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EFFECT OF TREATMENT WITH MICROALGAE EXTRACTS TO SOME MORPHOLOGICAL AND BIOCHEMICAL PARAMETERS OF TOMATO LEAVES

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ABSTRACT

The research aimed to the evaluation of some morphological and chemical parameters of tomato leaves under the treatment with microalgae extracts. In August 2023, tomato seedlings (cultivar 'Belle' F1, Enza Zaden) were planted in 25 L pots, filled with peat (producer Laflora LTd., pH_{KCl} 5.5) and grown in polycarbonate greenhouse of the laboratory of Horticulture and Beekeeping of the Latvia University of Life Sciences and Technologies. The plants were sprayed weekly (for a total of five times) with the solution of ethanol extractions of different microalgae species: *Spirulina*, *Dunaliella* and *Chlorella*, from 14th August till 11th September, till harvesting time. In total, nine plants per treatment were used. Two concentrations of the extracts were compared with sprays with corresponding ethanol solution as a control. In September, one week before first yield harvesting, for tomato leaves, such indices as length and width of leaf plate, pH, content of soluble solids, pigments and colour components of L*, a*, b* were determined. The obtained results showed that tomato leaves under the treatment with 10% *Spirulina* were shorter and narrower. Under the treatment with 10% *Dunaliella* as well as under 2% ethanol in control leaves were longer and wider, characterised with higher Brix, chlorophyll and carotenoids content, but decreased colour components of the L*, a*, b*. Despite the fact that tomato plants grew well and developed normally under all treatments, 10% *Dunaliella* extract is the most suitable for tomato leaves. Further research is needed to adjust treatment with microalgae extracts for tomato cultivation.

Keywords: *Tomato leaves, Microalgae extracts, Physical properties, Phytochemicals.*

INTRODUCTION

Horticultural plants, including the increasingly popular tomato (*Solanum lycopersicum* L.), which is a favored vegetable in Europe, are rich in bioactive compounds like flavonoids, phenolics, anthocyanins, and phenolic acids, as well as essential nutrients such as sugars, essential oils, carotenoids, vitamins, and minerals They pay attention also on sensory properties of products, including taste and colour (Raza et al., 2022; Duma et al., 2019; Ullah et al., 2020). Carotenoids, a class of secondary photosynthetic pigments synthesized in plants, are vital to survival and extremely important for plant health. Carotenoids are not only essential for harvesting light in leaves, but they are also precursors of molecules responsible for plant signaling and growth regulation (Payne et al., 2024; Sun et al., 2022). Chlorophyll is an important pigment for photosynthesis of plants and its content and composition directly affect the photosynthetic capacity, nutrient level, growth and development of plants. The contents of chlorophyll a and chlorophyll b as well as their proportion directly affect the selective light absorption and utilization in plants (Wang et al., 2015). Therefore, rapid quantitative measurement of the composition and content of chlorophyll in leaves is conducive to grasping the physiological status of plants, which can be used to guide plant production management (Wang et al., 2015). A number of authors emphasize the various biochemical factors, such as the content of pigment and nutrients, have been found to affect the optical properties of tissues. Leaf pigment content can reflect the nutrient content of the cultivation medium indirectly (Zhao et. al., 2023). Whereas chlorophylls can be considering as indicator of plant's quality, content of chlorophylls in plants indicates the nutritional value of them. Moreover, plants colour, is also an indicator of the vegetable pigment concentration (Ozola et al., 2019). Plants which are growing in polluted environment have decreased content of chlorophylls and carotenoids, the structure of their chloroplast membranes are changed so that intensity of photosynthesis is decreased (Ozola et al., 2019). Researcher from Bulgaria accepted that for centuries, farmers have known the condition of their crops by their appearance and colour alone (Atanasov et al., 2023).

Modern greenhouse horticulture is characterized by high crop yields and a stable year-round supply of high-quality fruits and vegetables. This high crop output is often accompanied by the intensive use of biocides, inorganic fertilizers and soilless cultivation techniques (Vox et al., 2010). In this context of sustainable vegetable production under cover, microalgae offer the potential to recover nutrients from the micro algal biomass as a slow release fertilizer (Coppens et al., 2016). Microalgae can be used to enhance the nutritional value of food owing to their chemical composition, they play a crucial role in aquaculture. The microalgal biotechnology only really began to develop in the middle of the last century. Nowadays, there are numerous commercial applications of microalgae. Moreover, they are cultivated as a source of highly valuable molecules, for example, polyunsaturated fatty acid oils (Spolaore et al., 2006). In the word most commercially available algae preparations for agriculture are made from ocean-

grown and often (68%) wild harvested algae (macro algae). However, microalgae, unlike macro algae, are easier to grow in closed systems at different scales close to where algae products are used such as near farms. Therefore, over 50% of European algae producers grow microalgae, though mainly for food or food supplements (Kampuss et al., 2024).

