Theses for doctoral dissertation (PhD)

EFFECT OF DIFFERENT CULTIVATION TECHNOLOGY FACTORS ON THE MORPHOLOGICAL AND QUALITY CHARACTERISTICS OF LEAFY VEGETABLES

Ágota Kovácsné Madar
PhD candidate

Supervisor:
Prof. Dr. Mária Takácsné Hájos
Professor

UNIVERSITY OF DEBRECEN
KERPELY KÁLMÁN DOCTORAL SCHOOL

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1. INTRODUCTION

Vegetable consumption is an essential part of a balanced. healthy diet. as vegetables contain many vitamins. minerals. dietary fibre and phytochemicals that are vital for the body. Their important role in the daily diet extends to maintaining gastrointestinal health. protecting vision. and reducing the risk of chronic diseases such as cardiovascular disease. stroke. diabetes and some malignant tumors (Dias. 2012). Leafy vegetables. in particular. stand out due to their significant nutritional value; they are essential sources of antioxidants and contain large amounts of fibre. vitamins and minerals (Settaluri et al.. 2015). Since they do not contain cholesterol and are naturally low in calories. they can be an important part of a balanced diet (Bunning & Kendall. 2012).

In the cultivation of vegetables. earliness is of particular importance due to higher selling prices. The length of the growing season is primarily determined by the genetic characteristics of the variety. environmental factors (especially light conditions and temperature). and cultivation technology (Slezák & Jezdinsky. 2013).

Among leafy vegetables. lettuce (Lactuca sativa L.) is one of the most popular and widely cultivated species worldwide. It is a species of significant economic and nutritional value. mainly used raw. on its own or as a component of ready-to-eat (fresh-cut) products (Putnam et al.. 2000; Kenny & O’Beirne. 2009).

According to the latest FAO data. in 2022. the world production of lettuce and chicory exceeded 29 million tonnes. China continues to lead global production with nearly 15 million tonnes. followed by the United States and India. The two largest European producers. Spain (1.1 million tonnes) and Italy (0.7 million tonnes). account for more than 30% of Europe’s total production of over 5.7 million tonnes.

Salad consumption contributes significantly to the intake of provitamin A. vitamins C and E. carotenoids and fibre (Agüero et al.. 2008). The importance of vegetable consumption is attributed to nutrients and various bioactive substances. including phytochemicals (phenols. flavonoids. carotenoids). minerals. vitamins and dietary fibre (Oz & Kafkas. 2017). The demand for leafy vegetables has increased significantly in the last decade. This can be mainly explained by their high calcium. iron. provitamin A. vitamin C and antioxidant content. as well as their beneficial effects on human health (Barickman et al.. 2018)."

The main objectives of the research were as follows:

- **Comparison of cultivation methods**: To evaluate the effect of different cultivation methods (unheated plastic tunnel. greenhouse. open field) on the morphological. physiological and nutritional properties of six lettuce varieties and five other leafy vegetables.
- **Investigation of seasonal effects**: To determine the effect of the cultivation period (spring vs. autumn/summer) on the yield. growth characteristics. physiological parameters (NDVI. SPAD). and the accumulation of bioactive substances (total polyphenol. flavonoid. vitamin C) and nitrate concentration of the tested plants.
- **Evaluation of genotypes**: To compare the quantitative and qualitative indicators of different lettuce types (loose-leaf. iceberg. butterhead. romaine lettuce) and other leafy vegetables (corn salad. arugula. spinach. baby beetroot leaves) under different cultivation conditions.
- **Analysis of bioactive substances and nitrate dynamics**: To examine in detail the accumulation of bioactive compounds (polyphenols. flavonoids. vitamin C) and nitrate in different varieties. depending on cultivation methods and periods. with special regard to environmental stress effects.
- **Determination of correlations**: To determine the relationships between the examined morphological. physiological and compositional parameters in different cultivation systems using correlation analysis.
- **Development of practical recommendations**: Based on the experimental results. to formulate practical recommendations for growers regarding optimal variety selection and cultivation technology. in accordance with the desired cultivation goal.

2. MATERIALS AND METHODS

2.1. Experimental conditions and plant materials

The experiments were conducted in the AKIT-DTTI Demonstration Garden and Arboretum of the University of Debrecen (2019–2022) on calcareous chernozem soil. The soil of the experimental plots was sandy loam. characterized by a near-neutral pH (avg. 7.3–7.6) and good phosphorus (P) and potassium (K) supply. while the humus content was variable. The spring cultivation in unheated plastic tunnels was characterized by optimal average temperatures (approx. 20–23 °C) and high solar radiation. In contrast. the autumn periods were cooler (10–14 °C in the plastic tunnel. 17–19 °C in the greenhouse) with significantly lower light intensity. During the summer open field cultivation. we recorded the highest radiation values (avg. 160–173 W/m²) and high average temperatures around 23 °C. Lettuce varieties were cultivated in unheated plastic tunnels during the spring growing cycle. while in the autumn period. they were grown in both unheated plastic tunnels and a greenhouse. Other leafy vegetable species were grown in unheated plastic tunnels in the spring period and in the open field during the summer.

Experiment 1 (lettuce. 2019–2021):

- **Genotypes (6 varieties):** 'Lungavilla' (Lollo Bionda). 'Cencibel' (Lollo Rossa). 'Great Lakes 659' (Iceberg Lettuce). 'Cortazar' (Romaine Lettuce). 'King of May' (Butterhead Lettuce). 'Kirke' (Oakleaf Lettuce).
- **Cultivation methods:** spring unheated plastic tunnel. autumn unheated plastic tunnel. autumn greenhouse.

Experiment 2 (leafy vegetables. 2019–2022):

- **Genotypes (5 species):** 'Cirilla' (Corn salad). 'Themisto' (Arugula). 'Matador' (Spinach). 'New Zealand Spinach' (*Tetragonia tetragonioides*). 'Bonel' (Beetroot).
- **Cultivation methods:** spring unheated plastic tunnel. summer open field.

2.2. Experiment design and treatments

The experiments were arranged in a randomized block design with four replications for both lettuce and leafy greens.

Lettuce seedlings. raised in 84-cell trays and treated with *Previcur Energy* (3.0 ml/m²). were transplanted at the 5–6 leaf stage (25×25 cm). Leafy greens were direct-sown in April and August with 25 cm row spacing.

Prior to planting and sowing. the soil was treated with microbiological preparations (*Trifender Pro* and *Artis Pro*) at a concentration of 0.8% to protect against soil-borne pathogens and pests. The nutrient supply for the lettuce varieties was provided using water-soluble fertilizers. adjusted to the plants' phenological phases. During the rooting phase. *Ferticare Starter* (15-30-15+2.5MgO+M.e.) was applied at a concentration of 0.15%. During the intensive growth phase. *Ferticare I* (14-11-25+Mg+M.e.) was applied weekly at 0.14%. Subsequently. *Ferticare II* (24-8-16+3.8MgO+M.e.) was applied as a 0.65% solution until the onset of heading to promote leaf development and head formation.

Water was supplied via a drip irrigation system. During the spring growing season. irrigation was performed 3–4 times a week with doses of 30–40 mm. In the autumn period. irrigation frequency was reduced due to higher relative humidity. Humidification was used in spring to regulate humidity. while ventilation was employed in autumn.

Integrated plant protection included *Actara* (0.1%) and *Amistar* (0.75%) treatments against pests and fungi (*downy mildew. rhizoctonia*). In the autumn period. protection against slugs was managed using BIOFITO and manual removal. Sampling for laboratory analysis was performed at harvest.

2.3. Research methodology

At harvest. morphological characteristics of the plants were measured. including head weight. leaf number. stem diameter. plant height. and shape index.

Physiological status was assessed using non-destructive handheld instruments. Relative chlorophyll content (SPAD) was determined using a Konica Minolta SPAD-502Plus device. while the vegetation index (NDVI) was measured with a GreenSeeker Model 505 instrument.

