Bulgarian Journal of Agricultural Science, 20 (No 6) 2014, 1361-1367
Agricultural Academy

THE EFFECT OF A COMPLETE FERTILIZER FOR LEAFY VEGETABLES PRODUCTION IN FAMILY AND URBAN GARDENS

R. BULGARI[1*], N. PODETTA[1], G. COCETTA[1], A. PIAGGESI[2] and A. FERRANTE
[1]*Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, Università degli Studi di Milano, 20133 Milan, Italy*
[2] *Valagro S.p.A., 66041 Piazzano di Atessa (Chieti), Italy*

Abstract

BULGARI, R., N. PODETTA, G. COCETTA, A. PIAGGESI and A. FERRANTE, 2014. The effect of a complete fertilizer for leafy vegetables production in family and urban gardens. *Bulg. J. Agric. Sci.*, 20: 1361-1367

The home vegetables cultivation in family or urban gardens for self -consumption is mainly performed as a hobby but without following the good agricultural practices. Home gardening is often carried out by inexperienced people, which may lead to wrong fertilization. In this work, a fertilizer was evaluated for providing the mineral nutrients required from vegetables and stimulate the nutrient use efficiency in the plant. The commercial product ONE®, commercialized by Valagro S.p.A., was tested on two different leafy vegetables, *Lactucasativa* L. and *Cichoriumendivia* L. In order to discriminate the nutritional effect from the biostimulant effects, ONE® treatments were compared to a control added with the minimum crops requirements and to solutions containing the same concentrations of macro-nutrients as in ONE®. Results showed that ONE® treatments were able to speed up the growing rate of plants that reached the commercial maturity earlier. The chlorophyll content was higher in ONE® treatments, showing a positive effect on the visual appearance of the vegetables. Nitrate content in lettuce was below 500 mg/kg fresh weight, a value much lower than the commercialization law limits. Treatment caused a higher increase of reducing sugars in lettuce respect to endive. In lettuce the sucrose content was higher in outer leaves compared to the inner leaves, while opposite results were found for total sugars. In endive sucrose and total sugar were lower in ONE® treatments compared to control. In conclusion, considering yield and quality parameters, ONE® was effective in improving the quality of the leafy vegetables tested.

Key words: fertilization, *Lactuca sativa* L., *Cichorium endivia* L., yield, quality, biostimulant

Introduction

Salad production in family gardens for self-consumption often is not performed following the good agricultural practices. The most part of the farming operations are approximate, especially the fertilization. In fact, inexperienced people (Tei and Gianquinto, 2010) often carry out vegetables cultivation in the urban area. Therefore, the urban gardens are performed almost exclusively as a hobby by employees, retired or disabled people (Tei, 1997) with the aim to satisfy the family needs, for spending part of the day in contact with nature, for physical exercise and for meeting with other people (La Malfa, 1997). Organic or slow-release fertilizers usually provide the nutrient supply. Unfortunately, the mineral nutrient release does not often coincide with the nutrient uptake rate of vegetables, especially during summer when the biological cycle is shorter. Moreover, high temperatures increase the mineralization of the organic matter, causing high leaf nitrate content. A wrong fertilization for vegetables can produce a nitrate excess in the leaves, beyond the EU regulation limits. Nitrate itself is relatively non-toxic but its metabolites may produce a number of negative health effects (Santamaria, 2006). Nitrate in human diet may cause gastrointestinal cancer and methemoglobinemia (Du et al., 2007). There are not quality controls on homemade vegetables and, therefore, a wrong fertilization might cause health problems to the unaware farmer-consumer. Hence, the existing commercial products are not only made to nourish plants but also

E-mail: roberta.bulgari@unimi.it*; nadia.podetta@unimi.it; giacomo.cocetta@unimi.it
antonio.ferrante@unimi.it; a.piaggesi@valagro.com

to stimulate the nutrient use efficiency in order to increase growing and quality production. A lot of these biostimulants are composed by unknown components, like some amino acids that stimulate the plant metabolism (Vernieri and Mugnai, 2003). The combined application of fertilizing elements and biostimulants might improve the nutrient use efficiency of the plant and at the same time reduce the fertilizers supply, with benefits for consumers and environment.

