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YIELD OF NITRATE ACCUMULATIONS IN SPINACH (*SPINACIA OLRACEA L.*), WITH DIFFERENT FERTILIZATION SYSTEMS

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ABSTRACT

Spinach production is based on a conventional cultivation system. This form of cultivation, due to excessive use of mineral fertilizers, pesticides and other inputs in production, can lead to an unbalanced relation and disruption of natural processes in the soil. Due to this fact, various spinach fertilization systems are being examined in order to ensure health value and environmental protection. A two-factorial (locality and fertilization) one-year study was conducted by random block schedule in four replications. The study examined the influence of different localities (Krizevci in Croatia and Velika Kladusa in Bosnia and Herzegovina) and fertilization systems (mineral, reduced mineral and organic) on yields and nitrate concentrations, as well as the amount of vitamin C in the spinach variety Norvak. Analysis of variance indicates a high and statistically justified ($p < 0.05$) influence of localities and fertilization on height, spinach yield and nitrate concentration. Due to economic and environmental justification, a reduced mineral fertilization system should be applied in further practice, because the obtained yield and yield components do not differ statistically from those obtained using mineral fertilization, and benefits are multiple (better product quality and less environmental pollution).

Key words: mineral fertilization system, organic fertilization system, quality, reduced mineral fertilization system, spinach, yield

INTRODUCTION

Spinach (*Spinacia oleracea L.)* is annual, herbaceous plant of moderate climate, which is grown for its leaf. It is native to central parts of Asia, where it is determined as annual plant (rarely biennial), (Okimura et al, 2003). It is one of the most important leafy vegetables, as well as an important source of minerals, antioxidants such as beta-carotene and lutein (Alessa et al, 2017). Spinach is highly valued seasonal vegetable that produce a leaf rosette in the vegetative stage of growth (Kansal et al, 1981; Morelock T.E. and Correll J.C, 2008). The root system grows rapidly, and the main root reaches the length of up to 180 cm, on which numerous lateral roots develop, most often in the arable layer, 15-30 cm deep, whose diameter corresponds to the diameter of the rosette (Matotan, 2004). In the vegetative phase, the spinach stem is short, overgrown with a rosette of leaves on longer and shorter petioles. According to the position of the rosette, it can be: sessile, semi-upright and upright, which is varietal characteristic, but it also depends on sowing density (Parađiković, 2009). Fertilization regime, lighting and moisture affect leaf size, color, but also elongation (Morelock T.E. and Correll J.C, 2008). The blade comes with various shapes, it can be round, oval, speared or elongated, with whole or wavy edge, smooth or wrinkled surface. Low temperatures, especially at night, affect the strength and plication of spinach leaves. The leaves are saggy and appear fleshy, light green to dark green in color, whose shape and form also depends on the climatic condition, as well as varietal characteristics and cultivation methods (Jessica, 2016; Ors and Suarez, 2016).

Spinach is an everyday food in human diet, and is a particularly prized vegetable. It is grown in gardens for the daily households` needs, but also in intensive cultivation for market needs. A short vegetation period makes spinach very interesting because it allows several harvests a year, which brings significant economic benefits. It is characterized by low caloric content, richness of minerals and vitamins C and B groups. The highest production of spinach in Europe is in Italy (7500 ha), France (5000 ha) and Germany (3750 ha), while spinach production in United States covers the area of 6800 ha for sales in fresh condition, and additional 7700 ha for processing purposes (Lešić et al, 2016). The production of spinach in the world (Faostat, 2017) covers the area of 929,758 ha, and the yield in 2017 amounted 27,885,841 tons (fao.org, 2019). According to the data of the Agency for Statistics of Bosnia and Herzegovina (bhas.gov.ba, 2019), the production of spinach in Bosnia and Herzegovina covered the area of 489 ha in 2018, while in 2019, the production was recorded in slightly larger area of 565 ha, which represents the increase by 15.5%. According to the data from the Statistical Yearbook of Federation of Bosnia and Herzegovina (fsz.ba, 2019), the total sales of spinach at retail markets 2018 were in the amount of 273 tons. There are no precise statistical data on the market production of spinach in the Federation of Bosnia and Herzegovina. The total turnover of spinach on green markets in Bosnia and Herzegovina in 2018 was in the amount of 325,688 kg, with total value of 841,314 BAM, which tells us that the average price was 2.60 BAM per kg (bhas.gov.ba, 2019). The average redemption price was 1.40 BAM.

