Bulgarian Journal of Agricultural Science, 23 (No 5) 2017, 806–815
Agricultural Academy

ABIOTIC FACTORS AND THEIR IMPACT ON GROWTH CHARACTERISTICS OF SPINACH (*SPINACIA OLERACEA*)

IVANKA MITOVA[1*]; LYUBA NENOVA[1]; NIDAL SHABAN[2]
[1] *Agricultural Academy, N. Pushkarov Institute of Soil Science Agrotechnologies and Plant Protection, BG-1331 Sofia, Bulgaria*
[2] *University of Forestry, BG-1756 Sofia, Bulgaria*

Abstract

Mitova, I., L. Nenova and N. Shaban, 2017. Abiotic factors and their impact on growth characteristics of Spinach (*Spinacia oleracea*). *Bulg. J. Agric. Sci.*, 24 (5): 806–815

This experiment was carried out under controlled conditions in phytotron chambers of artificial climate – KNER 2 and KNER 4, on two soil types – Luvisols and Vertisols. The Spinach plants (*Spinacia oleracea*) were grown at air temperature of 18°C, illuminance of 9000 lx and 18000 lx, duration of the day 9 hours and rising rates of nitrogen fertilization.

The aim of the study was establishing the influence of the factors soil type, nitrogen fertilization and light on the growth characteristics of spinach.

From the factors studied (soil type, fertilization and illuminance) the strongest impact (between 41.2 and 60.6%) on the development of the plants (number of leaves, height and biomass) was that of fertilization. The response of the test plants to the illuminance was not in one direction. The number of leaves in both soils and the leaf mass on Vertisols were greater at illuminance 18000 lx. With the increase of the nitrogen fertilization rates, the content of chlorophylls, total sugars, nitrates and nutrients (N, P, K) in plants were rising.

Key words: spinach (*Spinacia oleracea*); fertilization; illuminance; growth characteristics

Introduction

The knowledge and compliance with abiotic factors is directly related to the success of agricultural production. Their basic understanding and skillful use is a necessary condition for the development of crop production. Abiotic factors of importance for the growth and development of plants include: light, soil and air temperature, water, soil type and soil reaction (pH).

The vast variety of lighting effects, starting from the synthesis of substrates and finishing with the regulation of the growth and development of plants, puts light among the most powerful factors of the environment. Depending on the plant reaction to the light duration, the flowering response of many plants is controlled by the photoperiod (the length of uninterrupted darkness). Photoperiod responses could be divided into two or three types: a “long day” plants, flowering in response to short periods of night darkness. Examples include onion and spinach. A “short day” plants are flowering in response to long periods of night darkness. Examples include Poinsettias, Christmas cactus, Chrysanthemums, and single-crop strawberries. “Day neutral” plants are flowering without regarding the length of the night, but typically flowering earlier and more profusely under long day light regimes. Water regime and optimal water availability to plants are also main components of the “living” matter (Petrova and Kireva, 2016; Gadjalska et al., 2017). Different reactions in biological systems occur in a very narrow temperature range. At extremely low temperatures biological reactions are suppressed, due to the energy shortage, while at

**E-mail:* *smolyanovci@abv.bg, lyuba_dimova@abv.bg, nshaban@ltu.bg

very high temperatures a destruction of the complex protein structures occurs. The environmental factors have a huge impact on the nutrients uptake (of electrolytes) by plants. Acidification of the environment around the roots zone (rhizosphere) increases the absorption of anions and vice versa, alkalization around the roots zone stimulates the absorption of cations (Klechkovski and Petersburgsky, 1967).

The facilities, which create artificial conditions for optimal growth and development of plants (artificial climate chambers, phytotron) are especially suitable for simulation of climatic conditions – temperature, light intensity, photoperiod, humidity, where experimental crops respond to certain expectations.

Leafy crops (as spinach), such as high sensitivity to various environmental factors, compact growth and development with short growing season, because of their species characteristics are particularly suitable as “test” crops, due to their quick indicative reactions (Mitova and Stancheva, 2003; Mitova et al., 2005; Dinev and Mitova, 2011; Brechner and Villiers, http://www.cornellcea.com/attachments/Cornell%20CEA%20baby%20spinach%20handbook.pdf).

The aim of the study was establishing the influence of the factors soil type, nitrogen fertilization and light on the growth characteristics of spinach.

Materials and Methods

The test plants were grown in a growth chamber of artificial climate – KNER-2 and KNER–4 (Stoykov, 2006, 2009). These growth chambers were used by heating the useful volume of the chamber using a lamp which resembles the day light while during the “night” electric heaters were used. The following variants were tested (Table 1).

The plants from variants 1 to 4 and 9 to 12 were grown in a growth chamber KNER-4 with air temperature of 18°C, illumination of 18000 lx and day length (photoperiod) of 9 hours. The plants from variants 5 to 8 and 13 to 16 were grown in a growth chamber KNER-2, at air temperature of 18°C, the illumination was 9000 lx, and the duration of the day – 9 hours. In both chambers soil moisture was maintained at 75% of the field capacity, and the relative air humidity was 80%.

