Influence of the distribution of the fertilizer norm of nitrate nitrogen and irrigation regime on the yield and some morphological and biochemical parameters in peanuts variety Tsvetelina

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Abstract
During the period 2019-2020 at the Institute of Plant Genetic Resources - Sadovo, Bulgaria a comparative study was conducted on the influence of nitrate nitrogen and irrigation regime on the productive potential of peanuts of the Valencia type, variety Tsvetelina. This study aims to determine whether the distribution of nitrate nitrogen and water regime are interrelated factors in peanuts. The study showed that the fertilization whit nitrate nitrogen are effective up to the budding phase. Its deficiency before the onset of this phenophase leads to a decrease in yield and cannot be compensated by its later fertilization. The optimal water content in the soil contributes to better nitrogen absorption. Nitrogen and water lead to higher chlorophyll content in peanut leaves and efficiency of photosynthesis. The supply of nitrate nitrogen in the initial stages of peanut development, the content of chlorophyll in the leaves and the optimal water content of the soil lead to increased yield. Deficiency of nitrogen and water disrupts the synthesis of chlorophyll and leads to lower yields. The regularly irrigated variant with pre-sowing application of the whole nitrate norm is distinguished by the highest productive possibilities.

Key words: peanuts; mineral nutrition; water deficiency; photosynthesis; yield

INTRODUKTION

Among the environmental factors that can be controlled by farmers, water and nitrogen are the main determinants of plant growth. According to Neto et al. (2012), nitrogen is the element that is absorbed the most by plants, because in addition to being essential in the process of photosynthesis, it is present in amino acids that act in the synthesis of structural and functional proteins. There are several studies proving the need for nitrogen application in peanut culture (Mantovani et al., 2012; Rowland et al., 2012).

The level of damage caused to peanuts by water shortage is determined by the intensity, duration of stress and the phenological stage in which the crop is located (Duarte et al., 2013; Ezihe et al., 2017). According to de Azevedo et al. (2014) in irrigated agriculture it is necessary to determine the determining factors in the management of irrigation, which directly interfere with the greater or lesser water consumption, and to determine the water needs of crops according to the different phenological stages. The development cycle of Bulgarian peanut varieties varies from 125 to 135 days and depending on the climate their water needs vary from 280 to 320 mm. The syntheses of plastid pigments are essential for the photosynthetic activity of plants. The content of plastid pigments under normal and stressful conditions has been extensively studied (Mikiciuk et al. 2010; Aienl et al. 2011). Measurement of chlorophyll content provides direct information on the potential for photosynthesis. Together with the associated parameters, it provides an indirect measure of the plant's physiological stress (Arunyanark et al., 2008) and nutritional status (Gáborcík, 2003). CCM 200 plus (a measurer of chlorophyll content) is useful for improving nitrogen management, ideal for determining stress, leaf aging, health status determination and other research (Richardson et al., 2002). Many studies have shown that chlorophyll meter reading is useful as a selective parameter of drought resistance and peanut productivity (Madhava et al., 2003; Songsri et al., 2009; Arunyanark et al., 2008; Balota, 2012).

This study aims to identify how the distribution of nitrate nitrogen and irrigation during growing season affects the productive potential of peanuts. In addition, it will answer the question of whether there is a link between nitrogen and the presence of available soil moisture on the efficiency of photosynthesis.

MATERIAL AND METHOD

Experiment design

The study was carried in 2019-2020 at the Institute of Plant Genetic Resources - Sadovo, Bulgaria. The experiment was performed in three replications by the block method, with a total area for each of the variants of 30 m^2. For the analysis of the results, measurements were taken from 50 randomized plants of each variant. Soil sampling was done at a depth of 0-30 cm. 20 samples were made. The pH, stock and content of digestible forms of nitrogen, phosphorus, potassium, as well as the content of organic matter (humus) were analyzed.

