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INFLUENCE OF CULTIVAR, MICROBIOLOGICAL FERTILIZERS AND GROWING SEASONS ON NITRATE CONTENT IN LETTUCE

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Abstract

Lettuce belongs to a group of leafy vegetable crops with special importance in human nutrition. Along with healthy compounds lettuce can accumulate nitrate in leaves. The purpose of this study was to examine the effects of genotype, microbiological fertilizers and season on plant weight and nitrate content in outer and inner leaves. Green cultivars ('Kiribati', 'Aquino', 'Aleppo') were cultivated in a fertile soil, in a greenhouse trial at the company Iceberg Salat Centar, during three successive seasons (autumn, winter and spring). Microbiological fertilizers (EM Aktiv, Vital Tricho and combination of EM Aktiv and Vital Tricho) were applied in the soil before trials and foliar. In spring cultivar 'Aquino' showed the highest head fresh weight (360.3 g). All microbiological fertilizers led to increased head fresh weight in 'Aquino' during autumn trial. Mainly fresh weight was higher in spring and winter compared to autumn. Cultivar 'Aquino' showed the highest nitrate content (985.4 mg/kg, autumn) in outer leaves with application of Vital Tricho. Opposite to that, the lowest nitrate content was found in inner leaves of cultivar 'Aleppo' (35.4 mg/kg, spring) with combination of fertilizers. Generally, microbiological fertilizers significantly increased nitrate content in outer and decreased in inner leaves. In both leaves the lowest nitrate level was measured in spring season. During all trials nitrate content remained under allowed level of European Commission Regulation (563/2002) for protected lettuce.

Keywords: *Lettuce, Microbiological fertilizers, Season, Plant weight, Nitrate.*

Introduction

Lettuce is an annual, cool weather vegetable crop from *Asteraceae* family. Generally, lettuce has short vegetation period and in temperate climate it is feasible to accomplish multiple harvests all year-round. According to Food and Agriculture Organization (FAO) in 2017, total area harvested in European Union was 118.508 ha with leading countries Spain (34.508 ha), Italy (34.069 ha) and Germany (15.096 ha). Lettuce is consumed as a fresh vegetable and it is a component of fourth range products which are processed and packaged in modified atmosphere and come to market as ready for consumption (Borghi, 2003). It is rich in beneficial phytochemicals important in human diet (minerals, fiber, antioxidative compounds).

Leafy vegetables, including lettuce, tend to accumulate nitrate. Various factors like genetic, environmental (photoperiod, irradiance, humidity) and growing practice (use of fertilizers and pesticides) influence on nitrate accumulation in plants (Maynard et al., 1976; Reinink and Eenink, 1988). Major sources of human nitrate intake are from vegetables, drinking water and meat products (Wolff and Wasserman, 1972). Excessive use of nitrate in agriculture can have a negative effect on human health (transformation of nitrate into nitrite and its interaction with haemoglobin to form methaemoglobin). European Commission regulated maximum levels of nitrate that can be found in vegetables. It is allowed for lettuce grown under cover (excluding

'iceberg' type) harvested between 1 October to 31 March, 4500 mg/kg nitrates and between 1 April to 30 September 2500 mg/kg nitrates (EC regulation No 563/2002).

Effective microorganisms are a group of naturally found beneficial and compatible microorganisms that can be used as microbial inoculants in different preparations (fungi, lactic acid bacteria, photosynthetic bacteria, yeasts and actinomycetes). Application of effective microorganisms can have a positive effect on soil physical and chemical properties, improve plant growth, yield and quality (Babalola, 2010). Fungus genus *Trichoderma* spp. includes avirulent, cosmopolite, opportunistic species that can be used as biofertilizer, biopesticide and soil improver. They can colonize plant roots without evoking defense response in plants, ameliorate root growth and enhance nutrient uptake, improve plant growth and yield, enhance quality of vegetables and alleviate impact of different stresses (Lopez-Bucio et al., 2015). Effective microorganisms and *Trichoderma* preparations can be used in conventional, organic and integrated agriculture. With their application we can decrease the usage of inorganic fertilizers and synthetic pesticides and preserve natural environment (soil, water and air) from further pollution.

The aim of this study was to investigate the effect of genotype, microbiological fertilizers and season on plant weight and nitrate content in outer and inner lettuce leaves.

Material and Methods

Three green lettuce cultivars (oak 'Kiribati', multi-leaf butterhead 'Aquino' and lollo 'Aleppo'-Rijk Zwaan) were studied. Seedlings were grown in peat cubes, made from substrate Potgrond H (Klasmann - Deilmann) in a controlled glasshouse conditions. After transplanting plants were cultivated in the greenhouse without additional heating, in a fertile soil, covered with black mulch film. The experiments were conducted during three consecutive growing seasons autumn (11 October-7 December 2016), winter (27 December 2016-5 April 2017) and spring (27 April-3 June 2017) at the company Iceberg Salat Centar, Surcin.

