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Original Article

Effect of Bacterial-algal Biostimulant on the Yield and Internal Quality of Lettuce (*Lactuca sativa* L.) Produced for Spring and Summer Crop

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Abstract

Plant biostimulants can enhance crop nutrition status, stress tolerance, yield and quality in environment-friendly manner. The aim of this study was to determine the effect of an algae and bacteria preparations on the yield and nutritional parameters of leaf and romaine lettuce cultivated for spring and summer crop. The application of a combined biostimulant consisting of plant growth-promoting bacteria (*Bacillus licheniformis*, *Bacillus megatherium*, *Azotobacter* sp., *Azospirillum* sp., and *Herbaspirillum* sp.) and fresh water algae (*Chlorella vulgaris*) was done by watering the lettuce every 14 days, and a determination of the fresh weight, total antioxidant capacity, and total carotenoids content was performed. The result revealed that the application of bacterial-algal preparation significantly affected the plant weight of both romaine and leaf lettuce in spring and summer seasons. The highest increase in the weight of romaine lettuce reached 18.9% in the spring crop, while in the case of leaf lettuce, biostimulant treatment led to a 22.7% higher weight in the summer crop. Total antioxidant capacity and total carotenoids content showed increased values in the summer crop of romaine lettuce, while for the leaf lettuce there were no differences between treatments. Therefore, the positive effect of bacterial-algal treatment on lettuce yield, total antioxidant capacity and total carotenoids confirm that it could be applied for improving romaine lettuce yield quality and quantity, especially in stress, summer conditions.

Keywords: sustainable agriculture; microalgae; PGPB; total antioxidant capacity; total carotenoids

Introduction

Plant biostimulants, described as any substance or microorganism applied to plants with the aim of enhancing nutrition efficiency, abiotic stress tolerance, and/or crop quality traits (du Jardin, 2015), have a steadily growing market, according to market analysis reports (Marketsandmarkets.com, 2014). Microorganisms and their metabolites that are capable of enhancing soil fertility, crop growth, and/or yield could provide an alternative to agricultural chemicals (Von Bennewitz and Hlušek, 2006).

Freshwater microalgae are known to produce a broad spectrum of biologically active compounds such as phytohormones, enzymes, antibiotics, and vitamins (Tsavkelova *et al.*, 2006; Bertoldi *et al.*, 2008). In addition, microalgae extracellular enzymes can transform nutrients to more bioavailable forms that improve nutrient availability for both soil microflora and plants. Examples of this are the phytohormones produced by freshwater algae, which can directly affect the physiological processes, morphogenesis, reproduction, rhizogenesis, and growth of cultivated plants (Tarakhovskaya *et al.*, 2007).

Together with algal antimicrobial compounds, plant growth-promoting bacteria (PGPB) has been shown to protect plants from phytopathogenic fungi and bacteria. An important property of PGPB is its production of biopolymers, which improve the water-retention capacity of rhizosphere and are reported to be the protection mechanism of PGPB against drought stress (Noaman *et al.*, 2004). Bacteria interactions with plants can influence the supply of nutrients, increase nutrient use efficiency, induce disease resistance, enhance abiotic stress tolerance, and modulate morphogenesis through plant growth regulators (Ahmad *et al.*, 2008).

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Herbaspirillum sp. produces auxins (Yin *et al*., 2015), which support the root system of plants (Takahashi, 2013), and extracellular phosphatases (Pfeiffer, 1996), which exposes phosphorus to plants (Lee, 1988); *Chlorella vulgaris* helps to start initial bacterial growth (Cole, 1982; Watanabe *et al*., 2005); *Azotobacter* sp. produces extracellular biopolymers (Remminghorst and Rehm, 2006), which are necessary for aquaretence capacity cover (Or *et al*., 2007; Redmile-Gordon *et al*., 2015); while *Azotobacter* sp. and *Azospirillum* sp. fix nitrogen and produce siderophores (Steenhoudt and Vanderleyden, 2000; Jiménez *et al*., 2011).

