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The Influence of Organic Growing of Maize Hybrids on the Formation of Leaf Surface Area and Chlorophyl Concentration

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ABSTRACT

The goal of this study is to establish the impact of tillage (reversible to a depth of 25–28 cm and irreversible to a depth of 25–28, 15–18 and 5–8 cm) and the fertilizing products such as LEANUM and VITAMIN O7 (liquid and powdered, respectively) for growing Hemingway and Harmonium hybrids with FAO 280 and 380 on the leaf area duration (LAD) and the concentration of chlorophyll *a* and *b*. The leaf area duration was measured by the method of M. I. Orlovskyi and calculated using the formula. The content of chlorophyll *a* and *b* in maize leaves was determined using the ULAB 102 Spectrophotometer. In general, the effect of biofertilizer treatment on the leaf can be noted by increasing the leaf area duration. Thus, for Harmonium, with any variant of tillage, the leaf area duration increased on the variants of foliar treatment. And on irreversible tillage for three years, an increase in the leaf area duration was noted with the use of pre-sowing inoculation with the studied fertilizing products, one treatment with LEANUM foliar, and a combined treatment with LEANUM + 1 LEANUM. It is worth noting that disking to a depth of 5–8 cm only led to a decrease in the leaf area duration during the cultivation of Harmonium. However, when growing Hemingway, almost all variants had a positive effect on the leaf area duration, with the exception of inoculation (when using both fertilizing products). An interesting fact is that in most cases, an increase in the leaf area duration led to a decrease in the concentration of chlorophyll *a*, but did not lead to a decrease in the total concentration of chlorophylls *a* and *b*, due to an increase in the concentration of chlorophyll *b*. It should be noted that biofertilizer treatment and tillage significantly affected the concentration of chlorophyll *a* and chlorophylls *a* and *b*, but chlorophyll *b* was not affected by tillage. When growing Hemingway, neither biologics nor tillage had any effect, while other pigments had a significant effect.

Keywords: plant growth promoting bacteria, effective microorganisms, *Zea mays* L, soil tillage, biofertilizers, trace elements, climatic conditions, pre–sowing inoculation.

INTRODUCTION

Recent studies are increasingly raising the issue of using an alternative to chemical fertilizers. These include fertilizing products that contain effective microorganisms in their composition, or products of their vital activity and thus enable to increase plant productivity or improving soil quality (Ilchenko et al, 2019; Zakharchenko et al., 2023; Shelest et al., 2023). A plenty of studies have shown that the impact of effective microorganisms on plant organisms is extremely high (Talaat 2019; Jalal et al., 2020; Karbivska et al., 2020; Chaudhary et al., 2021). And it's not just plant yields (Raymond et al., 2021; Sousa et al., 2021; Khanna et al., 2019) or biometric properties (Gao et al., 2020; Hussain et al., 2021; Bada, 2022), but also a qualitative indicator such as chlorophyll content (Fitriatin et al., 2021; Kerubo et al., 2021; Kots et al., 2022; Hryhoriv et al., 2022). This also raises issues regarding the tillage in which these microorganisms will work most actively (Pohromska 2019; Datsko and Zakharchenko, 2022; Dudar et al., 2022; Radchenko et al., 2023). After all, it is very important to combine rational tillage, which will meet the requirements of the crop and create the best conditions for microorganisms contained in biofertilizers. It is an effective combination that enables to improve the biometric and qualitative indicators of the crop, which will result in an increase in yield. For example, chlorophyll, which is the main element of photosynthesis, is of key importance during crop formation (Zabolotna et al., 2021; Rieznik et al., 2021; Karbivska et al., 2023). However, the content of this pigment in a particular maize hybrid is not a constant value since its content varies depending on the environment and growing conditions (Palamarchuk 2019; Malynovska and Borko, 2021; Karpenko et al., 2022). An equally important factor is the leaf area duration, since it has been found that the larger it is, the faster the accumulation of organic matter takes place, which, accordingly, leads to an increase in yield (Savchuk et al., 2018; Trotsenko, et al., 2020; Hryhoriv et al., 2023; Kovalenko et al., 2024). Therefore, the goal of this study is to determine the leaf area duration of maize hybrids with different FAO and their chlorophyll content when using such fertilizing products as LEANUM (L) and VITAMIN O7 (V) subject to the four different types of tillage.