Before experiments chemical analysis showed sufficient level of macronutrients and humus (nitrogen-0.22 %; [phosphorus](#)-58.35 mg/100g; [potassium](#)-32.45 mg/100g and humus-5.02 %) and all experiments were carried out without application of inorganic fertilizers. Two different microbiological fertilizers and their combination were examined. EM Aktiv (EMA; Candor) is a liquid preparation of different groups of beneficial microorganisms isolated from natural surroundings: *Aspergillus oryzae*, *Azotobacter chroococcum*, *Bacillus subtilis*, *Bacillus megaterium*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Rhodopseudomonas palustris*, *Rodobacter sphaeroides*, *Saccharomyces carevisiae*, *Streptomyces albus*, *Streptococcus lactis*, *Streptomyces griseus*. Vital Tricho (VT; Candor) is a powder preparation of two *Trichoderma* species (*Trichoderma asperellum* and *Trichoderma viride*). The experiments were organized in a complete block design with four treatments (control - without fertilization (C), EM Aktiv (EMA), Vital Tricho (VT) and combination of EM Aktiv and Vital Tricho (EMA+VT)) and 3 replications. Each plot consisted of 32 plants with 25x25 cm density. Microbiological fertilizers were applied in the soil before planting (150 ml/10 l H₂O EMA, 21 g/10 l H₂O VT and 150 ml + 21 g/10 l H₂O EMA+VT) and four times foliar during vegetation period (30 ml/6 l H₂O EMA, 12 g/6 l H₂O VT and 30 ml + 12 g/6 l H₂O EMA+VT). Regular agricultural practices were applied for lettuce greenhouse production (ventilation, irrigation, weeding and preventive protection against diseases and pests).

During vegetation period air temperature and air relative humidity were measured for 24 hours using RC-4HC Data Logger. Monthly reviews of average air temperature and air relative humidity with minimum and maximum temperatures are represented in Table 1.

Table 1. Climate conditions during three growing seasons

	Average temperature (°C)	Average humidity (%)	Minimum temperature (°C)	Maximum temperature (°C)
October 2016	12.4	85.4	1.2	26.2
November 2016	8.0	87.7	-6.2	26.1
December 2016	2.3	89.3	-7.9	26.4
January 2017	-1.7	85.2	-16.6	20.8
February 2017	6.5	82.0	-9.8	38.0
March 2017	13.4	78.4	-2.1	38.8
April 2017	15.8	67.5	3.1	34.6
May 2017	21.1	74.5	5.0	40.4
June 2017	26.0	70.1	15.9	40.3

Plants were harvested when they achieved marketable size, by hand, same day. Rosette fresh weight was measured on scale. After that lettuce leaves were divided into two groups, outer and inner. Fresh leaves were dried for 72 h at 70 °C to constant weight to obtain dry weight, results are represented in grams (g). Nitrate content was determined colorimetrically by nitration of salicylic-acid described by Cataldo et al. (1975) with modifications (Jana and Moktan, 2013). Absorbance was recorded at 410 to 420 nm. Nitrate content in outer and inner leaves is represented in ppm FW. Three-way ANOVA with Tukey’s test for post-hoc comparison was used in order to test effects of genotype, treatment and season. All tests were performed at a significance level α of 0.05. Statistical analysis was performed using software SPSS Statistics for Windows (Version 22.0. Armonk, NY: IBM Corp) and Microsoft Office Excel 2007.

Results and Discussion

Results of rosette (head) fresh weight are represented in Table 2. Rosette (head) fresh weight varied between 90.7-171.7 g in autumn, 188.7-324 g in winter and 229-360.3 g in spring.

Table 2. The effect of genotype, microbiological fertilizers and season on rosette (head) fresh weight (g FW)