There is increasing interest in enhancing the nutritional quality of fresh produce. Leafy vegetables, mainly different varieties of lettuce, are commonly cultivated and consumed all over a world. The optimal temperature range for lettuce development is 15-20 °C, growth inhibition emerges under high temperatures in summer (Han *et al*., 2016). Summer production offered the lower glucose and fructose as well as higher nitrate content in lettuce as compared to spring cultivation (Fallovo *et al.,* 2009). There was also noted lower water use efficiency due to the high radiation and temperature that may have reduced the rate of photosynthesis and increased respiratory losses. The sensory properties of lettuce also fluctuated widely throughout the growing season, at higher temperatures and bitterness increased generally in relation to higher phenolic content (Bunning *et al.,* 2010). The effect of growing season on leafy lettuce yield and quality was more pronounced than the effect of nutrient solution composition (Fallovo *et al*., 2009). Additionally the proper mineral nutrition and chemical protection of lettuce is difficult because of short vegetation period and risk of high level of nitrate concentration and pesticide residues (Skovgaard *et al*., 2017).

Leafy vegetables are the most often eaten raw so they need specific attention with respect to safety, guaranteed, inter alia, by organic production technologies including application of beneficial microorganisms. A significant contribution of *Azotobacter* and vermicompost on lettuce yield was reported by Chatterjee (2015). According to Muymas *et al*. (2015), no significant differences in fresh weight and dry weight were observed between the control and lettuces treated with *Bacillus licheniformis*. The leaf number, width, and length of treated lettuces were slightly lower than the control plants in the first and third crop seasons. These results indicate that the application of *Bacillus licheniformis* alone did not promote the growth of lettuce in any crop season. Colla *et al*. (2015) stressed that the application of the biostimulant tablet containing *Glomus intraradices* and *Trichoderma atroviride* can promote transplant establishment and vegetable crop productivity in a sustainable way. There is already some evidence of increases in the total phenolics and flavonoids content and antioxidant activity in spinach after the application of algae extracts (Fan *et al*., 2011). Lettuce grown in stress conditions, treated with a microbial-based biostimulant containing *Rhizophagus intraradices* and *Trichoderma atroviride*, was characterised by higher chlorophyll content and photochemical activity of PSII, and a better nutritional status in the leaf tissue (Rouphael *et al*., 2017). According to mentioned references rhizosphere bacteria can have negative, neutral, or positive effects on plant growth. Most recommendations for the soil application of beneficial microorganisms in vegetable production do not take into account environmental conditions. Therefore, different temperature, water and solar radiation conditions may be good treatments variable determine possible interactions of environmental conditions and beneficial microorganisms application.

We hypothesised that the combination of freshwater algae and plant growth-promoting bacteria affect yield and nutritional value of leaf lettuce and romaine lettuce and the influence should be different depending on the term of cultivation. We assumed that beneficial microorganisms can promote lettuce growth especially in summer season when high temperature can be stressful for plants.

Materials and Methods

Biological material

Leaf lettuce (*Lactuca sativa* var. *crispa* L.) cv. 'Santoro' and romaine lettuce (*Lactuca sativa* L. var. *longifolia* Lam.) cv. 'Quintus' were grown as spring and summer crops in a commercial field on medium-heavy loam soil (N - min. 5.6 $mg{\cdot}kg^{-1}$) in a warm, slightly dry climate region (GPS 49°9'55"N, 16°38'14"E) in the Czech Republic (average annual temperature 8-9 °C, average rainfall 500-600 mm). Crops were grown using standard commercial management practices. The spring 4-week-old seedlings of both varieties were planted on April 25, with spacing of 0.3 × 0.25 m. The harvest was realised on June 6 (leaf lettuce) and June 16 (romaine lettuce). The summer 3-week-old seedlings of both types were planted on June 19, with of spacing 0.3 × 0.25 m. Fertilisation, included NPK (7-20-28) totally with 83 $kg{\cdot}ha^{-1}$ of N, was done 2 weeks before planting. Irrigation was applied by overhead sprinklers for 3 h, three times per week (10 mm each), started immediately after planting. The local meteorological station recorded a mean temperature and mean maximum temperature which is presented in Table 1. Mean temperature was within the optimal limit recommended for lettuce by Han *et al*. (2016) in spring season with the exception of 8th week of growing, but exceeded this limit in 5-8th week in summer season. Maximum temperature was above the optimal level during whole summer growing period.

