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The effect of biostimulant and fertilizer on "low input" lettuce production

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Abstract – Lettuce production in the winter on the Adriatic coast, especially in a non-heated plastic tunnel, requires longer cultivation and is characterised by lower head mass and yield. In these conditions, the effect of biostimulant Bio-algeen S-90 and fertilizer Megagreen on the production of the traditional winter lettuce cultivar 'Four Seasons' was tested. Both treatments showed a positive effect on the growth and total yield of winter lettuce, and decreased the share of non-marketable yield. Bio-algeen S-90 treatment increased the plant height by 61.5%, and foliar treatment with Megagreen by 60.9%, as compared to the control treatment. Equally, both treatments resulted in higher leaf numbers (47.7% for Bio-algeen S-90 and 37.2% for Megagreen). The head mass of lettuce treated with Bio-algeen S-90 and Megagreen was 30.3% and 25.0% higher than in the control treatment, respectively. Megagreen contributed more to chlorophyll and carotenoid content, while Bio-algeen S-90 elevated the amount of vitamin C and dry matter. The pH value of lettuce juice decreased after Bio-algeen S-90, while the mineral content (N, P and K) did not differ between the tested treatments. Lower nitrate content was detected after both treatments. The obtained results elucidate the effect of Bio-algeen S-90 and Megagreen on "low input" lettuce production.

Key words: Bio-algeen S-90, carotenoids, chlorophyll *a* and *b*, Megagreen, mineral content, non-heated plastic tunnel, vitamin C

Introduction

Growing vegetables such as winter lettuce, rocket, leek, onion, leafy brassicas or radishes, often of local types and traditional cultivars, in semi-Mediterranean climatic conditions during the winter period with poorer light and milder temperature conditions, extends the production season, provides fresh vegetables for local markets and ensures additional income for local producers. Such production is commonly carried out in non-heated high plastic tunnels without heating and lightning. Traditional varieties are today in increasing demand not only in produce markets but also in hotels and in the local gastronomy for tourist purposes, while worldwide they are being increasingly included in revitalisation and protection programmes for growing traditional varieties, for regional supply as niche products (Lissek-Wolf et al. 2009), but also in larger variety trials (Wenz and Wenger 2012) and in organic farming.

Traditional cultivars are cultivated in Istria (northern coastal Croatia) on small areas in response to the needs of local markets, where this type of cultivar is highly appreciated, recognizable and achieves good prices. One of the widely spread traditional lettuce cultivars in Istria is 'Four Seasons'. This cultivar belongs to the class of butterhead lettuce (*Lactuca sativa* var. *capitata* L.), which is predominantly well suited for protected cultivation during winter because of its ability to develop heads of acceptable quality and mass in poorer light and temperature conditions. Its leaves are dark reddish-brown, and it is one of the darkest butterhead types of lettuce when grown in optimal growing conditions, though the colour intensity as well as the compactness of the heads depends on the weather and temperature (Wenz and Wenger 2012). The 'Four Seasons' cultivar has a long growing tradition in organic farming in early spring or for autumn and winter harvest, due to its good cold-hardiness, and as its name says, it can be cultivated all year round. However, the heat and water shortage in summer, as well as the deficiency of light and emergence of frost in the winter on the northern Adriatic coast restrict the optimal yield of the lettuce.

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The use of biostimulants and fertilizers to enhance plant yield nowadays is a regular farming practice. Biostimulants include diverse substances and microorganisms that enhance plant growth (Calvo et al. 2014). The effects of several biostimulants on plant growth from spring until autumn in the Mediterranean region have been investigated so far (Vernieri et al. 2002, Amanda et al. 2009, Lucini et al. 2015). Regarding the winter season, Johnstone et al. (2005) and Tahsin (2010) investigated the effect of preplant phosphorous and nitrogen application, respectively, on lettuce yield, but this was not in the Mediterranean region.

Organic extracts of seaweed bioactive substances have been used for ages to enhance soil fertility and crop productivity. From among biostimulants prepared from seaweed extracts, Crouch et al. (1990) investigated the effect of *Ecklonia maxima* (Osbeck) extract application on *L. sativa* L. cv. Winter Crisp. One of the most commercial seaweed extracts is made from brown seaweed *Ascophyllum nodosum* (L.) Le Jolis. However, the extract composition may vary according to the *A. nodosum* source, the season of collection and the extraction process used (Calvo et al. 2014). The *A. nodosum* extract often used in Croatia is Bio-algeen S-90.

