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Evaluation of alternative substrates for hydroponics based on biological parameters of leaf lettuce (*Lactuca sativa* L.) and its stress response

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ABSTRACT

The study focusses on alternative substrates with the potential to replace common substrates, such as mineral wool and perlite, as the influence of these on ecosystems and resources is being debated. To this aim, wood fibre, sheep wool and coco peat substrates were selected for testing. Leaf lettuce (*Lactuca sativa* L. 'Lisboa') is taken as the model crop for the evaluation of alternative substrates. The closed hydroponic system ebb and flow with growing in pots was used for the experiment. The parameters of the nutrient solution were EC 1.5–2 mS and pH 6–7. Biological parameters, content of nitrates, plant stress indicators, antioxidant activity (AA) as % scavenging of DPPH (AA), glutathione (GSH), ascorbate peroxidase (APX), total phenol content (TPC) and the content of chosen elements in the drain from substrates were evaluated. According to the biomass production of lettuce, the substrates can be ranked from the lowest as follows: sheep wool < wood fibre < mineral wool < perlite < coco peat. The levels of GSH and APX did not affect AA. The TPC showed the greatest effect on AA and the stress response in general. The nitrate content ranged between 426 and 686 mg · kg^{-1} of fresh mass. Instead of mineral wool and perlite, coco fibre could be a promising alternative organic substrate for lettuce production in the ebb and flow hydroponics system. Wood fibre and sheep wool still have unresolved circumstances regarding their physical and chemical parameters.

Keywords: hydroponics, lettuce, organic substrates, renewable resources, stress response

Abbreviations: AA, antioxidant activity; APX, ascorbate peroxidase; AsA, ascorbic acid; DM, dry mass; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FM, fresh mass; GAE, gallic acid equivalents; GSH, glutathione; ROS, reactive oxygen species; RT, room temperature; TPC, total phenol content.

INTRODUCTION

Lettuce is one of the most important leafy vegetables in terms of production, and its global production is steadily on the rise (Shatilov et al., 2019; Ridder, 2022; Trenda, 2022). It is important not only due to its wide use in the food industry but also as a source of many bioactive compounds that improve the health of the population. These include polyphenols, carotenoids and chlorophylls (Shi et al., 2022).

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Mineral wool is the world's most used medium for growing fruit vegetables in greenhouses because of its advantages such as uniformity, inertness, easy handling and good physical parameters (Gruda et al., 2016). However, in the case of mineral wool, handling the used material as waste appears to be problematic. There have been efforts to reuse mineral wool mats for growing cucumbers. Unfortunately, the yield reduced when used the second time, so reusing it for growing is not recommended (Łaźny et al., 2021). The amount of mineral wool that is reused is very low, at approximately 10% (Bussell and McKennie, 2004). For these reasons, researchers are trying to find an alternative substrate that could provide good conditions for obtaining yields comparable to those using mineral wool while making the management of the resulting waste easier. Another commonly used substrate is perlite – aluminium silicate, which is produced from volcanic origin. Its advantages are low bulk density, high porosity, inertness and the fact that it does not undergo rapid decomposition (Awad et al., 2017). Unfortunately, according to the findings of Vinci and Rapa (2019), who investigated the impact of substrates (perlite, rockwool, coconut fibre, peat, etc.) on human health, ecosystems and resources, perlite was found to have worst outcomes. It is therefore necessary to find other alternatives.