Treatment and experimental design

Plant growth-promoting substances protected as utility model No. 29940 by the Czech Industrial Property Office, was used as the treatment. This product contains a mixture of the following bacterial and algal cultures: *Bacillus licheniformis* strain RAWAT 7C, *Bacillus megatherium* strain RAWAT 2A, *Azotobacter* sp., strain RAWAT 15C, *Azospirillum*, strain RAWAT 21A, *Azotobacter* strain RAWAT 13B, *Herbaspirillum* sp. strain RAWAT 31D and strain 35, *Chlorella vulgaris* strain Marsalek 85, at a concentration of 10^7 $cfu{\cdot}g^{-1}$. The composition follows the biologically active compounds produced by particular microorganisms. Bacterial-algal treatment (BA) was applied by watering in 0.4 l of substance per plant every 14 days, and a total of four applications were performed. In the control treatment, only fresh water was applied.

Table 1. Mean and maximum temperatures (°C) during spring and summer season of lettuce cultivation in the field

Spring growing season			Summer growing season		
Calendar week	Mean temp.	Max temp.	Calendar week	Mean temp.	Max temp.
21-27 April	14.8	19.1	16-22 June	17.9	22.8
28 April-4 May	13.5	19.4	23-29 June	18.7	24.9
5-11 May	13.5	18.7	30 June-6 July	19.4	24.7
12-18 May	10.7	14.1	7-13 July	20.5	26.0
19-25 May	20.0	25.5	14-20 July	24.1	30.7
26 May-1 June	15.2	20.0	21-27 July	22.1	28.5
2-8 June	18.3	23.8	28 July-3 August	22.5	27.1
9-15 June	22.3	27.8	4-10 August	21.8	26.8
16-22 June	17.9	22.8	-	-	-
Mean	16.24	21.24	Mean	20.88	26.44

The experiment evaluating the effect of BA treatment was arranged in a split-plot design containing 350 plants in each treatment, which consisted of four replicates. For the determination of lettuce weight, ten plants per replicate were selected according to quality standards for commercial production (UNECE Standard FFV-22, 2012).

Evaluation of yield, nutrition's and statistical analysis

The harvest was realised on August 4 (leaf lettuce) and August 8 (romaine lettuce) according to the market quality standards (UNECE Standard FFV-22, 2012). Whole rosettes were harvested by hand, the outer leaves with the symptoms of damage or deterioration were removed, plants were weighted to estimate the marketable yield. For nutrient analysis, three plants per a replicate were collected at harvest time. A 20 g sections of each head of lettuce, including inner and outer leaves were homogenised and used for subsequent analyses. Antioxidant activity was determined using the modified DPPH (2.2-diphenyl-1-picrylhydrazyl) assay (Brand-Williams *et al.*, 1995), with absorbance at 515 nm. The results were expressed as mM Trolox equivalents (TE) per 1000 g of fresh weight (FW). The total carotenoids were determined by spectrometry at a wavelength of 440 nm, using a Jenway 6100 (GB) spectrophotometer and expressed as $\mu g \cdot g^{-1}$ of FW, according to Holm (1954). Data were compared using analysis of variance (ANOVA) at a significance level of $p < 0.05$. Data were analysed using Statistica 12 (StatSoft).