The fertilizer rate of 17 kg/da nitrate nitrogen in the form of nitrate was introduced in three fertilization schemes and distributed as follows:

- The whole norm of 17.0 kg/da N imported before sowing;
- 1/2 of the norm imported before sowing and 1/2 during the beginning of peanut flowering, distributed according to the scheme 8.5 N + 8.5 N;
- 1/3 of the norm imported before sowing, 1/3 of the norm imported during the beginning of flowering of peanuts and 1/3 of the norm introduced in the phase of beginning of fruiting, distributed according to scheme 5.66 N + 5.66 N + 5.66 N;

The irrigation regime for peanuts was realized after the beginning of flowering phase, by drip irrigation. The used irrigation rate of 210 l/m^2 was realized with three irrigations. Variants with the three fertilization rates were irrigated without allowing the soil to dry out until the end of the flowering phase. In these variants, an optimal maximum soil moisture content of 75% was maintained. The same irrigation rate was used for the other three variants of the fertilizer rate, but the water was supplied after a period of drought, the maximum soil moisture content reached 60%.

The study includes 6/six experimental variants formed by two main factors. The first factor is the irrigation regime presented in two levels, and the second factor fertilization presented in three levels. During the vegetation before each feeding with nitrate nitrogen the morphological parameters were taken into account:

Shrub height, shrub width, leaf blade length and leaf blade width. The leaf area was reported according to the formula:

LP = DP * SHP * 0.56, where:

LP - leaf area in cm^2

DP - length of the sheet

SHP - width of the sheet

0.56 - coefficient for correction of the elliptical shape of the sheet.

The counting was performed by measuring 10 randomized plants of a variant.

Biochemical parameters

Determination of the nitrogen content in the leaves was performed by the Kendal method by distillation in a Parnassus-Wagner apparatus after wet burning of plant materials. The crude protein content is obtained by the formula:

SP = x * f, where

X - nitrogen content in the leaves;

f- correction factor (6.25)

The chlorophyll content of the leaves in the experiment variants was performed by the value of the index of chlorophyll content (CCI) in the leaves. It was measured using a portable Chlorophyll Content Meter-CCM 200 plus manufactured by Opti-Sciences, Inc., NH, USA. The measurements were made in six phases of peanut development for each variant. From each variant, 20 sheets were analyzed (n = 20).

The yield of fruits and nuts from the plant was reported from 20 randomized plants from each variant. The total biomass, the mass of the plant without fruits were taken from the same plants and with their help the harvest index was calculated according to the formula:

HI = BMwP / TB * 100, where: HI - harvest index; BMwP - biomass without fruits; TB- total biomass;

Statistical methods

One-factor and two-factor analysis of variance were used to prove the existing differences, using the LSD method by 0.05 a degree of freedom. The two-factor analysis of variance took into account the influence of the two controlled factors - nitrate nitrogen fertilization scheme and irrigation regime on the change in yield, morphological and biochemical parameters in peanuts.

The functional relationship in which a fixed change in the absolute value of the independent variable leads to a fixed proportional change (ie percentage increase or decrease) in the value of the function is reported by an exponential equation.

RESULTS AND DISCUSSION

Table 1 presents the results of the soil analysis. The data show that the content of mineral nitrogen is very low, the content of phosphorus is low, the content of potassium is high, and the content of humus is low. Such content suggests the effect of mineral nutrition with nitrogen.

After the germination of the peanuts until the budding phase occurs, the height of the bush in the individual variants varied from 3.6 to 4.9 cm, Fig. 1A. The highest is the height of the plants from the variant in which the whole fertilizer rate of nitrogen is imported before sowing. The differences are well secured and mathematically significant. The exponential curve shows a decrease in plant height with a decrease in nitrate nitrogen in this phase.