The aim of this work was to study the effect of ONE® on Iceberg lettuce and endive as nutrient supply and plant biostimulant. The efficiency of this product was evaluated considering some quantity and quality parameters.

Materials and Methods

Plant material, cultivation and fertilization

Iceberg lettuce plants (*Lactuca sativa* L., var. Capitata "Regina dei ghiacci") and endive (*Cichorium endivia* L., var. Latifolia) were transplanted in plastic pots with peat and fertilization was performed according to the plant nutrient uptake. The plant density was 9 plants/m^2 for lettuce and 8 plants/m^2 for endive. In each pot 14 g of fertilizer (N-P-K: 13-8-24) were mixed with peat to provide the 100% of plant nutrient requirements.

The ONE® fertilizer contains the following mineral elements: nitrogen (N) 5.5% subdivided in 0.5% organic and 5% ureic , phosphorus (P_2O_5) 5%, potassium (K_2O) 7.5%, organic carbon 4.5%, soluble iron in water 0.1% and chelate with EDDHSA 0.1%, soluble manganese in water 0.03% and chelate with EDTA 0.03%, water soluble zinc 0.04% and chelate with EDTA 0.04%. In order to evaluate the effect of ONE® as fertilizer and biostimulant a NPK solution containing the same mineral nutrient concentration of ONE® was prepared. In particular, 0.1 g of N-P-K 20-20-20 fertilizer and 0.1 g of K_2SO_4 were dissolved in 2 L of water. The application of treatments was performed one week after the transplanting, according to the following scheme:

- Control (400 mL/plant of water);
- Control fertilized with NPK at 50% dose (200 mL/plant of water and 200 mL/plant of NPK solution);
- ONE® at 50% dose (200 mL/plant of water and 200 mL/plant of ONE® solution);
- Control fertilized with NPK at 100% dose (400 mL/plant of NPK solution);
- ONE® at 100% dose (400 mL/plant of ONE® solution).

In the first application, 60 mL of ONE® were diluted in 3 L of water, as label instructions. From the second application, the treatments were performed every two weeks, using a 50% dose of both ONE and NPK (30 mL of ONE® diluted in 3L of water and 50 mg of fertilizer N-P-K 20-20-20 and of K_2SO_4 in 2 L of water). Vegetables were watered initially with 0.5 L/plant and then with 1L/plant considering the daily water consumption for each species.

Determination of head weight, chlorophyll, sucrose, reducing sugars and total sugars content

Each head of lettuce or endive was cut at the collar, released by outer damaged leaves and then was weighed. Dry weight was determined after over-dry desiccation at 80°C until a constant weight was reached.

Chlorophyll content was determined by chlorophyll meter (CL-01, Hansatech, UK). About 1-2 g of leaves was ground in distilled water for both sucrose determination and total sugars. Homogenate was centrifuged at 10000 rpm for 5 min. For sucrose determination, 0.2 mL of extract were added to 0.2 mL NaOH 2N and incubated at 100°C for 10 min; then 1.5 mL of hot resorcinol were added and incubated at 80°C for 10 min. A resorcinol solution was prepared by adding 35 mg of resorcinol and 90 mg of thiourea in 250 mL HCl 30%, mixed with 25 mL of acetic acid and 10 mL of distilled water. Samples were cooled at room temperature and spectrophotometer readings were performed at 500 nm and a calibration curve was built with sucrose standards at 0, 0.5, 1, 1.5, 2 mM.