The development and improvement of technological processes of spinach production in recent years assume industrial-conventional character, which implies the use of heavy machinery together with use of mineral fertilizers and chemical preservatives. Those efforts, aimed to increase average yields through intensive vegetable production, can result in environmental pollution and endangering consumers` health. An intensive cultivation using mineral fertilizers, will results in high yield in production of spinach, but over the long period of time, it can disrupt the biological cycle of substances in nature, which contributes imbalances and disturbances of natural processes in the soil. Unfortunately, spinach is also a nitrophilic plant, which has the ability to accumulate high amount of nitrates, which can potentially become harmful to human health. The solution that professional and scientific institutions must offer is how to produce sufficient quantities of food, without endangering our own survival. Moderate development, along with balanced cost-benefit ratio, is the way to achieve sustainable production as permanent commitment.

MATERIALS AND METHODS

The aim of this research is to determine the differences in market yield and quality of spinach (with emphasis on the concentration of nitrates and vitamin C) in the system of mineral, reduced mineral and organic fertilization at two different localities. In order to explore this issue, the experiment was set up on the open field in the northwestern part of Bosnia and Herzegovina (municipality of Velika Kladusa) and northern part of Croatia (municipality of Krizevci). The research was conducted in the period from April to June 2019, in which we tried to determine the impact of different fertilization systems, as well as different localities on overall biometric parameter of the spinach plant. A two-factor one-year study was implemented by using random block schedule in four replications, where factor A = locality and factor B = fertilization. The research was conducted on spinach of the Norvak variety, which belongs to fast-growing varieties, tolerant to temperature oscillations. The resistance it has to temperatures makes it suitable for cultivating in winter and summer. It forms a leaf rosette with large leaves, gives high yields, and is rich in vitamin C and iron. Fertilizer was applied to the soil manually, for each randomly selected plot in the following variants: mineral fertilization (1000 kg/ha NPK 7 : 14 : 21 and 300 kg/ha KAN in two supplementations); reduced mineral fertilization (500 kg/ha NPK 7 : 14 : 21 and 150 kg/ha KAN in supplementation); organic fertilization (5 t/ha of composted chicken manure). Sowing of spinach at both localities were done manually on April, 5th, 2019, with spacing of 25 cm between the rows, while the spacing within the row was 4 - 5 cm. The sowing depth was 3 - 4 cm. The spinach was harvested manually in the second week of June, after 6 to 8 leaves were formed in the leaf rosette, and the central rows of plants, from each experimental plot, were taken for analysis. After determining the total weight of spinach plant, the non-sellable leaves were removed (introduced and damaged), and the weight, height and diameter of spinach were re-measured. A titration method with 2.6-p-dichlorophenolindophenol (AOAC 2002) was used to determine the amount of vitamin C, and a colormetric method with salicylic acid (Cataldo et al, 1975) and reading on spectrophotometer were used to determine the nitrate concentration. Data collected by experimental research were processed in the statistical program SAS 9.4 (SAS, 2012).

RESULTS AND DISCUSSION

The total yield ranges between 2.05 and 4.92 kg/m² (Tables 1 and 1a). The lowest yield was gained at location Krizevci on organic fertilization, while the highest yield was obtained at the same locality by using mineral fertilization. The locality did not statistically significantly affect the yield of spinach, but fertilization did, so the average yield in mineral fertilization, regardless of location, was significantly higher (4.55 kg/m²) than in reduced mineral fertilization (3.41 kg/m²), and the lowest yield was in organic fertilization (2.20 kg/m²). Mineral fertilization resulted in twice the yield compared to organic fertilization (Tables 1 and 1a).

