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The effect of succinic acid on the productivity of *Lactuca sativa* L. in artificial agroecosystems

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Abstract. The research of the effect of the biostimulant on the growth and development of plants of the 'Robin' lettuce variety was carried out in 2019–2020 in the department of closed artificial agroecosystems for crop production on the basis of the Federal State Budgetary Scientific Institution «Federal Scientific Agroengineering Center VIM», Moscow (VIM, Russia). Succinic acid was used at the stage of inoculation of seed and with constant drip irrigation of plants throughout the growing period. Lettuce plants were grown using a low-volume technology in a climatic chamber produced by VIM (Russia). According to the studies carried out, inoculation of seeds stimulates the development of plants at the initial stages, reducing the period from sowing to germination and increasing the rate of plant growth. As the lettuce plants grew, the leaf surface area increased from 221.0 to 1511.9 cm² with the addition of succinic acid to the hydroponic nutrient solution. The use of succinic acid under controlled growing conditions of 'Robin' lettuce plants allowed to increase the performance of the leaf apparatus in relation to the control. It was experimentally established that productivity directly depended on the value of photosynthetic potential and net productivity of photosynthesis, which is confirmed by a strong degree of dependence with a correlation coefficient of 0.98 and 0.77, respectively. Seed treatment and adding succinic acid to the hydroponic solution increases the accumulation of dry matter in lettuce plants. With this method of using succinic acid, significant differences in the accumulation of dry mass of plants were established. The share of the effect of the factor of inoculation of seeds with succinic acid was 17.5%, the addition of hydroponics to the nutrient solution was 50.1%. The use of succinic acid increases the accumulation of plant biomass, increases the total leaf surface area, and also contributes to an increase in the parameters of photosynthetic activity of the leaf apparatus of the 'Robin' lettuce plants under controlled conditions of the climatic chamber.

Key words: salad, biostimulant, succinic acid, climatic chamber.

INTRODUCTION

The development of the agricultural sector is an important task for the world economy in many countries. Thus, according to FAO, around 820 million people suffer from hunger and malnourishment in the world, part of the population is affected by the problem of a lack of certain substances in food or an unbalanced diet that causes obesity and other diseases. According to the agricultural forecast for 2020–2029, about 85% of global growth in crop production over the next years is associated with higher yields as a result of more intensive use of resources, investments in production technologies and improved cultivation methods (OECD-FAO, 2020). As a result, agriculture requires a dynamic interaction of science and technology to address the global challenges of climate change, land degradation, and next-generation food for the world's growing population (Rangga et al., 2021).

In last year huge attention is devoted to researches and developments in the field of urban agriculture with a completely closed and artificially controlled agroecosystems, which aimed at the safe and constant production of high-quality and functional nutrition (Nguyen et al., 2019; Kumar et al., 2020a).

The development of resource-saving, environmentally friendly technologies for cultivation in greenhouses of promising varieties and hybrids of vegetable crops is possible thanks to the development of new cultivation systems. The Federal Scientific Agroengineering Center VIM develops climatic chambers of various modifications, with fully automatic control of the parameters of plant growth and development in the dynamics of ontogenesis (Izmailov et al., 2020). Climate chambers are used in biological and agro-engineering research, as well as for food production (Dost & Ferkl, 2014; Nishida & Okada, 2019).

The complex use of agrochemicals in the production of crop products is an integral part of the modern level of intensive production. The intensification of the cultivation of seed material of different crops is a perspective direction for improving agrobiotechnology (Coomes et al., 2019; McDougall et al., 2019). The use of physiologically active substances (biostimulants) for plant growth and development makes it possible to mobilize the genetic potential of crops depending on their phenology (Colla & Rouphael, 2015).

Italy is the European leader in leafy herbs. Over the past two decades, interest in *Diplotaxis tenuifolia* L. from the *Brassicaceae* family has increased due to the high content of useful elements and the creation of functional foods. Scientists Giordano, M., El-Nakhel, C., Caruso, G. and others from the University of Naples Federico II (2020) conducted an experiment on the effect of biostimulants based on extracts of tropical plants containing active compounds of aspartic acid. The results showed that using of Auxym (PE) and Trainer (PH) has increased the yield and total dry biomass of greenhouse plants of *Diplotaxis tenuifolia* by an average of 48% and 37% compared to untreated plants (Giordano et al., 2020).

