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Effect of Organic Fertilization on the Physiological Status of Tomatoes under Abiotic Stress

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Abstract **– During the vegetation period of the years 2020 and 2021 in the experimental field at Agricultural University - Plovdiv it was conducted a physiological study of tomato cultivar (Rugby) with determined growth under the treatment with chemical and organic fertilizers. The aims of the study were to determine the changes in physiological behaviour and productivity of field tomatoes depending on the fertilization and dates of planting. The physiological assessment included the measuring of some parameters of chlorophyll fluorescence and chlorophyll content index. Chlorophyll fluorescence has proved to have the potential to detect abiotic stress effects on photosynthetic efficiency. This technique was used to assess growth intensity in tomato plants (*Solanum lycopersicum*), grown in different types of fertilizer treatment and planted in three additional dates – 30.04, 07.05 and 14.05 in conditions of ambient heat stress. Results have shown the slight effect of the applied organic fertilizer upon fluorescence parameters but significantly improved chlorophyll content index compared to no treatment variant. Additionally, it was measured higher chlorophyll content index for the second and third planting dates. The highest average yield was calculated for the NPK variant, as the statistically significant differences of the yield between control and fertilization variants were calculated in 2021. It possessed the highest average yield for second planting compared to the third date of planting.**

Keywords **– Chlorophyll fluorescence; chlorophyll content index; organic fertilizer; photosynthesis; *Solanum lycopersicum***

1. Introduction

Climate change is increasingly accepted as a worldwide phenomenon with possibly significant consequences and accompanying with recurrent extreme weather events [1], [2]. The research focused on climate change and the balanced distribution of water resources will lead to effective irrigation water management [3]. This will be a basis for sustainability and providing enough water for various needs, including ecosystems [4]. The regime and amount of precipitation are major factors for various agricultural and economic activities [5]–[7] and for changes in the environment [8], [9]. In the last few decades, organic vegetable production has expanded its areas around the world. The biological plant system is also applied in greenhouse conditions. This process is a bit slow compared to organic farming, but there is a

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positive trend. So far, a wide range of research has been done around the world in organic production both outdoors and in greenhouses [10]–[15]. Many of them test different formulations of NPK organic fertilizers. With this experiment, we set ourselves the goal of achieving sustainable vegetable production during the process of climate change.

We tried the possibilities for testing highly accepted endemic tomato varieties (Rugby variety) with a combination of different fertilization approaches. Two different types of fertilization were studied: organic and mineral on three other planting dates [16]–[19].

Tomato is an important vegetable that is highly sensitive to drought stress which impairs the development of tomato seedlings [20]. Chlorophyll fluorescence is a commonly used technique in plant physiology. It has been used to evaluate the heterogeneous distribution of photosynthetic activities over a leaf surface and has thus been applied to the detection of photosynthetic dysfunctions caused by biotic and abiotic stress factors [21], [22]. These photosynthesis decreases could result from the inhibition of photosystem II (PSII) activity, which has been shown to be the most thermally labile component of the electron transport chain. The inhibition of PSII also leads to a decrease in the variable chlorophyll fluorescence. Thus, in vivo chlorophyll fluorescence has been shown to be a sensitive and reliable method for the detection and quantification of temperature-induced changes in the photosynthetic apparatus [23]. The CCM 200 plus is helpful for improving nitrogen and fertilizer management and is ideal for crop stress, leaf senescence, plant breeding, health determination, and other studies. Furthermore, the affordability and ease of use make it an exceptional teaching tool for botany and plant science courses [24]. The aims of study were to determine the changes of physiological behaviour and productivity of field tomatoes depending on the fertilization and dates of planting.