MATERIAL AND METHODS

Characteristics of experimental plots

The study was conducted in the experimental fields of Sumy National Agrarian University during 2020–2022. The soil of the experimental plots was typical low–humus medium–loamy black soil in the forest. The climatic conditions of the growing seasons of 2020–2022 are shown in Figure 1. The predecessor of each year was winter wheat. Pre-sowing tillage was the cultivation to a depth of 10–12 cm. The area of the experimental plots was 1726.4 m^2, while the length of the plot was 100 m, the width was 12.6 m, and the protective strips were 2 m on each side. The studied tillage was carried out in autumn.

Experimental design

The conducted experiment was three-factor, factor A – maize hybrids: (a) harmonium with

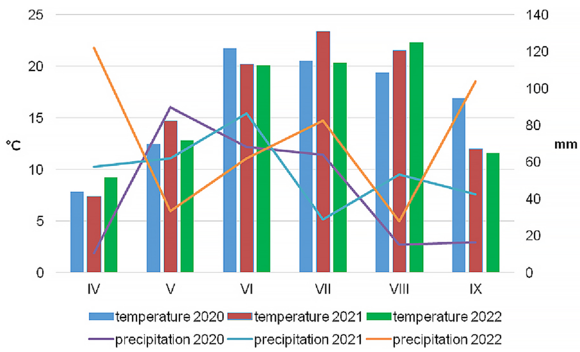

Figure 1. Climatic conditions for 2020–2022, reflected during the growing season of maize

FAO 380 and (b) hemingway, which had FAO 280, the producer of both was Euralis. Factor B – tillage: (1) reversible (plowing) to a depth of 25–28 cm (R 25–28) as a control; (2) irreversible (flat–cut tillage) to a depth of 25–28 cm (IR 25–28); (3) irreversible (disking) to a depth of 15–18 cm (IR 15–18); (4) irreversible (disking) to a depth of 5–8 cm (IR 5–8). Factor C – treatment using L and V fertilizing products before sowing ($BBCH_{00}$) and the leaf treatment with L in the critical phases of $BBCH_{13}$ and $BBCH_{17}$. Accordingly, the options are: (C) – without treatments; (1L) – with one L treatment per leaf; (2L) – with two L treatments per leaf; (L) – inoculation L before sowing; (L+1L) – inoculation L and one L treatment per leaf; (L+2L) – inoculation L and two L treatments per leaf; (V) – inoculation V before sowing; (V+1L) – inoculation V and one L treatment per leaf; (V+2L) – inoculation V and two treatments L on the leaf. It is worth noting that L is a fertilizing product in liquid form, which contains nitrogen-fixing, phosphate-mobilizing and nodule bacteria. In addition, the composition includes enzymes, amino acids, vitamins, trace elements in an accessible form, as well as fulvic and humic acids. V is similar in composition, but has a powdery form. Treatment of seed material with fertilizing products had been carried out 3 hours before sowing, according to the norm recommended by the manufacturer of biofertilizers.

Data and sample collection

To determine the leaf area duration in each variant, metric data of leaves on ten plants were measured in three times repetition according to the method of Orlovskyi. To determine its length, the distance from the leaf sinus to the end of the leaf was measured with a tape measure, and the width was measured in the widest part of the leaf, also using a tape measure, after which the area duration of each leaf was calculated using the formula:

$$S = k \cdot l \cdot n \quad (1)$$

where: S – leaf area duration, cm^2; k – average adjustment factor (for maize 0,75); l – leaf length, cm; n – leaf width at its widest point, cm (Pysarenko et al., 2015).

After that, the leaf area duration per hectare was calculated for each of the variants. To determine the chlorophyll concentration, 10 apical leaves were cut three times from each variant. In the laboratory, the leaves from each variant were crushed with scissors, so that the suspension included slices from each selected sample, while discarding petioles and large veins. The 0.3 g suspension, which was made on a KERN 600–2 scale (Germany), was placed in a mortar and ground with a small amount of quartz sand and $CaCO_3$ until smooth. After that, 10 ml of ethyl alcohol was added to the mortar and continued to grind until a dark green extract was obtained. The resulting ethyl extract was filtered through a dry paper filter into a test tube. The extract was selected using a special pipette, while not using the vegetable mass, and transferred to the filter. Another 5 ml of ethyl alcohol was added to the remaining vegetable mass in the mortar and grinding was continued. After that, the settled ethyl extract was transferred to the filter. Chlorophyll extraction was continued with small portions of alcohol until the plant material completely lost color. The mortar, pestle and filter were washed with small portions of alcohol until the green color completely disappeared from them. The volume of the extract was brought with pure ethyl alcohol to 30 ml. The extract had been put in a dark place before analysis on the ULAB 102 Spectrophotometer. Waves λ = 665 Nm were used to determine the content of chlorophyll *a*, and for chlorophyll *b* – λ = 649 Nm. After the analysis, the concentration of the studied pigments in the resulting volume of alcohol extract was calculated (formulas 2 and 3) as follows:

$$C_a = 13.70 \times A_{665} - 5.76 \times A_{649} \quad (2)$$

$$C_b = 25.80 \times A_{649} - 7.60 \times A_{665} \quad (3)$$

where: C – chlorophyll concentration in alcohol extract, mg/l; A_{665} – solution density (optical) for a wave of 665 Nm; A_{649} – solution density (optical) for a wave of 649 Nm.

Statistical data processing

Data processing and analysis were performed using Statistica 10.0 (StatSoft Inc., Tulsa, USA). ANOVA and an LSD test were used to establish the reliability of the results obtained.

RESULTS AND DISCUSSION

Leaf area duration

On average, over 3 years for Harmonium on tillage R 25–28, biologics used on the leaf surface showed a positive effect on the leaf area duration, in particular, variants 1L and 2L (Table 1). At the same time, for non–reversible tillage (IR 25–28 and IR 15–18), an increase in the leaf area duration was provided by inoculation V before sowing, 1L foliar and combined treatment L+1L. At the same time, the IR 5–8 tillage variant did not show a statistically significant increase in the leaf area duration, but only a significant decrease.

For Hemingway, on average, a significant increase in the leaf area duration was recorded in 2020–2022. Thus, only leaf treatments or combined treatments with fertilizing products had a positive effect on all types of tillage. Inoculation did not affect the leaf area duration in all tillage options, except for IR 15–18.

According to the results of the Fisher's least significant difference (LSD) analysis, all options for using fertilizing products except for L and L+2L were essential for growing Harmonium. At the same time, tillage options mostly did not significantly affect the leaf area duration, except for IR 5–8, which led to a significant decrease in the indicator. When growing Hemingway, all fertilizer treatment options except for L and V had a significant impact compared to the control. Instead, all tillage options had a significant impact on the leaf area duration.

Chlorophyll concentration

The concentration of chlorophyll *a* with tillage R 25–28 during the cultivation of Harmonium was higher than the confidence interval of Univariate Analysis of Variance ($p>0.05$), however, according to the LSD test, the difference between the control and the biofertilizer treatment option V was significantly lower than the control during 2020–2022 (Table. 2). A similar situation developed with the

Table 1. Average leaf area duration by variants in 2020–2022, thousand $m^2 \cdot ha^{-1}$, (x±SD)

Hybrid	Treatment options with biofertilizers	R 25-28	IR 25-28	IR 15-18	IR 5-8
Harmonium	C	40.5±14.3	40.0±11.2	38.9±10.4	33.7±5.6
	1L	45.1±10.9	45.2±10.4	46.7±14.3	37.3±5.8
	2L	44.0±10.4	41.9±9.9	42.1±8.5	38.8±7.6
	V	42.4±13.2	46.6±16.1	43.7±12.0	38.2±6.6
	V+1L	43.4±8.3	40.9±10.0	42.0±9.4	41.6±7.4
	V+2L	40.9±7.7	43.4±13.8	42.6±12.9	35.6±6.7
	L	40.3±10.4	38.0±10.6	40.8±13.0	37.8±9.1
	L+1L	42.1±10.9	40.9±11.4	45.8±14.9	40.0±10.8
	L+2L	41.0±7.8	37.5±8.0	36.6±8.6	34.5±7.4
	Duncan's Criterion	3.0			
Hemingway	C	40.4±11.8	47.1±17.4	44.7±15.6	39.5±9.9
	1L	46.4±10.6	46.8±11.5	47.9±11.0	43.3±6.1
	2L	48.5±11.7	51.6±15.1	48.7±12.0	45.5±7.3
	V	43.2±13.1	43.3±13.7	43.3±12.7	40.3±9.1
	V+1L	45.5±8.6	51.5±14.0	50.2±15.4	44.1±6.4
	V+2L	47.2±9.7	47.0±8.7	47.3±9.5	46.5±7.5
	L	44.1±14.1	42.7±12.6	46.9±32.3	41.3±10.6
	L+1L	46.8±14.1	46.6±8.7	45.7±10.5	45.5±7.9
	L+2L	48.9±8.7	48.6±9.2	50.6±16.0	47.1±6.9
	Duncan's Criterion	3.7			

Note: x is the average value, SE is the standard error.