The exogenous addition of plant growth regulators (gibberellic acid) to the mineral base of the hydroponic nutrient solution promoted biomass accumulation, increasing leaf area and efficient using of water and nitrogen in *Lactuca sativa* L. and *Eruca sativa* L. plants grown in a floating system (Vetrano et al., 2020).

The use of salicylic acid contributed to an increase in yield of *Solanum melongena* L. eggplants by 7.3–22.7% (Wakchaure et al., 2020), an increase in plant height and the weight of 1,000 Plantago ovata Forsk grains (Roumani et al., 2019). Potato tubers had treated with a growth stimulant from hydrolyzed collagen with an increased dose of glycine and they had an increased content of biologically active compounds from 15 to 50% (Murashev et al., 2020).

Amino acid solution (salicylic, gibberellic and ascorbic acids) improve the antioxidant state, increase the accumulation of pigments (2.6–10.8%) and dry matter (1.4–4.0%) in the treated plants of *Allium sativum* L. (Ulianych et al., 2020a). It was found that the use of the 'MaxiMarin' growth regulator in greenhouses when treating seeds of *Spinacia oleracea* L. increased the profitability of the crop by 83–102% (Ulianych et al., 2020b).

The purposes of the research were evaluating the effect of biostimulant (succinic acid) on productivity and developing technological methods for producing high-quality commercial products *Lactuca sativa* L., which has been grown in the substrate hydroponics of the climatic chamber.

MATERIALS AND METHODS

The research of the biostimulant influence on the growth and development of plants of the 'Robin' lettuce variety was carried out in 2019–2020 in the department of closed artificial agroecosystems for crop production on the basis of the Federal State Budgetary Scientific Institution "Federal Scientific Agroengineering Center VIM", Moscow, Russia (VIM).

Succinic acid was used at the stage of seeds inoculation (factor A) and with constant drip irrigation of plants (factor B) throughout all the vegetative period - 40 whole days. Lettuce seeds were treated (soaked) with an aqueous solution of succinic acid with a concentration of 2.5 mM for 3 hours, and also the studied biostimulator was added to the nutrient hydroponics solution. Seeds soaked in water with the same exposure and without adding amber acid to the nutrient solution served as control. The experiment included 4 variants, each variant with 25 plants in five replicates.

This experiment included the following variants:

1 Control - without inoculation (seeds soaked in water);

2 Seeds without inoculation but with the introduction of succinic acid into a hydroponic solution;

3 Inoculation of seeds with Succinic acid;

4 Inoculation of seeds by the Succinic acid with and also the introduction of succinic acid into a hydroponic solution.

After treatment, the seeds were sown in a substrate soaked in a nutrient solution. As a substrate, we used mineral wool mats of the SPELAND VEGA brand (Russia) with a horizontal structure of fibers that ensure optimal uniform distribution of water and necessary nutrients along the entire length of the mat. This substrate is close to soil in terms of chemical composition, the main component of which is silica (silicon dioxide).

Biometric parameters (number of leaves and leaf area) were measured using a LI-COR-LI-3100C device (USA). The main indicators of photosynthesis of lettuce plants were calculated twice: on the 20^{th} and 40^{th} days of cultivation. Photosynthetic potential was determined as the product of the average area of lettuce leaves by the length of the growing season. Net productivity of photosynthesis is the ratio of the average daily growth of dry biomass of lettuce to the average leaf area for a specified period of time (20 and 40 days). The determination of the crude mass fraction substance was carried out on an LA 230S analytical balance (Germany). Determination of dry matter was made by drying a sample to constant weight in a Memmert UN-450 drying chamber (Germany) in accordance with GOST 31640-2012. The quantitative content of the main pigments (chlorophyll a, b and carotenoids) was determined on a spectrophotometer (Speks SSP-705M, Russia) in the lettuce leafs. The pigment concentration was calculated for 100% acetone using the Holm-Wettschnein equation (Lichtenthaler, 1987; Tretyakov, 1990). The content of chlorophylls, carotenoids were determined twice during the growing period, as well as indicators of the vegetative mass in the lettuce on the 20 and 40 day of the observation.