Table 1. Average yield (kg/m²) depending on location and fertilization treatment

Location/ Treatment	Mineral fertilization	Reduced min. fertilization	Organic fertilization	$\bar{x}$
Krizevci	4.92	3.28	2.05	3.42 n.s.
Velika Kladusa	4.18	3.53	2.35	3.36 n.s.
$\bar{x}$	4.55 A	3.41 B	2.20 C	

n.s. – not significant; a, b, c - significant ($P < 0.05$); A, B C ($P < 0.01$)

Table 1a. Results of analysis of variance for yield according sources of variability

Source of variability	Yield		
	Degree of freedom	F	P
Location	1	0.01	0.9069
Fertilization	2	15.70	0.0001

The obtained results are in accordance with previous research by authors Lešić et al. (2016) and Matotan (2004), who states an average yield of 15 to 30 t/ha, depending on whether it was spring or autumn cultivation. Also, Canali et al. (2014) have revealed the average yield from 38.8 to 40.7 t/ha, depending on the year of cultivation, and from 31.2 to 46.6 t/ha, depending on the amount of added nitrogen. Further on, Abdelraouf (2016) have published that average yield was from 10.55 to 41.51 t/ha, using from 55 kg N/ha to as much as 224 kg N/ha.

As we assumed, a statistically significant difference was found at the average nitrate concentration, depending on the locality ($P < 0.05$) and fertilization ($P < 0.01$), as shown in tables 2 and 2a. As expected, the highest concentration was obtained with mineral fertilizers treatment (642 mg/100 g of fresh matter), on organic fertilizer treatment it was 46 mg/100 g of fresh matter, while the concentration measured on the treatment by reduced mineral fertilization amounted 81 mg/100 g of fresh matter, and is statistically different only from treatment by mineral fertilizer. The nitrate concentration in the spinach leaf was also statistically significantly infuenced by locality, so the measured values were on average higher in Krizevci (416 mg/100 g of fresh matter), than in Velika Kladusa (97 mg/100 g of fresh matter).

Table 2. Average nitrate concentration (mg/kg of fresh matter) depending on location and fertilization treatment

Location/ Treatment	Mineral fertilization	Reduced min. fertilization	Organic fertilization	$\bar{x}$
Krizevci	1097	95	56	416 a
Velika Kladusa	189	67	35	97 b
$\bar{x}$	642 A	81 B	46 B	

a, b, c - significant ($P < 0.05$); A, B C ($P < 0.01$)

Table 2a. Results of analysis of variance for nitrates according to sources of variability

Source of variability	Nitrates		
	Degree of freedom	F	P
Location	1	4.32	0.0495
Fertilization	2	9.80	0.0010

Koh et al. (2012) examined 27 varieties of spinach in conventional and organic production, and found that nitrate concentration was significantly higher in conventional production (from 96 to 245 mg/100 g of fresh matter) compare to organic (from 31 to 117 mg/100 g of fresh matter). These values are slightly higher than the values from our experimental studies. The nitrate concentration in spinach leaves is significantly higher in the research of Aisha et al. (2013) compared to our results, ranging from 889.67 to 1,477.57 ppm, depending on the amount of added fertilizer, so higher amounts of both organic and mineral fertilizers result in higher nitrate concentration. Canali et al. (2014) also found slightly higher nitrate values in spinach leaves. It ranged from 571 mg/kg for spinach with no fertilizer applied, up to 1,512 mg/kg for spinach treated with 300 kg N/ha. The well-known fact that the amount of added nitrogen directly affect the nitrate content in spinach leaves is confirmed by our research.

Abdelraouf (2016), who examined the effect of nitrogen fertilization on nitrate concentration, determined that average values ranged from 268 to 1,294 mg/kg in dry matter, while the amount of nitrogen applied for fertilization ranged from 56 to 224 kg N/ha. The obtained values are significantly higher compared to our research. They also state a strong correlation ($R^2 = 0.9984$) between the nitrate concentration in the spinach leaf and the dose of nitrogen applied. Although we did not calculate correlations (Table 2.), it can be concluded that nitrate concentration is higher with full mineral fertilization (642 mg/kg) compared to reduced mineral fertilization (81 g/kg), while the lowest value was in organic fertilization (46 mg/kg).

The average values of vitamin C concentration and statistical processing of these values are shown in tables 3. and 3a. The highest concentration of vitamin C was noticed in Velika Kladusa with reduced mineral fertilization (176 mg/100 g of fresh matter), while the lowest was measured in Krizevci on mineral fertilization (112 mg/100 g of fresh matter).