When searching for an alternative substrate for hydroponic crops, some authors focussed on waste materials such as compost from vegetable waste or groceries (Mazuela et al., 2005; Moschou et al., 2022), recycled plastics and almond shells (Kennard et al., 2020), sheep wool (Böhme et al., 2008; Dannehl et al., 2015; Jug, 2018) or rice hulls (Sambo et al., 2008; Buck and Evans, 2010). Other authors used substrate materials from renewable sources such as hemp (Dannehl et al., 2015; Li et al., 2021), wood materials as fibre, bark or sawdust (Allaire et al., 2004; Muro et al., 2004; Dorais et al., 2007; Maboko and Modise, 2018; Rahman et al., 2018) or coconut husk, which is presented in the literature as coir, coco fibre or coco peat (Böhme et al., 2008; Suvo et al., 2017). In the present study, three organic substrates, namely, coco peat, wood fibre and sheep wool, were used for testing in the hydroponic system. Coconut husks are used as a material to produce a substrate for hydroponic cultivation with a porosity similar to peat. Due to its high lignin content, it resists decomposition and is thus suitable for long-term cultures. The high water-holding capacity represents an ideal source of water for plants for a longer period than other substrates. The advantage from an environmental perspective is that coco peat is created as a co-product of the food and clothing industries, is compostable and is a renewable resource, unlike peat (Noguera et al., 2000; Ali, 2011). On the contrary, coconut shells naturally contain a high amount of salt due to their place of origin. Many buffer solutions, such as calcium nitrate and water, are needed for this purpose (Peano et al., 2012). During the soaking process of coconut husks, a large amount of harmful substances such as pectin, pentosan, tannins and polyphenols are produced, which are released into the sea. Due to this, the content of various chemical substances increases and the oxygen content decreases in the sea, which results in the death of many marine organisms (Narayanan, 1999). Wood fibre is produced by mechanically crushing wood or extrusion using hot steam (Carlile et al., 2015). It has been used in horticultural substrates for a long time as a substitute for peat to limit its extraction. The use of wood fibre in hydroponic cultivation, which functions similar to coco peat, and thus the need to import materials from other parts of the world would be eliminated, thereby reducing the carbon footprint and total impact on the global ecosystem (Vinci and Rapa, 2019). According to Gruda and Schnitzler (2004), wood fibre-based substrates possess favourable physical parameters such as total pore space, air content or water capacity. Using wood fibre as a substrate still has its pitfalls and several unanswered questions, especially in terms of phytotoxicity (Gruda et al., 2009). The problem of the phytotoxicity of wood materials used as a substrate is still being solved and warrants different methods of heat treatment during production (Yang et al., 2022). Sheep wool is a good fertiliser for plants (Zheljazkov, 2005; Komorowska et al., 2022). It contains some beneficial elements for plant nutrition, including calcium, magnesium, potassium, sodium, phosphorus and iron, and due to the structure of keratin, it slowly releases nitrogen. Furthermore, sheep wool fibres are hydrophilic and thus can retain water, which, in turn, is available to plants for a longer period (Petek and Logar, 2021). The number of sheep in the world shows a 20-year upward trend. The world population of sheep was 1.266 billion in 2021, with 59.55 million in Europe. The production of greasy sheep wool and clean sheep wool was 1.950 mkg and 1.0336 mkg, respectively (Eurostat, 2021; IWTO Market Information, 2022). Several studies focussed on using sheep wool as a growing medium or as a component of substrates for vegetables cultivated in greenhouses (Böhme et al., 2008; Górecki and Górecki, 2010; Dannehl et al., 2015; Jug, 2018). However, wool as a material for substrate production has not yet received enough attention to warrant further research and exploit the potential in the quantity in which wool is produced. Therefore, it was included among the substrates in the present study. The greatest challenge in terms of finding alternative substrates is to find one substrate that will meet the criteria of ideal physical and chemical properties and thus be a suitable medium for growing plants. The substrates should provide plants with sufficiently suitable conditions in terms of the availability of water, air and nutrients. Moreover, they should not possess characteristics that they many pose as obstacles to plant growth, such as phytotoxicity, immobilisation of nutrients from the solution or heavy metal content. Many of such deficiencies can be detected in substrate testing by monitoring stress factors.

The aim of this study is to conduct an experiment with a model lettuce crop grown in different substrates (rockwool, perlite, wood fibre, sheep wool and coco peat) and determine their yield and quality assumptions when growing lettuce hydroponically using the flood-and-drain method and compare biological and analytical parameters of lettuce. It is assumed that the use of individual substrates affects the growth parameters of lettuce, which will be reflected in the monitored stress indicators. A positive effect is expected from the physical and chemical properties of substrates of organic origin, which could thus replace mineral wool or perlite.