The reducing sugars analysis was performed using 0.2 mL of crude extract that were added to 0.2 mL of dinitrosalicylic acid (DNS). The reaction mixture was heated at 100°C for 5 min, then 1.5 mL of distilled water was added and absorbance readings were taken at 530 nm. The reducing sugars were expressed as glucose equivalent using a glucose standard curve (0, 1, 2, 3 and 4 mM).

Total sugars were calculated by anthrone method: 0.2 g of anthrone was melted in 100 mL of H_2SO_4 and shaked for 30-40 min. 1 mL of extract was added to 5 mL of anthrone solution, cooled in ice for 5 min and mixed thoroughly. Samples were incubated at 95°C for 5 min and then cooled on ice. Absorbance readings were measured at 620 nm and a calibration curve was built with glucose standards at 0, 1, 2, 3 and 4 mM.

Determination of nitrate content

Nitrate content in leaves was measured with the salicylsulphuric acid method (Cataldo et al., 1975). 1 g of leaves was ground in 4 mL of distilled water. The extract was centrifuged at 5000 rpm for 5 min and the supernatant was recovered and used for the colorimetric determination. 20 µL of sample were added to 80 µL of 5% salicylic acid in sulphonic acid and to 3 mL of NaOH 1.5 N. The samples were cooled at room temperature and the spectrophotometer readings were performed at 410 nm. Nitrate content was calculated referring to a standard calibration curve.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 6.

All data were reported as means ± standard errors (*n = 3*). One-way analysis of variance and two-way analysis of variance were used to analyse the data deriving from endive and lettuce treatments respectively. The differences among treatments were analysed by Bonferroni test (*P < 0.05*).

Results

Yield and chlorophyll content

Lettuce was harvested at the commercial maturity after 57 days following the transplanting. The production for each pot was determined by measuring the weight at the harvest; each head of lettuce weighed between 41 g and 323 g (Figure 1A). Both ONE® treatments increased the plants weight over 300 g/head of lettuce; NPK solutions did not show significant differences compared to the control.

With a density of 9.5 plants/m^2 the yield was 1.3 kg/m^2 in control and 3 kg/m^2 in the two ONE® treatments (Figure 1C). The dry matter (DM) was lower in plants treated with ONE®: in control DM was 5%, while in ONE® at 50% dose was 4.1% and in ONE® at 100% dose was 3.9% (Figure 1E).

The endive was harvested after 48 days following the transplanting. The weight of each head of endive was comprised between 168 g and 198 g (Figure 1B).