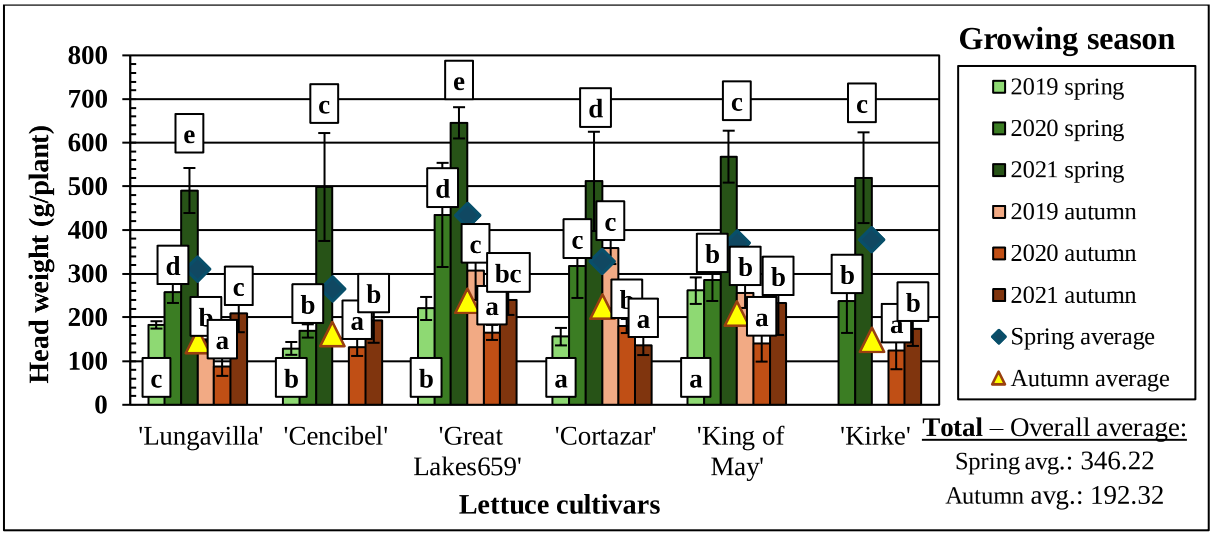
Analytical measurements were primarily performed in the accredited laboratory of the Agricultural Instrument Centre at the University of Debrecen. Selected chemical components (dry matter. total polyphenol. and flavonoid content) were analyzed in the institute's laboratory.

Dry matter content was determined according to the MSZ-08-1783-1:1983 standard by drying samples at 105 °C to constant weight. Total polyphenol content was measured using the Folin-Ciocalteu colorimetric method (Meda et al.. 2005; Abrankó et al.. 2011) at an absorbance of 760 nm. while flavonoid content was determined using a colorimetric method (Kim et al.. 2003) at 510 nm. Vitamin C concentration was determined by redox titration according to MSZ ISO 6557-2:1991. and nitrate content was analyzed using the flow injection analysis method (λ=540 nm) in accordance with MSZ EN 12014-7:1999.

3. RESULTS

3.1. The combined effect of growing season and lettuce varieties on morphological and nutritional parameters in plastic tunnel cultivation

The results regarding **lettuce yield** (head weight) showed significant differences between cultivation periods and technologies (Figure 1). Spring cultivation in unheated plastic tunnels. due to more favorable light and temperature conditions. resulted in a significantly higher yield (average 346.22 g/plant) compared to the autumn period (192.32 g/plant). Among the varieties. 'Great Lakes 659' (iceberg lettuce) achieved the highest head weight (646 g/plant). while 'Kirke' (oak leaf lettuce) had the lowest (237 g/plant). However. this exceptionally high value exceeds domestic market requirements (180–300 g).



* Means sharing the same letter do not differ significantly within species/cultivar ($p < 0.05$).

Figure 1. Head weight (g/plant) of lettuce varieties grown in unheated plastic tunnels during spring and autumn periods (Debrecen. 2019–2021)

The leaf number of the studied lettuce varieties was primarily determined by environmental factors (Figure 2). The significantly higher average leaf number in spring cultivation (29.88 leaves/plant) compared to autumn cultivation (19.16 leaves/plant) can be explained by the more favorable photoperiod and temperature conditions for vegetative development. A comparison of the varieties reveals that a higher leaf number did not necessarily result in a higher head weight (biomass). Genotypes with the highest leaf number. approaching 50 ('Cortazar'. 'King of May'). did not demonstrate a correspondingly higher head weight. In contrast. 'Great Lakes 659'. which achieved the highest biomass. had an average leaf number of only 30–40. This suggests that head weight is influenced more by the physical properties of the leaves (e.g.. thickness. tissue density) and their arrangement (i.e.. head compactness) than by the leaf number itself.

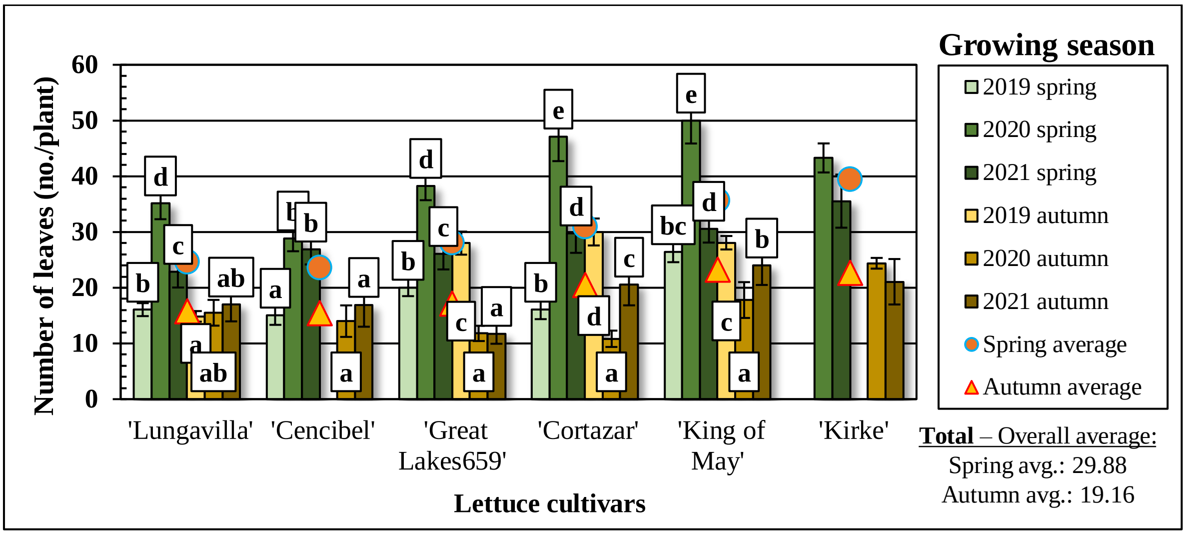


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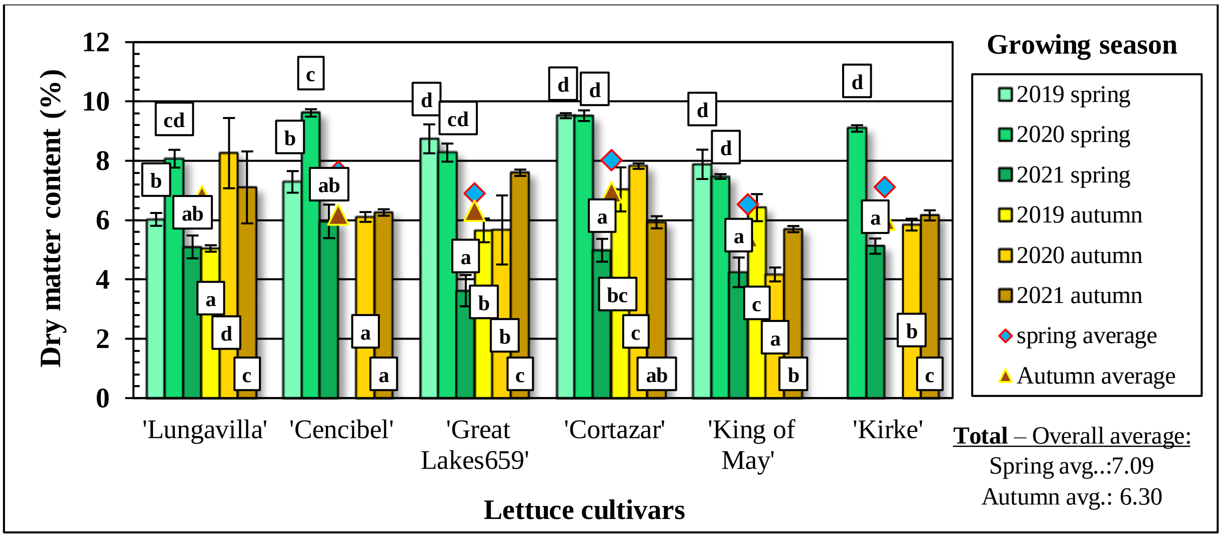
Figure 2. Leaf number development of lettuce varieties in spring and autumn growing seasons under plastic cover (Debrecen. 2019–2021)

A The growing season significantly influenced the dry matter content of the lettuce varieties. Spring values (avg. 7.09%) were higher than those measured in autumn (avg. 6.30%). as spring conditions are more favorable for biomass accumulation (Figure 3).