MATERIALS AND METHODS

Experiment design

The experiment was carried out in the spring of 2022 in the greenhouse in Krakow (Poland). A closed flood-and-drain growing system was used, and 21 lettuce plants (7 plants for one repetition) were grown in each of five different substrates (perlite, mineral wool, spruce wood fibre, raw sheep wool and fine fraction coco peat). The physical and chemical properties of the substrate are detailed in Table 1. *Lactuca sativa* L. 'Lisboa' (Rijk Zwaan) was chosen as the model crop. Lettuce seeds were sown on March 7 in mineral wool cells of size 2 cm × 1.5 cm × 1.5 cm and then germinated in greenhouse conditions. After 22 days, the seedlings were transplanted into pots of size 9 cm × 9 cm × 10 cm, filled with selected substrates and moved to a flood-and-drain table and kept at a distance of 12 cm × 12 cm. As soon as the leaves exceeded the borders of the pots, the plants were distanced 25 cm × 25 cm apart. The harvest and measurement of the lettuce biological parameters took place 64 days after planting (17 May). Climatic conditions in the greenhouse were set to 23/18 °C day/night and approximately 75% relative humidity. The CO_2 content in the air varied between 377 and 622 ppm. No artificial light was used, and the natural photoperiod was between 12 hr and 15 hr from the beginning to the end of the experiment. The growing system was closed with nutrient solution recirculation. The solution was completely changed every 3 days. As a fertiliser, a mix of Solinure GT 1 and Solinure GT 7 fertilisers was used in a 1:1 ratio, so that the N:P:K ratio was 4:1:6. EC of the nutrient solution was maintained between 1.5 and 2 mS with a pH of 6–7. The irrigation regime was determined according to the developmental stage of the plants and the climatic conditions in the greenhouse. At the beginning of cultivation, the frequency of watering was three times a day at an interval of 8 hr. In the later stages of cultivation and on sunny days, the frequency of watering was up to six times a day, with an interval of 4 hr. Water was filled by flooding up to a water level of 35 mm, and the flooding lasted 10 min.

Methodology for evaluating drain from substrates parameters

The content of selected elements in the drain from substrates was analysed using the ICP-OES method. As the sample for the analysis, 5 mL from the drain was mineralised in the Anton Paar Multiwave 3000 microwave system. After that, it was digested in a mixture of HNO_3 and H_2O_2 in a ratio of 5:1 vol. The measurement of the number of elements was performed by inductively coupled plasma atomic emission spectrometry using an Optima7600 instrument (Perkin Elmer, Akron, USA).

Methodology for evaluating lettuce parameters

The fresh leaf biomass, number of leaves and stem diameter were measured. The stem diameter was measured after cutting the plant using a caliper. The leaf area was measured 1 day before the end of the experiment using the LeafScan mobile application (by Carlos Anderson), which is a non-destructive method of measuring the leaf area of the entire head of lettuce.

Methodology for evaluating analytical parameters of lettuce

The analysis of content of nitrates, antioxidant activity (AA), ascorbate peroxidase (APX), total phenol content (TPC) and glutathione (GSH) were performed. The nitrate content in the lettuce was assayed following the method by Cataldo et al. (1975).

A total of 2 g of dry matter was put in 10 mL (4 × 2.5 mL) of hot water (90–95 °C). The extracts were then placed in a water bath for 30 min at 80 °C. After that, the solution was mixed for 3 min at 200 rpm and cooled to room temperature (RT). Then, the samples were centrifuged for 10 min at 4,500 rpm. A total of 0.2 mL of the extract was

Table 1. Physical and chemical parameters of substrates.

Substrate	Bulk density	Total pore space	Air capacity	Total water-holding capacity	Chlorides in drain	EC	pH
Unit	$kg \cdot L^{-1}$	%	%	$L \cdot m^{-3}$	$g \cdot L^{-1}$	$mS \cdot cm^{-1}$	
Mineral wool	0.064	97.13	15.6	815	0.006	0.247	6.6
Perlite	0.11	59.97	30	300	0.008	0.0828	7.99
Coco peat	0.081	77.48	4.4	730	0.029	0.528	4.85
Wood fibre	0.064	76	12.8	632	0.011	0.248	6.25
Sheep wool	0.048	96.77	77	198	0.094	6.24	7.97

The pH values and electrical conductivity were measured in soil:distilled water (1:2.5 = v:v) suspension by using an electrometric method. Mohr's method was used to determine the chloride ion concentration of a solution by titration with silver nitrate.

mixed with 0.8 mL of 0.5% salicylic acid (5 g in 100 mL 96% sulfuric acid), and then 19 mL of 0.5 M NaOH was added. After cooling to RT, the absorbance was measured at 420 nm on a spectrophotometer (UV/VIS Helios Beta).

An analysis of total AA was performed following the method by Molyneux (2004) using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The extract was prepared from 2 g of ground samples with 10 mL of 80% methanol in four portions and centrifuged (4,500 × *g*, 10 min, 4 °C). A total of 0.1 mL of supernatant was then mixed with 4.9 mL of 0.1 mM DPPH, dissolved in 80% methanol, then shaken and sustained in the dark at normal conditions for 30 min; after that, the absorbance was measured at 517 nm. The AA was calculated as DPPH [%] = [(A0 − A1)/A0] × 100, where A0 and A1 are the absorbance of the reference and test solutions, respectively.