Future research should focus on the improvement of production systems and the genetic modification of microalgae strains. Microalgae products would in that way become even more diversified and economically competitive.

The research aimed to the evaluation of some morphological and chemical parameters of tomato (cultivar 'Belle' F1, Enza Zaden) leaves under the treatment with different microalgae species: *Spirulina*, *Dunaliella* and *Chlorella*.

MATERIAL AND METHODS

In August 2023, tomato seedlings (cultivar 'Belle' F1, Enza Zaden) were planted in 25 L pots, filled with peat (producer Laflora LTd., pH_{KCl} 5.5) and grown in polycarbonate greenhouse of the laboratory of Horticulture and Beekeeping of the Latvia University of Life Sciences and Technologies. During experiment, plants were regularly watered and fertilized, phytosanitary measures were provided. Ventilation was carried out automatically, when the air temperature in the greenhouse exceeded +23 to +24 °C limit. Additional lighting was provided by high-pressure sodium lamps. Pruning of plants was made by traditional scheme.

The plants were sprayed weekly (for a total of five times) with the solution of ethanol extractions of different microalgae species: *Spirulina*, *Dunaliella* and *Chlorella*, from 14th August till 11th September, till harvesting time.

Determination of chlorophyll a, chlorophyll b and carotenoids.

The homogenized samples were accurately weighted (0.5±0.0001 g) in a glass test tube, 10 mL of 95% ethanol was added and the test tubes were held for 15 min with occasional shaking at room temperature. The extracts were filtered (paper No. 89) and extraction process was done in triplicate. The obtained solution was analyzed for chlorophyll *a* (c_{Cha}), chlorophyll *b* (c_{Chb}) and total carotenoids (carotenes and xanthophylls) c_{c+x} content by a spectrophotometer JENWAY 6300 at wavelengths 470, 649 and 664 nm using a glass cuvette. The content of chlorophylls and total carotenoids were calculated according to the following equations (Sumanta et al., 2014):

Chlorophyll *a* (mg ml^{-1}) $c_{Chla} = 13.36A_{664} - 5.19A_{649}$ (1)

Chlorophyll *b* (mg ml^{-1}) $c_{Chla} = 27.43A_{649} - 8.12A_{664}$ (2)

Total chlorophylls (mg ml^{-1}) $c_{a+b} = c_{Chla} + c_{Chlb}$ (3)

Ratio between chlorophyll *a* and *b* ($R_{a/b}$) = $\frac{c_{Chla}}{c_{Chlb}}$ (4)

Total carotenoids (mg ml^{-1})c_{c+x} = $\frac{1000A_{470} - 2.13c_{Chla} - 97.63c_{Chlb}}{209}$ (5)

Results were expressed as mg g^{-1} of fresh plant material.

Colour analysis.

Colour of samples were measured in CIE L*a*b* colour system using a colorimeter ColorTec PCM (Accuracy Micro sensors Inc., USA). Before the measurement, the colorimeter was calibrated using a white reference tile and a light trap (black tile). Ten random leaves were measured and the mean values were reported for each sample. In colour measurement, CIE L*a*b* coordinates show the degree of brightness (L), the degree of redness (+a), or greenness (–a), and the degree of yellowness (+b), or blueness (–b), respectively (Tarhan et al., 2010).

pH value measurements were determined by standard method LVS ISO 5542:2010 using JENWAY 3520 pH Meter.

Soluble solids (^{o}Brix) were determined at 20±2 ° temperature with a digital refractometer by standard method ISO 2173:2003.

Statistical analysis

Analyze of variance were used for data statistical processing, whereas the significance of differences between mean values was evaluated with p-value.

