are in agreement with our previous work [8] in which highest content of betanin was afforded by the bulbs of treatments 4 and 8 in the first stage. Thus, it could be concluded that the different organs of the plant (bulbs and leaves) responded similarly to the used fertilizers, regarding the betanin content.

In case of the polyphenolic content, significant pronounced increase could be observed in nearly all treatments, in both seasons when compared to control treatment. Results in table (3) revealed

that the highest significant polyphenolic contents were that produced by treatment 4 among treatments of biofertilizers and treatment 8 of the different yeast fertilizers. It was also observed that the polyphenolic content in the studied treatments increased in the leaves of the third stage than those of the first and the second stages. Thus, the highest polyphenolic values of all treatments were obtained in the third stage in both seasons. These results are in disagreement with our previous results [8] in which highest content of polyphenolic compounds was reached by the bulbs of treatments 4 and 8 in the second stage.

Also, such results are partially in agreement with Taie et al. [22] who reported that biofertilizers play a major role in determining the level of polyphenols in soybean, whereas Matter and ElSayed [23] stated that combination of chemical and yeast fertilizers produced the highest percentage of essential oil constituents in caraway.

From the previous results, it could be concluded that the highest values among all fertilizers were afforded by the treatments 4 and 8. Thus, these samples were harvested in the second stage, in which highest vegetative parameters, highest values of chlorophyll (a & b) and carotenoids were attained. Besides, reasonable amounts of bioactive metabolites of the plant *viz.* betanin and polyphenols were afforded. Thus, they were selected to be subjected to further biological evaluation in comparison to treatment 1 (control) and 2 (100% chemical fertilizer).

Table 2: Effect of Different Fertilizers on Chlorophyll a & b and Carotenoids of *Beta vulgaris* L. Leaves, During Two Seasons, at Age of 2, 3 and 4 Months after Planting