The APX analysis was conducted following the method of Nakano and Asada (1981) with some modification. A measure of 2 g of leaf sample was homogenised in 50 mM potassium phosphate buffer (pH 7.0) containing 1mM ethylene diamine tetra acetic acid (EDTA), 1% soluble polyvinyl pyrrolidone (PVPP), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 10 mM ascorbic acid (AsA). All extraction steps were carried out in ice at 4 °C. APX was measured according to the oxidation of ascorbate. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate and 0.1 mM hydrogen peroxide and 0.15 mL of the enzyme extract. The oxidation of AsA with hydrogen peroxide was measured continuously for 5 min by decreasing absorbance at 290 nm assuming an absorption coefficient of 2.8 mM · cm^{-1}. The APX activity was expressed as µg AsA · min^{-1} · g^{-1} of fresh weight.

The TPC was determined using the Folin–Ciocalteu method according to Djeridane et al. (2006). A measure of 2 g of the fresh sample was homogenised with 10 mL of 80% methanol in four portions (4 × 2.5 mL). The mixture was centrifuged (4,500 × *g*, 10 min, 4 °C). A total of 2 mL of 2% Na_2CO_3 was mixed with 0.1 mL of the supernatant. After 2 min, 0.1 mL diluted with water (distilled) Folin–Ciocalteu's reagent (1:1, v/v) was added, and the mixture was incubated at RT in the dark, for 45 min. The measurement was carried out using the UV-VIS Helios Beta spectrophotometer at 750 nm. The total phenolics content was calculated using the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE) per 1 g of fresh weight.

The GSH analysis was performed using the method by Guri (1983) with some modifications. A measure of 2 g of the fresh sample was homogenised with 10 mL of 0.5 mM EDTA and 1% trichloroacetic acid (TCA) in an ice bath (4 °C). After centrifugation (13,986 × *g*, 10 min, 4 °C), 2 mL of the supernatant was mixed with 5 mL of potassium phosphate buffer (pH = 7.0). Subsequently, 2 mL of the sample was taken and mixed with 1 mL of potassium phosphate buffer and Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid) in the amount of 0.1 mL. The measurement was carried out using the spectrometric method against the blank without Ellman's reagent at absorbance 412 nm and expressed as an equivalent of µg gluthatione per 1 g fresh mass (FM).

Statistical analysis

The experiment was performed in three repetitions for each variant of the substrate, and the data were presented as the mean supplemented with standard deviation. Differences in the biological parameters of the lettuce fresh leaves' biomass, number of leaves, stem diameter and leaf area, as well as the analytical parameters of the dry matter, nitrate content, TPC, AA, APX and GSH, were evaluated using an analysis of variance with Statistica 12 (TIBCO Software Inc., Palo Alto, USA). Significant differences were calculated using Scheffé's test at a significance level of $p \leq 0.05$, where different small letters denote significant differences. Furthermore, a multiple regression analysis was performed to evaluate the dependence of selected substances (StatSoft).

RESULTS

Content of elements in drain from substrates

The substrates can be ranked according to the total content of all measured elements in the drain of the substrate in descending order, starting from sheep wool and followed by coco peat, mineral wool, perlite and wood fibre. There was no statistical difference in the total elements content in the drains from perlite and from wood fibre. Looking at the individual elements, the content of all monitored elements was significantly higher in the drain from sheep wool (see Table 2). The most represented elements in general were potassium (12–2,996 mg · L^{-1}), sodium (5–58 mg · L^{-1}) and calcium (5–127 mg · L^{-1}). The least represented element in the drain was zinc (0–1.2 mg · L^{-1}) for each substrate. The order of individual elements in the drain of substrates was as follows:

Perlite K > Na > Ca > Fe > Mg > S > P > Zn
Mineral wool S > Na > Ca > K > Mg > Fe > P > Zn
Wood fibre K > Ca > Na > P > Mg > S > Fe > Zn
Sheep wool K > Ca > S > Na > Mg > P > Fe > Zn
Coco peat K > Na > Ca > Mg > S > P > Fe > Zn

Biological parameters of lettuce

All monitored biological parameters were significantly affected. After a statistical analysis using Scheffé's test ($p \leq 0.05$), the biomass and number of leaves, stem diameter and leaf area were the highest for lettuce that was grown in the coco peat substrate.