2. METHODS AND METHODOLOGY

2.1. Plant material and growth conditions

In 2020 and 2021 an experiment was set in the experimental field of the Agricultural University of Plovdiv. The following three variants were tested: 1. Not fertilized – Control; 2. Mineral fertilization ($N_{34}P_{50}K_{46}$); 3. Organic fertilization (Arcobaleno). The nitrogen, phosphorus and potassium were imported in the form of ammonium nitrate, triple superphosphate and potassium sulfate in the optimal norms for this crop, and the organic fertilizer Arcobaleno in the norm recommended by the manufacturer. With the last tillage before planting, fertilizers were applied. The experiment was set on the block method in 4 repetitions with a plot size of 16 m^2. Prior to planting, the beds were mulched with plastic wrap. Twenty plants were planted in repetition according to the 160/50 cm scheme. Rugby tomatoes with three planting dates were used – April 30, May 7 and May 14. A drip system was used for irrigation. The occurrence of individual phenophases was reported. The physiological status of the plants was determined by the phenophase of mass fruiting. Vegetative manifestations of tomato plants (*Solanum lycopersicum* L.) were observed. *Arkobaleno* organic fertilizer containing nitrogen (N) – 4.5 %, phosphoric anhydrite (P_2O_5) – 3.5 %, calcium (CaO) – 5–8 %, magnesium (MgO) 0.8–1 %, organic carbon (C) – 30 %, organic matter 55–60 %; moistened organic matter 12–14 %, trace elements Fe, B, Cu, Zn. pH 6–8.

2.2. Chlorophyll fluorescence measurements

Chlorophyll fluorescence transients of tomato leaves were measured using a portable device. PAR-FluorPen FP 110/D. The chlorophyll fluorescence transients were measured on the same day in the morning. The dates of measurement were 15.07.2020 and 09.08.2020 for the first year, the 16.07.2021 and 05.08.2021 for the second year, when the tomato plants had reached in phenophase mass fruiting. The nine leaves from each variant were dark adapted for about 30 min by detachable leaf-clips prior each measurement. The fluorescence measurement protocol uses short (30 µs) measuring flashes to measure zero level fluorescence (Fo) followed by a strong saturating flash (duration 0.8 s, intensity about 3000 µmol m^{-2} s^{-1}) to measure the maximum fluorescence (Fm). Photosynthetically Active Radiation (*PAR*) is measured as Photosynthetic Photon Flux Density (*PPFD*), which is indicated by units of quanta (photons) per unit time per unit surface area. Three strong flashes of saturating light probed the effective quantum yield Fv/Fm of PSII during the actinic light exposure [25], [26].

2.3. Determination of chlorophyll content index (CCI)

Chlorophyll content index-*CCI* of the leaves was measured using a portable apparatus CCM 200 plus. The physiological determination was carried on the field. Measurements have been made in two dates of a sample of leaves when the tomato plants had reached in mass fruiting vegetation stage. The dates of measurement were 17.07.2020 and 10.08.2020 for the first year, and 15.07.2021 and 04.08.2021 for the second year. The plant assessments were made of each replication of variants. From each plant there were analysed 20 leaves by readings on the central part of the leaf.

2.4. Statistical analysis

Data are presented as mean values ± Standard error and the ANOVA test has been used for the comparison of mean values from two independent factors. For mathematical data processing it was used program Excel for Windows 10.

3. RESULTS

3.1. Meteorological conditions

Fig. 1 shows the daily solar radiation (W/m^2) and the average daily temperature (°C) in July and August for the measurement period in 2020 and 2021. At the beginning of the period (between the 1^{st} and the 5^{th} of July), temperatures in 2020 are several degrees higher than those in 2021. The deviation reaches 3.8 °C. From 6^{th} to 15^{th} July, the temperature will be reversed with higher values in 2021 as the deviation at the end of the period exceeds 6.0 °C. The average daily values of the air temperature determine 2021 as warmer compared to 2020 with more sunny hours are the weather in the first half of July is warmer 2021. In the first half of August, it is warmer in 2021 as the deviation from 2020 is between 0.9 °C and 5.9 °C on the 3^{rd} of August – 7.1 °C. In 2021, all average values are >25 °C, which determines the period of flowering-fertilization and fruit formation as unfavourable for tomatoes. The solar radiation (W/m^2) is higher in the first period and slightly lower in August. In conclusion, the measurement period in 2021 is warmer with higher daily temperatures and sunshine is close in intensity in both years and stronger positive peaks in 2021 on 4^{th} ,5^{th}, 7^{th}, 13^{th} July and 7^{th} and 8^{th} August.