Mineral fertilizers are products containing nutrients essential for the normal growth and development of plants (Isherwood 2000). For some period in Croatia NPK (nitrogen, phosphorus, potassium) were the most used fertilizers; however new preparations with other elements alongside N, P and K emerged and gave promising results as well. One such fertilizer is Megagreen, a Ca fertilizer with micronutrients.

Bio-algeen S-90 and Megagreen, which are both implemented in this study, are on the list of permitted fertilizers and soil conditioners for organic farming (Ministry of Agriculture RC 2013). So far, Bio-algeen S-90 has been applied on several crops, among them: cereals (Kolbe and Blau 1998), soybean (Redžepović et al. 2007), basil (Kwiatkowski and Juszczak 2011) and tomato (Mikiciuk and Dobromilska 2014). The effect of Megagreen was also studied on several cultures, like potatoes (Horvat et al. 2014) and sugar beet (Artyszak et al. 2014). Both products were revealed to have a positive effect on the investigated crops. As far as we know, the possible application of these two preparations in the winter production of leafy vegetables, including lettuce has not been tested so far. As for the production of lettuce, it has been proven that organic fertilizers are more suitable than inorganic ones in lettuce cultivation in river sand (Masarirambi et al. 2010), that toxic nitrate concentration in romaine-type lettuce increases after fertilization with sewage sludge (Castro et al. 2009), and that mineral fertilization increases the nitrate concentration in the lettuce cultivar Mantecosa (Premuzic et al. 2001).

The aim of this study was to examine the effects of Bio-algeen S-90 biostimulant and the Megagreen fertilizer on winter production of the traditional lettuce cultivar 'Four Seasons'. For that purpose we determined the effects of both treatments on: a) plant height, leaf number, head mass, marketable and non-marketable yield of lettuce, b) chlorophyll *a* and *b* content, c) carotenoid content, d) vitamin C content, e) pH of lettuce leaf juice, f) nitrate content, and g) mineral content (N, P, K).

Materials and methods

Plant material and growth condition

The experiment was carried out during 2012 – 2013 in a non-heated high plastic tunnel at the Agricultural Department in Poreč, Polytechnic of Rijeka, Croatia. The traditional cultivar of lettuce 'Four Seasons' (seeds Franchi, Italy) was sown in Klasmann 1 horticultural substrate (Klasmann-Deilmann, Germany) and transplanted into 60 cell flats (532 mm x 323 mm x 60 mm, Koplast, Croatia).

The experiment included three treatments: double watering with 1.0% Bio-algeen S-90 (200 mL per plant), double foliar treatment with 0.3% Megagreen (100 mL m^{-2}) and control. The basic unit in the experiment was the plot, 3.5 m long and 2.5 m wide (8.75 m^2), and each treatment was represented by three replicates, i.e. three plots. Plots were arranged according to a random schedule. The experiment consisted of 9 plots (in total, 78.75 m^2), the distance between plots (the track) was 0.5 m, while the total area of the experiment amounted 99 m^2. In total, 112 lettuce plants per plot were planted at a distance of 0.30 m x 0.30 m, and the total number of plants in the trial was 1008. The soil was *terra rossa* whose nutrient content and pH values were determined according to standard soil analysis methods. Soil was additionally enriched with 2.5 kg per 10 m^2 of pelleted organic compost Hortyflor Stallatico (Fomet, Italy).

All the treatments were carried out manually, using small bottles with sprayers and a container for watering with a dosing cup. Every plant in the plot was treated separately, watered or foliar treated according to the planned trials and treatments. The first application was done 6 days after planting and the second 14 days later. During frost, all variants of lettuce treatments and the control variants were additionally protected with agrotextile (17 g m^{-2}).