Leaf biomass

Lettuce grown in coco peat had the highest leaf biomass, with an average of 75 g per plant (see Figure 1). The lowest biomass was recorded for sheep wool and wood fibre substrates. Leaf biomass measured for perlite and mineral wool was significantly lower than that for coco

Table 2. Elements content in the drains from the substrates (mg · L^{-1}).

Element	Perlite	Mineral wool	Wood fibre	Sheep wool	Coco peat
P	0.4 ± 0.0 a	0.2 ± 0.0 a	2.6 ± 0.0 b	22.5 ± 0.5 d	4.3 ± 0.1 c
K	30.4 ± 1.1 c	12.1 ± 0.2 a	22.6 ± 0.7 b	2,995.9 ± 1.5 e	103.0 ± 3.0 d
S	0.9 ± 0.1 a	45.3 ± 1.8 c	1.6 ± 0.0 a	69.3 ± 1.4 d	10.8 ± 0.4 b
Ca	4.6 ± 0.5 a	14.6 ± 0.5 b	7.0 ± 0.5 a	126.6 ± 1.2 d	32.6 ± 1.4 c
Na	4.8 ± 0.3 a	43.9 ± 1.9 c	6.1 ± 0.2 a	57.5 ± 2.0 d	33.7 ± 0.8 b
Fe	1.7 ± 0.5 b	0.3 ± 0.1 a	0.2 ± 0.0 a	10.9 ± 0.2 d	4.2 ± 0.1 c
Mg	1.1 ± 0.2 a	4.8 ± 0.0 a	2.5 ± 0.1 a	30.1 ± 2.6 c	12.5 ± 0.2 b
Zn	0.0 ± 0.0 a	0.0 ± 0.0 a	0.1 ± 0.0 a	1.2 ± 0.1 c	0.2 ± 0.0 b
Sum	44.0 ± 0.8 a	121.2 ± 3.5 b	42.8 ± 1.8 a	3,314.0 ± 7.8 d	201.4 ± 5.4 c

The table shows the average values with standard deviation.
Different letters for individual values indicate significant differences at $p \leq 0.05$.

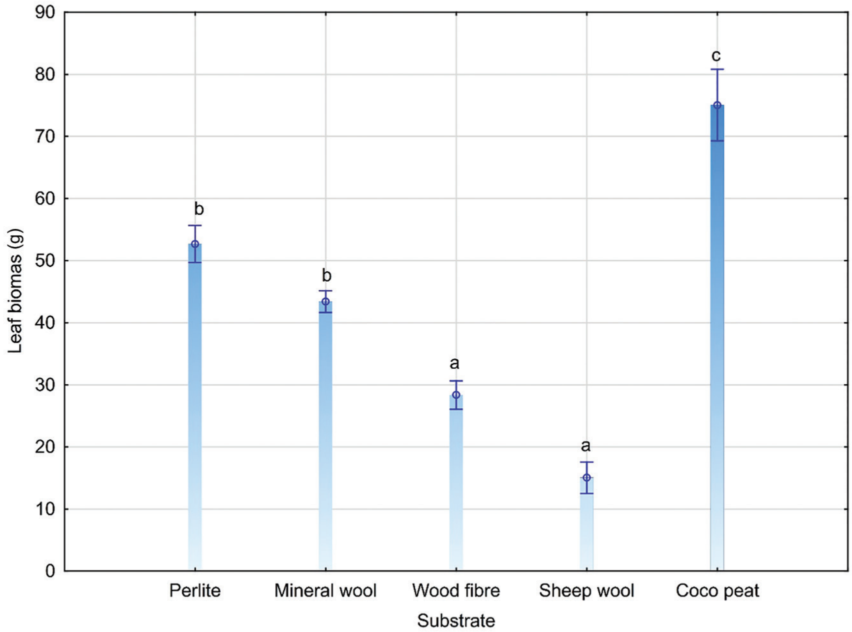

Figure 1. Leaf biomass (g). Different letters above the individual columns indicate significant differences at $p \leq 0.05$. Error bars represent standard deviation.

peat but significantly higher than that for sheep wool and wood fibre.

Number of leaves

In the number of leaves (Figure 2), there was a similar trend to the leaf biomass. The average number of leaves for lettuce grown in coco peat was 16.9, which was significantly higher than that for all substrates that were used in the experiment. The lowest number of leaves per plant was recorded for wood fibre and sheep wool substrates at 10 and 11.6, respectively.