RESULTS AND DISCUSSION

Modern agricultural farming requires precise, quick and non-destructive methods for detection of plant physiological parameters. One of the commonly used parameters is the chlorophyll content in plant leaves. Chlorophyll concentration varies in plant leaves depending on plant species background, environmental mineral elements content and different stress factors (Novac et al., 2022). The major chlorophylls in plants include chlorophyll *a* and chlorophyll *b*, which are usually present at a ratio of 3 (Straumite et al., 2015). Chlorophyll *a* and *b* differed between all the tested samples (Table 1). The highest content of chlorophyll *a*, chlorophyll *b* and total chlorophyll in the tomato leaves treated with 10% *Dunaliella* sp. extract was observed 12.15; 7.17 and 19.32 mg 100 g^{-1} respectively as well as in the tomato leaves treated with 10% *Spirulina* sp. extract 11.10; 7.33 and 18.43 mg 100 g^{-1}. In the tested tomato leaves a ratio between chlorophyll *a* and chlorophyll *b* ranged from 8.42 to 12.18, meaning that chlorophyll *a* is the main form of chlorophyll in the leaves. Chlorophyll, a materials base for photosynthesis, is most important photosynthetic pigment. The content of chlorophyll is one of main index reflecting leaf photosynthesis ability and plant health condition (Jiang et al., 2017).

Table 1. Chlorophyll *a*, chlorophyll *b* and carotenoids in tomato leaves.

Plant material	Total carotenoids, mg 100 g^{-1}	Chlorophyll *a*	Chlorophyll *b*	Total chlorophylls, mg 100 g^{-1}	Ratio between chlorophyll *a* and *b*
Drinking water	4.10	9.49	5.10	14.59	11.39
Control, ethanol, 2%	4.71	10.95	5.50	16.46	12.18
Control, ethanol, 4%	3.90	9.01	4.95	13.96	11.30
10% *Spirulina* sp. extract	4.43	11.10	7.33	18.43	9.60
20% *Spirulina* sp. extract	3.94	9.89	6.89	16.77	9.07
10% *Dunaliella* sp. extract	4.91	12.15	7.17	19.32	10.44
20% *Dunaliella* sp. extract	3.41	8.59	4.94	13.53	11.22
10% *Chlorella* sp. extract	3.32	9.13	6.73	15.86	8.42
20% *Chlorella* sp. extract	4.02	9.81	6.70	16.51	9.20

The basic pigments of green plants are chlorophylls, always accompanied by carotenoids. Carotenoid content differed between all the tested tomato leaves samples. In part of samples significantly higher ($p < 0.05$) concentration of carotenoids in the tomato leaves was observed for 10% *Dunaliella* sp. extract (4.91 mg 100 g^{-1}), control variant with ethanol, 2% (4.71mg 100 g^{-1}) and 10% *Spirulina* sp. extract (4.43 mg 100 g^{-1}) (Table 1). The decline in chlorophyll content under stress conditions could be due to impaired biosynthesis or accelerated pigment degradation. However, for all samples differences in total carotenoids content were not significant ($p>0.05$).

Colour parameters of the tomato leaves samples presented not significant differences between brightness or L* values ($p>0.05$) (Table 2). However, plant material showed slightly lower indicators 32.92±1.80 for 10% *Dunaliella* sp. extract and 32.73±1.11 for control with ethanol, 2%, thereby tomato leaves are slightly darker (lower L* value) in turn tomato leaves treated with 10% *Chlorella* sp. extract is lighter 36.11±2.05 (higher L* value) (a* parameter is more negative). On the other hand, colour parameters of the tomato leaves samples presented the significant differences between a* and b* values ($p<0.05$). More green (a* parameter is more negative) -10.31±0.75 (20% *Chlorella* sp. extract) and -10.03±0.82 (Control, ethanol, 4%) and significantly higher ($p<0.05$) numerical value of yellow color intensity (b*) 19.13±0.81 more yellow (higher value parameter b*) 20% *Chlorella* sp. extract compared to other samples.

Table 2. Colour characteristics of the tomato leaves.

Plant material	Colour parameter values		
	L*	a*	b*
Drinking water	33.08±1.33a	-9.35±0.52ab	15.23±0.57ab
Control, ethanol, 2%	32.73±1.11a	-9.10±0.64ab	14.42±0.58a
Control, ethanol, 4%	35.06±2.03a	-10.03±0.82a	17.40±0.71ab
10% *Spirulina* sp. extract	33.19±1.35a	-8.82±0.59ab	15.28±0.68ab

20% *Spirulina* sp. extract	33.42±1.49a	-8.17±0.45b	13.94±0.54a
10% *Dunaliella* sp. extract	32.92±1.80a	-8.44±0.32b	13.99±0.54a
20% *Dunaliella* sp. extract	33.75±1.08a	-9.13±0.38ab	16.32±0.64ab
10% *Chlorella* sp. extract	36.11±2.05a	-9.70±0.43ab	18.38±0.72ab
20% *Chlorella* sp. extract	35.98±2.12a	-10.31±0.75a	19.13±0.81b

**Values, marked with the same letter, are not significantly different (p>0.05).*

Total soluble solids (°Brix) data is represented in Figure 1. Soluble solids of tomato leaves were significantly affected by different microalgae species levels and their application of and their interaction was found significant effect. Maximum value of total soluble solids (10.27 °Brix) were found in tomato leaves treated with 10% *Dunaliella* sp. extract, while minimum value of total soluble solids (5.73 and 6.1 °Brix) was noted in 10% *Chlorella* sp. extract and control variant with ethanol, 4%.