Bio-algeen S-90 and Megagreen preparations

Bio-algeen S-90 (Shulze & Hermsen GmbH, Germany) is an organic biostimulant derived from marine alga *Ascophyllum nodosum,* a natural algae extract, with macro- and micronutrients, containing natural chemical compounds including vitamins, amino acids and alginic acid. According to the label, Megagreen (Velebit Inf. International d.o.o., Croatia) is a Ca fertilizer with micronutrients for foliar implication: 44.1% CaO, 2.2% MgO, 1.2% Fe_2O_3, 0.7% Al_2O_3, 9.1% SiO_2, 0.11% SO_4, 132 mg kg^{-1} Mn, 60 mg kg^{-1} Zn, 22.5 mg kg^{-1} Cu, 11.5 mg kg^{-1} Pb, 3.3 mg kg^{-1} Ni, 3.25 mg kg^{-1} Cr, 0.8 mg kg^{-1} Cd and Hg in traces.

Analysis of qualitative and quantitative yield

The following parameters were monitored 82 days after the second application: plant height, leaf number, head mass, total yield, marketable yield, the share (%) of non-marketable yield (head mass lower than 100 g, visible symptoms of diseases and/or physiological disorders), chlo-

rophyll *a* and *b* content, carotenoid content, nitrate content in fresh leaves, pH value of fresh lettuce juice, vitamin C content in fresh leaves, dry matter content of leaves, and total N, P and K content in leaves.

Chlorophyll *a*, chlorophyll *b* and carotenoid content were analysed spectrophotometrically by measuring absorbance at 665, 649 and 480 nm, respectively, according to Wellburn (1994). Nitrate content was determined spectrophotometrically according to Cataldo et al. (1975). The pH value was determined in fresh lettuce juice using a pH meter (Sartorius Croatia – Libra Elektronik d.o.o., Zagreb, Croatia), vitamin C content using 2,6-dichloroindophenol titrimetric method and dry matter content gravimetrically at 105 °C until the mass became constant. Total N, P, K contents were determined according to standard ISO 11261 (2004), ISO 6878 (1998) and ISO 9964-3 (1993) procedures, respectively.

Statistical analysis

Obtained data were statistically analysed using ANOVA and post hoc Tukey test for multiple comparisons between groups on the basis of $p \leq 0.05$. Due to homogeneity of error variance, a synthesis of the results of two year studies was carried out. The obtained data was processed using SPSS v. 17.0, and using IBM SPSS Statistics Software Version 22.0 for principal component analysis.

Results

Climatic conditions in the cultivation period at the location in Poreč were characterised by mild temperatures and the emergence of frost, mostly of short duration. The average air temperature was in the range 4.8 – 7.8 °C, while soil temperature was between 5.0 and 8.7 °C (Fig. 1).

Nutrient content and pH values of *terra rossa* soil are presented in Tab. 1. The soil contained approximately 69 mg kg^{-1} K_2O, 50 mg kg^{-1} P_2O_5 and 4 mg kg^{-1} $CaCO_3$. The pH value of soil water was slightly alkaline, approximately 7.8; and of KCl approximately 7.3. The share of humus in the soil was around 4.0%.

Double watering at the start of cultivation with Bio-algeen S-90 increased the plant height of the lettuce by 61.5%, and double foliar treatment with Megagreen by 60.9%, compared to the control treatment (Tab. 2). Equally, the lettuce treated with Bio-algeen S-90 and Megagreen yielded a significantly higher leaf number compared to the control treatment (increase by 47.7% and 37.2%, respectively). The difference in leaf number between Bio-algeen S-90 and Megagreen applications was on average 7.1% and was not significantly confirmed.

Lettuce reached a head mass between 255 and 330 g (Tab. 2). Application of either Bio-algeen S-90 or Megagreen significantly increased lettuce head mass. The highest mass of lettuce head was reached after Bio-algeen S-90 treatment. It was 30.3% higher than in the control treatment, while Megagreen treatment resulted in head mass increase of 25.0%. There was no significant difference between Bio-algeen S-90 and Megagreen effects on head mass (Tab. 2).

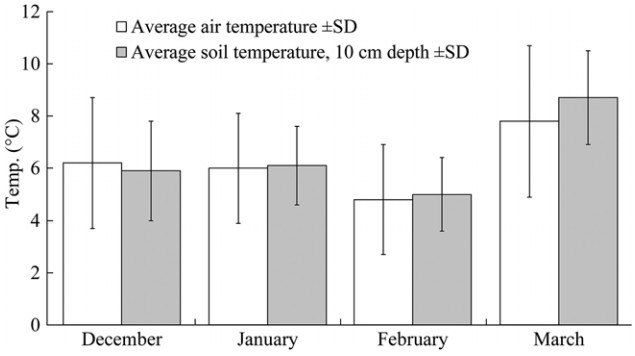

Fig. 1. Average air and soil temperature for cultivation period on location in Poreč, Croatia (December – March), (Croatian Meteorological and Hydrological Service, 2013). SD – standard deviation.