Stem diameter

The largest diameter of the stem that was measured in the root neck was for lettuces from coco peat, with an average of 11.7 mm. No differences were found between the stem diameter of wood fibre and sheep wool and between that of perlite and mineral wool (see Figure 3). The smallest stem diameter was measured in sheep wool, with 7.3 mm on average.

Leaf area

The largest leaf area, as measured by using the non-destructive method focussing on whole heads of lettuce before harvest, was recorded for lettuce grown in coco peat, with an average leaf area of 836.2 cm^2. The smallest leaf area was measured for lettuce grown in sheep wool at only 268.7 cm^2 (Figure 4). Furthermore, a significant difference was found between the leaf area of lettuce grown in wood fibre and that grown in perlite. However, the leaf area of lettuce grown in mineral wool did not differ from that grown in wood fibre and perlite.

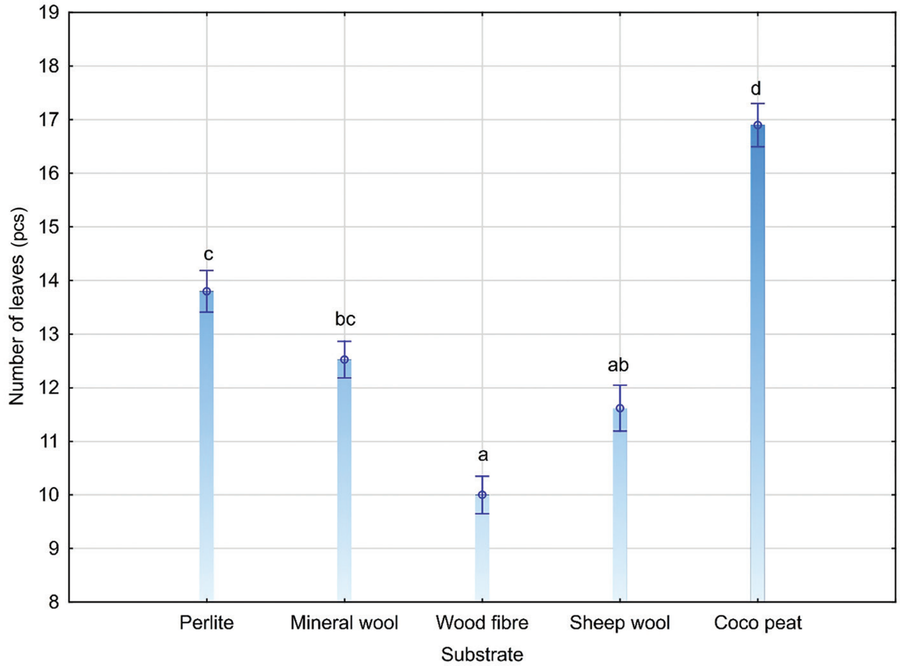

Figure 2. Number of leaves (pcs). Different letters above individual columns indicate significant differences at $p \leq 0.05$. Error bars represent standard deviation.