Results and Discussion

The effect of bacterial-algal preparation on lettuce yield

The application of the BA preparation significantly affected the romaine and leaf lettuce weight (Fig. 1). Average romaine lettuce weight varied between 526-690 g, which is higher than the range of 243-356 g reported by Hoque *et al.* (2010) for romaine lettuce under different N fertilisation, and lower than that of Thorp *et al.* (2016), who obtained an average lettuce weight of 715-998 g. Average weight of leaf lettuce varied between 268 and 415 g which is in line with the results of Esringu *et al.* (2015), who obtained an average leaf lettuce weight of around 346 g. The application of BA led to a significantly higher lettuce weight compared to the control; the increase of lettuce weight was 18.9% and 12.9% for roman lettuce and 16.5% and 22.7% for leaf lettuce, in the spring and summer crops, respectively. The lower yield harvested in summer season indicated the temperature stress which was significantly moderated by BA application in both lettuce varieties. Numerous studies have indicated that the application of combined microbial preparations (polyvalent inoculum) can sometimes give better results than untreated control (Jarak *et al.*, 2006), which is in line with our findings. The combined preparation used in this trial increased the lettuce weight for both varieties. The results of our experiment show that the combination of selected bacteria and fresh water algae can be promising way to positively influence crop yield especially in stress conditions.

The effect of bacterial-algal preparation on lettuce total antioxidant capacity

Lettuce may serve as potential dietary sources of natural phenolic antioxidants (Liu *et al.*, 2007). The TAC for the spring and summer crop was generally higher in leaf lettuce as compared to romaine. In the spring crop of romaine lettuce, the TAC for BA treatment was 23% lower but in the summer crop 2.5 times higher as compared to the control (Fig. 2). For leaf lettuce differences were not significant. TAC values noted for both lettuce varieties were higher than those of Reyes *et al.* (2007). Being a cool-season crop, lettuce is particularly vulnerable to heat stress. According the meteorological data (see M&M), it is obvious that the summer crop was grown under a higher temperature, compared to the spring crop. Certain plant stress incidence could be assumed. Almeselmani *et al.* (2006) and Babu *et al.* (2008) stated that tolerance to high temperature stress in crop plants has been associated with an increase in antioxidative capacity. In the case of romaine lettuce, low antioxidant capacity in the summer crop was significantly improved by BA treatment, leading to increase the tolerance against heat stress and better yielding as compared to the control treatment in this season of cultivation. According to Liu *et al.* (2007) leaf lettuce possessed the highest TPC and highest DPPH scavenging ability, followed by romaine, butterhead and Batavia. In the conditions of present experiment, the TAC of leaf lettuce remain high and stable independently on season and treatment. For this variety not TAC but the other mechanisms could be involved in positive reaction on BA treatment.