Tab. 1. Analysis of minerals (P_2O_5, K_2O and $CaCO_3$), pH value and humus content in *terra rossa* soil. Results are mean values of three replicates ± standard deviation (SD).

Depth 0–30 cm	Values±SD
P_2O_5 (mg kg^{-1})	49.98±0.45
K_2O (mg kg^{-1})	68.80±1.21
$CaCO_3$ (mg kg^{-1})	3.61±0.70
pH H_2O	7.77±0.01
pH KCl	7.26±0.01
Humus (%)	4.06±0.29

Tab. 2. The effect of Bio-algeen S-90 and Megagreen treatments on plant height, leaf number and head mass of 'Four Seasons' lettuce. Results are mean values of three replicates ± standard deviation (SD). Different letters in the same column indicate significant difference (Tukey's test, $p \leq 0.05$). LSD – least significant difference.

Treatment	Plant height (cm)	Leaf number	Head mass (g)
Bio-algeen S-90	10.87±2.97 a	19.20±4.52 a	332.10±30.87 a
Megagreen	10.83±3.19 a	17.83±3.43 a	318.74±29.33 a
Control	6.73±2.11 b	13.00±4.07 b	254.92±21.71 b
LSD, $p \leq 0.05$	3.21	4.53	35.96

Biostimulant and fertilizer treatment significantly affected total yield as well as marketable yield and share (%) of non-marketable lettuce heads, compared to the control variant. Control treatment had a significantly lower total yield and marketable yield as well as a significantly higher percentage of non-marketable heads in total yield (Tab. 3).

The influence of biostimulant and fertilizer on pigment content is shown in the Tab. 4. Chlorophyll *a* and *b* and carotenoid content varied significantly between Bio-algeen S-90 and Megagreen treatments. Compared to control, chlorophyll *b* level was significantly higher after Megagreen treatment, but chlorophyll and carotenoids did not differ significantly. Bio-algeen S-90 treatment did not change significantly the amount of any of the tested pigments.

Tab. 3. The effect of Bio-algeen S-90 and Megagreen treatments on yield, marketable and non-marketable yield of 'Four Seasons' lettuce. *Non-marketable lettuce heads with head mass lower than 100 g, visible symptoms of diseases and/or physiological disorders like loose heads or bolting. Results are mean values of three replicates ± standard deviation (SD). Different letters in the same column indicate significant difference (Tukey's test, $p \leq 0.05$). LSD – least significant difference.

Treatment	Yield (kg m^{-2})	Marketable yield (kg m^{-2})	Non-marketable yield* (%)
Bio-algeen S-90	3.1±0.12 a	2.54±0.32 a	18.02±0.61 a
Megagreen	2.9±0.08 a	2.31±0.11 a	20.22±1.11 a
Control	2.0±0.07 b	1.26±0.13 b	36.91±1.19 b
LSD, $p \leq 0.05$	0.56	0.99	12.30

Tab. 4. The effect of Bio-algeen S-90 and Megagreen treatments on chlorophyll *a* (Chl a), chlorophyll *b* (Chl b) and carotenoid (Car) contents in 'Four Seasons' lettuce leaves. Results are mean values of three replicates ± standard deviation (SD). Different letters in the same column indicate significant difference (Tukey's test, $p \leq 0.05$). FM – fresh mass. LSD – least significant difference.