Parameter	Season	Cultivar	Treatment			
			C	EMA	VT	EMA+T
Rosette (head) fresh weight (g FW)	Autumn	Kiribati	110.5±2.0 aAx	117.7±4.1 a,bAx	133.3±19.8 aAx	150.0±10.6 a,bAx
		Aquino	102.3±10.7 aAx	148.3±5.2 bBx	147.0±11.6 aBx	171.7±2.7 bBx
		Aleppo	90.7±12.1 aAx	104.0±14.2 aAx	119.7±14.08 aAx	131.0±8.6 aAx
	Winter	Kiribati	261.5±0.9 bAy	266.0±1.2 bAy	290.0±21.6 aAy	270.7±18.0 aAy
		Aquino	275.0±8.4 bAy	261.3±11.4 bAy	324.0±38.0 aAy	286.3±16.6 aAy
		Aleppo	188.7±21.9 aAy	197.0±17.3 aAx,y	274.3±41.4 aAy	276.7±18.1 aAy
	Spring	Kiribati	328.0±22.3 aAz	294.0±4.6 aAz	299.7±30.4 aAy	287.7±7.8 aAy
		Aquino	360.3±15.2 aBz	313.3±17.5 aA,By	290.3±16.3 aA,By	249.0±27.7 aAx,y
		Aleppo	321.7±6.3 aAz	261.0±30.4 aAy	257.3±22.3 aAy	229.0±13.2 aAy

Values followed by the same letter aren’t significantly different at the 0.05% level of probability according to Tuckey’s test. Symbols are a,b - differences between cultivars; A,B - differences between treatments; x,y,z - differences between seasons

Cultivar 'Aquino' showed the highest head fresh weight in spring (control treatment, 360.3 g) and with application of VT in winter (324 g). In all treatments cultivar 'Aleppo' showed the lowest rosette fresh weight in autumn (control, 90.7 g; EMA, 104 g). Literature data indicate positive impact of different effective microorganisms and *Trichoderma* preparations on yield, fresh and dry weight of different vegetables (Björkman et al., 1998; Tošić et al., 2016). Effective microorganisms can synthesize vitamins, hormones, organic acids, enzymes, siderophores and other compounds that can stimulate plant growth. They contribute to enhanced nutrient availability and uptake, improve plant growth and yield, ameliorate soil structure, contribute to microbial diversity in the soil and suppress plant pathogens. In our study, all microbiological fertilizers significantly increased head fresh weight in cultivar 'Aquino' in autumn. On contrary, in spring combination of microbiological fertilizers led to significantly decreased head fresh weight in aforementioned cultivar. Some experiments didn't show positive effect on yield of different crops with application of microbiological fertilizers (Poldma et al., 2001; Van Vliet et al., 2006). The effectiveness of microbiological fertilizers, including *Trichoderma*, depends on genotype, potential root colonization by different *Trichoderma* strains, soil characteristics, method and frequency of application. Our results showed that in autumn and winter season microbiological fertilizers led to increased rosette fresh weight in different cultivars but this wasn't statistically significant. This can be probably explained with particular interaction of genotype and effective microorganisms (*Trichoderma*) or inadequate quantity of applied fertilizers. Season significantly affected rosette (head) fresh weight in all cultivars and treatments. The present study pointed out to increased rosette (head) fresh weight in spring and winter compared to autumn season. This can be probably linked to influence of day length (autumn-short day 11-9 h, winter-growing day 9-13 h, spring-long day 14-15 h) and temperature. Optimal temperature for lettuce planting is 21-23 °C (day) and 15-18 °C (night). Our study confirmed previous reports in lettuce (Pavlou et al., 2007; Konstantopoulou et al., 2012) regarding to reduced plant weight and yield in autumn compared to spring and winter production.

Results of nitrate content in outer and inner leaves are represented in Table 3. Results of nitrate content varied between 202.7-985.4 ppm in autumn, 210.0-932.0 ppm in winter and 35.4-316.0 ppm in spring. In control treatment, the highest nitrate content in outer and inner leaves showed cultivars 'Aquino' and 'Aleppo' (autumn 908.2 ppm, winter 701.5 ppm). Siomos et al. (2002) found higher nitrate content in butterhead type compared to leaf lettuce. In both leaves, the lowest nitrate content was found in spring in cultivars 'Kiribati' and 'Aleppo' (control 62.4 ppm, 35.4 ppm with combination of fertilizers). According to Sorensen et al. (1994) nitrate accumulation in lettuce depends on genotype (variety) and environmental factors. Application of EMA and combination of fertilizers led to significantly enhanced nitrate content in outer leaves of cultivar 'Kiribati' in autumn and spring, VT in cultivar 'Aquino' in spring and EMA in cultivar 'Aleppo' in spring. On contrary, combination of fertilizers led to significantly decreased nitrate content in cultivar 'Aquino' in winter. In spring, application of all microbiological fertilizers led to significantly increased nitrate content in inner leaves in cultivar 'Kiribati' and application of EMA in cultivar 'Aleppo'. In autumn fertilizer VT significantly decreased nitrate content in cultivars 'Kiribati' and 'Aquino' and in winter in cultivar 'Aleppo'. Generally, in outer leaves application of microbiological fertilizers led to increased nitrate content as well in spring in inner leaves. These results probably indicate contribution of effective microorganisms to increased nutrient availability. Experiments with lettuce and rocket showed enhanced nitrogen uptake from the soil using *Trichoderma* fertilizers (Fiorentino et al., 2018). Season significantly affected nitrate content in both leaves of all cultivars and treatments with exception of cultivar 'Aquino' with application of VT in inner leaves. The lowest nitrate content in both leaves was measured in spring. Short day and low light intensity can influence on increased nitrate content