Table 2. Chlorophyll concentration in leaves for R 25–28 in 2020–2022, x±SD

Variant	Harmonium			Hemingway		
	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}
C	15.33±1.10	40.74±0.37	56.07±0.79	14.33±0.52	41.25±0.23	55.58±0.44
C+1L	14.81±0.85	40.47±0.26	55.28±0.67	11.48±1.42	40.66±0.16	52.15±1.37
C+2L	15.32±0.98	40.59±0.35	55.92±0.66	11.23±1.31	40.89±0.21	52.12±1.23
V	11.36±2.17	36.83±0.65	48.20±2.80	11.93±1.43	41.59±0.41	53.52±1.06
V+1L	15.57±1.21	41.02±0.42	56.60±0.77	10.99±1.51	40.48±0.33	51.48±1.50
V+2L	13.77±1.81	41.58±0.69	55.36±1.15	12.39±1.55	40.51±0.24	52.91±1.38
L	14.93±0.85	40.68±0.28	55.62±0.67	11.94±1.48	40.55±0.20	52.49±1.32
L+1L	14.97±0.92	40.88±0.31	55.85±0.73	12.11±1.19	41.69±0.41	53.81±0.84
L+2L	17.39±0.81	40.34±0.37	57.73±0.46	12.52±1.16	41.34±0.33	53.86±0.93
Duncan's Criterion	3.8	1.5	3.4	3.5	1.1	3.4

Note: x is the average value, SE is the standard error.

concentration of chlorophyll *b*, variant V also had significantly lower indicators. While for growing Hemingway, the LSD test did not show any significant differences compared to the control.

In 2020–2022, on the IR 25–28 tillage for the cultivation of Harmonium, two treatment options with C+2L and V biologics were distinguished in comparison with the control (Table 3). Interestingly, the concentration of chlorophyll *a* in these variants increased, while the concentration of chlorophyll *b*, on the contrary, decreased. However, their sum was on par with other options, and did not have a significant statistical difference. And when growing Hemingway, the highest concentration of chlorophyll *a* in three years was increased with the use of V+1L, which

Table 3. Chlorophyll concentration in leaves for IR 25–28 in 2020–2022, x±SD

Variant	Harmonium			Hemingway		
	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}
C	13.29±1.60	40.63±0.40	53.92±1.27	13.19±1.58	40.96±0.47	54.16±1.14
C+1L	12.95±1.28	40.82±0.38	53.78±1.01	13.48±0.81	40.99±0.26	54.48±0.61
C+2L	17.62±0.48	38.63±0.70	56.25±0.69	13.40±1.80	40.92±0.50	54.32±1.33
V	17.54±1.21	36.93±0.50	54.47±1.65	14.08±1.58	40.97±0.38	55.06±1.22
V+1L	14.80±1.62	40.75±0.49	55.56±1.15	16.98±0.82	40.87±0.44	57.85±0.55
V+2L	13.19±1.64	40.74±0.37	53.93±1.41	14.25±1.61	40.78±0.51	55.04±1.11
L	15.02±1.30	40.48±0.36	55.51±0.97	11.74±1.39	40.64±0.25	52.39±1.28
L+1L	15.01±0.88	40.97±0.30	55.99±0.60	15.16±0.49	40.97±0.18	56.14±0.31
L+2L	15.41±1.53	40.31±0.41	55.73±1.17	14.49±0.28	41.30±0.16	55.80±0.20
Duncan's Criterion	3.8	1.5	3.4	3.5	1.1	3.4

Note: x is the average value, SE is the standard error.

had a positive effect on the concentration of both chlorophylls, but the concentration of chlorophyll *b* in either of the variants was not significantly higher than the control.

For IR 25–28 tillage in 2020–2022, the concentration of chlorophyll *a* for the cultivation of Harmonium was lower compared to the control for all variants except L+1L (Table 1; Table 4). Whereas the concentration of chlorophyll *b*, on the contrary, increased in the C+1L, V+1L, V+2L, and L+2L variants. In general, the concentration of chlorophyll in plant leaves was higher compared to the control in variants C+1L, C+2L, V+1L, V+2L, and lower in variant L. A similar pattern was observed when growing Hemingway on biologics C+1L, V+1L, V+2L, and L+1L had significantly lower concentrations of chlorophyll *a* compared to the control. While the LSD test did not show a significant difference between the control and treatment options using biologics in any of the options. However, in general, the concentrations of chlorophylls *a* and *b* in the C+1L, V, V+1L, V+2L, L, and L+1L variants were lower compared to the control.