Treatments (no.)	Chlorophyll "a" (mg/100g F.wt.)						Chlorophyll "b"(mg/100g F.wt.)						Carotenoids (mg/100g F.wt.)					
	First season			Second season			First season			Second season			First season			Second season		
	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage
Control (1)	66.40 ± 1.27	79.40 ± 3.52	56.70 ± 3.64	67.50 ± 1.96	80.40 ± 0.4	58.90 ± 1.96	44.20 ± 1.04	52.90 ± 1.27	37.50 ± 0.92	47.70 ± 1.50	80.80 ± 2.60	65.50 ± 1.56	23.70 ± 1.62	28.10 ± 1.39	19.90 ± 1.39	25.90 ± 1.96	29.00 ± 2.14	20.80 ± 1.85
100% Chemical fertilizer(2)	105.90 ± 2.37*	121.70 ± 1.91*	93.40 ± 1.44*	103.50 ± 0.98*	119.40 ± 2.37*	95.30 ± 2.54*	71.30 ± 1.85*	81.33 ± 1.57*	62.30 ± 1.04*	75.30 ± 2.83*	86.30 ± 2.42	72.50 ± 1.96	38.23 ± 1.56*	43.70 ± 1.21*	33.40 ± 1.50*	40.20 ± 2.08*	46.90 ± 1.79*	39.6 ± 2.14*
100% Bio-fertilizer (3)	95.70 ± 1.91*	111.30 ± 2.25*	83.70 ± 1.79*	98.80 ± 1.9*1	118.40 ± 1.21*	85.70 ± 1.33*	63.60 ± 1.56*	74.30 ± 2.02*	55.60 ± 2.42*	70.50 ± 3.12*	80.50 ± 1.96	69.80 ± 2.25	36.10 ± 1.27*	40.50 ± 0.92*	30.20 ± 1.04*	38.00 ± 1.91*	44.50 ± 1.56*	37.90 ± 1.21*
75% Bio+ 25% chemical fertilizer(4)	101.30 ± 4.39*	112.30 ± 3.58*	89.30 ± 2.37*	105.20 ± 1.56*	120.20 ± 0.75*	90.50 ± 1.91*	67.60 ± 1.50*	75.00 ± 1.21*	59.60 ± 2.31*	68.00 ± 1.50*	78.40 ± 1.21	65.40 ± 2.48	40.70 ± 1.27*	43.60 ± 1.62*	32.40 ± 1.62*	45.30 ± 3.00*	48.20 ± 1.56*	39.50 ± 1.96*
50% Bio+ 50% chemical fertilizer(5)	89.60 ± 2.54*	105.20 ± 5.43*	74.50 ± 2.89*	90.30 ± 3.12*	110.40 ± 0.98*	80.90 ± 2.54*	59.60 ± 1.91*	70.30 ± 2.25*	49.50 ± 1.85*	65.70 ± 2.77*	75..50 ± 2.14	56.40 ± 1.27*	32.40 ± 1.62*	38.50 ± 0.87*	26.90 ± 0.69*	40.70 ± 1.85*	43.30 ± 2.45*	35.40 ± 1.62*
25% Bio+ 75% chemical fertilizer(6)	81.30 ± 2.42*	94.20 ± 2.14*	62.40 ± 2.83	87.50 ± 3.00*	98.20 ± 0.81*	75.80 ± 2.02*	54.10 ± 1.30*	62.73 ± 1.63*	41.50 ± 1.28	60.40 ± 1.91*	70.80 ± 2.48*	52.30 ± 1.85*	29.70 ± 1.21	34.10 ± 1.50*	22.60 ± 0.98	30.90 ± 2.60	35.20 ± 2.02	29.00 ± 1.91*
100% Yeast fertilizer(7)	92.50 ± 1.91*	102.90± 1.27*	76.40 ± 1.85*	95.90 ± 1.33*	103.20 ± 1.91*	80.50 ± 2.14*	61.60 ± 1.21*	68.30 ± 1.27*	51.00 ± 2.48*	65.70 ± 2.02*	72.00 ± 1.91*	65.20 ± 2.60	33.50 ± 1.21*	37.40 ± 0.87*	28.10 ± 1.50*	34.50 ± 1.39	39.70 ± 2.19*	28.90 ± 0.87*
75% yeast+ 25% chemical fertilizer(8)	98.60 ± 2.08*	108.30 ± 1.44*	84.60 ± 2.71*	100.60 ± 2.54*	105.60 ± 1.39*	95.70 ± 1.67*	65.60 ± 2.14*	72.30 ± 1.85*	59.20 ± 1.59*	69.50 ± 2.42*	80.60 ± 1.91	68.40 ± 0.92	35.70 ± 2.77*	39.60 ± 1.85*	30.80 ± 0.92*	39.40 ± 1.62*	42.40 ± 1.50*	35.30 ± 1.85*
50% yeast+ 50% chemical fertilizer(9)	83.70 ± 1.62*	94.30 ± 1.79*	71.80 ± 2.02*	90.30 ± 2.08*	95.80 ± 2.83*	75.30 ± 2.08*	55.50 ± 2.63*	62.90 ± 2.60*	47.50 ± 1.44*	59.50 ± 2.08*	65.90 ± 1.62*	50.60 ± 2.14*	30.20 ± 2.02	34.10 ± 1.27*	25.80 ± 1.33*	35.60 ± 2.54*	39.00 ± 1.50*	32.10 ± 1.91*
25% yeast+ 75% chemical fertilizer(10)	73.60 ± 1.10	91.30 ± 2.60	61.70 ± 1.04	85.60 ± 2.48*	93.40 ± 0.92*	70.60 ± 2.08*	48.90 ± 1.85	60.90 ± 1.33*	40.80 ± 1.56	50.40 ± 2.02	63.80 ± 1.39*	45.20 ± 2.77*	26.40 ± 1.85	33.00 ± 2.42	22.10 ± 1.85	29.70 ± 1.50	36.70 ± 1.62	29.70 ± 1.56*

Values are mean of three results ± SE; * statistically significant from control at P≤0.05.