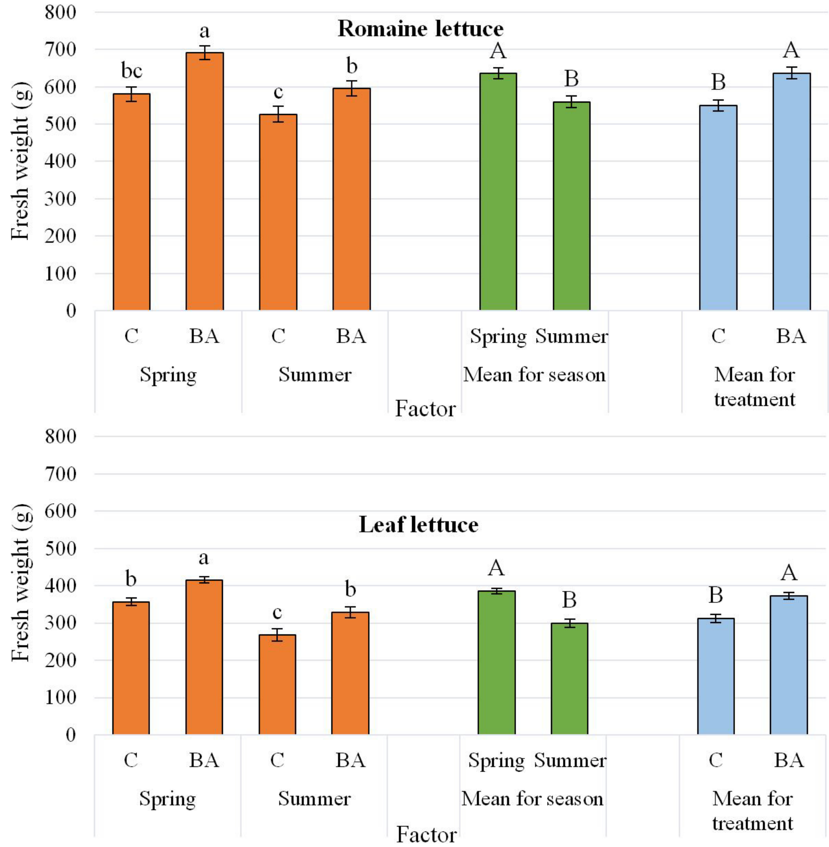

Fig. 1. Effect of bacterial-algal application, term of cultivation and their interactions on the fresh weight (g) of two lettuce varieties. Note: different lowercase letters represent significant ($p < 0.05$) differences in the interaction effects, while capital letters – in the main effects as determined by a two-way ANOVA and Fisher's LSD test, bars represent standard errors (± SE). BA - bacterial-algal preparation, C – control

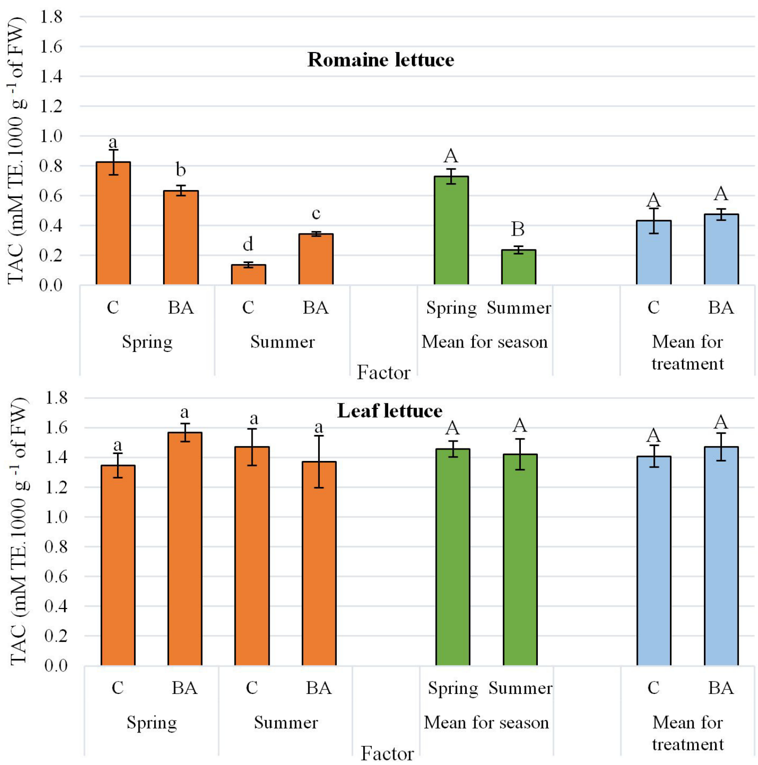

Fig. 2. Effect of bacterial-algal application, term of cultivation and their interactions on the total antioxidant activity (mM Trolox·1000g^{-1} FW) of two lettuce varieties. Note: different lowercase letters represent significant ($p < 0.05$) differences in the interaction effects, while capital letters – in the main effects as determined by a two-way ANOVA and Fisher's LSD test, bars represent standard errors (± SE). BA - bacterial-algal preparation, C – control