Plant material

The research material was the seeds of the leaf lettuce (*Lactuca sativa* L.) of the 'Robin' variety, included in the State Register of Breeding Achievements, approved for use on the territory of the Russian Federation. The 'Robin' variety is a late ripening, leafy lettuce, bred by the Czech company Agrofirma moravoseed (Registry, 2021).

Mineral nutrition

The plants were feed with Flora Series mineral fertilizers from General Hydroponics Europe in accordance with the regulations for growing lettuce plants in hydroponic systems from William Texier. The EC of the solution was maintained within 0.9–1.2 mS cm^{-1}, pH 5.8–6.0. The mineral base of the nutrient solution remained the same throughout the growing period of the plants (General Hydroponics Europe, 2018).

Experimental conditions

Lettuce plants were growing using a low-volume technology in a climatic chamber produced by VIM (Fig. 1).

Figure 1. Climatic chamber VIM.

A chamber are used specially for growing crops up to 1.5 m on an area of 3.8 m^2. The chamber is equipped with a control unit with a touch panel for the operator, through which information is collected from sensors with a configurable interval from 1 to 30 minutes with data saving to a removable flash drive.

Control is carried out using the operator's touch panel. The display shows the current parameters and operating modes, with the ability to enter data. The chamber is equipped with a forced air circulation system. Plant nutrition is carried out independently through two channels from two containers with a volume of 100 liters by means of a drip irrigation system. During the experiment, the following conditions were maintained in the climatic chamber (Table 1).

The climatic chamber is equipped with LED irradiators manufactured by VIM (Russia) with a customizable spectral composition and a function of dimming the radiation intensity, as well as the ability to adjust the lighting operation according to a given program and simulate the day-night function (Smirnov et al., 2018).

Table 1. Characteristics of the main parameters of the climatic chamber during the experiment

Parameter	Set values
Duration of daylight hours, h	14
Air temperature, °C	26
Relative humidity, %	70
Watering interval for plants, min	2
Adjustable pause between waterings, min	286

At the stage of germination of lettuce seeds, light with the presence of a far red range was chosen to create stress (700–780 nm - 600 mW m^{-2}, 122 mmol m^{-2} s) and then switch to red to provoke active growth and induce a reverse reaction of phytochrome. At the stage of vegetative mass formation, the illumination spectrum presented in Fig. 2 was used, with illumination at the plant level equal to 8,900 lux and total PAR - 232 mmol m^{-2} s: blue - 39 mmol m^2 s; green - 50 mmol m^2s; red - 137 mmol m^2s; far red - 5 mmol m^{-2} s (Proportions B: G: R ~ 13:22:65).

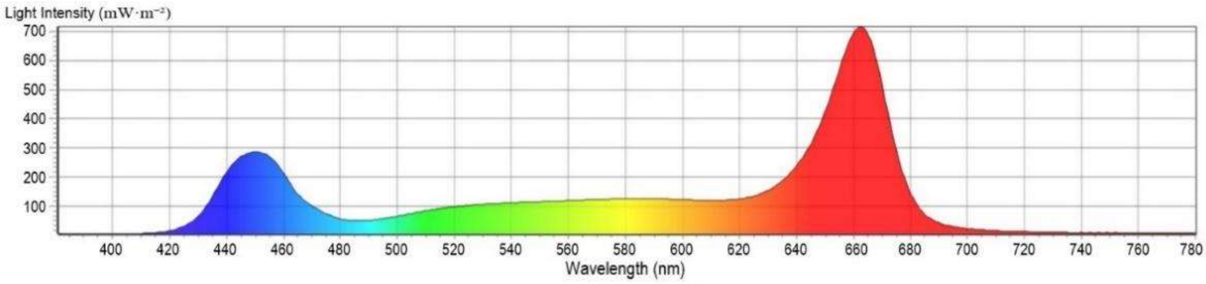

Figure 2. Spectral composition of optical radiation at the stage of vegetative mass formation.