A year effect was also observed; plants exhibited increased dry matter accumulation (avg. 8.67% across all varieties) during the spring cycle of 2020. when favorable environmental and soil conditions facilitated intensive growth. Among the varieties. 'Cencibel' and 'Cortazar' showed the highest dry matter content (exceeding 9.5% in spring 2020). suggesting superior genetic potential.



* Means sharing the same letter do not differ significantly within species/cultivar ($p < 0.05$).

Figure 3. Dry matter content (%) of lettuce varieties in spring and autumn growing seasons under plastic cover (Debrecen. 2019–2021)

The total polyphenol content (TPC) of plants is determined by both defense mechanisms triggered by environmental stress and genetics (e.g.. higher anthocyanin content in red varieties) (Figure 4).

The growing season significantly influenced TPC. Autumn cultivation resulted in higher values (avg. 50.80 mg GAE/100 g) compared to the spring cycle (avg. 42.42 mg GAE/100 g). indicating that the autumn period imposed greater stress on the plants. The stress response of the varieties differed significantly. The inherently higher polyphenol levels of the red-leaved 'Cencibel' (50–55 mg GAE/100 g) increased dramatically (>180 mg GAE/100 g) under stress. while the green-leaved varieties ('Great Lakes 659'. 'King of May') showed consistently low values (20–40 mg GAE/100 g). indicating a weaker stress response.

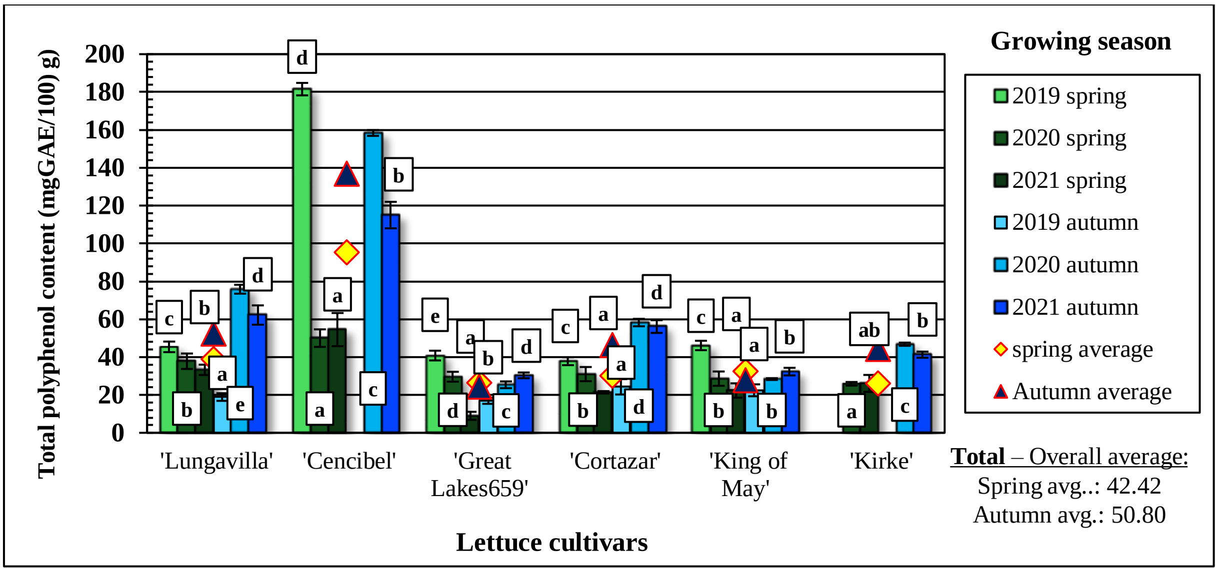


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Figure 4. Total polyphenol content (mg GAE/100 g) of lettuce varieties in spring and autumn growing seasons under plastic cover (Debrecen. 2019–2021)

Flavonoids are a key subgroup of polyphenols with strong antioxidant activity. Their content in the lettuce varieties showed a pattern similar to total polyphenol content (TPC). but the responses to environmental influences were even more pronounced (Figure 5).

The ranking of the varieties correlated with the TPC values. The exceptionally high values of the red-leaved 'Cencibel' were due to a dual effect (genetically high anthocyanin content and sensitive stress response). which further enhanced the baseline level (peak value: ~14 mg CE/100 g). In contrast. the flavonoid content of the green-leaved 'Great Lakes 659'and 'King of May'. with lower genetic potential. remained low under all conditions (<5 mg CE/100 g in spring. <11 mg CE/100 g in autumn).

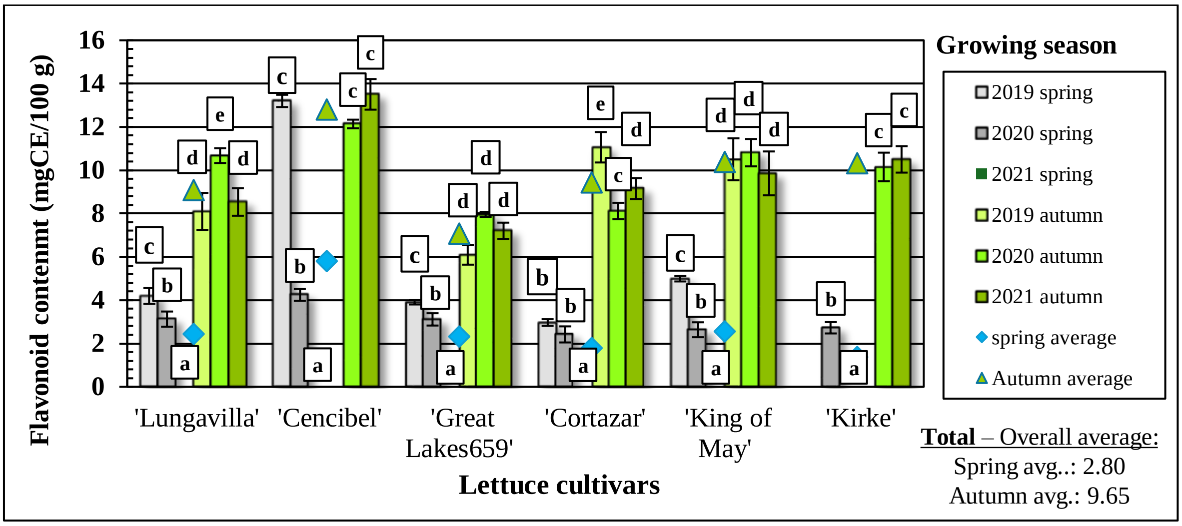

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Figure 5. Flavonoid content (mg CE/100 g) of lettuce varieties under plastic cover during spring and autumn growing seasons (Debrecen. 2019–2021)

Nitrate concentration is primarily determined by soil nitrogen availability and the light intensity required for nitrate assimilation. Autumn values fluctuated. whereas spring values remained consistently high. a difference likely explained by the varying limiting factors of the two periods (Figure 6). The nitrate accumulation potential of the varieties differed significantly. 'Cortazar' and 'King of May' showed the highest tendency for nitrate accumulation. In spring. their concentrations ranged from 520 to 640 mg/kg. whereas in autumn. average nitrate levels exceeded 1000 mg/kg. In contrast. 'Kirke' and 'Great Lakes 659' exhibited significantly lower nitrate accumulation. Their levels remained moderate even during the period with the highest nitrogen availability (spring 2021) (e.g.. 'Great Lakes 659': 624 mg/kg). suggesting more efficient nitrogen assimilation or restricted uptake.