The width of the bush in the experimental variants varies from 8.8 to 9.7 cm, but without mathematically provided differences. The size of the leaf area by changing the amount of nitrate nitrogen in this phase of plant development are presented in Picture 1. The size of the leaf by changing the amount of nitrate nitrogen is obvious in this phase of plant development. The largest are the leaf of the variant in which the whole norm of nitrate nitrogen is imported before sowing. Their length is 2.36 cm versus 1.9 cm in the variant in which 1/3 of the norm nitrate nitrogen is imported. The differences are well secured and mathematically significant. The exponential curve shows a decrease in leaf length with decreasing nitrate norm. The high coefficient of determination $R^2 = 0.915$ indicates the linear character of the curve, Fig. 1B. Leaf width also decreases with decreasing nitrate nitrogen and varies from 1.9 to 1.54 cm with statistically significant differences. The decrease in leaf width is also exponential in nature with a high coefficient of determination, Fig. 1C. The functions of the size of the leaf are the leaf area. Quite naturally, it also changes in the same dependencies as in the size of the leaves, Fig. 1D. The content of total nitrogen in the leaves is high and varies between 4.31 and 4.69% in the individual variants without statistically significant differences.

The chlorophyll content index has values from 28.09 to 29.06 and is without significant differences in the individual variants.

Lack of nitrate nutrition to the budding phase has a negative effect on plant growth. The height of the bush varies between 4.2 to 6.8 cm (Fig. 2A). The growth retardation of plants in height is mathematically significant and decreases exponentially. The differences in the width of the bush are also significant between the different variants and vary between 11.0 to 15.8 cm. Exponentially, the width of the bush decreases with the lack of nitrate nitrogen, Fig. 2B. The width of the leaf varies mathematically signifi-

Table 1. Soil analysis

Soil layer, cm	pH (H_2O)	Nitrate nitrogen, NO_3-N mg/kg	Ammonium nitrogen, NO_4-N mg/kg	Mineral nitrogen, NO_3N+NH_4-N mg/kg	Assimilate phosphorus, P_2O_5 mg/kg	Exchanged potassium, K_2O mg/kg	Hummus, %
0-30	6.46	3.73	2.86	6.59	25.76	265.06	1,36