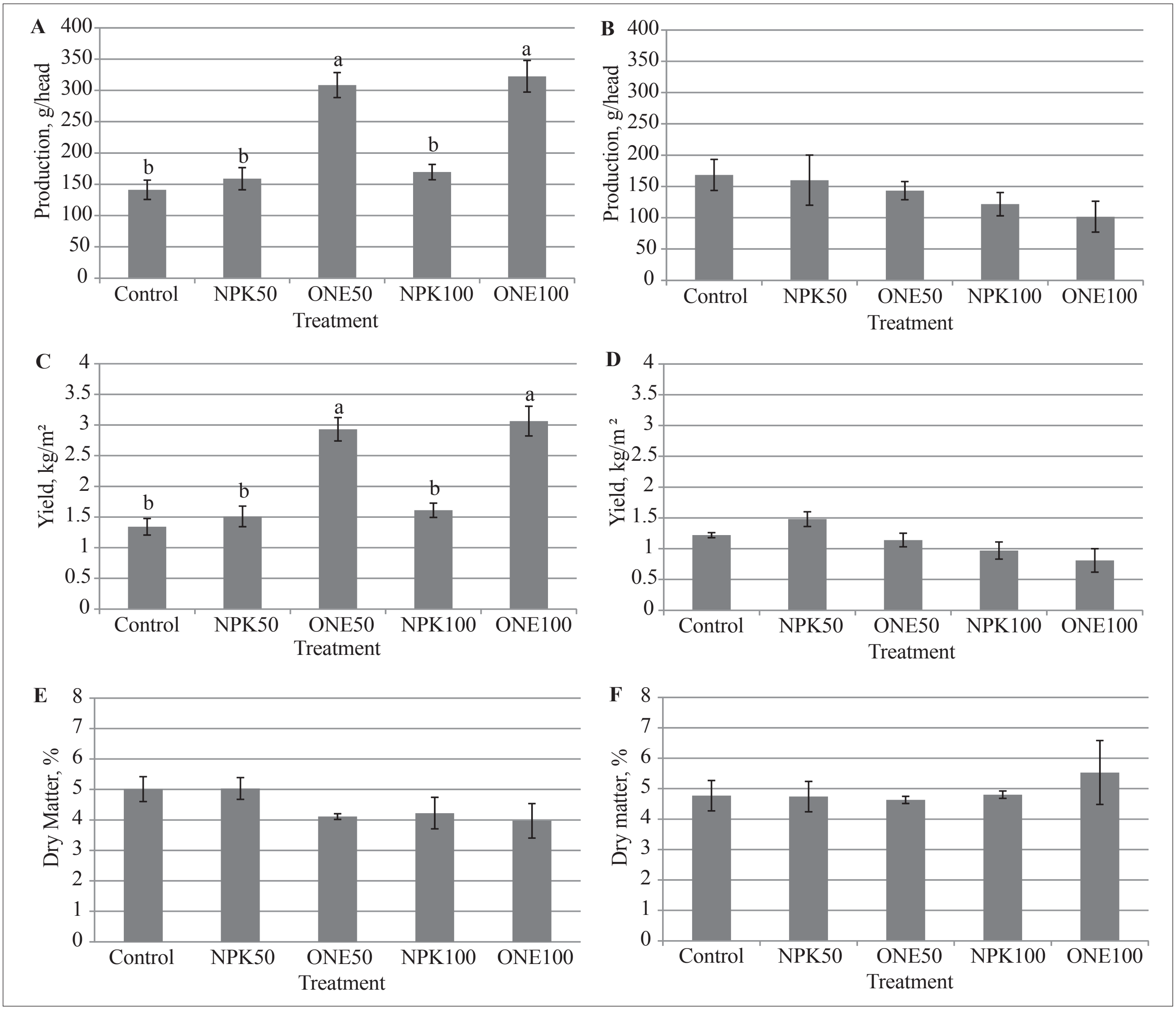

Fig. 1. Production (A, B), yield (C, D) and dry matter % (E, F) of lettuce and endive in response to different treatments. Values are means with standard errors (*n = 3*). Different letters indicate statistical differences among treatments (*P < 0.05*)

Considering a density of 8 plants/ m^2 the yield was higher in plants treated with NPK at 50% dose (Figure 1D).

The dry matter (DM) was higher in endive plants treated with ONE® at 100% dose but no significant differences were observed (Figure 1F). The values ranged from 4-6%.

The chlorophyll content showed the same trend: both ONE® treatments increased leaf pigments but statistically differences were observed between ONE® at 50% and 100% dose (Figure 2A). The effect of the biostimulant on the chlorophyll concentration in the endive was visible in plants treated with ONE® at 100% dose (Figure 2B); in fact the pigments were almost doubled, with a value of 12.48 r.u., which was statistically different compared to other treatments.

Nitrate content

Lettuce nitrate content was measured both in outer and inner leaves; results showed that nitrate content was lower in the outer leaves (100-200 mg/kg FW) compared to the content in the inner leaves (200-300 mg/kg FW). ONE® at 100% dose increased nitrate content (500 mg/kg FW) but any significant differences were observed among inner and outer leaves. The NPK treatment at 100% dose was the only treatment that induced less nitrate content in inner leaves than other treatments (Table 1). The nitrate content in the endive salad ranged from 221 to 1239 mg/kg FW. Lowest nitrate content was observed in the control and the highest in the ONE 100 treatment (Table 1).