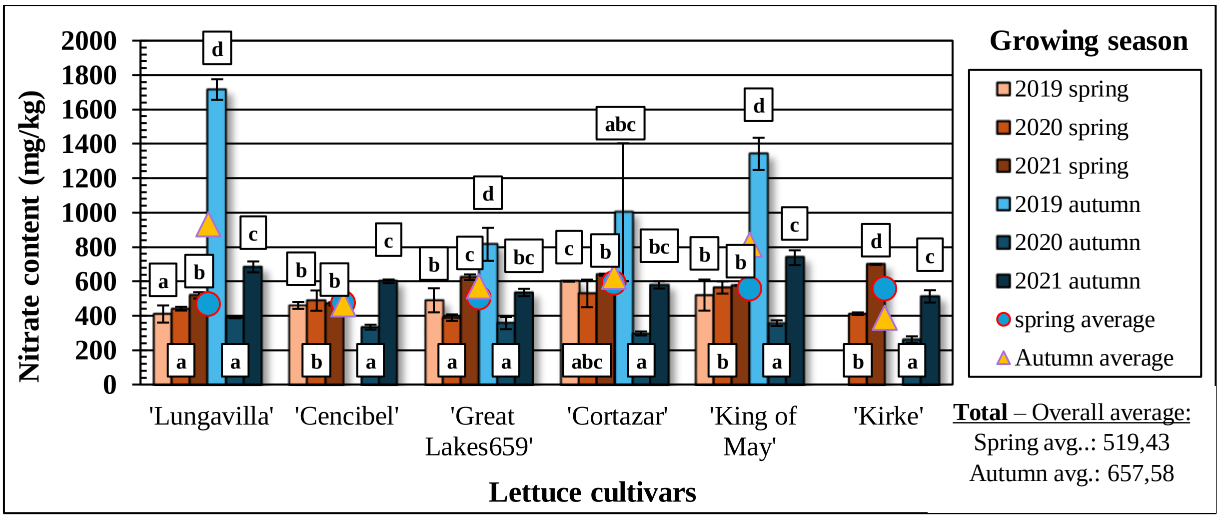

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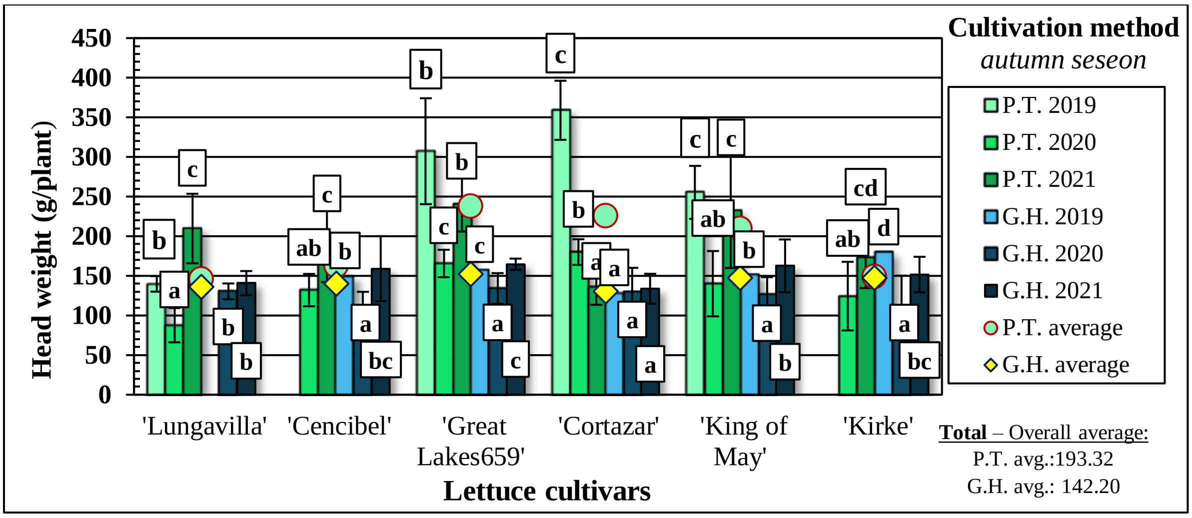
Figure 6. Nitrate accumulation (mg/kg) of lettuce varieties under plastic cover during spring and autumn growing seasons (Debrecen. 2019–2021)

3.2. The effect of cultivation method (greenhouse vs. plastic tunnel) and year on morphological and compositional parameters of lettuce varieties

In the autumn experiment. head weight was primarily determined by the cultivation technology and annual climatic conditions. particularly the level of solar radiation (Figure 7). Significant differences were observed in the yield of the varieties. The highest head weight was achieved by 'Great Lakes 659'. 'Cortazar'. and 'King of May'. 'Cortazar' produced an exceptionally high weight of 358.93 g/plant during the favorable 2019 season in the unheated plastic tunnel. However. this variety proved to be highly sensitive to low light intensity; its head weight dropped by half in 2020. In contrast. the more balanced performance of varieties grown in the greenhouse. such as 'Cencibel' (149. 110. 159 g/plant). indicates that a stable environment mitigates the expression of genetic differences between varieties.



* Means sharing the same letter do not differ significantly within species/cultivar ($p < 0.05$); P:T.-plastic tunnel; G.H.-Greenhouse

Figure 7. Head weight (g/plant) of different lettuce varieties grown in plastic tunnel and greenhouse cultivation during the autumn growing season (Debrecen. 2019–2021)

Autumn leaf number was determined by genotype and environmental stress. though the limiting factors differed between the two cultivation systems. In plastic tunnels. light deficiency was the primary constraint. whereas in the greenhouse. unfavorable soil parameters were decisive (Figure 8).

Significant differences were observed among the varieties. 'King of May' and 'Kirke' produced stably high leaf numbers (20–33 leaves/plant) in the greenhouse. In contrast. 'Cortazar' and 'Great Lakes 659' were the most productive in plastic tunnels (28–30 leaves) in the first season. but responded to the subsequent light deficiency with a severe decrease (10–11 leaves). 'Lungavilla' exhibited a consistently low leaf number (14–17 leaves/plant) under all conditions. reflecting its compact habit.

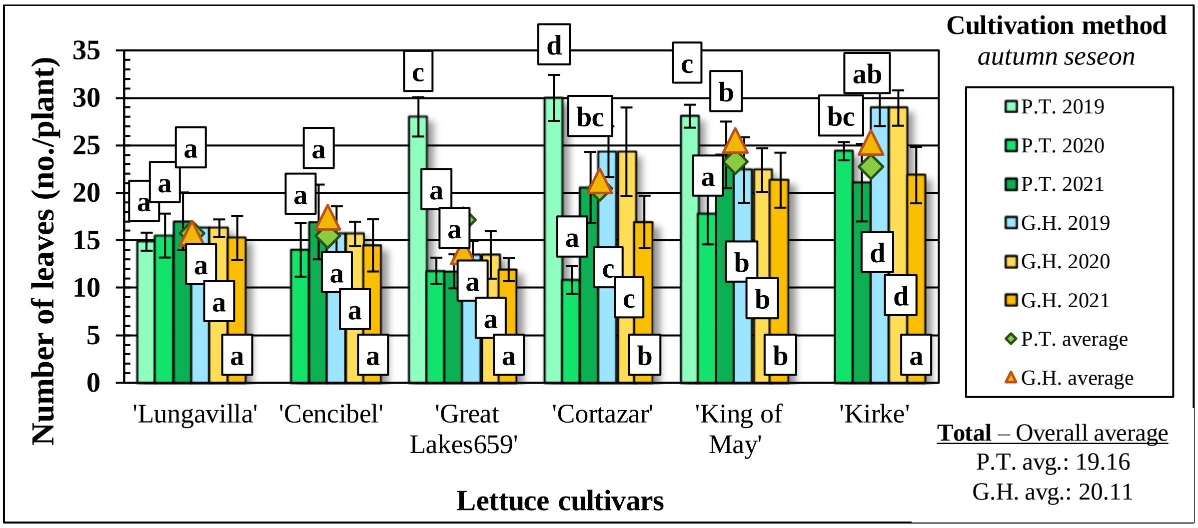


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Figure 8. Number of leaves (leaves/plant) of different lettuce varieties grown in plastic tunnel and greenhouse cultivation during the autumn growing season (Debrecen. 2019–2021)

Dry matter content was influenced by the cultivation system and annual environmental effects. particularly soil and light conditions (Figure 9).

Significant differences were observed in the stress tolerance and dry matter accumulation of the varieties. 'Lungavilla' proved to be the most stress-tolerant. reaching the highest value (8.26%) in the unfavorable. light-deficient 2020 season in the plastic tunnel. In contrast. the dry matter content of 'Great Lakes 659' decreased from 6.65% to 4.18% due to stress conditions in the greenhouse. while its performance remained stable in the more favorable environment of the plastic tunnel (5.65% and 5.67%). This suggests that favorable soil conditions reduced the sensitivity of the variety.