Table 1
Scheme of the variants in the experiment

Soil type	Illumination	
	18 000 lx	9000 lx
Luvisols	1. $N_0P_0K_0$	5. $N_0P_0K_0$
	2. $N_{200}P_{300}K_{377}$	6. $N_{200}P_{300}K_{377}$
	3. $N_{400}P_{300}K_{377}$	7. $N_{400}P_{300}K_{377}$
	4. $N_{600}P_{300}K_{377}$	8. $N_{600}P_{300}K_{377}$
Vertisols	9. $N_0P_0K_0$	13. $N_0P_0K_0$
	10. $N_{200}P_{300}K_{377}$	14. $N_{200}P_{300}K_{377}$
	11. $N_{400}P_{300}K_{377}$	15. $N_{400}P_{300}K_{377}$
	12. $N_{600}P_{300}K_{377}$	16. $N_{600}P_{300}K_{377}$

Nitrogen was applied in the form of NH_4NO_3. Phosphorus and potassium were added as KH_2PO_4. Nitrogen rates and background rates of phosphorous and potassium were based on the data from previous experiments (Mitova and Stancheva, 2003; Mitova et al., 2005; Mitova and Stoykov, 2008).

The study was conducted with spinach of Matador variety with a vegetation period of two months. Experimental plants were grown in six replications on two soil types – Luvisols and Vertisols (Koinov, 1987).

The humus content in the surface horizon of Luvisols was 1.0%, which characterized them as poor. The availability of total nitrogen and total phosphorus was poor, too. The soil reaction (pH) was neutral to slightly alkaline – pH_{H2O} – 7.6; pH_{KCl} – 6.1. The contents of mineral nitrogen (NO_3-N + NH_4-N) and phosphorus (P_2O_5) were low – 19.0 $mg.kg^{-1}$ and 4.4 mg.100 g^{-1}, and the content of K_2O – 10.4 mg.100 g^{-1} was very low. The humus content in the surface horizon of Vertisols was 3.6%, and characterized them as medium. The soil reaction was neutral to slightly alkaline – pH_{H2O} – 7.8; pH_{KCl} – 6.6. The availability of mineral nitrogen was good – 23.0 $mg.kg^{-1}$ and when regarding mobile phosphorus and potassium it was very good (P_2O_5 – 22.8 $mg.100g^{-1}$ and K_2O – 55.7 $mg.100g^{-1}$).

The pots with the test plants were placed on the perforated bottom of the chamber uniformly spaced from each other. Through the openings of the bottom, air entered at the desired temperature, flowed around the vessels and plants (leaves and stems) passed near the fluorescent lamps wherein its temperature increased, and then left the working volume of the chamber, through the slots of the ceiling. The air temperature in the chamber was controlled by means of an electronic thermostat. Due to the hysteresis of this device, during the night the chamber temperature was about 1.5 – 2°C and it was lower than during the day. That was good for the development of plants in this type of growth chamber.

The illumination of the plants was adjusted by changing the distance between the surface of the leaves and the lamps. The lamps were designed so they could be moved up and down. The duration of the photo period (modes “day” – “night”) was regulated by contact clock, which set the duration of the regime “day” in which lights should illuminate.

The content of macro elements in the soil was determined by standard methods (Arinushkina, 1970). Ammonium and nitrate nitrogen were determined colorimetrically. The mobile forms of phosphorus and potassium were determined by the method of Ivanov (1984). The total nitrogen in the plants was determined by the Kjeldahl method that is digestion with concentrated N_2SO_4 and 30% N_2O_2. Other macro – and trace elements were determined by "dry" burning in muffle furnaces and subsequent dissolution in 20% HCl considering the atomic absorption spectrophotometer (Mincheva and Brashnarova, 1975). Chlorophyll content was determined in fresh weight (Annex – 2), spectrophotometric (Annex – 3) in the extract by 80% acetone by the method of Vernon (Moskov et al., 1975). From the plant samples of spinach after drying at 60°C, the dry matter (%) was determined. The content of total sugars was determined by refractometer – (%) (Digital refractometer – 32,145). Nitrate was defined by apparatus RQ flex plus 10 Merck.

Experimental data were analyzed by Statgraphics centurion statistical package (One-way- ANOVA, Multifactor ANOVA).

Results and Discussion

Influence of abiotic factors on growth characteristics and yield of spinach

The number of germinated and developed plants is counted in triplicate at different key stages of spinach growth (Table 2). According to Bulgarian State Standard BDS- 2456-79 for spinach, plants with number of leaves in the rosette over 8 and a diameter of over 16 cm are first quality. When the number of leaves is over 6 and the diameter – over 12 cm, the plants are classified as second class (Mitova et al., 2005). In this type of experiment (conducted in a growth chamber) it is difficult to discuss quality, because the number of leaves of the plants correspond to the quality of the standard, but the diameter of the leaves cannot reach the standard values because of the limited area on which the plants were grown.

During the vegetation period when plants were grown on Luvisols in the growth chamber with 18000 lx illumination, they were infected with damping off disease. Therefore invariants 1.$N_0P_0K_0$ and 4.$N_{600}P_{300}K_{377}$ the number of plants in phase 6-8th leave were more than at the later phase 8-10th leave (Table 2). In most of the plants on this soil, but at lighting – 9000 lx, in the early stages of development etiolation was observed. Variants with a rate of fertilization – $N_{400}P_{300}K_{377}$ and $N_{600}P_{300}K_{377}$ had the greatest number of seedlings on both soil types. There was a trend, the number of plants in phase 8-10th leave grown on Vertisols to be greater than that those grown on Luvisols. On Luvisols under low light condition,

Table 2
Dynamic of germination (number of plants) depending on soil type, illumination level and nitrogen fertilization norm