The concentration of chlorophyll *a* during the cultivation of Harmonium with IR 5–8 tillage was significantly higher compared to the control on the treatment options with L+1L and L+2L biologics (Table 5). Whereas the concentration of chlorophyll *b* was higher than the control variant for C+1L and L treatments. The total concentration of both pigments exceeded the control for the

Table 4. Chlorophyll concentration in leaves for IR 15–18 in 2020–2022, x±SD

Variant	Harmonium			Hemingway		
	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}
C	21.70±0.71	38.82±0.26	38.82±0.49	17.31±0.58	40.88±0.43	58.20±0.24
C+1L	15.79±1.30	40.50±0.53	40.50±0.86	13.61±1.74	41.13±0.53	54.74±1.30
C+2L	12.57±2.31	38.90±0.27	38.90±2.36	17.35±1.13	40.32±0.37	57.68±0.77
V	18.53±0.44	39.74±0.22	39.74±0.30	14.76±1.04	40.92±0.39	55.69±1.10
V+1L	13.54±1.31	40.93±0.35	40.93±0.97	12.37±1.22	41.41±0.30	53.79±0.95
V+2L	14.48±0.96	41.31±0.35	41.31±0.65	12.73±1.47	41.10±0.41	53.83±1.22
L	14.44±0.63	38.46±0.68	38.46±1.13	14.39±0.46	41.12±0.15	55.52±0.36
L+1L	18.44±0.78	39.74±0.32	39.74±0.54	11.36±0.78	41.44±0.14	52.80±0.74
L+2L	17.57±1.33	41.04±0.42	41.04±1.33	14.51±0.31	41.25±0.26	55.76±0.32
Duncan's Criterion	3.8	1.5	3.4	3.5	1.1	3.4

Note: x is the average value, SE is the standard error.

Table 5. Chlorophyll concentration in leaves for IR 5–8 in 2020–2022, x±SD

Variant	Harmonium			Hemingway		
	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}	Concentration of chlorophyll *a* pigments, mg l^{-1}	Concentration of chlorophyll *b* pigments, mg l^{-1}	Concentration of chlorophyll *a* and *b*, mg l^{-1}
C	12.03±2.07	39.45±1.05	51.49±2.32	14.82±0.67	41.26±0.36	56.09±0.42
C+1L	12.88±1.18	41.76±0.53	54.64±0.79	10.35±1.34	40.18±0.52	50.53±1.53
C+2L	13.40±1.49	41.37±0.58	54.78±1.00	12.40±1.79	40.59±0.43	52.99±1.42
V	12.43±2.08	40.30±0.73	52.74±1.78	15.57±0.96	41.29±0.61	56.86±0.45
V+1L	14.65±1.86	40.19±0.84	54.85±1.24	13.34±0.49	41.47±0.20	54.81±0.37
V+2L	13.64±2.08	40.36±0.67	54.00±1.67	10.70±1.61	40.45±0.74	51.16±1.68
L	14.01±1.23	41.82±0.59	55.83±0.74	10.87±1.72	40.34±0.37	51.22±1.74
L+1L	17.93±0.73	40.96±0.48	58.89±0.28	11.20±1.79	40.20±0.44	51.41±1.83
L+2L	18.02±0.86	38.55±1.27	56.58±1.76	10.81±1.72	40.96±0.24	51.77±1.59
Duncan's Criterion	3.8	1.5	3.4	3.5	1.1	3.4

Note: x is the average value, SE is the standard error.