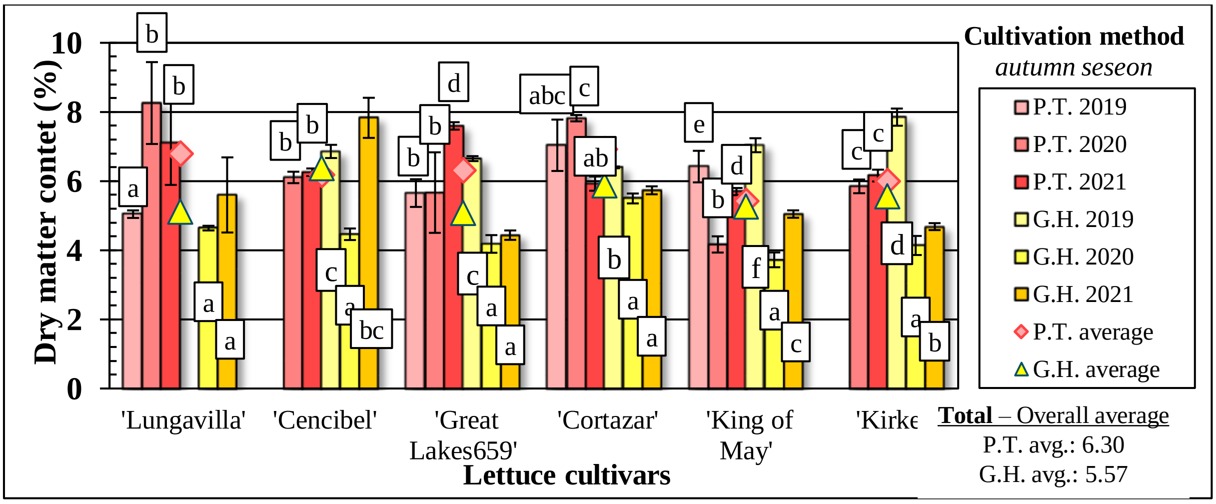

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Figure 9. Dry matter content (%) of lettuce varieties grown in plastic tunnels and greenhouses during the autumn periods (Debrecen. 2019–2021)

Total polyphenol values showed considerable fluctuation in plastic tunnels (avg. 65.49 mg GAE/100 g). whereas significantly lower but more stable levels (avg. 28.55 mg GAE/100 g) were measured under greenhouse cultivation (Figure 10).

Polyphenol accumulation and stress response differed significantly among the varieties. 'Cencibel' reached the highest value during the stressful 2020 season in the plastic tunnel (158.37 mg GAE/100 g). confirming its high genetic potential. 'Cortazar' responded to climatic stress in the plastic tunnel with enhanced accumulation (increasing from 24.29 to 58.23 mg GAE/100 g). whereas it showed low values in response to unfavorable soil conditions in the greenhouse. The sensitivity of 'Kirke' was highlighted by the fact that TPC declined from 35.40 to 10.35 mg GAE/100 g. paralleling the deterioration of soil conditions in the greenhouse.

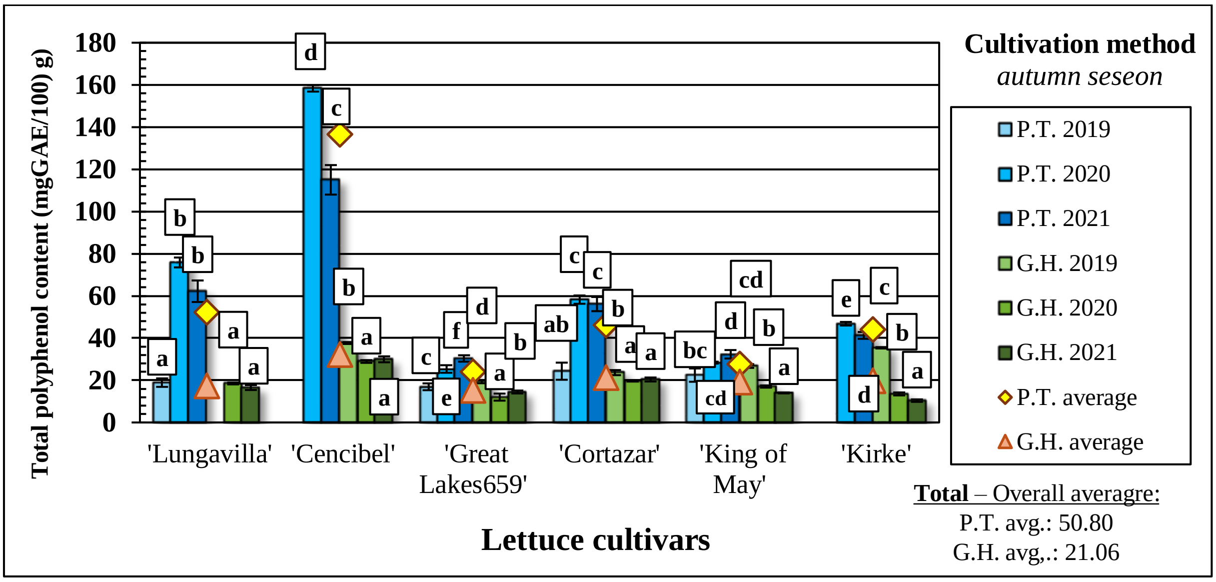

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Figure 10. Total polyphenol content (mg GAE/100 g) of lettuce varieties grown in plastic tunnels and greenhouses during the autumn periods (Debrecen. 2019–2021)

A Flavonoid accumulation in the lettuce varieties showed contrasting trends at the two cultivation sites. differing from the pattern of total polyphenol content observed in the plastic tunnel (Figure 11).

Significant differences were found among the varieties. 'Cencibel' again exhibited the highest flavonoid content (12.14 mg CE/100 g) during the 2020 season in the plastic tunnel. whereas 'Cortazar' maintained stably high flavonoid levels under all conditions. The sensitivity of 'Kirke' to soil deterioration in the greenhouse was confirmed by a drastic decline in flavonoid content. dropping from 12.10 mg CE/100 g (2019) to 7.69 mg CE/100 g (2020).

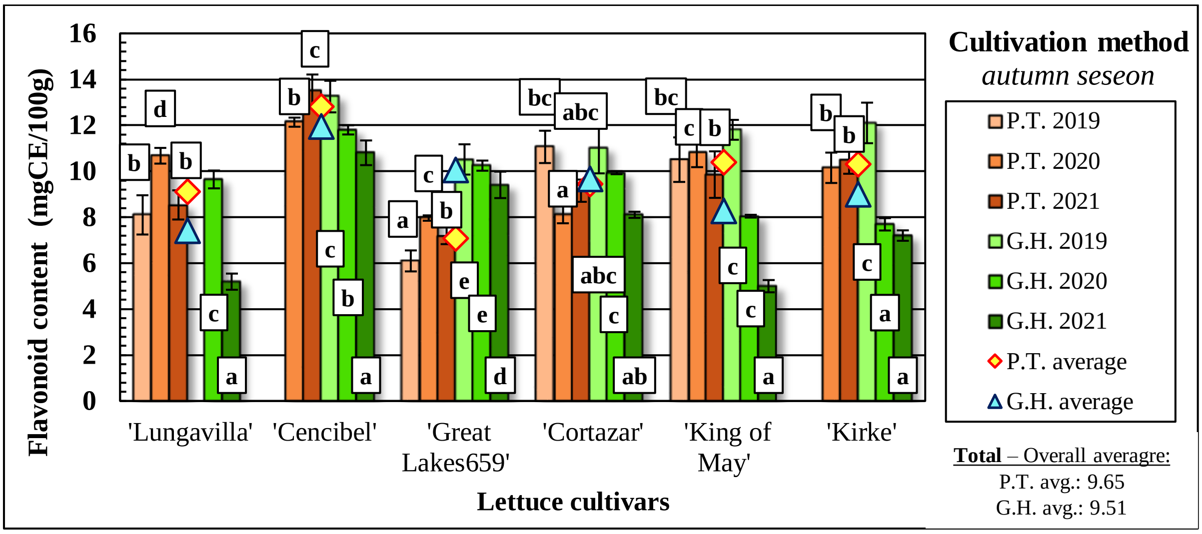

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Figure 11. Flavonoid content (mg CE/100 g) of lettuce varieties grown in plastic tunnels and greenhouses during the autumn periods (Debrecen. 2019–2021)

Nitrate accumulation showed contrasting trends at the two cultivation sites. driven primarily by light and soil nitrogen availability (Figure 12). While a fluctuating but overall decreasing trend was observed in the plastic tunnels. nitrate concentrations in the greenhouse increased continuously over the study period.