Variants	Ph. 4-6 $^{-th}$ leave	Ph. 6-8^{-th} leave	Ph. 8-10^{-th} leave
	Luvisols		
	Illumination level 18 000 lx		
1. $N_0P_0K_0$	1.0	1.3	1.0
2. $N_{200}P_{300}K_{377}$	1.2	1.3	1.7
3. $N_{400}P_{300}K_{377}$	1.5	1.7	2.3
4. $N_{600}P_{300}K_{377}$	1.3	1.2	1.0
	Illumination level 9000 lx		
5.$N_0P_0K_0$	1.0	1.0	1.7
6. $N_{200}P_{300}K_{377}$	1.0	1.1	2.0
7. $N_{400}P_{300}K_{377}$	1.0	1.2	2.7
8. $N_{600}P_{300}K_{377}$	1.0	1.3	2.0
	Vertisols		
	Illumination level 18 000 lx		
9. $N_0P_0K_0$	1.2	1.2	2.3
10. $N_{200}P_{300}K_{377}$	1.3	1.7	2.3
11. $N_{400}P_{300}K_{377}$	1.3	2.7	3.0
12. $N_{600}P_{300}K_{377}$	1.3	1.8	2.7
	Illumination level 9000 lx		
13. $N_0P_0K_0$	0.5	1.4	2.0
14. $N_{200}P_{300}K_{377}$	0.7	1.4	2.0
15. $N_{400}P_{300}K_{377}$	1.0	1.5	3.0
16. $N_{600}P_{300}K_{377}$	1.0	1.5	2.7

at the end of the study, more developed plants were reported, while on Vertisols no differences in the number of plants at different illumination were established.

The number of plants is one of the most important growth parameters of spinach, which was investigated in relation with the soil type, illumination level and nitrogen fertilization norm (Table 3). Statistical analyses of the results (by the method of Multi-factor ANOVA) showed that the plant number was affected significantly by the soil type and fertilization rate – by 21.5% and 19.3% of the variability in the experiment respectively. Illumination rate had no influence on the plant growth.

The average number of plants in the growing containers varied between 1.0 and 3.0 units (Table 3). In the number of plants grown on Luvisols, at both illumination levels, statistically significant differences were reported only between control variants (1.$N_0P_0K_0$, 5.$N_0P_0K_0$) and plants fertilized with $N_{400}P_{300}K_{377}$ (3.$N_{400}P_{300}K_{377}$ and 7. $N_{400}P_{300}K_{377}$). There was a

Table 3
One-way ANOVA (P=95%) of the plants number depending on the variants in the experiment, LSD=1.1

Variants	Average	Homogenous groups
1. $N_0P_0K_0$	1.0	A
4. $N_{600}P_{300}K_{377}$	1.3	A B
5. $N_0P_0K_0$	1.7	A B C
2. $N_{200}P_{300}K_{377}$	1.7	A B C
6. $N_{200}P_{300}K_{377}$	2.0	A B C D
14. $N_{200}P_{300}K_{377}$	2.0	A B C D
8. $N_{600}P_{300}K_{377}$	2.0	A B C D
13. $N_0P_0K_0$	2.0	A B C D
3. $N_{400}P_{300}K_{377}$	2.3	B C D
10. $N_{200}P_{300}K_{377}$	2.3	B C D
9. $N_0P_0K_0$	2.3	B C D
7. $N_{400}P_{300}K_{377}$	2.3	B C D
12. $N_{600}P_{300}K_{377}$	2.7	C D
16. $N_{600}P_{300}K_{377}$	2.7	C D
15. $N_{400}P_{300}K_{377}$	3.0	D
11. $N_{400}P_{300}K_{377}$	3.0	D

notable trend showing that the largest number of plants was formed in variants fertilized with $N_{400}P_{300}K_{377}$, but this is not statistically significant. On Vertisols no proven differences between non-fertilized and fertilized plants were observed. On this soil type the variants fertilized by rate of $N_{400}P_{300}K_{377}$ had the greatest number of plants. There was statistical difference between the control variant on Luvisols at 18000 lx and the plants in the same light, grown on Vertisols. Statistical differences were not established between similar variants of fertilization in different soil types and light conditions. As a conclusion, for this indicator (number of plants) it could be said that it is greater on Vertisols than on Luvisols. On Luvisols the number of plants is greater at low light (9000 lx), while on Vertisols the differences in the number of plants depending on the intensity of the light is less noticeable. There was a depressing effect on the third level of fertilization ($N_{600}P_{300}K_{377}$) in both soil types compared to the variants fertilized with $N_{400}P_{300}K_{377}$.

The number of leaves per plant is another indicative parameter, which could give information on the influence of abiotic factors. The analysis showed that it depended statistically on the three investigated abiotic parameters – soil, light and fertilization. From these three factors, fertilization manifested the strongest impact, responsible for 57.1% of the variability in the assay. Statistically proven differences between the variants of the experiment were presented in Table 4.