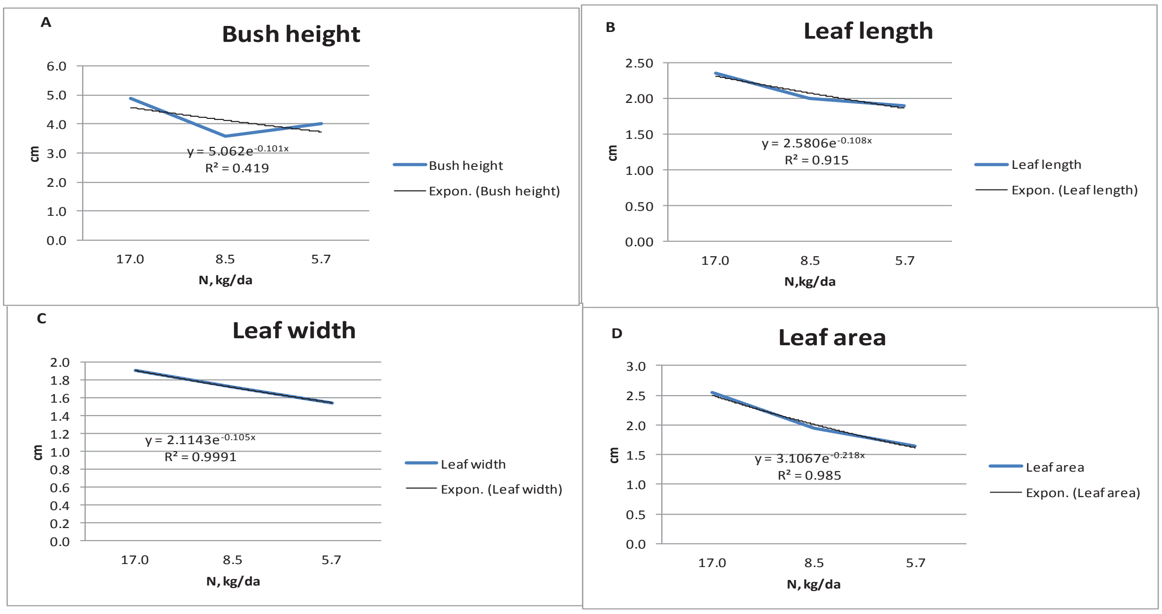

Figure 1. Morphometric parameters in the germination phase

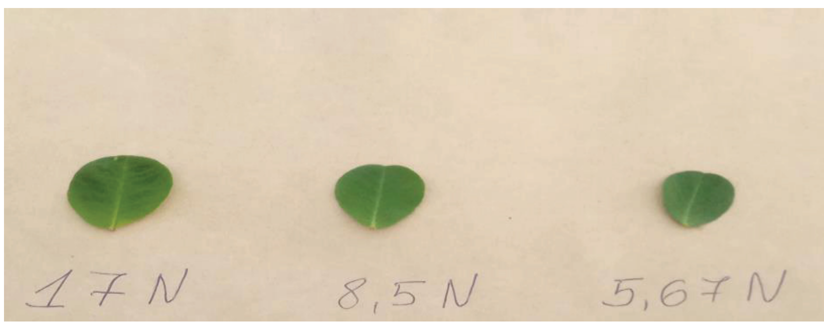

Photo 1. Changes in the size of the leaf blade depending on the different fertilizer rates

cant between the different variants and it are between 1.9 and 2.3 cm and decreases exponentially, Fig.2C. The significant change in the width of the leaf leads to mathematically significant differences in the leaf area, Fig. 2D. Leaf area decreases exponentially with the lack of nitrate nitrogen in the soil.

The content of total nitrogen in the leaves after germination of plants and the appearance of true leaves varies from 4.31 to 4.69. In the budding phase, in the case of a variant fertilized pre-sowing with the whole nitrogen norm, the nitrogen content in the leaves is optimal 3.15%. In the other two variants, it reaches the critical minimum and is in the range of 2.84 to 2.87%. The values of the chlorophyll content index are in the range of 11.01 to 14.0.3 and the differences are mathematically non-significant.

The growth of the vegetative mass is a direct function of the fertilization whit nitrate nitrogen in the initial stages of peanut development. Its deficiency in this phase is reflected in the depression of the vegetative organs.

During mass flowering, after the first feeding of the plants, the morphological parameters between the different variants are without significant differences. Nitrogen nitrate feeding leads to an increase in nitrogen in the leaves and in the fed variants it reaches from 2.94 to 3.01%. There is a decrease in the variant with pre-sowing imported the whole nitrate norm - 2.87%. The index of chlorophyll content also changes its value and the lowest it in the variant with pre-sowing nitrogen application - 18.7. Chlorophyll content index is higher in the nutri-