Reducing sugars, sucrose and total sugars

Reducing sugars in lettuce were determined in the inner and outer leaves, since the lettuce is closed to form a ball head. The reducing sugars in the outer leaves ranged from 1700 to 2080 mg/kg FW, while the inner leaves showed higher concentrations ranging from 3120 to 5250 mg/kg FW. No statistical differences were found among treatments (Figure 3A). The endive showed a reducing sugars content similar to the concentrations observed in the outer leaves of lettuce. Contrary to the lettuce, the treatments affected the

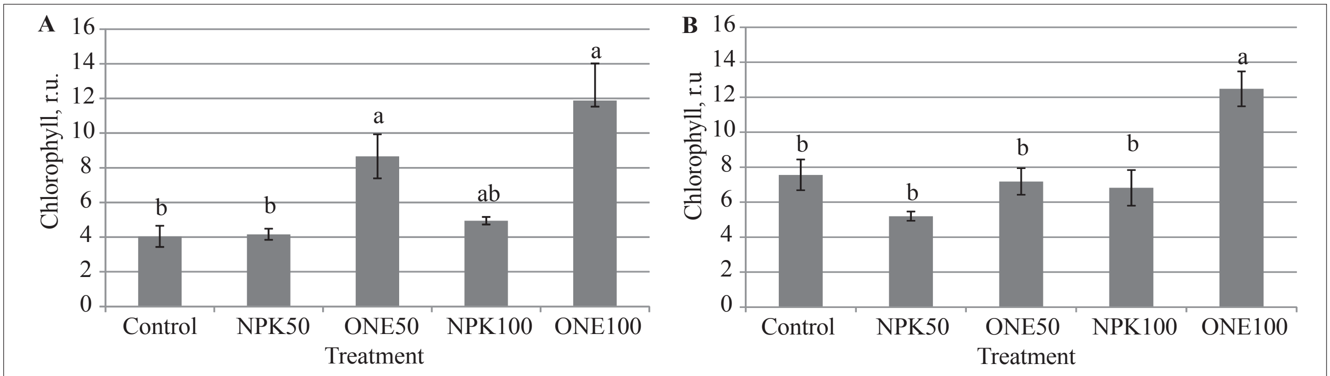

Fig. 2. Chlorophyll content in lettuce (A) and endive (B) in response to different treatments. Values are means with standard errors (*n = 3*). Different letters indicate statistical differences among treatments (*P < 0.05*)

Table 1
Nitrate content in lettuce and endive leaves in response to different treatments

Treatment	Nitrate content, mg/kg FW		
	Lactuca sativa L.		*Cichorium endivia L.*
	Inner leaves	Outer leaves	
Control	146.22±14.485b	179.51±18.146b	221.19±29.36
NPK50	193.83±58.443ab	269.50±45.453ab	607.58±29.52
ONE50	202.51±31.637ab	242.49±28.592ab	552.85±155.22
NPK100	195.08±42.506ab	291.95±105.449ab	552.82±110.03
ONE100	498.96±165.455a	497.70±143.676a	1239.11±424.88

Values are means with standard errors (*n = 3*). One-way analysis of variance and two-way analysis of variance were used to analyse the data deriving from endive and lettuce treatments respectively. Differences among means were determined using Bonferroni's post-test. Different letters indicate statistical differences among treatments (*P < 0.05*) reducing sugars content in endive with lower concentration in treatments compared with control (Figure 3B). In treatments the values were in average below 2000 mg/kg FW, while in the control the average was above 3000 mg/kg FW. Sucrose content in endive leaves was higher in control compared with treatments, which showed half concentration (Figure 3D). Sucrose concentration was 3-4 times higher in outer leaves (Figure 3C). Inner leaves had a sucrose content of 400 mg/kg FW, while outer leaves showed variable values from 600 to 1200 mg/kg FW. Sucrose concentration was higher in plants treated with ONE® than other treatments.