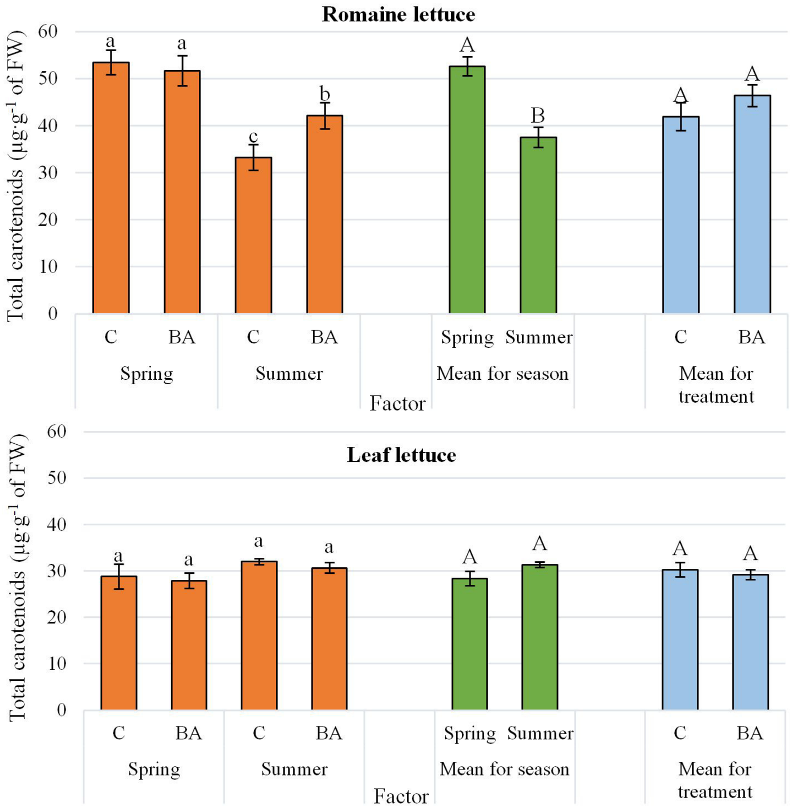

Fig. 3. Effect of bacterial-algal application, term of cultivation and their interactions on the total carotenoids content (μg·g^{-1} FW) of two lettuce varieties. Note: different lowercase letters represent significant ($p < 0.05$) differences in the interaction effects, while capital letters – in the main effects as determined by a two-way ANOVA and Fisher's LSD test, bars represent standard errors (± SE). BA - bacterial-algal preparation, C – control

The effect of bacterial-algal preparation on lettuce total carotenoids content

Total carotenoids content varied between 33.21 and 53.47 μg·g^{-1} FW for romaine lettuce but significant differences between treatments were observed only during summer time of cultivation (Fig. 3), BA treatment led to a significant increase of total carotenoids, of 26.7%. These values are lower than those reported by Saini *et al.* (2016), where the average content of total carotenoids in green romaine lettuce reached 88.4 μg·g^{-1} FW. The results obtained for leaf lettuce are slightly below the level 37.75 μg·g^{-1} FW reported by Amanda *et al.* (2009), where the summer crop grown under a plastic tunnel reached a generally higher total carotenoids content compared to the spring crop, despite their being no difference between the treatments. Romaine and leaf lettuce head structure determine the penetration of the sunlight and therefore accelerating the synthesis of light-dependent metabolites. This conclusion can be supported by the results of Mou and Ryder (2004) on relationship between the nutritional value and the head structure of lettuce. Subsequent investigations of Mou (2005) on genetic variability in carotenoid contents of 52 genotypes of lettuce allow for recognise leaf and romaine lettuce as the best carotenoids source, followed by butterhead and crisphead varieties. Additionally, our results let to conclude that summer high temperatures and insolation seem to promote decreasing of total carotenoids content in romaine but not leaf lettuce.

Conclusions

The application of bacterial-algal preparation of lettuce can be an environmentally friendly manner of improving the yield and biological quality, especially in stress conditions of summer cultivation. Microbial inoculation applied to alleviate stresses in plants could be more cost-effective and available in a shorter time, compared to the development of a stress tolerant crop genotypes. There is a need for further studies with more vegetable species included as well as different production periods to find out which factors (climatic, agronomic, etc.) enhance the BA effect on plant nutritional quality.

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