Significant differences were also observed among the varieties. 'Lungavilla' exhibited the highest accumulation potential. reaching 1715.67 mg/kg during the 2019 season in the plastic tunnel. In contrast. the low nitrate levels of 'Cencibel' indicate more efficient nitrogen assimilation. Meanwhile. the sensitivity of 'Kirke' was confirmed by the fact that its highest concentration (945.00 mg/kg) was measured in the greenhouse under deteriorating soil conditions.

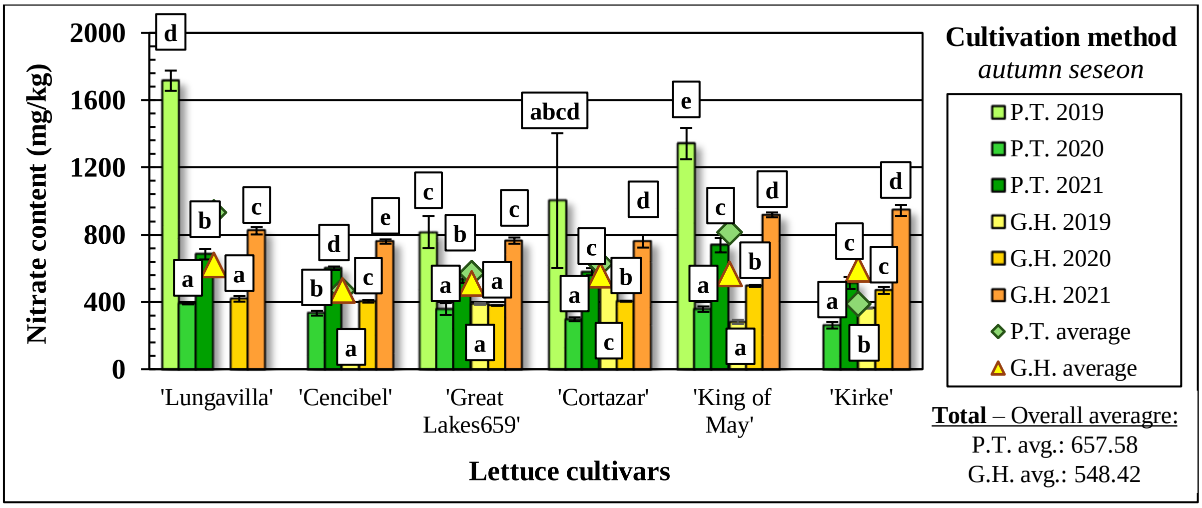

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Figure 12. Nitrate content (mg/kg) of lettuce varieties grown in plastic tunnels and greenhouses during the autumn periods (Debrecen. 2019–2021)

3.3. The effect of cultivation systems (plastic tunnel. open field) on the agronomic and quality characteristics of selected leafy vegetable species

A The development of morphological and yield characteristics of the examined leafy vegetables in the two cultivation systems – spring unheated plastic tunnel and summer open field – can be interpreted through the complex interaction of environmental factors (Table 1).

Spring yields in unheated tunnels decreased despite improved microclimate (irradiance: 135→172 W/m²; temp: 20.35→23.47 °C). due to humus loss (2.88%→1.76%) and nutrient imbalance. The most pronounced change was the decrease in the yield of arugula (57.60→5.26 g/plant) and spinach (40.80→25.32 g/plant).

In summer open field cultivation. the highest irradiance (173 W/m²) and temperature (23 °C) in 2019 resulted in peak yields for all species: arugula ('Themisto': 37.58 g). spinach ('Matador': 16.04 g). baby beetroot ('Bonel': 15.33 g). and lamb's lettuce ('Cirilla': 5.64 g).

Nutrient imbalance in plastic tunnels caused variations in leaf number. 'Matador' decreased to 9.50 leaves/plant despite excessive nitrogen (244 mg/kg). resulting in small leaves. In contrast. 'New Zealand Spinach' peaked at 7.90 leaves/plant in the warmest year (2022). demonstrating good heat tolerance.

In summer open field. leaf numbers followed climatic trends. 'New Zealand Spinach' remained high (15–20 leaves/plant) due to its growth habit. while other species peaked in the favorable 2019 season. consistent with head weights.

Table 1. Green mass (g/plant) and leaf number (no./plant) of various leafy vegetable species under different cultivation technologies (Debrecen. 2019–2022)

Measured parameter	Leafy vegetable variety/type	Plastic tunnel, spring 2019	Plastic tunnel, spring 2021	Plastic tunnel, spring 2022	Open field, summer 2019	Open field, summer 2020	Open field, summer 2021
Green mass *(g/plant)*	'Cirilla' Corn salad	6.40^{c}	3.80^{b}	2.85^{ab}	5.64^{c}	3.20^{b}	2.81^{ab}
	SD	*2.07*	*0.27*	*0.31*	*1.30*	*0.28*	*1.43*
	'Themisto' Arugula	57.60^{d}	10.30^{b}	5.26^{ab}	37.58^{c}	7.23^{ab}	3.62^{a}
	SD	*11.91*	*1.42*	*1.44*	*6.17*	*2.88*	*0.91*
	'Matador' Spinach	40.80^{d}	30.50^{c}	25.32^{bc}	16.04^{ab}	20.75^{bc}	10.88^{a}
	SD	*7.69*	*2.01*	*11.28*	*6.69*	*6.36*	*3.29*
	'Bonel' Beetroot	30.00^{d}	19.20^{c}	6.36^{a}	15.33^{bc}	5.09^{a}	12.42^{b}
	SD	*6.60*	*0.82*	*3.15*	*6.86*	*2.53*	*1.16*
	'New Zealand' Spinach	–	10.85^{a}	11.67^{a}	12.58^{a}	13.45^{a}	14.74^{a}
	SD	–	*0.85*	*4.85*	*4.50*	*7.12*	*3.54*

Leaf number *(no./plant)*	'Cirilla' Corn salad	13.60[c]	12.10[bc]	6.10[a]	12.80[bc]	10.00[b]	11.70[bc]
	SD	*2.97*	*1.20*	*0.74*	*3.16*	*1.41*	*3.97*
	'Themisto' Arugula	28.80[d]	9.30[b]	7.30[ab]	22.50[c]	7.70[ab]	5.60[a]
	SD	*1.64*	*1.34*	*1.49*	*3.24*	*1.64*	*0.84*
	'Matador' Spinach	17.00[d]	13.20[c]	9.50[ab]	13.30[c]	10.80[bc]	7.00[a]
	SD	*3.54*	*0.92*	*1.51*	*3.27*	*2.66*	*1.15*
	'Bonel' Beetroot	12.60[c]	9.60[b]	5.00[a]	5.80[a]	4.80[a]	9.10[b]
	SD	*1.82*	*1.26*	*0.67*	*0.63*	*1.48*	*3.03*
	'New Zealand' Spinach	–	7.10[a]	7.90[a]	20.20[c]	18.20[bc]	15.00[bc]
	SD	*–*	*0.99*	*1.29*	*4.42*	*4.71*	*3.27*

* Values within a species/cultivar followed by the same letter are not statistically different ($p < 0.05$)

The analysis of total polyphenol and flavonoid concentrations. serving as indicators of plant stress response. reveals how limiting factors in the two cultivation systems influenced the content of leafy vegetable species (Table 2).

In the plastic tunnels. initially high total polyphenol levels declined in parallel with the decrease in humus content (2.88% → 1.72%). The content in lamb's lettuce ('Cirilla') decreased from 311 to 154 mg GAE/100 g. and in spinach ('Matador') from 231 to 75 mg GAE/100 g. indicating reduced efficiency of defense mechanisms. In 2022. a renewed increase in polyphenol accumulation was observed ('Cirilla': 305 mg; 'Matador': 145 mg GAE/100 g). Thus. soil deterioration adversely affected not only yield but also defense mechanisms and nutritional quality.

A In open field cultivation. phytochemical content was primarily determined by climatic factors. The high temperature (23.04 °C) and irradiance (173.02 W/m²) in 2019 induced a classic abiotic stress response. In lamb's lettuce. total polyphenol content reached 284.67 mg GAE/100 g and flavonoid content 99.13 mg CE/100 g. whereas in 'Matador' spinach. these values were 197.92 mg GAE/100 g and 29.86 mg CE/100 g. respectively.

Since the highest bioactive content coincided with the highest yields. environmental conditions in 2019 remained optimal for growth despite the stress pressure. The elevated polyphenol and flavonoid concentrations thus indicate an effective defense mechanism. which. facilitated by stable soil conditions. was not accompanied by a yield penalty.