With the exception of ONE® at 100%, all treatments induced a higher (Figure 3E) content of total sugars in inner leaves of head of lettuce. Significant differences were observed only in outer leaves of NPK at 100% dose and in inner leaves of ONE® at 100% dose, both presenting very low values. The total sugar concentration had the same trend of sucrose with highest value in the control (5800 mg/kg FW) and in other treatments the concentration was almost one third (Figure 3F).

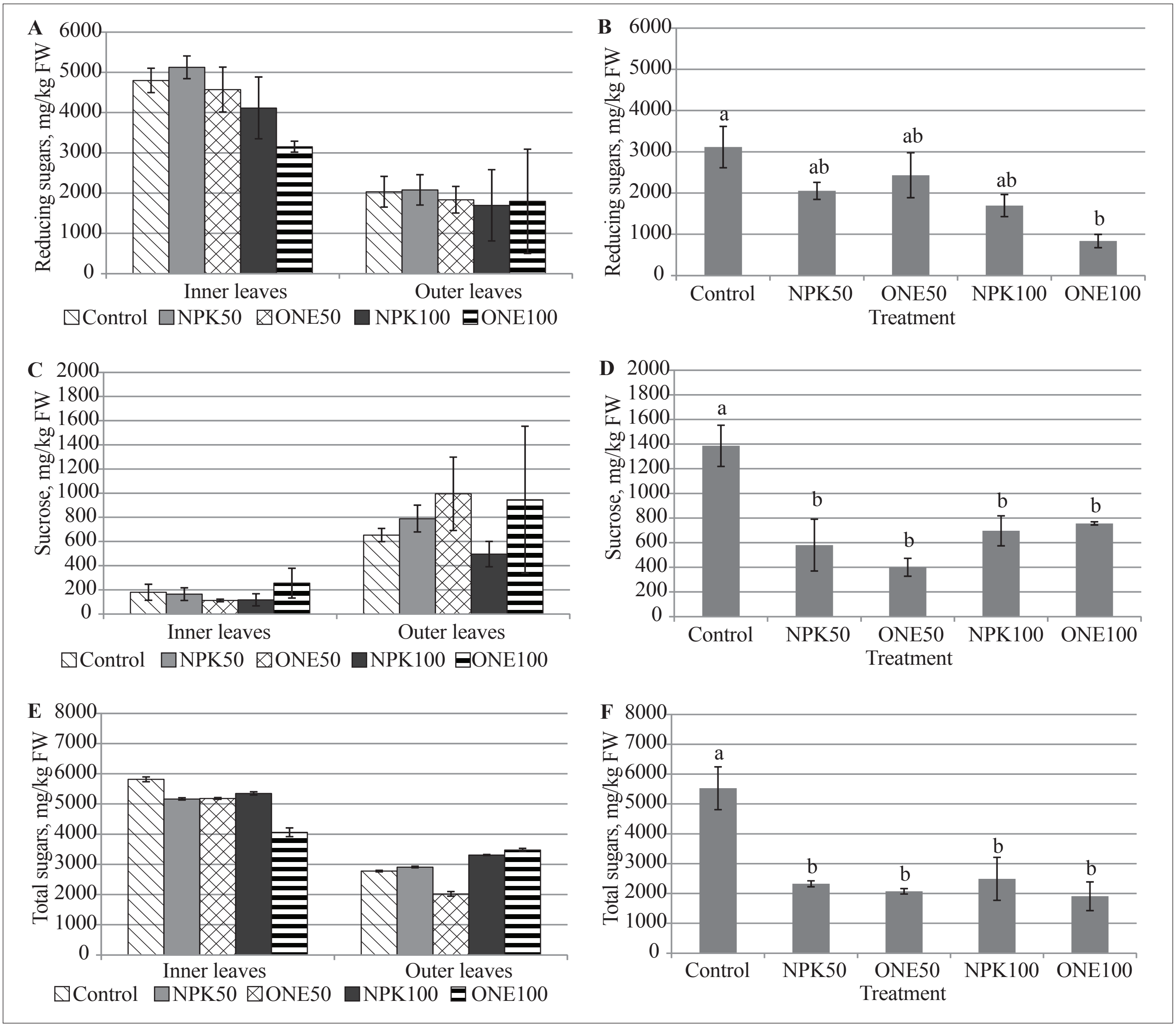

Fig. 3. Reducing sugars (A, B), sucrose (C, D) and total sugars (E, F) of lettuce and endive in response to different treatments. Values are means with standard errors (*n = 3*). Different letters indicate statistical differences among treatments (*P < 0.05*)

Discussion

Since vegetables production in urban environment is spreading in many cities, the scientific interest to improve cultivation practices and quality parameters has been growing. At the same time the use of biostimulants is gaining a worldwide interest because they represent an environmental friendly method for stimulating crop productivity, inducing stress resistance and improving yield or chemical composition of the plants (Grabowska et al*.,* 2013).

In this work a complete fertilizer containing biostimulants was tested to supply nutrient needs of leafy vegetables.

Results showed that ONE® treatments speeded up the production: the formation of head of lettuce and the commercial stage of maturity were earlier in ONE® treatments than in other. These effects on production were already observed in vegetables (Vernieri et al*.,* 2005) and in ornamental plants (De Lucia and Vecchietti, 2012). By enhancing the chlorophyll content, ONE® treatments improved the lettuce and endive visual appearance and probably the photosynthetic activity of plants. High concentration of leaf pigments deriving from biostimulant treatments was previously observed in other vegetables, such as rocket (Vernieri et al., 2005; Vernieri et al., 2006). Nitrate content in lettuce and endive was very low in all treatments and in control though a slight increment of nitrates in ONE® at 100% dose was found. This result could depend on total sugars reduction in the leaves. In fact deficiency of carbon skeletons could