The locality of cultivation did not affect the examined parameter, as well as the quantity and type of fertilizer used. The highest average values at both localities were noticed in organic fertilization (157 mg/100 g of fresh matter), while in reduced mineral fertilization these values were 156 mg/100 g of fresh matter. The lowest values (131 mg/100 g of fresh matter) were noticed with mineral fertilization, but they did not differ statistically significant from the other two.

Table 3. Average concentration of vitamin C (mg/100 g of fresh matter) depending on location and fertilization treatment

Location/ Treatment	Mineral fertilization	Reduced min. fertilization	Organic fertilization	$\bar{x}$
Krizevci	112	136	169	139 n.s.
Velika Kladusa	150	176	146	157 n.s.
$\bar{x}$	131 n.s.	156 n.s.	157 n.s.	

n.s. – not significant

Table 3a. Results of analysis of variance for vitamin C according to sources of variability

Source of variability	Vitamin C		
	Degree of freedom	F	P
Location	1	3.17	0.0889
Fertilization	2	2.96	0.0735

Parađiković Nada (2009), in her book General and Special Vegetable Growing, states that the average value of vitamin C in spinach are 72 mg/100 g of green matter. Citak and Sonmez (2009) conducted a two-years experimental research with three different types of organic fertilizers, used as such in organic production, and compared them with results obtained with mineral fertilizers. The obtained values of vitamin C with organic fertilization were 48.3 mg/100 g of fresh matter in autumn production, and 154 mg/100 g of fresh spinach in winter production. In mineral fertilization, the value of vitamin C was lower as expected, ranging from 21.1 to 44.9 mg/100 g of fresh spinach in autumn compared to winter production. Koh et al. (2012) examined 27 varieties of spinach in conventional and organic production, noticed that the average concentrations of vitamin C are higher in organic (40.48 mg/100 g) compared to conventional production (25.75 mg/100 g), which are significantly lower values compared to our research.

CONCLUSIONS

Based on conducted one-year research of spring spinach cultivation on two localities and with three different fertilizations, the following can be concluded: Fertilization had a statistically significant impact on some yield components, as well as spinach yield itself, while the impact of localities was statistically significant on certain yield components, but not for the yield itself. The yield and market mass of the plant were significantly affected by fertilizers, but not by locality of cultivation. The highest market mass and yield of spinach was gained with mineral fertilization (0.069 kg/4.55 kg/m²), significantly lower on reduced mineral fertilization (0.051 kg/3.41 kg/m²), while the lowest was gained on organic fertilization (0.033 kg/2.20 kg/m²). Fertilization had statistically significant influence on dry matter content, phosphorus and nitrate concentration, while the locality affected nitrogen, phosphorus and nitrate concentration. Different cultivation localities, as well as the types of fertilization applied, showed statistically significant impact on the average nitrate content in the fresh spinach matter. The average nitrate concentration at Krizevci was 416 mg/100 g of fresh matter, while at Velika Kladusa it was significantly lower, and amounted 97 mg/100 g of fresh matter ($P < 0.05$). The highest average nitrate concentration was measured in mineral fertilization (642 mg/100 g of fresh matter), while the lowest average nitrate concentration was measured in organic fertilization (46 mg/100 g of fresh matter), which is almost 14 times less than mineral fertilization. The differences were statistically justified at the probability level $P < 0.01$. Different fertilization, as well as location of cultivation, did not statistically significantly affect the concentration of vitamin C in spinach leaves. The average content of vitamin C at Krizevci was 139 mg/100 g of fresh leaves, while at Velika Kladusa it amounted 157 mg/100 g of fresh leaves, but the differences are not statistically justified. The highest average concentration of vitamin C was with organic fertilization (157 mg/100 g of fresh leaves), and the lowest concentration was measured at mineral fertilization (131 mg/100 g of fresh leaves). The differences were also not statistically significant.

The use of organic fertilizers, in addition to positive effect on nutritional characteristics of vegetables, enriches the soil with organic matter. Organic fertilizers contain a large percentage of organic matter, the nutrients which are available to the plant throughout the vegetation, a high concentration of microorganisms, and particularly well affect the physical characteristics of the soil (loosens the soil, heating makes easier, hold water better and have better air capacity). In this way, we improve the biological, physical and chemical characteristics of the soil, which in the long run contributes to the preservation of soil from pollution and erosion.

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