Statistical analysis

Data analysis was evaluated by methods of variance and correlation analyzes using the STADIA 8.0 software. Least significant difference (LSD) was used to check the significance of the data obtained at a probability level of $p < 0.05$.

RESULTS

According to the research data, inoculation of the seeds stimulated the development of plants at the initial stages, reducing the period from sowing to germination and increasing the rate of plant growth. In the variants of the experiment with the seeds inoculation with an aqueous solution of amber acid, germination was observed 2 days earlier, on the 4th day, than in the other variants (on the 6th day) without the effect of the growth regulator on the seeds. Besides, the biostimulant promoted a more intensive formation of the plants vegetative mass and the formation of a rosette of 5–6 leaves in comparison with the control variant - 3–4 leaves. The additional supply of amber acid to the nutrient solution of hydroponics allowed plants to assimilate nutrients more actively throughout the growing season, which ultimately makes it possible to obtain high-quality and environmentally friendly products with greater productivity.

As it can be seen from Table 2 in all variants of the experiment, the biometric indicators of lettuce plants exceeded the control variant. The greatest indicator of the leaf surface area on the 20th day of cultivation was noted in variants 3 and 4 with inoculation of the seeds - 205.4 and 221.0 cm², exceeding the control variant of the experiment by 1.5 times.

Table 2. Biometric indicators of the assimilation apparatus of *Lactuca sativa* variety 'Robin'

Varient of experiment	Growing period			
	20 day of the observation		40 day of the observation	
	Number of leaves, pcs.	Leaf area, cm^2 $plant^{-1}$	Number of leaves, pcs.	Leaf area, cm^2 $plant^{-1}$
1	7.0 ± 0.4	137.0	16.2 ± 0.7	888.8
2	7.0 ± 0.5	178.6	17.8 ± 0.5	1399.7
3	8.4 ± 0.7	205.4	17.6 ± 0.5	1,266.4
4	7.2 ± 0.5	221.0	19.6 ± 0.9	1,511.9
	$LSD_{0.5}$ ob	36.20	-	1,71.48
	$LSD_{0.5}$ A	25.60	-	1,21.25
	$LSD_{0.5}$ B	25.60	-	1,21.25

During growing of the lettuce plants, the leaf surface area was increasing to 1,399.7–1,511.9 cm² in options 2 and 4 with the addition of succinic acid to the nutrient solution by the end of the vegetation period. Throughout the entire growing season, the number of leaves changed slightly as a result of the operation of the biostimulator in relation to the control variant. According to the analysis of variance calculation, it was noted that on the 20th day of cultivation, the share of the influence of factor A on the leaf area was 46.9%, the addition to the nutrient solution of hydroponics was 12.4%, on the 40th day of observation were 22.8 and 54.4%, respectively.

The efficiency of the photosynthetic activity of lettuce plants was estimated by the volume of work of leaves (photosynthetic potential) and the increase in dry matter per 1m² per day (net productivity of photosynthesis). As a result of the researches, it was found that the confirmed productivity directly depends on the value of photosynthetic potential and net productivity of photosynthesis which is confirmed by a strong degree of dependence with correlation coefficients of 0.98 and 0.77, respectively. The use of succinic acid under controlled growing conditions of lettuce varieties 'Robin' allowed to increase the performance of the leaf apparatus in relation to the control: photosynthetic potential from 17.13 (control) to 216.60 m^2 m^{-2} days (variant 4), net productivity of photosynthesis - from 1.66–2.30 g m^{-2} days (Fig. 3).

The optimal way to grow 'Robin' lettuce is the complex application of succinic acid at the stage of pre-sowing seed treatment in the climatic chamber with constant watering. A significant increase in the aboveground mass was noted both at the initial stages of growth (50.6%) and during further development of plants (52.6%) (Fig. 4).

There was a significant increasing in aboveground mass both in the initial stages of growth with an weight gain of 50.6%, and in the further development of plants with an weight gain of 52.6% (Fig. 4).