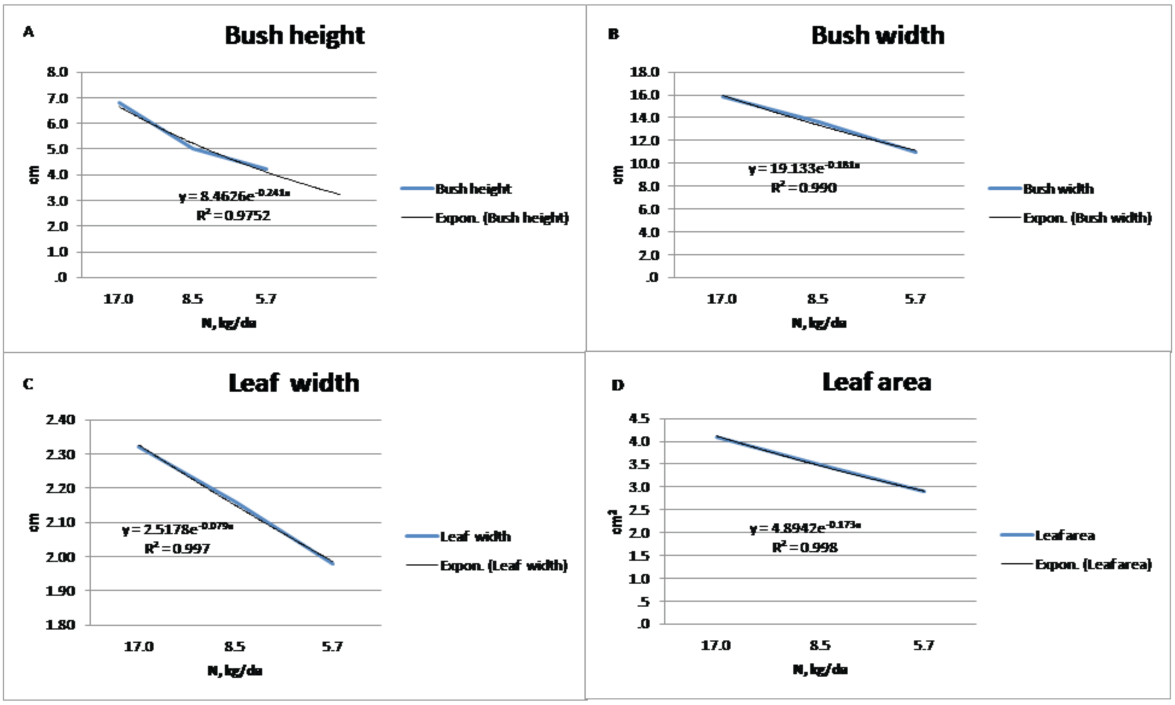

Figure 2. Morphometric parameters in the budding phase

tion variants, the values are between 20.9 and 22.6, which have been significant to be superior to the first variant. The exponential equation significant this dependence, Fig.3.

The needs of peanuts from irrigation begin after the phase of mass flowering and fruiting quote (Georgiev & Bencheva, 2011). After the onset of this phase, the irrigation of the crop begins. The chlorophyll content index undergoes significant differences between the studied variants. In this phase with the highest value of CCI - 24.2 is characterized the variant with double application of the nitrogen

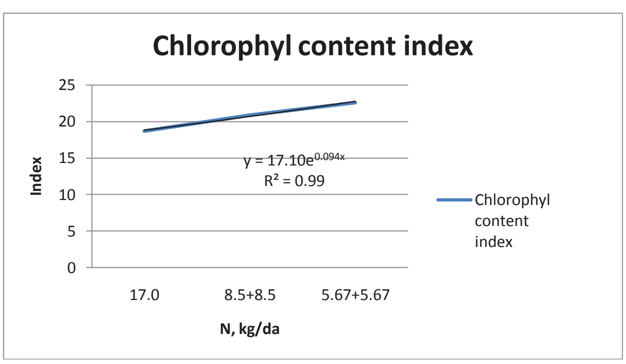

Figure 3. Change in chlorophyll content index depending on fertilizer levels of nitrate nitrogen

fertilizer rate in the variants with disturbed irrigation rate. The obtained increase in the variants with disturbed water supply is shown with an exponential increase, but with a low coefficient of determination $R^2 = 0.281$, Fig. 4. This means the presence of a nonlinear dependence of one of the two studied factors. The analysis of the results shows that the water in the soil and the combination between water and the nitrate nitrogen fertilization scheme show a significant influence on this indicator. Only the fertilization scheme is insignificant influence on the chlorophyll content, Table 2.

In this phase, the thickening of the cell sap leads to an increase in chlorophyll content.

In the beginning of fruit formation, the nitrogen content in the leaves is without significant differences in the individual variants. CCI increases significantly in the variants whit optimally irrigation and is highest in the double-fed variant. The low coefficient of determination indicates the insignificant influence of one of the tested factors, Fig. 5. Analysis of the results shows that the main influence on CCI is exerted by the presence of soil moisture and the combination between the nitrate nitrogen fertilization scheme and the irrigation regime, Table 3.