Treatment	Chl a	Chl b	Car
	mg g$^{-1}_{FM}$		
Bio-algeen S-90	0.725±0.08 b	0.670±0.09 b	0.155±0.03 b
Megagreen	1.011±0.02 a	1.038±0.05 a	0.216±0.00 a
Control	0.849±0.01 ab	0.829±0.07 b	0.182±0.02 ab
LSD, $p \leq 0.05$	0.167	0.199	0.043

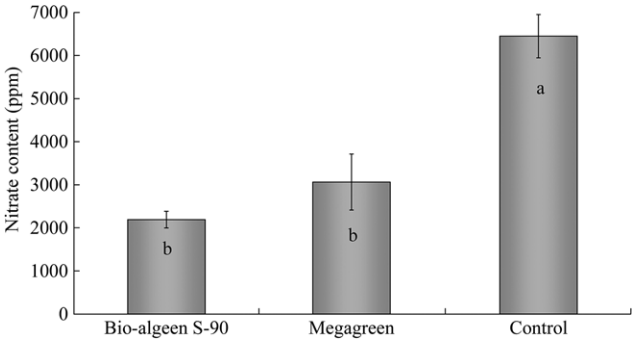

Fig. 2. Nitrate content (ppm) in fresh leaves of lettuce treated with Bio-algeen S-90 and Megagreen. The means labelled by different letters are significantly different (Tukey's test, $p \leq 0.05$).

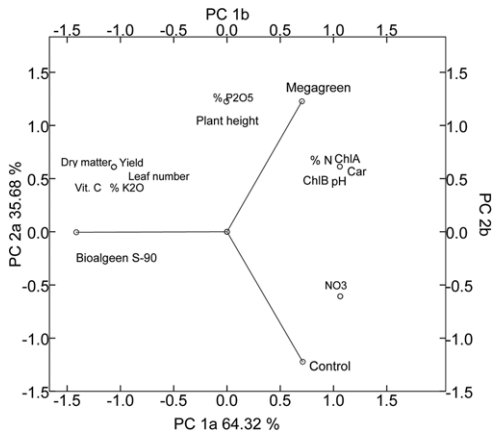

Fig. 3. Biplot of the principal component analysis based on ten tested compounds and three yield components.

The amount of nitrates in lettuce fresh leaves significantly decreased after both Bio-algeen S-90 and Megagreen treatment (Fig. 2) and was 47.5% and 34.0%, respectively of the value in control plants. There was no significant difference in treatments with Bio-algeen S-90 and Megagreen preparations with respect to their effectiveness on the level of nitrate decrease.

The pH value of fresh lettuce juice was significantly decreased only after Bio-algeen S-90 treatment (Tab. 5). Vitamin C and dry matter content increased significantly after both of the treatments (Tab. 5); however a higher increase was caused by Bio-algeen S-90 treatment.

The content of N, P and K minerals was not affected by either of the treatments (Tab. 5).

A biplot constructed by two principal components showing treatments and tested yield components is presented in Fig. 3. Principal component 1 (PC1) and principal component 2 (PC2) provided significant indications of 100% of the total variance in the data and showed clear separation of the treatments into three groups (Fig. 3).

Discussion

Agricultural growing practices have been evolving towards organic, sustainable or environmentally friendly systems. Fertilizers and lately biostimulants have increasingly been used as a tool with the potential to enable a more sustainable agriculture production (Bulgari et al. 2015). The global market for plant biostimulants and fertilizers is growing fast; according to the study mentioned by Calvo et al. (2014) the largest market for biostimulants in 2012 was Europe. The greatest increase in fertilizer consumption in Europe occurred in the second half of the 20th century (Isherwood 2000). About ten years ago the biostimulant Bio-

Tab. 5. The effect of Bio-algeen S-90 and Megagreen treatment on pH value of fresh lettuce juice, vitamin C (Vit C) content, percentage of dry matter and mineral content in 'Four Seasons' lettuce leaves. Results are mean values of three replicates ± standard deviation (SD). Different letters in the same column indicate significant difference (Tukey's test, $p \leq 0.05$).

Treatment	pH juice	Vit C mg100 g$^{-1}_{FM}$	Dry matter %	N %	P_2O_5 %	K_2O %
Bio-algeen S-90	6.27±0.05 b	17.38±2.45 a	5.93±0.82 a	3.76±0.75 a	1.09±08 a	8.42±1.67 a
Megagreen	6.43±0.03 a	15.48±1.11 b	4.43±1.41 b	4.24±0.83 a	1.23±04 a	6.66±1.55 a
Control	6.40±0.08 a	13.90±0.51 c	3.93±2.12 c	4.13±0.09 a	0.98±01 a	4.85±1.39 a

algeen S-90 and the fertilizer Megagreen appeared on the market; however there are no data about their effects on the qualitative and quantitative yields of leafy vegetables, including lettuce. Therefore, in this work the biological effects of the two preparations on "low input" lettuce cultivation in winter season were investigated.