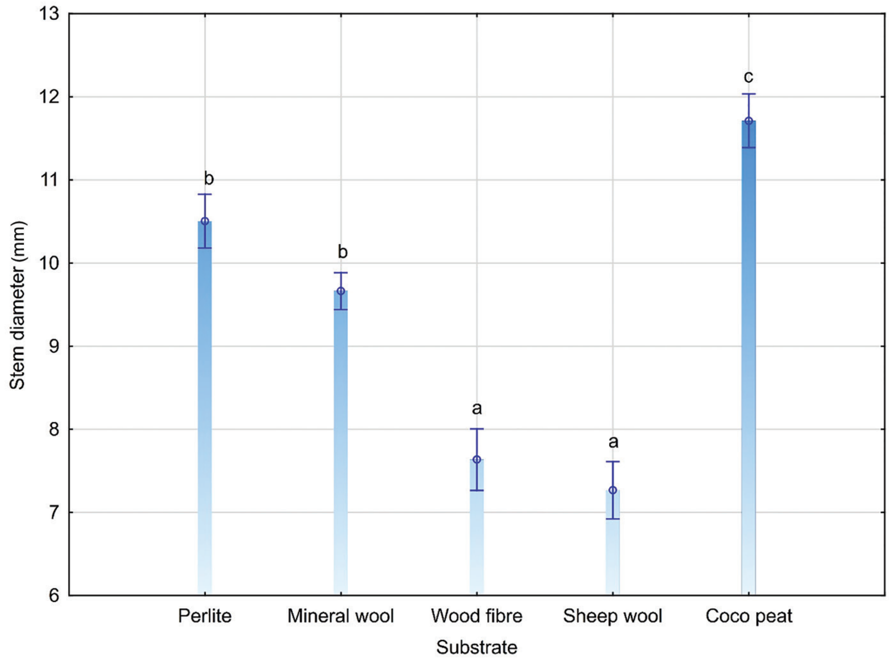

Figure 3. Stem diameter (mm). Different letters above individual columns indicate significant differences at $p \leq 0.05$. Error bars represent standard deviation.

Analytical parameters of lettuce

Significant differences were found in the parameters of dry matter, content of nitrates, TPC and AA, as shown in Table 3. Measurements of APX and GSH did not show significantly different values.

Antioxidant activity

Within the measurement of total AA expressed as % scavenging of DPPH, conclusive differences were found. The lowest DPPH scavenging activity was recorded in lettuce growing in perlite, and the highest was recorded

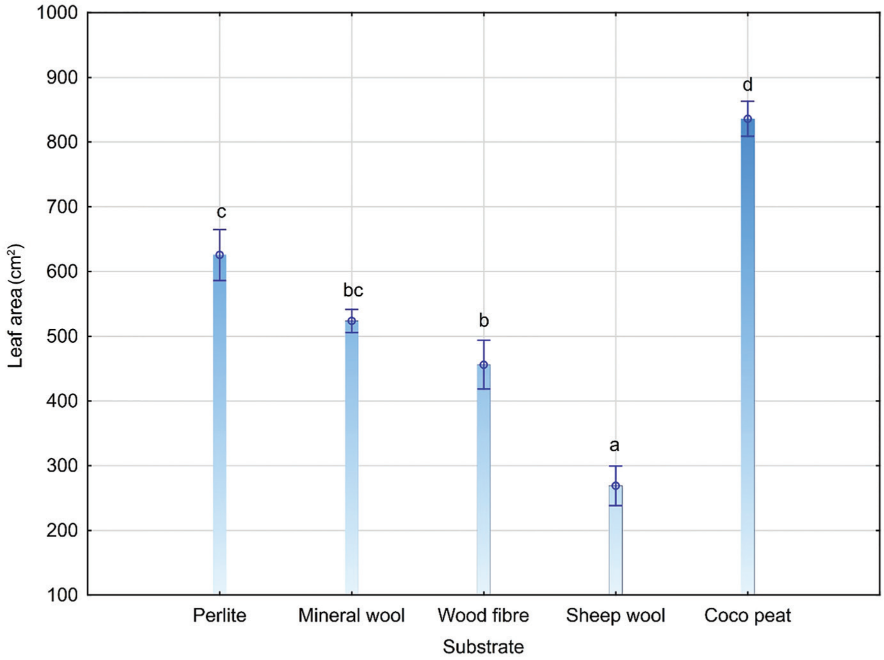

Figure 4. Leaf area of the entire head of lettuce (cm^2). Different letters above individual columns indicate significant differences at $p \leq 0.05$. Error bars represent standard deviation.

Table 3. Analytical parameters of lettuce. The table shows average values with standard deviation.

Substrate	AA (% scavenging of DPPH)	GSH ($mg \cdot g^{-1}$ DM)	APX (mg AsA $\cdot g^{-1}$ FM $\cdot min^{-1}$)	TPC (mg GAE $\cdot g^{-1}$ FM)	Nitrates ($mg \cdot kg^{-1}$ FM)
Perlite	21.6 ± 4.9 a	44.0 ± 19.7 a	108.8 ± 58.5 a	43.3 ± 0.9 a	604.2 ± 110.3 ab
Rockwool	32.6 ± 3.0 ab	22.5 ± 12.1 a	129.6 ± 25.6 a	40.6 ± 1.6 a	555.1 ± 58.1 ab
Wood fibre	46.1 ± 5.9 bc	31.0 ± 4.9 a	105.0 ± 32.2 a	55.4 ± 7.9 b	447.6 ± 38.2 a
Sheep wool	54.9 ± 11.5 c	23.1 ± 5.2 a	135.9 ± 24.5 a	74.0 ± 1.3 c	686.2 ± 86.5 b
Coco peat	40.3 ± 2.7 abc	19.9 ± 2.5 a	126.4 ± 25.5 a	48.0 ± 1.6 ab	552.6 ± 69.9 ab

Different letters for individual values indicate significant differences at $p \leq 0.05$.
AA, antioxidant activity; APX, ascorbate peroxidase; AsA, ascorbic acid; DM, dry mass; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FM, fresh mass; GAE, gallic acid equivalents; GSH, glutathione; TPC, total phenol content expressed as GAE.

in sheep wool. Significant differences were observed between these two substrates. The rest of the evaluated substrates did not differ.