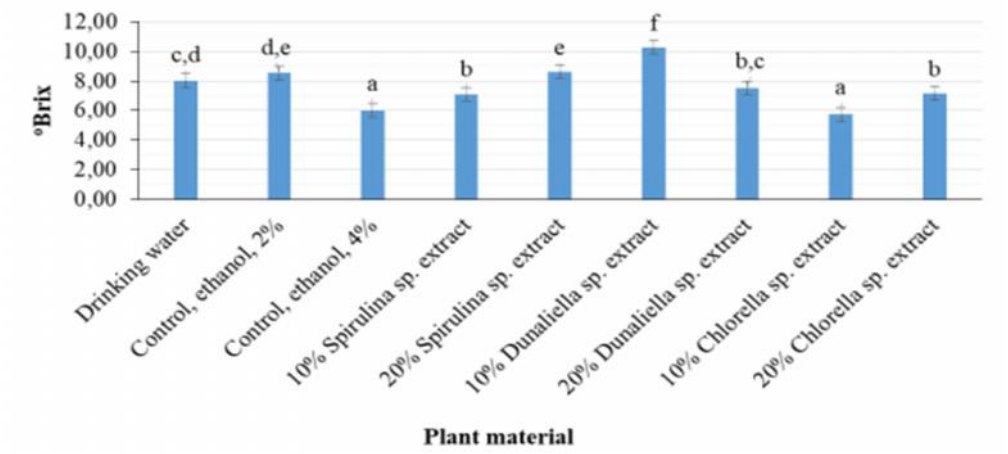

Figure 1. Soluble solids content in tomato leaves.
**Values, marked with the same letter, are not significantly different (p>0.05).*

Also, microalgae species 20% *Spirulina* sp. extract exhibited maximum value of total soluble solids (8.63 °Brix) compare as other tomato leave samples which exhibited minimum value of total soluble solids (from 7.07 to 8.53 °Brix) (Figure. 1). The results of pH level performed on the fresh tomato leave samples formulation revealed that the pH ranged from 5.30 to 5.55. The difference between the lowest and highest pH was not significant – just 0.25 units (p>0.05).

The data in Table 3 regarding length and width (cm) of tomato leaf plate was significantly affected by extractions of different microalgae species and their interaction was found significant effect. The maximum value of length of tomato leaf plates (55.27 cm) was found in control plants with ethanol, 2%), while the minimum value of length of tomato leaf plate (45.75 cm) was noted in leaf plates treated with the 10% *Spirulina* sp. extract.

Table 3. Length and width of tomato leaf plate.

Plant material	Leaf length, cm	Leaf width, cm
Drinking water	51.07	54.80
Control, ethanol, 2%	55.27	60.27
Control, ethanol, 4%	51.07	54.80
10% *Spirulina* sp. extract	45.75	52.17
20% *Spirulina* sp. extract	49.77	55.87
10% *Dunaliella* sp. extract	51.70	58.30
20% *Dunaliella* sp. extract	51.40	57.13
10% *Chlorella* sp. extract	48.15	54.95
20% *Chlorella* sp. extract	50.77	61.73

Regarding extractions of different microalgae species foliar application, maximum value of tomato leaf plate's width (60.27 cm) was noted in plants treated with control, with ethanol, 2%, while minimum value (52.17 cm) was found in plants treated with 10% *Spirulina* sp. extract and their interaction was found significant effect.

CONCLUSIONS

The beneficial effects obtained in this study demonstrate that microalgae extracts can be used for tomato cultivation. The obtained results showed that tomato leaves under the treatment with 10% *Spirulina* were shorter and narrower. Under the treatment with 10% *Dunaliella* as well as under 2% ethanol in control leaves were longer and wider, characterized with higher Brix, chlorophyll and carotenoids content, but decreased colour components of the L*, a*, b*. Despite the fact that tomato plants grew well and developed normally under all treatments, 10% *Dunaliella* extract is the most suitable for tomato leaves. Further research is needed to adjust treatment with microalgae extracts for tomato cultivation. The microalgal extracts increase some morphological and biochemical parameters of tomato leaves and it is possible that improve the quality of the fruits.

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