3.2. Chlorophyll fluorescence

In the first year of measurements, the highest average value of quantum yield Fv/Fm = 0.814 of dark adaptation leaves was calculated in the organic fertilizer *Arkobaleno* (Table 1). The lowest ratio Fv/Fm = 0.795 was calculated for the control variant, as difference is significant compared to the organic fertilization variant (Table 1). The initial fluorescence (Fo) in oxidized reaction centres of PSII after dark adaptation had the highest average value at NPK variant of the fertilization. The measured average photosynthetically active radiation (PAR) had similar values in all variants, which indicated for stable ambient condition during measurement. In terms on the factor date of planting, do not observe difference in plant reaction on their photosynthetically activity.

The values of the measured chlorophyll fluorescence parameters showed the same plant reaction for both factors, respectively fertilization variants and different sowing dates during next year with no proved statistically difference (Table 1). The highest average values for quantum yield Fv/Fm = 0.786 were estimated for both no fertilization variant and the second planting date variant. In accordance with these data, the values of the other recorded chlorophyll fluorescence parameters did not show significance difference from each other.

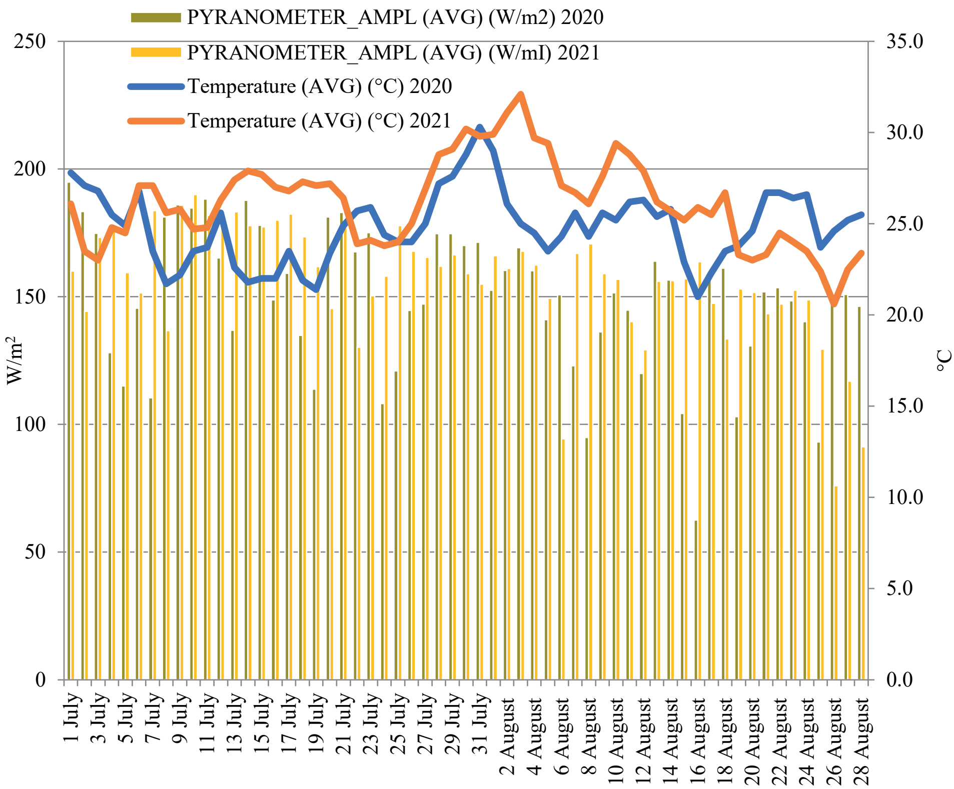

Fig. 1. Daily solar radiation (W/m²) and the average daily temperature (degrees Celsius) in July and August for the measurement period in 2020 and 2021.