L, L+1L and L+2L variants. For Hemingway, a significant decrease in the concentration of chlorophyll *a* was observed in the C+1L and V+2L variants. The concentration of chlorophyll *b* did not significantly decrease or increase compared to the control variant. After the MANOVA analysis, it should be noted that for the cultivation of Harmonium, tillage and treatment with biologics significantly affected the concentration of chlorophyll *a* and chlorophylls *a* and *b* ($p>0.05$), however, for chlorophyll *b*, only treatment with biologics showed a significant effect, and tillage had no effect ($p = 0.09$). The lowest values of chlorophyll *a* and the total value of both chlorophylls were observed with treatment IR 15–18, and a similar situation was observed when growing another hybrid. At the same time, when growing Hemingway, tillage and treatment with biologics did not affect chlorophyll *b*, while this effect was significant on other indicators.In general, it can be noted that an increase in the leaf area duration may effect the treatment with biologics applied on the leaf. Thus, for Harmonium, with any variant of tillage, the leaf area duration increased on variants 1L and 2L. And on non–reversible ones (IR 25–28 IR 15–18), an increase in the leaf area duration was observed over three years when using V, 1L foliar and combined L+1L treatment. The positive effect of one or two leaf treatments on all variants can be explained by the use of a biological product in critical phases of culture. While the increase in the leaf area duration during inoculation V can be explained by the active work of microorganisms populating due to seed inoculation and the mobilization of macro- and microelements of the soil, due to the active work of the biota. However, when growing Hemingway, none of the inoculation variants had a positive effect, except for IR 15–18 and L inoculation.Regarding the correlation of the leaf area duration and the concentration of the analyzed pigments, the analysis performed using the Statistica 10.0 Program did not show any correlation between these indicators. However, it is worth noting that when cultivating the soil IR 25–28 and inoculating V before sowing Harmonium, the leaf area duration of the hybrid increased, as did the concentration of chlorophyll *a*, however, the concentration of chlorophyll *b* decreased. A similar result was obtained when treating IR 15–18, for example, the leaf area duration of most biological treatment options increased, and the concentration of chlorophyll *a* decreased, however, the concentration of chlorophyll *b*, on the contrary, increased. However, when IR 5–8 treatment was applied to the L+1L variant, the concentration of chlorophyll *a* and the sum of both chlorophylls increased along with the leaf area duration.When growing Hemingway, V + 1L treatment with IR 25–28 tillage resulted in an increase in the leaf area duration and the concentration of chlorophyll *a* and the total concentration of chlorophylls. While when the soil was treated with IR 25–28 and variant V+1L, the leaf area duration increased, and the concentration of chlorophyll *a*, on the contrary, decreased. A similar situation was observed with the variants 1L and V+2L in IR 5–8 tillage, while the leaf area duration also increased and the concentration of chlorophyll *a* decreased.

The effect of effective microorganisms contained in biofertilizers on the leaf area duration and chlorophyll content has been proven by many scientists. Thus, Iraqi scientists conducted a study exanining the combined effect of phytohormones and effective microorganisms on maize plants. The results showed that the use of biofertilizers with microorganisms on the leaf led to an increase in the flag leaf, while the chlorophyll content was positively affected by the option with application on the leaf and soil inoculation (Kadhim 2020). Scientists from China studied the effect of inoculation of Rahnella aquatilis JZ–GX1 depending on its concentration. It was found that the highest chlorophyll content was achieved at a concentration of 107 cfu/mL (Li et al., 2021). At the same time, Brazilian scientists have proven the positive effect of Azospirillum brasilense on the chlorophyll content (Silva et al., 2022; Tsyuk et al., 2022). It is worth noting that Enterobacter cloacae of the PM23 strain for studying their effects had a positive effect on the content of the studied pigments in maize plants, as well as an increase in the leaf surface area for growing on saline soil (Ali et al., 2022). Many similar examples have been described in studies by Naik (2020). Moreover, it is worth adding that not only effective microorganisms affected the obtained indicators of chlorophyll and the leaf area duration, but also soil treatment. This is confirmed not only by this study, but also by other research (Sun et al., 2018; Stępień-Warda 2020).

CONCLUSIONS

In the light of the foregoing, after a detailed analysis of the data of 2020–2022, it can be concluded that in general, at any tillage, among the studied ones, an increase in the leaf area duration was provided for the use of a biological product on the leaf. Thus, for Harmonium, the magnification was provided by the 1L and 2L variants, and for Hemingway 2L. At the same time, for irreversible tillage during the cultivation of Harmonium, an increase in the leaf area duration was observed on variant V, 1L foliar and combined treatment L+1L. An interesting fact is that in most cases, an increase in the leaf area duration led to a decrease in the concentration of chlorophyll *a*, but did not lead to a decrease in the total concentration of chlorophylls *a* and *b*, due to an increase in the concentration of chlorophyll *b*. In addition, it is worth adding that treatment with biologics had a significant impact on the cultivation of both hybrids, but tillage significantly affected the leaf area duration only when growing Harmonium. It should be noted that treatment with biologics and tillage significantly affected the concentration of chlorophyll *a* and chlorophylls *a* and *b*, but chlorophyll *b* was not affected by tillage. When growing Hemingway, neither biologics nor tillage had any effect, while other pigments had a significant effect.

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