Table 4
One-way ANOVA (P=95%) of the number of leaves per plant depending on the variants in the experiment, LSD=1.02

Variants	Average	Homogenous groups
1. $N_0P_0K_0$	5.3	A
13. $N_0P_0K_0$	6.2	A B
9. $N_0P_0K_0$	6.3	A B C
5. $N_0P_0K_0$	7.0	B C D
8. $N_{600}P_{300}K_{377}$	7.3	C D
6. $N_{200}P_{300}K_{377}$	7.4	D E
14. $N_{200}P_{300}K_{377}$	7.5	D E
2. $N_{200}P_{300}K_{377}$	7.5	D E
7. $N_{400}P_{300}K_{377}$	7.5	D E
10. $N_{200}P_{300}K_{377}$	8.3	E F
16. $N_{600}P_{300}K_{377}$	8.6	F
15. $N_{400}P_{300}K_{377}$	8.7	F
3. $N_{400}P_{300}K_{377}$	9.3	F G
11. $N_{400}P_{300}K_{377}$	9.8	G
12. $N_{600}P_{300}K_{377}$	10.0	G
4. $N_{600}P_{300}K_{377}$	10.3	G

The number of leaves per plant in the trial ranged between 5.3 and 10.3 (Table 4). The lowest number of leaves was established at non fertilized variants, on both studied soils, at both light levels (variants 1.$N_0P_0K_0$, 13.$N_0P_0K_0$, 9.$N_0P_0K_0$ and 5.$N_0P_0K_0$). The greatest number of leaves was formed in variants – 12.$N_{600}P_{300}K_{377}$ and 4.$N_{600}P_{300}K_{377}$ – these variants were with the highest fertilization rate, at illumination of 18000 lx, on both soil types. In the same homogeneous group (marked with the capital G, in Table 4) were variants 3.$N_{400}P_{300}K_{377}$ and 11.$N_{400}P_{300}K_{377}$ – with lower fertilization rate, at 18000 lx illumination again, on both soil types.

Statistical differences between the control and fertilized variants on both soil types, at two levels of illumination were found. Regarding the number of leaves per plant, the same patterns were observed on Vertisols as on Luvisols. At illumination of 18000 lx the number of leaves per plant increased with the increase of nitrogen rate and reached maximum number at the variant $N_{600}P_{300}K_{377}$. At low light conditions the number of leaves decreased between N_{400} $P_{300}K_{377}$ and N_{600} $P_{300}K_{377}$ fertilization rate. Generally, the number of leaves per plant at fertilized variants was greater on Vertisols. On both soils, greater number of leaves had plants in the chambers of higher illuminance.

Statistical analysis of the plant height (cm) showed the effect of the three main factors – soil, light and fertilization (Table 5). Here, the fertilization had the greatest influence on the variability of the data – 60.6%, followed by soil type – 18.8%, and illumination in the chamber – 3.8%.

Table 5
One-way ANOVA (P=95%) of the plant height (cm) depending on the variants in the experiment, LSD=1.66

Variants	Average	Homogenous groups
1. $N_0P_0K_0$	4.3	A
5. $N_0P_0K_0$	4.7	A
9. $N_0P_0K_0$	4.9	A
13. $N_0P_0K_0$	5.4	A B
2. $N_{200}P_{300}K_{377}$	6.1	B
3. $N_{400}P_{300}K_{377}$	6.3	B C
6. $N_{200}P_{300}K_{377}$	6.4	B C
8. $N_{600}P_{300}K_{377}$	7.3	C D
10. $N_{200}P_{300}K_{377}$	7.5	C D E
4. $N_{600}P_{300}K_{377}$	8.2	D E
14. $N_{200}P_{300}K_{377}$	8.3	D E
11. $N_{400}P_{300}K_{377}$	8.3	D E
7. $N_{400}P_{300}K_{377}$	8.4	E
12. $N_{600}P_{300}K_{377}$	10.0	F
16. $N_{600}P_{300}K_{377}$	10.6	F G
15. $N_{400}P_{300}K_{377}$	11.2	G

The height of the plants varied between 4.3 cm at non-fertilized plants in variant with high illumination on Luvisols (1.$N_0P_0K_0$) and 11.2 cm in variant 15.$N_{400}P_{300}K_{377}$ – low light conditions on Vertisols. With all plants, regardless of soil type and illumination level, statistical differences between non-fertilized and fertilized ones were obtained. With the increase of the nitrogen rate the plant height increased on both soils types and illuminations, with the exception of the low light on Vertisols where at fertilization rate of N_{600} $P_{300}K_{377}$ an inhibiting effect was proved. Plants grown on Vertisols, on both illumination levels were with higher values of height in comparison with those grown in similar conditions on Luvisols.

The average fresh weight of one plant was one of the most important indicators in this experiment. The analysis of the data by the method of multifactor ANOVA established the significant effect of the three main factors – soil type, light and fertilization on the accumulation of the plant biomass. The greatest one was the effect of fertilization – 41.2% of the variability of the data was caused by that factor, followed by soil type – 17.7% and 14.3% for illumination.

Statistical analysis using the method of one-way-ANOVA indicated that there were statistical differences between the average fresh weight of the plants by variants (Table 6). That table showed the great diversity in the distribution of variants in separate homogeneous groups, a total number of 8 (from A to H).

Table 6
One-way ANOVA (P=95%) of the plant fresh weight (g) depending on the variants in the experiment, LSD=1.34

Variants	Average	Homogenous groups
13. $N_0P_0K_0$	0.86	A
1. $N_0P_0K_0$	1.31	A B
5. $N_0P_0K_0$	1.36	A B
9. $N_0P_0K_0$	1.56	A B C
6. $N_{200}P_{300}K_{377}$	2.34	B C
7. $N_{400}P_{300}K_{377}$	2.58	B C
8. $N_{600}P_{300}K_{377}$	2.80	C
4. $N_{600}P_{300}K_{377}$	4.37	D
14. $N_{200}P_{300}K_{377}$	4.64	D E
3. $N_{400}P_{300}K_{377}$	5.31	D E
10. $N_{200}P_{300}K_{377}$	5.38	D E
15. $N_{400}P_{300}K_{377}$	5.51	D E F
2. $N_{200}P_{300}K_{377}$	5.78	F
16. $N_{600}P_{300}K_{377}$	6.84	F
11. $N_{400}P_{300}K_{377}$	10.46	G
12. $N_{600}P_{300}K_{377}$	11.82	H