The obtained positive effect of both Bio-algeen S-90 and Megagreen on winter production of lettuce is in accordance with similar studies on lettuce. Wenz and Wenger (2012) accordingly have obtained a positive effect of the organic chitin fertilizer Biosol®, produced on the basis of *Penicillium chrysogenumon,* on lettuce head mass, and also reported the relationship between head mass and seed origin in the same cultivar. The application of NPK-based fertilizer ONE® in the study of Bulgari et al. (2014) was as efficient in plant weight increase of lettuce as the applications we investigated. In the experiment conducted on lettuce cv. Mathilda, Sternecker and Balas (2014) achieved a head mass increase of 31.0% by using the biostimulant Plantasalva, extract from 21 plant species with associated *Lactobacillus* and yeast, which is in good accordance with our results. Amanda et al. (2009) confirmed the increase of baby leaf lettuce yield after treatment with Actiwave® biostimulant with betaine, alginic acid, and caidrine, a derivative of vitamin K1. Recently, Lucini et al. (2015) have shown that *L. sativa* L. cv. Regina di Maggio treated with Trainer, a biostimulant with plant (L) amino acids, was more resistant to yield and biomass reduction caused by stress conditions.

Treatments with both Bio-algeen S-90 or Megagreen increased plant height and number of leaves. Since both preparations affect plant height almost twice as much as head mass and leaf number, we assume that their effects could be associated with synthesis, transport and/or accumulation of auxins in lettuce. Other studies also confirmed the positive effect of biostimulants on plant growth. Vernieri et al. (2002) showed that the application of the biostimulant Radifarm, a complex of plant extracts with polysaccharides, aminoacids, betains and enriched in vitamins and micronutrients, had a positive effect on plant growth in lettuce and spinach and improved the root/shoot ratios. In lettuce, the biostimulant strongly stimulated the root growth and showed also an increase of the leaf area.

We found that Bio-algeen S-90 and Megagreen effects on chlorophyll and carotenoid contents differ significantly. Namely, Megagreen yielded higher amounts of pigments than Bio-algeen S-90 treatment. This is not surprising if we take into account that the core of each chlorophyll molecule is the Mg^{2+} ion for which Megagreen is a far better source than Bio-algeen S-90 (Megagreen contains 2.2% MgO, but Bio-algeen S-90 only 0.021% Mg; see http://www.agro-klub.com/gnojiva/shulze-i-hermsen-gmbh-284/bio-algeen-s-92-313/). Compared to control plants, only plants treated with Megagreen had increased chlorophyll *b* content. Therefore, we would recommend the use of Megagreen for the production of lettuce with higher pigment content. With regard to the treatments with Megagreen and Bio-algeen S-90, there are no comparable results in the literature for lettuce production. However, some other studies revealed the effect of the same preparations on pigment content in other cultures, e.g. the effect of the fertilizer Megagreen on potato (Horvat et al. 2014) and the effect of the seaweed extract Bio-algeen S-90 on tomato (Mikiciuk and Dobromilska 2014). Although Megagreen treatment did not influence total chlorophyll content, it increased the photosynthetic intensity and larger sized tuber yield. Mikiciuk and Dobromilska (2014) proved the increased chlorophyll and carotenoid content in small-sized tomato leaves after multiple spraying with Bio-algeen S-90 during summer cultivation in a high plastic tunnel. It also contributed to early harvest and increased the clusters' length and the number of tomato fruits in the cluster. Although results from tomato cultivation are not directly comparable with lettuce, they can serve as an orientation. Furthermore, they are not in accordance with our results observed in winter cultivation, probably due to different light conditions. As previously mentioned, the chlorophyll content depends on the environmental conditions, especially light quality and quantity (Dias et al. 2007, Kosma et al. 2013). In suboptimal conditions, those environmental factors could influence the chlorophyll content more than the treatment with biostimulants. Other reasons for the different results obtained could be the method and frequency of biostimulant application.