(Govedarica-Lučić and Perković, 2013). In all seasons nitrate content varied 35.4-985.4 ppm and remained under allowed level for protected lettuce (EC regulation No 563/2002).

Table 3. The effect of genotype, microbiological fertilizers and season on nitrate content in outer and inner leaves (ppm FW)

Parameter	Season	Cultivar	Treatment			
			Control	EMA	VT	EMA+T
Nitrate content outer leaves (ppm FW)	Autumn	Kiribati	315.2±39.2 aAy	552.7±15.8 aBy	507.8±36.2 aA,By	705.9±77.5 aBy
		Aquino	908.2±127.8 bAy	887.0± 120.8 aAy	985.4±34.5 bAy	751.9±115.8 aAy
		Aleppo	651.3±14.9 a,bAy	854.6± 76.6 aAy	781.4±123.0 a,bAy	956.4±139.8 aAy
	Winter	Kiribati	380.8±25.0 aAy	495.5± 50.5 aAy	518.6±51.4 a,bAy	385.11±20.1 bAx
		Aquino	451.97±25.7 aB,Cx	529.8±34.9 aCx	395.7±14.6 aBx	210.0±27.2 aAx
		Aleppo	866.7±63.0 bAz	932.0±14.4 bAy	775.1±89.0 bAy	859.4±13.0 cAy
	Spring	Kiribati	62.4±8.7 aAx	90.1±2.3 aAx	94.2±11.0 aAx	211.9±36.5 a,bBx
		Aquino	216.9±16.1 bAx	287.9±17.1 bA,Bx	316.0±16.0 bBx	271.8±23.2 bA,Bx
		Aleppo	98.7±5.1 aA,Bx	249.1±8.6 bCx	63.4±1.2 aAx	117.2±18.1 aBx
Nitrate content inner leaves (ppm FW)	Autumn	Kiribati	441.4±58.1 aBy	477.4±73.4 aBy	202.7±23.7 aAy	462.5±39.4 aBy
		Aquino	593.7±45.9 aBz	410.8±44.4 aA,By	309.1±37.0 aAx	476.1±74.0 aA,By
		Aleppo	409.11±14.1 aAy	462.4±72.9 aAy	574.1±55.3 bAz	488.3±75.9 aAy
	Winter	Kiribati	296.6±39.7 aAy	315.8±9.1 aAy	212.1±28.3 aAy	225.0±33.5 aAx
		Aquino	305.4±19.6 aAy	254.5±42.2 aAx,y	276.4±11.2 a,bAx	232.0±18.2 aAx
		Aleppo	701.5±36.1 bBz	650.1±54.8 bBy	347.9±22.2 bAy	560.0±93.0 bA,By
	Spring	Kiribati	54.8±7.6 aAx	114.6±2.1 aBx	103.3±7.9 aBx	109.4±15.0 bBx
		Aquino	166.9±22.8 bAx	246.9±14.4 bAx	213.8±31.0 bAx	226.9±7.8 cAx
		Aleppo	72.4±8.9 aAx	178.8±24.9 a,bBx	64.3±0.1 aAx	35.4±3.1 aAx

Values followed by the same letter aren't significantly different at the 0.05% level of probability according to Tuckey's test. Symbols are a,b,c - differences between cultivars; A,B,C - differences between treatments; x,y,z - differences between seasons

Conclusions

The study showed that genotype, microbiological fertilizers and season significantly affected rosette (head) fresh weight and nitrate content in both leaves. Cultivar 'Aquino' showed the highest head fresh weight in spring. In autumn season, all microbiological fertilizers significantly enhanced head fresh weight in cultivar 'Aquino'. The lowest nitrate content was measured in cultivar 'Aleppo', in spring, using combination of fertilizers. Mainly, in outer leaves microbiological fertilizers led to increased nitrate content whereas in inner leaves microbiological fertilizers led to significantly decreased nitrate content. Application of VT and combination of fertilizers had a great influence on tested parameters. In all seasons nitrate content stayed bellow maximum allowed level for protected lettuce. Authors can recommend spring season to obtain the highest rosette fresh weight and the lowest nitrate content.

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