The lowest average weight was determined in the control variant on Vertisols at lowest illumination (variant 13. $N_0P_0K_0$) – 0.86 g and the highest weight – 11.82 g was determined in variant 12. $N_{600}P_{300}K_{377}$ plants grown under illumination of 18000 lx on the same soil type (Vertisols). With the exception of the variants at low illumination on Luvisols, all other variants in the experiment had statistically proved differences between control and fertilized variants, and between variants fertilized with $N_{400}P_{300}K_{377}$ and those fertilized with $N_{600}P_{300}K_{377}$, as in all the variants the biomass was greater at the highest level of fertilization. There weren't differences between the weights of the plants in the control variants of the experiment (homogeneous group A).

Plants developed on Vertisols had significantly greater fresh weight than those on Luvisols at both illumination levels. The fresh weight of spinach plants in cameras with 18000 lx illumination was greater than that obtained in 9000 lx, on both soil types.

Physiological and biochemical parameters of spinach plants depending on soil type, light and nitrogen rate

In the plastids of green leaves there is a group of pigments with a very important effect in the process of photosynthesis. Green colored pigments are chlorophylls and yellow – carotenoids. From the green pigments in higher plants, the blue-green chlorophyll "a" and the yellow-green chlorophyll "b" are the main optically active components. The amount of chlorophyll represents about 0.5 – 1.3% of the dry weight of the leaves. Plastid pigments are involved in photosynthesis and play a role in the growth and development of plants. Therefore the study of the composition and the quantity of pigments in plants under different conditions of development, and their role in the biochemical processes is of great scientific and practical interest. The values for the content of chlorophyll "a", chlorophyll "b" and carotenoids obtained in the experiment were significantly higher than in other studies (Ratterman, 2006; Dinev and Mitova, 2011), which could be explained by the good nutritional regime and microclimate conditions (Table 7). The content of chlorophyll "a" in the assay was between 6.1 and 18.6 mg /%, and that of chlorophyll "b" – between 4.5 and 12.7 mg /% (Table 7). As it could be expected the lowest chlorophyll content measured was in the plants developed in the control variants on Luvisols (of lower nutrient content). On this soil type, chlorophylls measured at the end of the experiment were significantly less than those of spinach plants grown on Vertisols. This is a logical consequence of ontogenesis, where with the improvement of climatic conditions the plant nutrition improves, too (Mengel and Kirkby 1982).

Comparing the difference between chlorophyll contents of the plants depending on the illumination level it could be seen, that there were differences in priority of the variants with a higher illuminance. With the increase of nitrogen rate, chlorophyll and carotenoids content generally had increased on Luvisols and in the variants of low illumination on Vertisols. In plants, developed at 18000 lx of illumination on Vertisols, the rate of 600 $mgN.kg^{-1}$ soil had caused inhibition in pigment synthesis (Table 7).

Table 7
The effect of soil type, fertilization rate and illumination level on plastid pigment content in spinach plants (mg/%)

Variants	Chl "a" (mg/%)	Chl "b" (mg/%)	Carotenoids (mg/%)	Chl"a"/ Chl"b"	Chl"a"+Chl"b"
			Luvisols		
			Illumination level 18 000 lx		
1. $N_0P_0K_0$	6.1	4.5	1.9	1.3	10.6
2. $N_{200}P_{300}K_{377}$	11.1	5.3	4.2	2.1	16.3
3. $N_{400}P_{300}K_{377}$	14.5	8.9	4.5	1.6	23.5
4. $N_{600}P_{300}K_{377}$	18.6	12.7	7.0	1.5	31.3
			Illumination level 9000 lx		
5. $N_0P_0K_0$	6.0	5.4	4.3	1.1	11.4
6. $N_{200}P_{300}K_{377}$	8.9	4.6	4.8	1.9	13.5
7. $N_{400}P_{300}K_{377}$	9.6	4.3	5.5	2.3	13.9
8. $N_{600}P_{300}K_{377}$	10.8	6.3	6.6	1.7	17.2
			Vertisols		
			Illumination level 18 000 lx		
9. $N_0P_0K_0$	10.4	4.7	6.2	2.2	15.1
10. $N_{200}P_{300}K_{377}$	10.1	4.6	5.9	2.2	14.8
11. $N_{400}P_{300}K_{377}$	14.0	7.9	8.4	1.8	21.9
12. $N_{600}P_{300}K_{377}$	12.7	5.6	7.3	2.2	18.5
			Illumination level 9000 lx		
13. $N_0P_0K_0$	6.5	3.1	2.2	2.1	9.5
14. $N_{200}P_{300}K_{377}$	12.7	6.3	4.1	2.0	18.9
15. $N_{400}P_{300}K_{377}$	10.7	5.3	3.4	2.0	15.9
16. $N_{600}P_{300}K_{377}$	12.6	7.4	3.8	1.7	19.9

Typically, the ratio of chlorophyll “a” / chlorophyll “b” is 3: 1 (Pochinok, 1976). According to Berova et al. (2007), the ratio of chlorophyll “a” / chlorophyll “b” is 2- 3: 1, but it is not constant and depends on both the internal and external factors (Hendry and Grime, 1993). In this experiment, the ratios between chlorophylls “a” and “b” were between 1.1 and 2.3. The lowest ones were recorded in the control variant at 9000 lx illumination on Luvisols, and the highest – in variant 7.$N_{400}P_{300}K_{377}$ under the same abiotic conditions.