Table 2. Total polyphenol and flavonoid content of leafy vegetable species in different cultivation systems (Debrecen. 2019–2022)

Measured parameter	Leafy vegetable variety/type	Plastic tunnel spring 2019	2021	2022	Open field summer 2019	2020	2021
		Cultivation system / season					
Total polyphenol *(mg GAE/100 g)*	'Cirilla' Corn salad	311.01d	154.76a	305.00cd	284.67c	284.67c	235.00b
	SD	*10.69*	*6.84*	*4.00*	*9.28*	*11.37*	*13.23*
	'Themisto' Arugula	202.52^{bc}	107.82^{a}	118.00^{a}	187.47^{b}	226.87^{c}	210.00^{bc}
	SD	*24.06*	*2.54*	*2.65*	*2.82*	*8.19*	*13.23*
	'Matador' Spinach	231.33^{e}	74.78^{a}	145.00^{b}	197.92^{d}	161.48^{bc}	183.33^{cd}
	SD	*23.80*	*0.75*	*5.00*	*2.39*	*14.10*	*3.79*
	'Bonel' Beetroot	165.28^{d}	73.52^{a}	110.33^{b}	144.24^{cd}	122.20^{bc}	135.33^{bc}
	SD	*14.78*	*2.29*	*1.53*	*14.32*	*10.87*	*0.58*
	'New Zealand' Spinach	–	48.00^{a}	86.00^{b}	112.23^{c}	88.93^{b}	96.00^{bc}
	SD	*–*	*2.79*	*3.00*	*0.85*	*14.45*	*2.00*
Flavonoid content *(mg CE/100 g)*	'Cirilla' Corn salad	112.84c	0.04a	103.00c	99.13bc	8.25a	83.00b
	SD	*12.93*	*0.00*	*2.65*	*9.26*	*0.25*	*2.65*
	'Themisto' Arugula	24.05^{d}	0.01^{a}	8.10^{b}	17.82^{c}	8.89^{b}	19.30^{c}
	SD	*2.82*	*0.00*	*0.26*	*1.98*	*0.26*	*0.10*
	'Matador' Spenót	37.79^{e}	0.01^{a}	6.60^{b}	29.86^{d}	11.24^{c}	10.60^{c}
	SD	*1.58*	*0.00*	*0.53*	*2.92*	*0.40*	*0.36*
	'Bonel' Beetroot	31.05^{d}	0.02^{a}	9.23^{b}	23.48^{c}	11.45^{b}	18.20^{c}
	SD	*3.52*	*0.00*	*0.25*	*4.85*	*0.51*	*0.10*
	'New Zealand' Spinach	–	0.02^{a}	3.20^{b}	14.88^{d}	13.14^{d}	10.20^{c}
	SD	*–*	*0.00*	*0.36*	*1.97*	*0.33*	*0.20*

* Values within a species/cultivar followed by the same letter are not statistically different ($p < 0.05$).

A The analysis of quality-indicating parameters demonstrates the impact of stress factors associated with plastic tunnel and open field cultivation on crop quality (Table 3).

Crop quality in plastic tunnels was characterized by lower dry matter and vitamin C levels. attributed to deteriorating soil conditions. inhibited transpiration within the enclosed environment. and reduced light intensity. The decrease in vitamin C content of lamb's lettuce (from 12.88 to 4.85 mg/100 g) was driven by unfavorable soil conditions.

The highest nitrate concentrations were measured in arugula ('Themisto': 4352.74 mg/kg) and spinach ('Matador': 7261.14 mg/kg). The nitrate level in spinach. which exceeded the regulatory limit (5000 mg/kg) set by the EU (2013). is a consequence of impaired nitrogen assimilation. The plant was unable to convert the abundant nitrogen into protein. leading to its accumulation.

In open field cultivation. climatic factors significantly influenced quality. Due to environmental stress (2019: 23.04 °C; 173 W/m²) and intense transpiration. plants exhibited higher dry matter content. 'Matador' spinach peaked at 15.70% (2020). exceeding the 8.38% maximum observed in plastic tunnels. Conversely. Vitamin C levels were highest in the cooler year of 2021 for most species. suggesting that antioxidant production depends on specific factors (e.g.. temperature fluctuations. water deficit) beyond average heat/light stress. While nitrate content was low (10–212 mg/kg) in favorable years. it spiked in 2019. reaching 5346 mg/kg in spinach and 3980 mg/kg in arugula.

Table 3. Dry matter. vitamin C. and nitrate content of leafy vegetable species in different cultivation systems (Debrecen. 2019–2022)

Measured parameter	Leafy vegetable variety/type	***Cultivation system / season***					
		Plastic tunnel spring			**Open field** summer		
		2019	***2021***	***2022***	***2019***	***2020***	***2021***
Dry matter content *(m/m)%*	'Cirilla' Corn salad	10.18^{c}	7.30^{b}	6.51^{a}	9.20^{bc}	9.20^{bc}	12.86^{d}
	SD	*0.96*	*0.10*	*0.62*	*1.31*	*1.31*	*0.61*
	'Themisto' Arugula	9.93^{b}	8.75^{a}	9.93^{b}	8.37^{a}	13.71^{d}	12.40^{c}
	SD	*0.71*	*0.02*	*0.14*	*0.31*	*0.34*	*0.32*
	'Matador' Spinach	8.38^{ab}	7.68^{a}	8.28^{ab}	10.36^{bc}	15.70^{d}	11.46^{c}
	SD	*0.50*	*0.08*	*0.13*	*0.34*	*1.79*	*0.52*
	'Bonel' Beetroot	8.57^{b}	6.74^{a}	9.27^{bc}	8.95^{bc}	9.69^{c}	11.33^{d}
	SD	*0.50*	*0.24*	*0.12*	*0.03*	*0.58*	*0.07*
	'New Zealand' Spinach	–	4.71^{a}	5.95^{b}	10.27^{e}	8.03^{c}	9.30^{d}
	SD	*–*	*0.11*	*0.60*	*0.15*	*0.25*	*0.17*
Vitamin C content *(mg/100 g)*	'Cirilla' Corn salad	12.88^{b}	4.85^{a}	11.20^{b}	11.05^{b}	6.81^{a}	6.28^{a}
	SD	*1.66*	*0.11*	*0.20*	*1.09*	*0.25*	*0.03*
	'Themisto' Arugula	2.59^{ab}	3.63^{ab}	4.05^{b}	2.30^{a}	8.84^{c}	43.50^{d}
	SD	*0.35*	*0.06*	*0.15*	*0.58*	*0.67*	*1.10*
	'Matador' Spinach	9.24^{b}	6.09^{a}	5.90^{a}	10.30^{b}	13.04^{c}	25.87^{d}
	SD	*0.30*	*0.06*	*0.10*	*1.70*	*0.56*	*1.21*
	'Bonel' Beetroot	9.30^{de}	6.09^{b}	7.50^{bc}	10.60^{e}	8.19^{cd}	3.65^{a}
	SD	*0.55*	*0.05*	*0.30*	*1.40*	*0.68*	*0.05*
	'New Zealand' Spinach	–	3.77^{a}	4.13^{a}	9.39^{b}	14.30^{c}	16.13^{d}
	SD	*–*	*0.13*	*0.23*	*0.56*	*0.53*	*0.76*

Nitrate content *(mg/kg)*	'Cirilla' Corn salad	3787.34^{e}	676.00^{b}	1546.67^{c}	3220.00^{d}	15.84^{a}	110.67^{a}
	SD	*212.90*	*5.29*	*51.32*	*52.92*	*0.92*	*6.66*
	'Themisto' Arugula	4352.74^{c}	596.67^{a}	1690.00^{b}	3980.33^{c}	14.17^{a}	212.00^{a}
	SD	*338.37*	*3.06*	*17.32*	*519.50*	*0.40*	*8.00*
	'Matador' Spinach	7261.14^{d}	770.00^{a}	1809.00^{b}	5346.00^{c}	11.57^{a}	160.00^{a}
	SD	*515.21*	*10.00*	*6.00*	*714.00*	*0.51*	*11.14*
	'Bonel' Beetroot	3025.46^{e}	768.33^{b}	1205.00^{c}	2450.00^{d}	12.16^{a}	110.33^{a}
	SD	*180.58*	*4.04*	*6.24*	*250.00*	*1.00*	*4.51*
	'New Zealand' Spinach	–	604.00^{c}	1485.00^{d}	3181.22^{e}	10.67^{a}	142.33^{b}
	SD	*–*	*5.29*	*21.79*	*51.09*	*0.41*	*4.04*

*Values within a species/cultivar followed by the same letter are not statistically different ($p < 0.05$).