At the end of the flowering period, the CCI is again higher in the optimally irrigated varieties. Its

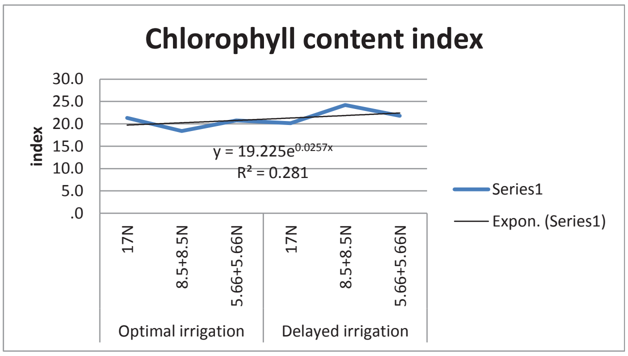

Figure 4. Change in chlorophyll content depending on fertilizer rates nitrate nitrogen and irrigation in the mass flowering phase

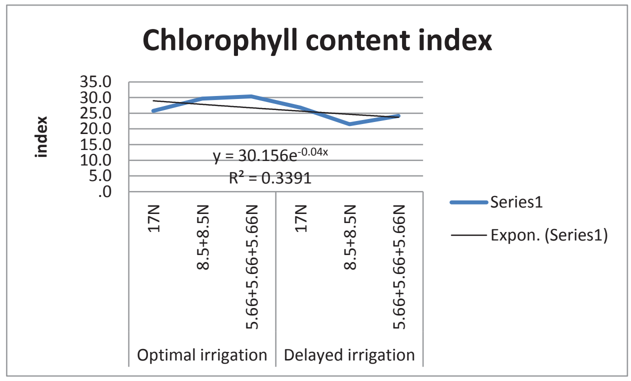

Figure 5. Change in the chlorophyll content depending on the fertilizer norms nitrate nitrogen and irrigation in the phase of onset of fruiting

Table 2. Test for the interaction of the studied objects in the flowering phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	278.173	5	55.635	2.784	.022
Intercept	40098.445	1	40098.445	2006.293	.0001
Irrigation*	79.336	1	79.336	3.970	.049
Fertilizer scheme	6.550	2	3.275	.164	.849
Irrigation x Fertilizer scheme*	192.287	2	96.143	4.810	.011
Error	1678.852	84	19.986		
Total	42055.470	90			
Corrected Total	1957.025	89			

Table 3. Test for the interaction of the studied objects in the fruiting phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	836.992	5	167.398	5.493	.0001
Intercept	62641.948	1	62641.948	2055.712	.0001
Irrigation*	442.668	1	442.668	14.527	.0001
Fertilizer scheme	42.670	2	21.335	.700	.499
Irrigation x Fertilizer scheme*	351.654	2	175.827	5.770	.004
Error	2559.660	84	30.472		
Total	66038.600	90			
Corrected Total	3396.652	89			

value decreases exponentially in the variants with disturbed water supply, but again with a low coefficient of determination, Fig. 6.

The analysis of the results shows that only the irrigation is important for the change of CCI in this phase of the development of the culture, Table 4.

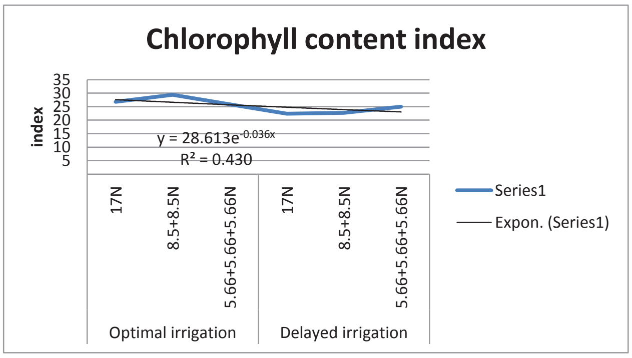

Figure 6. Change in chlorophyll content depending on fertilizer rates nitrate nitrogen and irrigation in the late flowering phase

Table 5. Mass of fruit from a plant

Irrigation	Nitrate nitrogen	Mean
Optimally irrigated	17N	35.4500
	8.5N+8.5N	24.9600
	5.66N+5.66N+5.66N	23.2833
Whit impaired irrigated	17N	18.7400
	8.5N+8.5N	16.9571
	5.66N+5.66N+5.66N	18.5000

The mass of the fruits of a plant is shown in Table 5. It can be seen that it decreases in the fed variants in comparison with the pre-sowing application of the whole nitrate norm and those with disturbed water supply.