Leafy vegetables are characterized by relatively low dry matter content, but their characteristics are also high vitamin content and ability to accumulate nitrates, especially green leafy vegetables such as spinach (Mihov et al., 1980; Mitova et al., 2005; Shaban et al., 2014). Dry matter content is a key indicator in determination of the quality of vegetables. It is a relatively stable, genetically determined parameter and it is regarded as a substance with its own special properties. Dry matter content is synthesized expression of the valuable components of the product of vegetable crops (Peev, 1985). Relatively low dry matter content has been reported by (Rankov et al., 2004), regarding fruit vegetables (tomato, cucumber, eggplant). However, the contents of the absolute dry matter obtained in this experiment with spinach plants were high (Table 8). The high content of dry matter in variants without fertilization, on both soil types was due to the inhibition in plant development, “dilution effect” (Krastev, 1983; Mitova and Stoykov, 2008), which results in an increased concentration of the cell sap in cases of intensive changes of the growing conditions (temperature, humidity, concentration of nutrients, etc.). The dry matter contents measured on Luvisols were higher than those on Vertisols. On Luvisols with the increase of nitrogen fertilization the dry matter content of plants increased, too. On Vertisols similar trends were not observed (Table 8). On Vertisols at both illumination levels, in the variants 12.$N_{600}P_{300}K_{377}$ and 16.$N_{600}P_{300}K_{377}$ the dry matter content of the spinach plants decreased. On Vertisols, dry matter contents at high illumination were higher, while on Luvisols soil such differences were not observed.

It is reported (Peev, 1985) that the sugars content has a good correlation relationship with the dry matter content. In the present study, the content of total sugars ranged from 7.2% in the variant without fertilization on Vertisols with high illumination to 16.9% for the plants fertilized with $N_{600}P_{300}K_{377}$ on the same soil type, but at low illumination level. On both soils, with the increase of nitrogen rate total sugar contents increased. It was higher in chambers with 9000 lx of light in comparison with 18000 lx. While at the high illuminance the differences between the content of total sugars on the two soils types were minor, at 9000 lx of illuminance the plants grown on Vertisols had a significantly higher content of total sugars than those grown on Luvisols.

Table 8
Effect of soil type, fertilization rate and illumination level on some quality parameters of spinach plants

Variants	Absolutely dry weight (%)	Total sugars (%)	Nitrates (mg. kg^{-1})
	Luvisols		
	Illumination level 18000 lx		
1. $N_0P_0K_0$	18.75	8.2	25.6
2. $N_{200}P_{300}K_{377}$	13.61	8.7	245.8
3. $N_{400}P_{300}K_{377}$	14.58	8.9	305.1
4. $N_{600}P_{300}K_{377}$	15.25	9.4	415.3
	Illumination level 9000 lx		
5 .$N_0P_0K_0$	15.06	9.4	48.0
6. $N_{200}P_{300}K_{377}$	13.64	12.8	272.6
7. $N_{400}P_{300}K_{377}$	14.24	14.3	325.9
8. $N_{600}P_{300}K_{377}$	16.42	15.0	759.5
	Vertisols		
	Illumination level 18 000 lx		
9. $N_0P_0K_0$	13.38	7.2	50.9
10. $N_{200}P_{300}K_{377}$	13.06	7.5	115.6
11. $N_{400}P_{300}K_{377}$	13.68	8.1	211.2
12. $N_{600}P_{300}K_{377}$	11.57	9.6	356.9
	Illumination level 9000 lx		
13. $N_0P_0K_0$	11.65	14.0	72.5
14. $N_{200}P_{300}K_{377}$	11.41	16.3	186.7
15. $N_{400}P_{300}K_{377}$	11.58	16.6	263.5
16. $N_{600}P_{300}K_{377}$	10.96	16.9	578.4

Nitrate accumulation in the leafy vegetables production is genetically determined (Stoyanov, 1997; Abu-Dayeh, 2006; Genkova, 2009). The nitrate content in leaves of spinach varied between 25.6 and 759.5 mg.kg^{-1} fresh weight (Table 8). The control of the maximum permissible levels for contaminants in food is performed by European Commission Regulation (EC)No. 194/97. For spinach limit concentration for nitrates from 1 October to 31 March are 3000 mg.kg^{-1} fresh weight, and from 1 April to 30 September – 2500 mg.kg^{-1} fresh weight (ordinance by the Ministry of Agriculture and Food – 31 from 29.07.2004).

With the increase of nitrogen level, nitrate content in the variants of the experiment increased. In the experiment however, in none of the variants limit concentrations of 2500 and 3000 mgNO_3. kg^{-1} fresh weight were exceeded. Regardless of the weak availability of mineral nitrogen, on Luvisols of increasing nitrogen rate, the plants accumulated more nitrate than the same variants on Vertisols. This could be due to the low sorption capacity of Luvisols, whose absorbing complex retains weakly the nitrate anions (NO_3^-) and they were assimilated quickly by plants. On both soil types, the highest nitrate content was measured at the lower illuminance level in the chambers (Table 8). Obviously, higher illumination favors more full inclusion of NO_3- N in cellular metabolism of plants.

Relatively low levels of nitrates in spinach plants in the experiment were probably due to the optimal lighting and temperature conditions, and the optimum soil and air humidity maintained in the chambers. These conditions are suitable for intensive utilization of nitrate nitrogen by plants and including it in the plant metabolism.