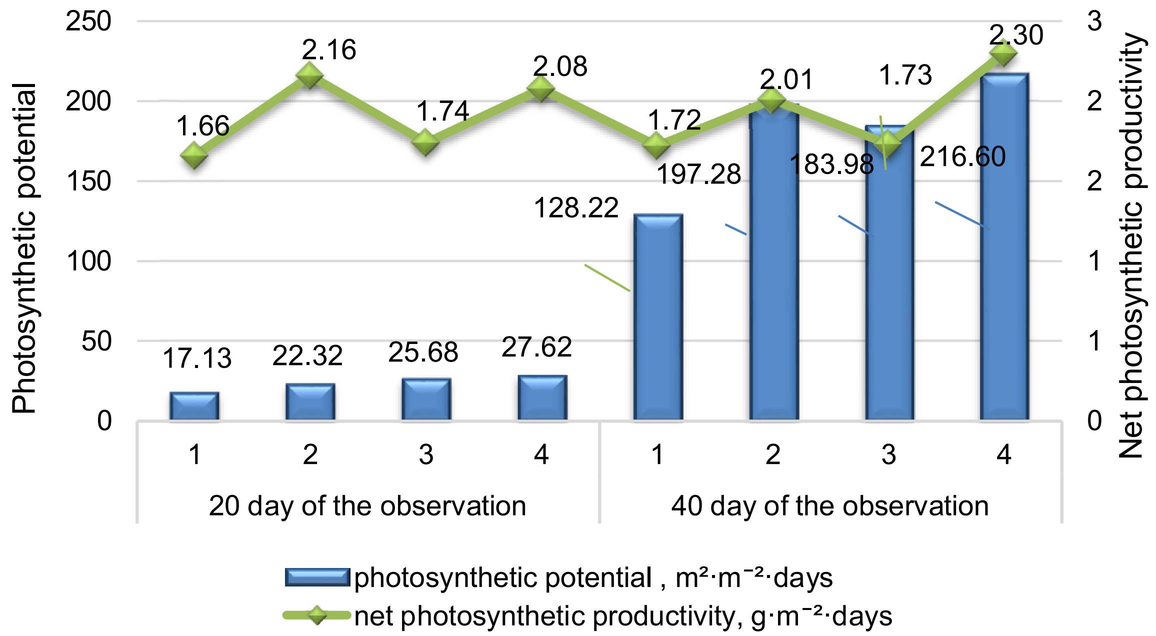

Figure 3. Indicators of photosynthetic activity of the leaf apparatus of *Lactuca sativa* variety 'Robin'.