The analysis of the results shows that both the irrigation and the nitrate nitrogen fertilization scheme, as well as the combination between them, have a relation to the yield of fruits from a plant, Table 6.

The exponential decrease in fruit yields is shown in Figure 7. The high coefficient of determination $R^2 = 0.8282$ shows that all tested factors influence fruit yield.

The results of nut yields are quite similar, where both the fertilization schemes and the irrigation regime affect the yield of nuts. The yield of nuts is highest when the entire dose of nitrate nitrogen is applied once and have the optimal water regime (Tables 7 and 8, and Figure 8).

The mass of the plant with the fruits or the total biomass is positively affected by the one-time application of the whole nitrate norm and the optimal irrigation regime similar to the yields of fruits and nuts, Fig. 9.

The influence on this parameter is reflected only through the fertilization scheme and irrigation regime. The combination of the two factors is statistically insignificant, Table 9.

The influence of the tested factors on the indicator of plant mass without fruits is insignificant. The differences in this indicator between the different variants are insignificant. The calculated harvest index shows that it is positively affected only by the water factor and interaction Irrigation x Fertilizer scheme, Table 10.

Table 4. Test for the interaction of the studied objects in the end of flowering phase

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	514.814	5	102.963	4.768	.001
Intercept	57780.268	1	57780.268	2675.813	.0001
Irrigation*	353.628	1	353.628	16.377	.0001
Fertilizer scheme	33.030	2	16.515	.765	.469
Irrigation x Fertilizer scheme	128.156	2	64.078	2.967	.057
Error	1813.857	84	21.594		
Total	60108.940	90			
Corrected Total	2328.672	89			

Table 6. Test for the interaction of the studied objects on fruit yield

Corrected Model	1435.605	5	287.121	10.542	.0001
Intercept	18658.386	1	18658.386	685.093	.0001
Irrigation*	853.763	1	853.763	31.348	.0001
Fertilizer scheme*	287.849	2	143.924	5.285	.011
Irrigation x Fertilizer scheme*	220.187	2	110.093	4.042	.028
Error	817.044	30	27.235		
Total	20889.450	36			
Corrected Total	2252.650	35			

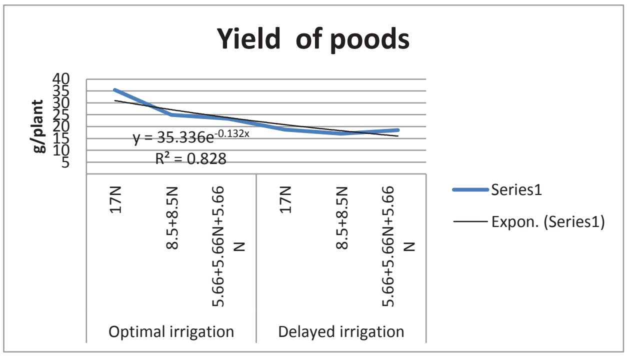

Figure 7. Yield of fruits from a plant depending on the fertilizer norms nitrate nitrogen and irrigation in the phase of end of flowering

Table 7. Mass of a nut from a plant

Irrigation	Nitrate nitrogen	Mean
Optimally irrigated	17N	24.9500
	8.5N+8.5N	17.4200
	5.66N+5.66N+5.66N	15.7667
Whit impaired irrigated	17N	19.4941
	8.5N+8.5N	12.8600
	5.66N+5.66N+5.66N	11.0857