Nutrient content in spinach, depending on the soil type, light and nitrogen rate

The content of nutrients in plants is dependent on baseline content in soils included in the experiment. The concentration of nitrogen in plants on Luvisols varied between 1.5 and 4.8% (Table 9).

With the increase of nitrogen rate applied, the nitrogen content in plants grown on Luvisols increased. At low illumination level nitrogen content in spinach was higher than at 18000 lx illumination. On Vertisols there was higher nitrogen content in plants grown in the camera of lower illumination, but in variant fertilized with $N_{600}P_{300}K_{377}$ the nitrogen content decreased. Apparently, the rate of 600 mgN.kg soil^{-1} applied to Vertisols is too high and had a depressing effect on the development of spinach, which is supported by both the chlorophyll content and the content of nitrogen in plants. Phosphorous content in the spinach plants varied between 0.52 and 0.96% and, like that the nitrogen, in both soil types, it was higher at the lower illuminance. On Vertisols in accordance with the higher output of phosphorous, the content of this macroelement in plants was higher than on Luvisols. The situation was similar concerning potassium. On Vertisoil the contents of potassium in plants were very high in variants fertilized with $N_{600}P_{300}K_{377}$ – 12.5% in plants with

Table 9

Effect of soil type, fertilization rate and illumination level on some macro elements content in spinach plants, %

Variants	N%	P%	K%	Ca%	Mg%
	Luvisols				
	Illumination level 18 000 lx				
1. $N_0P_0K_0$	1.5	0.54	3.7	0.83	1.46
2. $N_{200}P_{300}K_{377}$	2.6	0.75	4.2	0.80	1.42
3. $N_{400}P_{300}K_{377}$	3.8	0.52	4.5	0.74	1.35
4. $N_{600}P_{300}K_{377}$	4.2	0.57	6.0	0.66	1.18
	Illumination level 9000 lx				
5. $N_0P_0K_0$	2.1	0.78	6.5	1.35	1.28
6. $N_{200}P_{300}K_{377}$	4.3	0.89	8.6	1.34	0.71
7. $N_{400}P_{300}K_{377}$	4.4	0.88	8.7	1.21	0.83
8. $N_{600}P_{300}K_{377}$	4.8	0.65	9.5	1.20	0.75
	Vertisols				
	Illumination level 18 000 lx				
9. $N_0P_0K_0$	2.4	0.50	8.0	1.55	1.48
10. $N_{200}P_{300}K_{377}$	2.8	0.66	11.5	1.09	1.29
11. $N_{400}P_{300}K_{377}$	4.6	0.63	12.0	1.55	1.02
12. $N_{600}P_{300}K_{377}$	3.8	0.67	12.5	1.37	1.06
	Illumination level 9000 lx				
13. $N_0P_0K_0$	2.5	0.82	8.4	1.44	1.15
14. $N_{200}P_{300}K_{377}$	4.4	0.87	12.9	1.37	0.76
15. $N_{400}P_{300}K_{377}$	4.9	0.91	12.9	1.28	0.84
16. $N_{600}P_{300}K_{377}$	4.5	0.96	13.4	1.22	0.91

18000 lx illumination and 12.9% at 9000 lx. When studying the Ca content in plants (Table 9) an interesting pattern was observed. On both soil types, at both illuminations the calcium content was the highest in the plants of the control variants and decreased with the increase of the nitrogen rate. On Luvisols the calcium content was higher at low illuminance, while on Vertisols no such tendency was established. When Mg content was observed, there was a similar tendency as at calcium – the highest content of magnesium was measured at non-fertilized plants. On both soils, magnesium content was lower in plants of low illuminance, and between the variants of fertilization consequential differences were missing. High baseline potassium content of Vertisols and additional potassium fertilization in Luvisols inhibited translocation of K, Ca and Mg in plants. This is most probably due to the competitive relationship of these elements in plants (Neubert et al., 1970; Mengel and Kirkby, 1982).

Conclusion

On Vertisols when compared with Luvisols plants of higher and greater fresh weight and number of leaves were formed. From the factors studied (soil type, fertilization and lighting) fertilization was with the most strongly proven impact on the development of plants (between 41.2 and 60.6%), concerning the number of leaves, height and weight of the plants.

The reaction of the experimental plants to the illuminance was in different directions. On Luvisols more and higher plants were formed under low illumination level (9000 lx). The number of leaves in both soils and the leaf fresh weight on Vertisols were larger at illuminance of 18000 lx.

With the increase of nitrogen level, the contents of chlorophylls, total sugars, nitrates and nutrients (N, P, K) increased. On Vertisols at the fertilization level $N_{600}P_{300}K_{377}$, inhibition of the chlorophyll synthesis and nitrogen uptake was observed.

On Vertisols compared with Luvisols, at both illuminations (as well as the content of total sugars in the illumination of 9000 lx.) the absorption of nitrogen, phosphorus and potassium from the spinach plant was greater, chlorophylls were synthesized more, and the nitrate content was lower.

References

Abu-Dayeh, A., 2006. Determination of nitrate and nitrite content in several vegetables in Tulkarm district. Thesis for the degree of master in environmental sciences, *An-Najah National University*, Nablus, Palestine, 95 pp.

Arinushkina, E., 1970. Guidance on Chemical Analysis of Soil, *Moscow State University*, 487 pp (Ru).

Berova, M., H. Stoeva, A. Vasilev and H. Zlatev, 2007. Manual for Exercises of Plant Physiology, *Academic Publishing*, AU Plovdiv, pp. 42-45 (Bg).