Table 3: Effect of Different Fertilizers on Betanin and Polyphenolic Contents in *Beta vulgaris* L. Leaves, During Two Seasons, at Different Stages

Treatments (no.)	Betanin(mg/100g F.wt.)						Polyphenolic content (mg/100g F.wt.)					
	First season			Second season			First season			Second season		
	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage	1st stage	2nd stage	3rd stage
Control (1)	8.63 ± 0.15	2.66 ± 0.59	1.49 ± 1.04	6.04 ± 1.40	1.21 ± 0.07	0.67 ± 0.02	72.60 ± 1.15	76.00 ± 0.58	158.93 ± 4.92	27.19 ± 0.44	54.53 ± 0.53	62.94 ± 0.70
100% Chemical fertilizer (2)	17.60 ± 2.51*	15.61 ± 1.38*	10.53 ± 1.82*	18.57 ± 0.11*	9.18 ± 0.77*	4.46 ± 0.09*	74.77 ± 0.65	121.83 ± 3.50*	173.45 ± 2.74*	60.66 ± 0.81*	84.96 ± 0.68*	114.61 ± 0.79*
100% Biofertilizer (3)	.869 ± 0.24	2.83 ± 0.32	.188 ± 0.07	8.66 ± 0.05	1.77 ± 0.05	0.68 ± 0.01	82.50 ± 0.29*	99.67 ± 0.80*	260.20 ± 1.43*	27.99 ± 0.86	55.98 ± 0.79	80.06 ± 2.36*
75% Bio+ 25% chemical fertilizer (4)	30.29 ± 0.16*	20.12 ± 0.15*	15.58 ± 0.78*	17.70 ± 0.14*	10.51 ± 0.14*	6.28 ± 0.32*	100.00 ± 0.58*	130.00 ± 0.06*	615.85 ± 2.92*	56.48 ± 2.41*	67.79 ± 1.53*	123.92 ± 0.82*
50% Bio+ 50% chemical fertilizer (5)	17.55 ± 1.27*	18.49 ± 2.40*	11.24 ± 0.73*	14.69 ± 0.15*	1.96 ± 0.20	0.89 ± 0.11	75.67 ± 0.84	79.63 ± 0.30	472.73 ± 0.47*	33.57 ± 0.44*	40.09 ± 0.40*	59.43 ± 0.35
25% Bio+ 75% chemical fertilizer (6)	17.87 ± 3.02*	11.51 ± 1.44*	10.03 ± 2.91*	6.74 ± 0.52	3.68 ± 0.15*	1.56 ± 0.07*	97.97 ± 3.84*	128.10 ± 0.10*	466.47 ± 4.13*	53.14 ± 0.31*	53.47 ± 0.15	72.25 ± 0.39*

100% Yeast fertilizer (7)	12.83 ± 0.28	4.58 ± 0.04	4.06 ± 0.05	7.95 ± 1.17	2.68 ± 0.07*	2.50 ± 0.02*	87.43 ± 0.67*	106.17 ± 0.35*	304.00 ± 4.76*	53.69 ± 0.43*	57.42 ± 0.59	64.67 ± 0.97
75% yeast+ 25% chemical fertilizer (8)	18.20 ± 1.57*	15.60 ± 0.15*	14.39 ± 1.92*	14.41 ± 0.79*	9.21 ± 0.09*	5.73 ± 0.17*	123.77 ± 0.55*	197.43 ± 0.50*	711.00 ± 0.98*	60.99 ± 0.78*	83.07 ± 0.57*	143.99 ± 0.77*
50% yeast+ 50% chemical fertilizer (9)	18.06 ± 2.25*	15.20 ± 0.21*	11.73 ± 1.35*	13.91 ± 0.07*	3.84 ± 0.19*	1.06 ± 0.05	103.03 ± 0.83*	155.00 ± 3.61*	685.43 ± 1.41*	44.05 ± 0.73*	64.67 ± 0.71*	88.92 ± 0.75*
25% yeast+ 75% chemical fertilizer (10)	16.63 ± 0.49*	15.19 ± 3.74*	13.09 ± 2.57*	9.76 ± 0.33*	5.51 ± 0.08*	2.62 ± 0.10*	82.60 ± 3.70*	121.00 ± 0.58*	167.53 ± 3.39	35.13 ± 0.88*	71.97 ± 0.54*	94.44 ± 0.63*

Values are mean of three results ± SE; * statistically significant from control at P≤0.05.