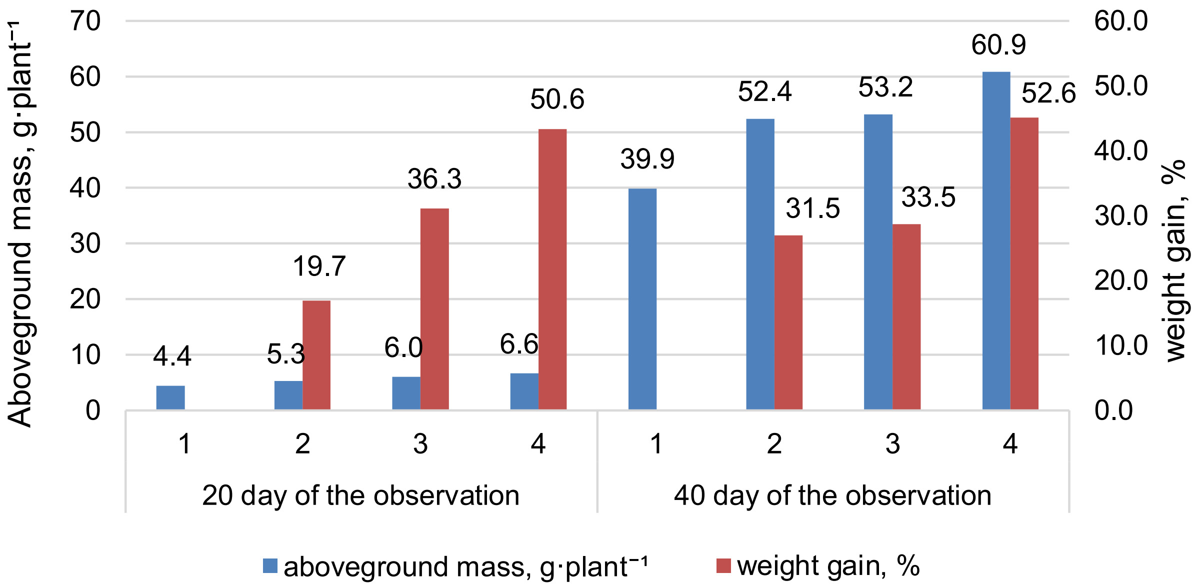

Figure 4. Growth rates of the aboveground mass of *Lactuca sativa* variety 'Robin'.

Seed treatment and adding succinic acid to the solution increases the accumulation of dry matter in lettuce plants. The dry mass from sprouting to the receipt of finished marketable products continuously increased. The most intense increase in dry matter mass was noted in variant 4–0.45 g plant^{-1} (on day 20 of observation) and 4.42 g plant^{-1} (on day 40 of observation) (Fig. 5). With this method of using succinic acid, significant differences in the accumulation of dry mass of plants were established. Analysis of variance showed that the use of succinic amber acid reliably affects the accumulation of dry plant biomass ($Ff > F_{0.5}$). The share of the effect of the factor of A seeds inoculation of amber acid was 17.5%, the addition of hydroponics to the nutrient liquor was 50.1%.

Lettuce 'Robin' variety, grown under controlled conditions of climatic chamber, corresponded to the quality of commercial products on 40 th day of the observation in accordance with GOST 33985-2016.

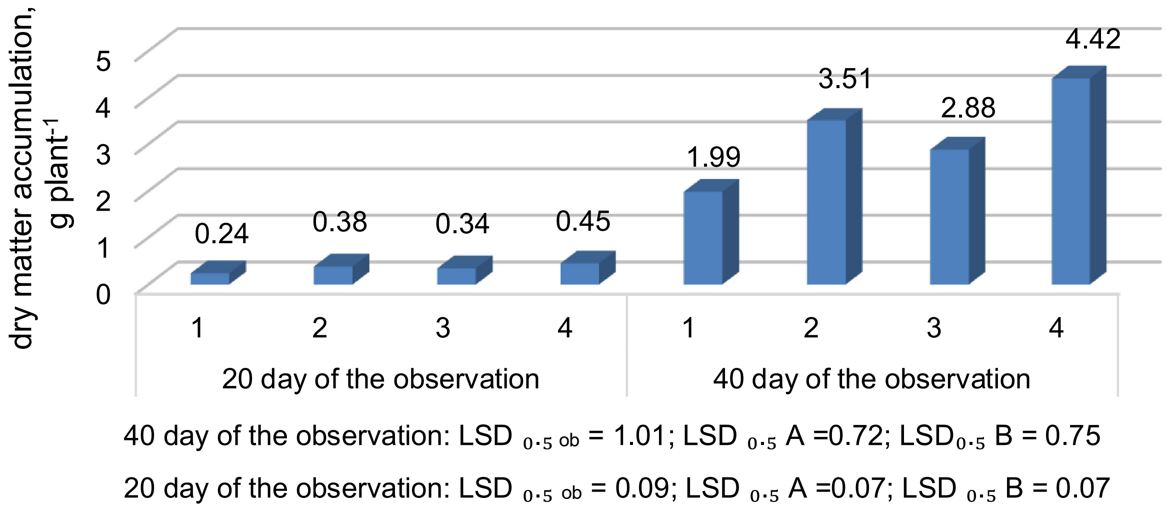

Figure 5. Dynamics of dry matter accumulation in *Lactuca sativa* variety ‘Robin’, g plant^{-1}.

When we are studying the quantitative content of pigments, it was found that the pre-sowing treatment of seeds and the addition of succinic acid to the nutrient solution had little effect on the accumulation of total chlorophyll and carotenoids in lettuce leaves (Table 3).