3.3. Chlorophyll content index

From the ANOVA analysis of the reported average values for relative leaves chlorophyll content from the different fertilization variants, it can be concluded that there is no significant difference on both measuring dates in 2020 (Table 2). For the other factor, statistical analysis indicated significance difference between first and third dates of planting at the second measurement. Also, the results showed a stable trend of increase of relative chlorophyll content from first date to third date of planting. The highest results were estimated in first measurement for variants third date of planting +NPK and third date of planting+control in the second measurement.

The reaction of plants on this parameter was different during next year, because was observed significance increase of relative chlorophyll content for organic fertilization compared to the control at the first measurement (Table 3). The similar results of relative chlorophyll content increase were measured for NPK and organic variant compared to control at the second measurements. In this case the date of planting factor does not affect the relative chlorophyll content, but showed same reaction in first measurement, as a previous vegetation year.

TABLE 1. RESULTS FOR CHLOROPHYLL FLUORESCENCE OF TOMATO LEAVES

		2020			2021		
Indices	**Variant of treatment**	**First planting date 30.04**	**Second planting date 07.05**	**Third planting date 14.05**	**First planting date 30.04**	**Second planting date 07.05**	**Third planting date 14.05**
Fo	1. No fertilizer (control)	3600±287.3	4150±378.2	3720±378.2	3714±159.5	3587±186.1	3571±145.9
*F*v/*F*m		0.801±0.007	0.778±0.007	0.805±0.008	0.788±0.008	0.795±0.007	0.775±0.012
PAR		288±25.0	260±15.2	248±15.9	211±14.4	185±12.3	203±11.4
*F*o	2. NPK fertilizer	3711±295.2	3889±279.3	4215±388.1	3846±202.1	3575±158.1	3912±185.8
*F*v/*F*m		0.805±0.008	0.812±0.008	0.801±0.007	0.776±0.005	0.779±0.009	0.780±0.010
PAR		270±18.0	255±12.2	252±24.3	205±9.3	182±14.4	207±10.92
*F*o	3. Organic fertilizer *Arkobaleno*	3825±270.3	3480±195.0	3690±195.6	3844±123.3	3841±164.1	3910±197.3
*F*v/*F*m		0.809±0.009	0.821±0.009	0.811±0.008	0.780±0.008	0.783±0.008	0.784±0.012
PAR		295±18.2	248±10.9	221±12.3	201±12.1	192±10.6	191±12.4

LSD factor fertilization		**2020**	**2021**	**LSD factor date of planting**	**2020**	**2021**
*F*v/*F*m	p 0.05	0.017	0.024	p 0.05	0.024	0.036
	p 0.01	0.023	0.032	p 0.01	0.033	0.047
	p 0.001	0.031	0.041	p 0.001	0.044	0.061
*F*o	p 0.05	342.6	537.6	p 0.05	419.6	498.3
	p 0.01	469.4	710.4	p 0.01	574.9	658.5
	p 0.001	639.5	915.2	p 0.001	783.2	848.4

3.4. Yield

The NPK and organic fertilization in the field slightly affected to yield compared to the control in 2020 (Table 4). The different date of planting lead to advance in productivity, but the difference was not statistically proved. Statistically significant differences of the yield between control and fertilization variants were found in 2021, when the highest yield was calculated for NPK variant. Regarding applied factor date of planting, it was possessed the highest average yield for second planting, significant at p 0.05 compared to the third date of planting (Table 4).