3.4. Biological evaluation

Data in table (4) showed that highest antioxidant activities in the selected treatments were exerted by the treatments 4 and 8. These high levels of antioxidant activity were associated with high levels of chlorophylls (a & b), carotenoids, betanin and polyphenols, which were known for their pronounced antioxidant activity [24-27].

Table 4:*In Vitro* Antioxidant Activity of the Selected *Beta vulgaris* L. Leaves

No. of treatment	Antioxidant activity (IC_{50}: mg/ml F.wt.)
1	5.18
2	1.17
4	0.28
8	0.84

Results of the antimicrobial activity outlined in table 5, showed that the tested microorganisms are moderately sensitive to the lyophilized juice (concentration = 100 mg/ml in DMSO) of treatment 4. Moreover, the control group (treatment 1) displayed an activity less broad than that of treatment 4. However, the juices of treatments 2 and 8 were shown to be inactive. The antimicrobial activity of beet leaves against the tested microorganisms is in line with the results reported by previous authors [28-29] who stated a nearly similar activity for beet root pomace extracts. Such similarity in their antimicrobial activity could be explained due to the resemblance of their bioconstituents. However, the absence of any antimicrobial activity in treatments 2 and 8 does not demonstrate the absence of bioactive constituents, but they may be present in insufficient quantities to inhibit the growth of the microorganisms. Also the presence of other metabolites which could exert antagonistic or negative effects against the bioactive metabolites, could be another reason for the inactivity [30].

Table 5: Antimicrobial Activity of the Selected *Beta vulgaris* L. Leaves

Microorganisms	Zone of inhibition (mm)					
	DMSO	1	2	4	8	Ciprofloxacin
Escherichia coli	-	-	-	15.3	-	32.0
Pseudomonas aeruginosa	-	19.0	-	17.7	-	29.0
Bacillus subtilis	-	15.0	-	15.0	-	35.0
Staphylococcus aureus	-	16.3	-	12.0	-	27.0

Values are mean of 3 replicates; zone included the well (10 mm)

The *in vitro* cytotoxic activity exhibited by the lyophilized juice of the four treatments was highest in case of treatment 4 and moderate in case of treatments 2 and 8, while the sample of treatment 1 showed the least activity. This activity is previously reported for the red beets against Ehrlich ascites carcinoma [29] and MCF-7 [31]. Nowacki et al. [31] described that red beetroots highly inhibited cancer cell proliferation and induced MCF-7 cell death but had no obvious effect towards normal cells, the activity could be due to the presence of betanin and its isomer; isobetanin. These findings could clarify our results as the sequence of the cytotoxic activity of the examined samples is in accordance with that of their betanin content i.e. the highest values of cytotoxic activity and betanin content are exhibited by leaves of treatment 4.

Table 5: Cytotoxic Activity of the Selected *Beta vulgaris* L. Leaves on MCF-7

Concentration(µg/ml)	Surviving fraction				
	1	2	4	8	Doxorubicin (Standard)
0	1.00	1.00	1.00	1.00	-
5	0.95	0.79	0.75	0.82	-
12.5	0.92	0.64	0.49	0.78	-
25	0.66	0.56	0.36	0.60	-
50	0.46	0.42	0.33	0.32	-
IC_{50} (µg/ml)	44.30	35.00	12.10	33.30	5.00

4. Conclusion

In conclusion, application of combination treatments of biofertilizers / yeast with chemical fertilizers as substitutes for full dose of inorganic fertilizers is recommended for healthy growth of red beet plant. The use of these combinations is favoured over 100% chemical fertilizers to maintain the crop and soil productivity, reduce more than 50% of recommended dose of mineral fertilizer and reduce the soil pollution, in addition to improving human health. Finally, beet leaves should be cut at the suitable time to afford satisfactory growth values and a considerably high content of biologically active metabolites.

This work could provide a starting point in the selection of beet leaves as a valuable source for bioactive metabolites and to further investigate their different extracts to isolate the constituents responsible for their biolgical activity.

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