The presence of nitrates in vegetables, water and other food is a serious threat for human health, especially the part converted to the more toxic nitrites (Santamaria 2006), which account for approximately 5.0% of all ingested nitrates (Spiegelhalder et al. 1976, Pannala et al. 2003) but can reach to 20% for individuals with a high rate of conversion (Thomson et al. 2007, Correia et al. 2010). In our experiment, we found that nitrate content in the control plants, with a value of 6,446 ppm, exceeded the permissible limit of nitrate in leafy vegetables (Commission Regulation 1258/2011). However, after either Bio-algeen S-90 or Megagreen treatment of fresh lettuce leaves, the amount of nitrates was significantly decreased, to 34.0 and 47.5% of the control value, respectively (Fig. 2). Since in lettuce *L. sativa*, protected or open grown, harvested in the period from October 1 to March 31 the limit amount of nitrates is 4,500 ppm expressed on the fresh weight basis, Bio-algeen S-90 or Megagreen are obviously great treatments for nitrate-related toxicity reduction of lettuce. Compared to the sewage sludge used by Castro et al. (2009), and mineral (94% $Ca(NO_3)_2$ + 6% NH_4NO_3) and organic (biostabilised compost) fertilizers used by Premuzic et al. (2001), in terms of nitrate concentration, Megagreen fertilizer would be a better choice for lettuce production.

The pH value of fresh lettuce juice, as expected, varied within the slightly acidic range, between 6.27 and 6.43. US FDA/CFSAN (2007) indicates approximate pH values for lettuce in general between 5.80 – 6.15, noting that pH values of vegetable juices can vary according to the cultivar, season, growing conditions or processing methods. In our experiment, the pH value of fresh lettuce juice was decreased after Bio-algeen S-90 treatment, and we presume this could be due to amino acids and alginic acid present in this preparation (see "Materials and methods" section), as well as due to a significantly higher amount of vitamin C (ascorbic acid) detected after this treatment (Tab. 5).

Both of the treatments significantly increased the amounts of vitamin C and dry matter content, however a higher increase was caused by Bio-algeen S-90 treatment. This was expected since Bio-algeen S-90 preparation already contains natural chemical compounds, including vitamins. Thus, even though after Bio-algeen S-90 and Megagreen treatments the total yield increased similarly, in case of need for higher dry mass, we would recommend the use of Bio-algeen S-90 preparation.

While vitamin C differed significantly between the tested treatments, mineral contents in lettuce leaves did not change (Tab. 5) and were in the range indicated in literature (Ismail and Fun 2003, Koudela and Petříková 2008, Masamba and Nguyen 2008). Since Bio-algeen S-90 preparation contains amino acids, we expected that this treatment would increase the concentration of N in lettuce leaves; however, this was not the case. We speculate this could be due to limited root absorption of amino acids from the soil and/or retention of amino acids in the lettuce root. Compared to the result of Premuzic et al. (2001) who found that N or biostabilised compost fertilization does not change lettuce vitamin C content, we found that Megagreen fertilizer increased vitamin C content in lettuce and in this term suggest it as a better choice for lettuce treatment.

The principal component analysis (PCA) based on ten tested compounds and three yield components enabled visualization of correlations between analysed data. Principal component 1 (PC1) and principal component 2 (PC2) analyses provided significant indications of 100% of the total variance in the data and showed clear separation of the treatments into three groups (control, Bio-algeen S90 and Megagreen).

Based on the obtained results we conclude that application of either Bio-algeen S-90 or Megagreen in winter cultivation conditions showed a significant positive effect on the growth, head mass and yield of lettuce fresh leaves compared to the control treatment. The content of lettuce chlorophylls *a* and *b* and carotenoids was significantly higher after Megagreen treatment compared to Bio-algeen S-90 treatment; however, compared to control plants, Megagreen treatment significantly increased only chlorophyll *b* amount. The content of lettuce vitamin C and dry matter was significantly increased after both treatments (more after Bio-algeen S-90 and less after Megagreen treatment). Both treatments significantly decreased toxic nitrate content and the share of non-marketable yield in total lettuce yield. The pH value of lettuce juice decreased after Bio-algeen S-90 treatment. Mineral content (N, P, K) of lettuce leaves was not affected by either biostimulant or fertilizer application. Finally, since the potent biostimulant Bio-algeen S-90 is a preparation derived from the northern Atlantic Ocean alga *A. nodosum*, and Croatia is a maritime state with various types of algae in its Adriatic sea, we propose investigation of Adriatic algae extracts as possible biostimulants.

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