Brechner, M. and D. Villiers, Hydroponic Spinach Production Handbook.pdf, Cornell Controlled Environment Agriculture, *Cornell University,* CEA Program http://www.cornellcea.com/attachments/Cornell%20CEA%20baby%20spinach%20handbook.pdf

Dinev, N. and I. Mitova, 2011. Effects of mineral and organic fertilization on the accumulation of contaminants in plants of spinach. *Soil Science, Agrochemistry and Ecology*, (1- 4): 104-110 (Bg).

Gadjalska, N., V. Petrova, V. Kancheva **and** T. Tashev, 2017. Adaptive Models for Irrigation of crops using water saving technologies. *Water Affairs*, 1/2: 21-29 (Bg).

Genkova, I., 2009. Intensive Vegetable Production. *Enyovche*, 334 pp (Bg).

Hendry, G. and J. Gime, 1993. Methods in Comparative Plant Ecology, *Chapman and Hall*, The Hague, 167 pp.

Ivanov, P., 1984. New acetate-lactate method for determining plants available phosphorus and potassium in soil. *Soi Science and Agrochemistry*, (4): 88-98 (Bg).

Klechkovski, V. and A. Peterburgsky, 1967. Agrochemistry. *Colossus*, *M.*, pp. 65-73 (Ru).

Koinov, C., 1987. Correlation between soils in Bulgaria and basic soils identified by the World Soil Classification. *Soil Science, Agrochemistry and Plant Protection*, (5): 5-13 (Bg).

Krastev, S., 1983. Fertilization of sugar beet with nitrogen, phosphorus and potassium in conditions of calcic chernozems. Doctoral dissertation, *"N. Poushkarov" Institute*, Sofia, Bulgaria, 138 pp (Bg).

Mengel, K. and E. Kirkby, 1982. Principles of Plant Nutrition. *International Potash Institute*, Bern, Switzerland, pp. 168-184.

Mihov, A., M. Yordanov and C. Karaivanov, 1980. Problems of Modern Horticulture, *Hristo Danov*, Plovdiv, 351 pp (Bg).

Mincheva, M. and A. Brashnarova, 1975. Certain ways of mineralization of plant material by routine analysis to define K, Ca, Mg, Na, Zn, Mn, Cu and Fe by the methods of contemporary spectrophotometry. *Soil Science and Agrochemistry*, (1): 114-122 (Bg).

Mitova, I. and I. Stancheva, 2003. Far- reaching effects of increasing rates of the organic and mineral nitrogen fertilizer on the yield and some quality parameters in spinach. *Ecology and Future*, (3- 4): 99- 100 (Bg).

Mitova, I., I. Stancheva, E. Atanasova and R. Toncheva, 2005. Influence of organic, mineral and foliar fertilization on spinach yield and soil fertility. *Soil Science Agrochemistry and Ecology*, (2): 32-37 (Bg).

Mitova, I. and H. Stoykov, 2008. Light and the soil humidity as factors determining growth processes and some of the quality indicators in Chinese cabbage *Brassica pekinensis* (Lour.), Seventh International Symposium "Ecology and sustainable development", 23- 25 October 2008, Vratza, Research Papers, pp. 291-294 (Bg).

Moskov, I., V. Vasev, L. Bozova, V. Kolev and D. Mileva, 1975. A Laboratory Manual of Plant Physiology, 118 pp. (Bg).

Neubert, P., W. Wrazidlo, H. Vielemeyer, Y. Hundt, Fr. Gollmick and W. Bergmann, 1970. Tabellen zur Pflanzen Analyse. Erste Orientierende Ubersicht- Erarbeitet Und Zusammengestellt., Inst. Fur pflanzen 69 Jena, Berlin, GDR, 29 pp.

Peev, Hr., 1985. Agrochemical and Physiological Bases of the Quality of the Crop Production. *Zemizdat*, Sofia, pp. 263-283 (Bg).

Petrova, V. and R. Kireva**,** 2016. Optimization on laying depth for subsurface drip irrigation of intensive crops and vegetables. *Journal of Mountain Agriculture on the Balkans*, **19** (5): 170-184 (Bg).

Pochinok, H., 1976. Methods of Biochemical Analysis of Plants, *Naukova Dumka*, Kiev, pp. 192- 195 (Ru).

Rankov, V., Hr. Boteva and D. Stoyanov, 2004. Influence of nitrogen fertilization on yield and quality of spinach production. Proceedings of the Vth International Technical conference "Ecology and Health", pp. 205- 209 (Bg).

Ratterman, D., 2006.The effect light intensity has on the photosynthesis of spinach chloroplasts. *Saint Joseph's University Archives*, https://adamcap.com/schoolwork/the-effect-light-intensity-has-on-the-photosynthesis-of-spinach-chloroplasts/

Shaban, N., S. Bistrichanov, C. Moskova, E. Kadum, I. Mitova and M. Tityanov, 2014. Vegetable Production, Sofia, *Forest University*, 490 pp (Bg).

Stoyanov, S., 1997. Nitrates, Nitrites, Nitrosamines. *Pensoft*, 102 pp (Bg).

Stoykov, Hr., 2006. A series of growth chambers with low energy consumption. *Agricultural Machinery*, (1): 15-17 (Bg).

Stoykov, Hr., 2009. New version of growth chamber with low energy consumption – KNER-4. Jubilee International Conference "Science Engineers for Sustainable Development of Agriculture" for the 60th anniversary of IMM, 5 – 7 November 2009 (Bg).

Received April, 12, 2017; accepted for printing September, 15, 2017