lead to a reduction in nitrate assimilation (Ervin et al., 2004). This condition was more evident in endive: the higher level of nitrate was recorded in plants treated with ONE® at 100% dose, in which the content of total sugars, sucrose and reducing sugars was lower. The greater concentrations of sucrose and the lower concentration of reducing sugars in the outer leaves of lettuce are probably determined by the enhanced photosynthetic activity due to higher light received (Yamaguchi et al., 1990). Outer leaves are green and rich in chloroplast, while inner leaves are etiolated and with a lower concentration of photosynthesis products. The lower concentrations of reducing sugars are used for amino acids biosynthesis and this explains the lower concentrations of nitrates. Moreover, the nitrate assimilation in plants is light dependent and shaded leaves or with lower light exposure usually show higher nitrate contents (Blom-Zandstra and Lampe, 1985; Antonacci et al., 2007). Both nitrates and sugars in plants undergo diurnal changes, therefore the sampling time is very important because may lead to different conclusions.

Conclusions

The collected data suggested that ONE® at 50% dose in Iceberg lettuce could increase production and improve quality parameters. In endive the treatment with ONE® at 100% dose had an effect especially on the visual appearance of plants, rather than on production parameters; Gajc-Wolska et al. (2012) investigated the effect of biostimulants on the yield and quality of curly endive, showing that these products did not affect the endive yield.

Generally the biostimulant had positive effects on leafy vegetable production but these were different among the two species that were studied; this is consistent with what was shown in other works demonstrating that the effects of biostimulant applications were dependent on plant species, cultivar, environmental factors, dose and time of application (Kunicki et al., 2010).

References

Antonacci, S., T. Maggiore and A. Ferrante, 2007. Nitrate metabolism in plants under hypoxic and anoxic conditions. *Plant Stress,* **1:** 136-141.

Blom-Zandstra, M. and J. E. M. Lampe, 1985. Osmoregulation of lettuce (*Lactuca sativa* L.) grown at different light intensities. *J. Exp. Bot.*, **36:** 1043-1052.