TABLE 2. RESULTS FOR CHLOROPHYLL CONTENT INDEX-CCI OF TOMATO LEAVES REPORTED AT THE 2020

	17.07.2020				**10.08.2020**			
factors	**control**	**NPK**	***Arkobaleno***	**Mean**	**control**	**NPK**	***Arkobaleno***	**mean**
30.04	29.48±1.46	29.65±1.66	28.96±1.99	**29.4**	26.19±2.15	27.10±1.43	27.73±1.32	**27.0**
07.05.	31.74±1.76	29.16±1.97	30.09±1.41	**30.3**	31.43±2.06	29.04±2.10	26.66±1.21	**29.0**
14.05	31.93±2.07	34.57±2.08	29.86±1.57	**32.1**	34.92±1.82	30.09±1.77	32.97±1.80	**32.7**
mean	**31.0**	**31.1**	**29.6**		**30.8**	**28.7**	**29.1**	
LSD fertilization			p 0.05	3.53	LSD fertilization		p 0.05	3.62
			p 0.01	4.84			p 0.01	4.97
			p 0.001	6.59			p 0.001	6.77
LSD date of planting			p 0.05	6.12	LSD date of planting		p 0.05	6.28
			p 0.01	8.38			p 0.01	8.60
			p 0.001	11.4			p 0.001	11.7

TABLE 3. RESULTS FOR CHLOROPHYLL CONTENT INDEX-CCI OF TOMATO LEAVES REPORTED AT THE 2021

	15.07.2021				**04.08.2021**			
factors	**control**	**NPK**	***Arkobaleno***	**Mean**	**control**	**NPK**	***Arkobaleno***	**mean**
30.04	23.35±1.61	31.90±1.92	32.63±2.15	**29.2**	24.91±1.21	27.31±1.87	32.73±1.60	**28.3**
07.05.	36.63±2.00	32.98±2.28	40.55±2.14	**36.7**	24.36±1.19	28.70±1.68	27.10±1.49	**26.7**
14.05	36.09±1.43	36.92 ±1.95	41.33±1.89	**38.1**	23.67±1.11	33.11±1.56	26.74±1.60	**27.8**
mean	**32.0**	**33.9**	**38.2**		**24.3**	**29.7**	**28.8**	
LSD fertilization			p 0.05	3.46	LSD fertilization		p 0.05	3.05
			p 0.01	4.74			p 0.01	4.14
			p 0.001	6.45			p 0.001	5.69
LSD date of planting			p 0.05	5.99	LSD date of planting		p 0.05	5.28
			p 0.01	8.20			p 0.01	7.23
			p 0.001	11.2			p 0.001	9.85

TABLE 4. RESULTS FOR COMMON YIELD IN KG/DA OF THE TOMATO CULTIVAR RUGBY

factors	2020 control	2020 NPK	2020 *Arkobaleno*	2020 Mean	2021 control	2021 NPK	2021 *Arkobaleno*	2021 mean
30.04	4892	5437	5140	**5156.4**	4632	5104	5754	**5163.6**
07.05	4225	4722	4987	**4644.7**	4831	5738	5929	**5499.5**
14.05	4598	5764	5231	**5197.6**	4098	5743	4605	**4816.1**
mean	**4571.7**	**5307.7**	**5119.3**		**4520.6**	**5528.8**	**5429**	
LSD fertilization			p 0.05 = 615.9 p 0.01 = 933.2 p 0.001 = 1499.9		LSD fertilization		p 0.05 = 666.7 p 0.01 = 1010.1 p 0.001 = 1623.5	
LSD date of planting			p 0.05 = 754.3 p 0.01 = 1142.9 p 0.001 = 1837.0		LSD date of planting		p 0.05 = 816.5 p 0.01 = 1237.2 p 0.001 = 1988.4	