4. NEW SCIENTIFIC RESULTS

1. It was found that the growing season significantly influenced the yield of the lettuce cultivars. Due to favorable light and temperature conditions, spring cultivation in unheated plastic tunnels resulted in a head mass more than 250–300% higher ('May King': 568 g/plant; ’Kirke’: 519 g/plant) than the 130–180 g/plant average typical of the autumn cycle. The yield increase was also reflected in the leaf count, which reached 50 leaves/head for the cultivar 'May King'.

2. We established that the growing season (spring/autumn) and the cultivar type (green/red) jointly determine the polyphenol accumulation in lettuce. While spring cultivation favored biomass yield, autumn cultivation in unheated plastic tunnels led to a significant increase in the total polyphenol content (TPC) of the plants. In the cultivar ’Cencibel’ (Lollo Rossa), concentrations of 115–158 mg GAE/100 g were measured during the autumn cycle, compared to the spring value of 50 mg GAE/100 g, representing an increase of over 200%.

3. The differential genetic sensitivity of the studied lettuce cultivars to nitrate accumulation induced by the low-light conditions of the autumn period was demonstrated. The 'Lungavilla' (Lollo Bionda) cultivar proved to be the most sensitive, with its nitrate concentration rising from a spring average of 457 mg/kg to 1715 mg/kg during autumn cultivation under plastic tunnels, representing a nearly 300% increase. In contrast, the nitrate content of 'Cencibel' (Lollo Rossa) remained stable regardless of the season, averaging 470 mg/kg.

4. It was established that the antioxidant content of red-pigmented cultivars ('Cencibel', 'Kirke') was significantly higher during the autumn cultivation cycle compared to green-leaved cultivars. In unheated plastic tunnels during autumn, the TPC value of 'Cencibel' (Lollo Rossa) reached 158.37 mg GAE/100 g—more than double the maximum value recorded for 'Lungavilla' (Lollo Bionda) at 75.84 mg GAE/100 g. These findings support the conclusion that the enhanced synthesis of red pigments (anthocyanins), which belong to the polyphenol group, is the determining factor behind the autumn increase in total phenolic content. This demonstrates that the intensification of pigmentation triggered by

environmental stress (low-light and cooler periods) is not merely an aesthetic change, but results in a direct and significant enhancement of the bioactive polyphenol content.

5. A significant negative correlation was established between biomass yield and bioactive compound content across the evaluated cultivation strategies. Comparing the two extremes of the experiment: the maximum yield of 568.20 g/plant (cv. 'May King', spring) was associated with a low TPC value of 22.34 mg GAE/100 g. Conversely, the highest TPC value of 181.53 mg GAE/100 g (cv. 'Cencibel', spring) was coupled with a head mass of only 129.0 g/plant. This implies that achieving the maximum yield involved a nearly 88% trade-off in quality compared to the maximum potential phenolic concentration.

6. A significant positive correlation (r = 0.603) was demonstrated between yield and nitrate content under plastic tunnel cultivation. At the same time, the lack of correlation between nitrate concentration and SPAD values (r = 0.018$)—which serve as an indicator of chlorophyll status—statistically confirms that nitrate accumulation was not driven by spring light deficiency or resulting assimilation disturbances. The exceptionally high nitrate value of 7261.14 mg/kg recorded for spinach (cv. 'Matador') is therefore presumably not attributable to light conditions; rather, it may be explained by cultivar-specific nitrate accumulation capacity and physiological stress induced by relatively high soil salinity, which can hinder the incorporation of absorbed nitrogen into organic matter.

7. Our results demonstrated a significant negative relationship between vegetative biomass production and the synthesis of secondary metabolites (defense compounds) during summer open-field cultivation. This was further confirmed by the significant negative correlation of plant height with both total phenolic content (r = -0.464) and flavonoid content (r = -0.558). These findings indicate that climatic stress (heat, UV radiation) induced the synthesis of defensive (antioxidant) compounds in the plant at the expense of vegetative growth.

5. PRACTICAL USE OF RESULTS

1. For industrial processing and the production of raw materials for salad mixes – adiming for maximum biomass and leaf number – cultivation in unheated plastic tunnels during spring is recommended. The most suitable varieties are 'May King' (butterhead). 'Kirke' (oak leaf). and 'Cencibel' (Lollo Rossa). provided that soil nutrient supply is carefully managed.

2. For the production of crops with high bioactive content suitable for the fresh market. cultivation in unheated plastic tunnels during autumn is ideal. For this purpose. the recommended varieties are 'Cencibel' (Lollo Rossa). due to its exceptionally high antioxidant content. and 'Cortazar' (romaine). which combines high nutritional quality with a favorably low tendency for nitrate accumulation.

3. To enhance production stability. especially in the spring season. the cultivation of the consistently high-yielding 'Great Lakes 659' (iceberg lettuce) is recommended.

4. In order to ensure low nitrate levels. it is advisable to reduce or eliminate nitrogen top-dressing in the autumn production cycle – particularly for varieties prone to nitrate accumulation (e.g.. 'Lungavilla' – Lollo Bionda; 'May King' – butterhead).

5. To increase market flexibility. the cultivation of dual-purpose varieties such as 'Kirke' (oak leaf lettuce) is recommended. This variety is suitable for producing high biomass in spring to meet the demands of the processing industry. while in autumn. it yields a high-quality product suitable for the fresh market.

6. In consumer communication. it is worth emphasizing the outstanding antioxidant content of autumn-grown. red-leaved varieties (e.g.. 'Cencibel'. 'Lollo Rossa').

7. For summer open-field leafy vegetable production – especially during warming periods associated with climate change – 'New Zealand Spinach' offers the best yield stability. Regardless of seasonal fluctuations. this species demonstrated stable yield (12–14 g/plant). leaf number (15–20 leaves/plant). and reliable nutritional quality due to its excellent heat tolerance.

8. To produce crops with high vitamin C content. the cultivation of open-field arugula ('Themisto') is recommended. particularly in cooler years (e.g.. 2021). This variety responded to moderate climatic stress with exceptionally high vitamin C accumulation (43.50 mg/100 g).

9. The cultivation of spinach ('Matador') in both systems (open field and plastic tunnel) poses a significant food safety risk. Measured peak nitrate values (5346–7261 mg/kg) exceeded the EU regulatory limit of 5000 mg/kg; therefore. its cultivation is recommended only under strictly controlled. low-nitrogen regimes.

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7. PUBLICATIONS RELATED TO THE DISSERTATION

DEBRECENI EGYETEM
EGYETEMI ÉS NEMZETI KÖNYVTÁR
H-4002 Debrecen, Egyetem tér 1, Pf.: 400
Tel.: 52/410-443, e-mail: publikaciok@lib.unideb.hu

Nyilvántartási szám: DEENK/575/2025.PL
Tárgy: PhD Publikációs Lista

Jelölt: Kovácsné Madar Ágota
Doktori Iskola: Kerpely Kálmán Doktori Iskola
MTMT azonosító: 10068646

A PhD értekezés alapjául szolgáló közlemények

Magyar nyelvű könyvrészletek (2)

1. **Kovácsné Madar, Á.**, Takácsné Hájos, M., Fehér, M., Stündl, L.: Különbözö salátafajták értékelése eltérő vízikultúrás termesztéstechnológia mellett.
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Magyar nyelvű tudományos közlemények hazai folyóiratban (1)

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Idegen nyelvű tudományos közlemények hazai folyóiratban (2)

4. **Kovácsné Madar, Á.**, Takácsné Hájos, M.: Agronomic evaluation of different lettuce (Lactuca sativa L.) varieties under unheated plastic tunnel.
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   DOI: http://dx.doi.org/10.34101/actaagrar/1/10449

DEBRECENI EGYETEM
EGYETEMI ÉS NEMZETI KÖNYVTÁR
H-4002 Debrecen, Egyetem tér 1, Pf.: 400
Tel.: 52/410-443, e-mail: publikaciok@lib.unideb.hu

Idegen nyelvű tudományos közlemények külföldi folyóiratban (2)

6. **Kovácsné Madar, Á.**, Takácsné Hájos, M.: Evolution of quality parameters of different lettuce (Lactuca sativa L.) varieties under unheated plastic tunnel.
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Magyar nyelvű konferencia közlemények (3)

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Idegen nyelvű absztrakt kiadványok (4)

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