Table 3. The content of the main pigments in plants of *Lactuca sativa* variety ’Robin’, mg g^{-1}

Varient of experiment	Growing period							
	20 day of the observation				40 day of the observation			
	ch. a[1]	ch. b[2]	ch.(a+b)[3]	car.[4]	ch. a	ch. b	ch. (a+b)	car.
1	2.1 ± 0.3	0.7 ± 0.2	2.5 ± 0.2	0.7 ± 0.1	1.7 ± 0.1	0.7 ± 0.1	2.5 ± 0.1	0.6 ± 0.1
2	2.6 ± 0.1	1.2 ± 0.2	3.8 ± 0.3	0.9 ± 0.1	2.4 ± 0.2	1.0 ± 0.1	3.3 ± 0.3	0.8 ± 0.1
3	2.2 ± 0.1	0.9 ± 0.1	3.3 ± 0.2	0.7 ± 0.1	2.0 ± 0.2	0.8 ± 0.1	2.8 ± 0.3	0.7 ± 0.1
4	2.2 ± 0.1	0.9 ± 0.1	3.1 ± 0.2	0.8 ± 0.1	2.1 ± 0.2	0.8 ± 0.1	2.8 ± 0.3	0.7 ± 0.1

[1]chlorophylls a, [2]chlorophylls b, [3]chlorophylls (a+b), [4]carotenoids.

The average, accumulation of the main pigments of chlorophylls a and b increased to 41.2% and 42.9%, respectively, and carotenoids increased to 33.3% under the influence of the biostimulant succinic acid in lettuce leaves by the 40th day of growing.

The use of succinic acid increases the accumulation of plant biomass, the total leaf surface area, as well as the accumulation of the main photosynthetic pigments in plants of ‘Robin’ lettuce throughout the growing season in the VIM climatic chamber.

DISCUSSION

According to the researches 1^{ts}, it was found that the use of various options for treating lettuce plants with a solution of succinic acid made it possible to improve the morphometric characteristics and quality of marketable products in comparison with the control variant.

Recently, succinic acid has been produced from wastes of fruit and vegetable products (Jiang et al., 2017; Saxena et al., 2017; Kumar et al., 2020b). When inoculating seeds and during the growing season of crops, aqueous solutions of succinic acid are used at ultra-low concentrations (Vereshchagin & Kropotkin, 2010; Cao et al., 2018).

Previous researches have shown that growing plants using succinic acid is an environmentally friendly and effective way to increase the productivity of most crops (Marenych et al., 2019; Kovaliova et al., 2020; Pivovarov et al., 2020). At the stage of pre-sowing seed treatment, succinic acid activates the nutrients contained in the seeds, thereby contributing to an increase in productivity throughout the development of plants. It was found that the use of succinic acid with a solution concentration of 10^{-3} M increases the leaf surface area of wheat by 17–20%, the mass fraction of dry matter by 33%, the photosynthetic potential by 17–20% in comparison with the option without pre-sowing treatment (Tsyganova et al., 2019). Similar results on the stimulating effect of succinic acid on seeds of other crops were obtained by scientists Buntsevich et al. (2015) Oliva, et al. (2018) and Chursinov & Kovaleva (2019). The presented results indicate the effectiveness of the use of succinic acid at the stage of pre-sowing treatment of seeds of various crops. Our results also support research carried out by numerous scientists.

CONCLUSIONS

As a result of evaluating the efficiency of using succinic acid in order to increase the productivity of 'Robin' lettuce in artificial agroecosystems was identified technological method of using a biostimulant as a pre-sowing treatment of seeds and adding it to a nutrient solution (variant 4). The use of succinic acid allows increasing the crop yield from 4.9 (control) to 7.6 kg m^{-2} (variant 4) in the growing lettuce under controlled conditions of a climatic chamber. In this variant, a significant increase in plant biomass was noted by 52.6% (wet weight), 122.1% (dry weight) and the total leaf area by 70.1% compared to the control variant. The use of succinic acid is very perspectivly for growing 'Robin' lettuce plants in artificial agroecosystems.

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