Table 8. Test for the interaction of the studied objects on the yield of nuts

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	744.698	5	148.940	9.946	.0001
Intercept	9005.469	1	9005.469	601.400	.0001
Irrigation*	411.457	1	411.457	27.478	.0001
Fertilizer scheme*	147.480	2	73.740	4.924	.014
Irrigation x Fertilizer scheme*	149.194	2	74.597	4.982	.014
Error	449.225	30	14.974		
Total	10196.770	36			
Corrected Total	1193.923	35			

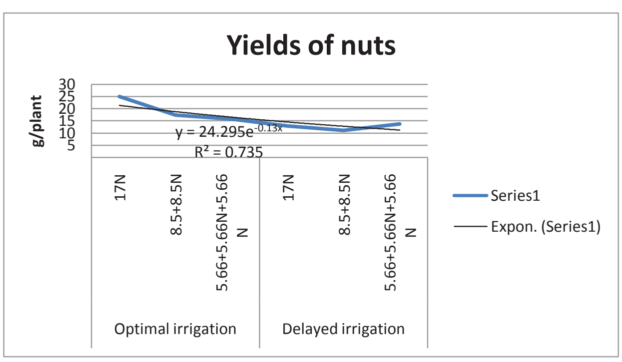

Figure 8. Yields of plant nuts depending on fertilizer rates nitrate nitrogen and irrigation in the late flowering phase

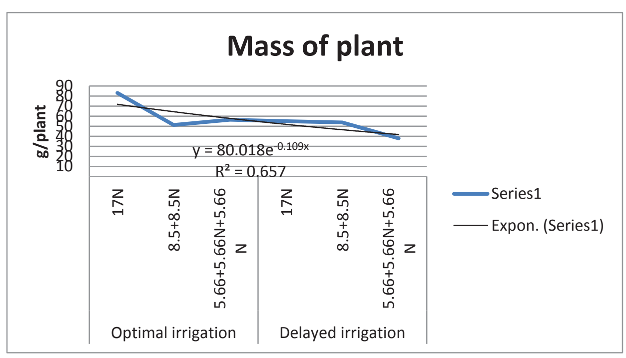

Figure 9. Mass of the plant with fruits depending on the fertilizer norms nitrate nitrogen and irrigation in the phase of end of flowering

Table 9. Test for the interaction of the studied objects on the total biomass of the plant

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6827.975	5	1365.595	4.036	.006
Intercept	111599.020	1	111599.020	329.826	.0001
Irrigation*	1900.019	1	1900.019	5.615	.024
Fertilizer scheme*	3001.436	2	1500.718	4.435	.021
Irrigation x Fertilizer scheme	1391.531	2	695.765	2.056	.146
Error	10150.725	30	338.357		
Total	129069.740	36			
Corrected Total	16978.700	35			

Table 10. Test for the interaction of the studied objects on the harvest index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1669.617	5	333.923	4.281	.005
Intercept	64015.175	1	64015.175	820.697	.000
Irrigation*	329.931	1	329.931	4.230	.049
Fertilizer scheme	226.707	2	113.353	1.453	.250
Irrigation x Fertilizer cheme*	1055.977	2	527.988	6.769	.004
Error	2340.030	30	78.001		
Total	68958.170	36			
Corrected Total	4009.648	35			

CONCLUSIONS

- Nitrate nitrogen deficiency in the early stages of plant development to the budding phase is a critical period in terms of fruit and nut yield.
- The content of total nitrogen in the leaves of the pre-sowing fertilized variant with the whole nitrogen norm is optimal 3.15%. In the other two variants it reaches the critical minimum and is in the range of 2.84 to 2.87%. Nitrogen fertilization after this phase is inefficient and does not compensate for the loss of productive potential in peanuts.
- The optimal water content of the soil is crucial for the size of the yield.
- The yield of peanuts is a direct function of the chlorophyll content in the leaves. It is affected in an identical way by the two factors tested.

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