Cataldo, D. A., M. Haroon, L. E. Sehrader and V. L. Youngs, 1975. Rapid colorimetric determination of nitrate in plant tissue by titration of salicylic acid. *Commun. Soil Sci. Plant Anal.,* **6:** 71-80.

De Lucia, B. and L. Vecchietti, 2012. Type of bio-stimulant and application method effects on stem quality and root system growth in L. A. lily. *Eur. J. Hortic. Sci.*, **77:** 10–15.

Du, S. T., Y. S. Zhang and X. Y. Lin, 2007. Accumulation of nitrate in vegetables and its possible implications to human health. *Agric. Sci. China,* **6:** 1246-1255.

Ervin, E. H., X. Z. Zhang, S. D. Askew and J. M. Goatley, 2004. Trinexapac-ethyl, propiconazole, iron and biostimulant effects on shaded creeping bentgrass. *Hort. Technology,* **14:** 500-506.

Gajc-Wolska, J., K. Kowalczyk M. Nowecka, K. Mazur and A. Metera, 2012. Effect of organic – mineral fertilizers on the yield and quality of endive (*Cichorium endivia* L.). *Acta Sci. Pol. Hortorum Cultus,* **11:** 189-200.

Grabowska, A., E. Kunicki, A. Sekara, A. Kalisz and R. Wojciechowska, 2012. The effect of cultivar and biostimulant treatment on the carrot yield and its quality. *Vegetable Crops Research Bulletin,* **77:** 37-48.

Hänsch, R., D. G. Fessel, C. Witt, C. Hesberg, G. Hoffmann, Walch, P. Liu, C. Engels, J. Kruse, H. Rennenberg, W.

M. Kaiser and R. R. Mendel, 2001. Tobacco plants that lack expression of functional nitrate reductase in roots show changes in growth rates and metabolite accumulation. *J. Exp. Bot*., **52:** 1251-1258.

Kunicki, E., A. Grabowska, A. Sekara and R. Wojciechowska, 2010. The effect of cultivar type, time of cultivation, and biostimulant treatment on the yield of spinach (*Spinacia oleracea* L.). *Folia Hort*., **22:** 9-13.

La Malfa, G., 1997. Principali profili dell'orticoltura amatoriale. III Giornate Tecniche SOI "Orto-Floro-Frutticoltura amatoriale" (Proceedings, Cesena, 13-14 November, 1997), pp. 7-26.

Santamaria, P., 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. *J. Sci. Food Agr*., **86:** 10-17.

Tei, F., 1997. Orti urbani per anziani. Atti III Giornate Tecniche SOI 1997 "Orto-Floro-Frutticoltura amatoriale", (Proceedings, Cesena, 13-14 November, 1997), pp. 114-118.

Tei, F. and G. Gianquinto, 2010. Origini, diffusione e ruolo multifunzionale dell'orticoltura urbana amatoriale. *Italus Hortus*, **17:** 59-73.

Vernieri, P., E. Borghesi, A. Ferrante and G. Magnani, 2005. Application of biostimulants in floating system for improving rocket quality. *J. Food Agric. Environ*., **3:** 86-88.

Vernieri, P., E. Borghesi, F. Tognoni, G. Serra, A. Ferrante and A. Piaggesi, 2006. Use of biostimulants for reducing nutrient solution concentration in floating system. *Acta Hort*., **718:** 477-484.

Vernieri, P. and S. Mugnai, 2003. L'uso di biostimolanti nella produzione di piante fiorite annuali da bordura. *L'Informatore Agrario*, **24:** 51-54.

Yamaguchi, T., Y. Kujira, K. Nakajima, K. Yokokawa and M. Yokonishi, 1990. The diurnal variation of the levels of sucrose, glucose, fructose and protein in green asparagus and lettuce. *J. Agric. Hort*., **65:** 637-640.

Received December, 10, 2013; accepted for printing July, 2, 2014.