4. DISCUSSION

The climate change affects the conditions of growth and development of vegetable crop [27]. There are significant differences in the hydrothermal coefficients in the region of the Upper Thracian lowland and in particular in Plovdiv [28], which is the area of the present study. The higher quantum yield of PSII in organic fertilization variant compared to control variant was normal plant reaction to ambient heat stress condition. Reference [29] reported that CO_2 and N_2 increased photosynthesis and water use efficiency (WUE) of tomato plants. According to [30], [31] the effect provoked by heat stress on the photosynthetic apparatus of the tomato plants were evidenced through analysis of chlorophyll fluorescence. Functional and structural damage on PSII were achieved in susceptible cultivars subjected to heat stress, thus the increased Fo value and a decreased quantum yield Fv/Fm. In our study the calculated Fo values showed similarity in both years, despite higher, but no significance difference values for NPK variant and organic fertilizations variants. The lower quantum yield of PSII in control variant compared to organic fertilization variant during first year was result from positive effect of fertilization and mild impact of the meteorological conditions. For that reason, during 2020 the average values of ratio Fv/Fm of the other variants are close to optimal value for healthy leaves – 0.83 [32]. This relation between chlorophyll fluorescence and other factors do not observe at more severe meteorological conditions next year. Nitrogen is one of the essential nutrients that affects the plant growth and physiology. Nitrogen is the key component of amino acids, proteins, nucleic acids and chlorophyll [33]. In this regard the increase of the relative leaf chlorophyll content in mineral and organic fertilization variant compared to a control was expected. On the other hand, higher relative leaf chlorophyll content for fertilization variants compared to the control was not estimated during 2020, from which it concluded that plant reaction is not stable by this indicator. More stable reaction was revealed at date of the planting, when relative leaf chlorophyll content increase from first data to third data. The reported behaviour indicates for better plant viability from the third date.

The higher common yield calculated for NPK and organic fertilization variants are in medium correlation with chlorophyll content index, as this relation is more expressed during 2021. The organic *Arkobaleno* was characterized with similar activation effect to plant, such as NPK fertilizer. The fertilization with *Arkobaleno* has led to equalization of the values of the reported parameters with mineral fertilization, as this organic fertilizer provided the needs of nitrogen, phosphorus and potassium. The same results for organic fertilizers with eggplant published reference [34]. The effect of the factor date of planting did not strong regarding of the productivity, but still the highest average yield for the two study years was received at the second planting date (07.05). The ability of the applied organic fertilizer to gradually restore soil fertility and humus in the second year of the study resulted in a higher yield compared to the application of mineral fertilizer on the first two planting dates. After the introduction of *Arkobaleno*, the fruits have retained their quality and more standard production has been obtained compared to the variant grown with mineral fertilizers. At the third planting date, abiotic stress is strongest and the highest yield is obtained after conventional NPK fertilization.

5. CONCLUSION

The measurement period in 2021 is warmer than that of 2020, with higher daily temperatures and sunshine is close in intensity in both years and stronger positive peaks in 2021.

During first year it was read the higher quantum yield of PSII in organic fertilization variant compared to control variant. The lower quantum yield of PSII in control variant compared to organic fertilization variant during first year was result from positive effect of fertilization and mild impact of the meteorological conditions. The values of the measured chlorophyll fluorescence parameters for both factors, respectively fertilization variants and different sowing dates during 2021 year were characterized with no proved statistically difference. It can be concluded that there is no significant difference on both measuring dates in 2020 for factor fertilization. For the other factor, statistical analysis indicated significance difference between first and third dates of planting at the second measurement.

It was estimated a significant increase of the relative leaf chlorophyll content in mineral and organic fertilization variant compared to a control during 2021. Stable reaction was revealed at the factor date of the planting, when relative leaf chlorophyll content increase from first data (30.04) to third (14.05) data. The reported behaviour indicates for better plant viability from the third date.

The highest average yield was calculated for NPK variant, the statistically significant differences of the yield between control and fertilization variants were calculated in 2021. It was possessed the highest average yield for second planting, significant at p 0.05 compared to the third date of planting.

In summary it can be said, that biological fertilization at the three planting dates ensures a high market yield, comparable to mineral fertilization, despite extreme climatic conditions (abiotic stress) and provides good values of basic physiological indicators. In practice, the 1st and 2nd planting dates can be recommended for both cultivation technologies. Based on care for nature, biological cultivation is preferable and recommended by research authors.

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