Glutathione

When comparing the content of GSH in lettuce of all varieties, certain tendencies were observed, but the difference was not significant.

Ascorbate peroxidase activity

The average APX activity ranged between 105 and 136 mg AsA $\cdot$ min^{-1} $\cdot$ kg^{-1} of fresh weight. The activity of APX in lettuce grown in perlite and wood fibre was lower by 14%–23% than that grown in mineral wool, sheep wool and coco peat, but the differences were not significant.

Total phenol content

The content of total phenols, expressed as GAE, was significantly higher in the lettuce grown in sheep wool. The increase was 33%–82% compared with other substrates. Lettuce grown in perlite and mineral wool substrates showed a significantly lower TPC than lettuce grown in wood fibre but did not differ to lettuce from coco peat.

Nitrates

The level of nitrates varied between 448 and 686 mg $\cdot$ kg^{-1} of FM. The influence of the substrate on the content of nitrates in lettuce was confirmed; however, a significant difference was observed only between wood fibre and sheep wool, where sheep wool had the highest content of nitrates and wood fibre the lowest.

DISCUSSION

Substrate and biological parameter evaluation

When testing alternative substrates in a hydroponic flood system, significant differences were found in all biological parameters of lettuce, which was used as a model crop. From the perspective of the values of the biological parameters measured for salads, the individual substrates can be ranked from the lowest value of sheep wool, followed by wood fibre, mineral wool, perlite and coco peat. Several factors could have affected the observed results, the most important of which are the physical and chemical parameters of the substrates. Many authors have studied the substrate parameters that would provide plants with ideal conditions for growth. The finding was that a suitable substrate should meet the following requirements: bulk density ≤0.4 kg · L^{-1}, total pore space 75%–85%, air capacity 10%–30%, water-holding capacity 600–1,000 L · m^{-3}, EC 0.75–3.49 mS and pH 5.2–6.3 (de Boodt and Verdonck, 1972; Boertje, 1983; Abad et al., 1993; Abad and Noguera, 2000). From the substrate parameters listed in Table 1, it is obvious that not all substrates meet the given requirements. The significantly highest leaf biomass, number of leaves, stem diameter and leaf area were achieved in lettuce grown in coco fibre. Sarkar et al. (2021) cultivated *L. sativa* L. in mixtures of coco peat, sawdust and rice husk substrates, where one of the variants contained an equal amount of individual substrates 1:1:1, and in the other three, one component prevailed in a ratio of 3:1:1. From the results of their experiment, the coco peat-based medium appeared to be the best variant, while lettuce grown in sawdust showed lower fresh biomass values. The results confirm the findings of the present study as the fresh biomass of leaves in wood fibre was 63.3% lower than that observed for coco peat. Wiggins et al. (2020) reached different results when comparing substrates based on coco peat and material from wood. For the purposes of their study, they used a mixture of perlite and coco peat in a ratio of 30:70 and two variants of pine bark with compost in ratios of 70:30 and 90:10, respectively. In the two growing seasons, a higher fresh weight of plants was recorded for lettuce grown in the wood-based substrate. The authors explain this by saying that the coco peat substrate had a very small percentage of air space and a very high water-holding capacity. The air capacity of coco peat used in the present study was only 4.4%. However, it should be considered that in the present study, a single-component substrate was used, rather than a mix. Lettuce grown in wood fibre and sheep wool ranked the lowest in terms of leaf biomass, stem diameter and number of leaves. Only in the case of leaf area, statistically better results were recorded for lettuce grown in wood fibre. In the study by Allaire et al. (2004), organic substrates were tested and compared with mineral wool, including three wood materials (sawdust, wood shavings and bark). When growing tomatoes under hydroponic conditions in a greenhouse, the yield was reduced by 17.7% when grown in wooden materials. By comparison, in the present study, leaf biomass was reduced by 30.6% for wood fibre. The authors suggest that the tomatoes may have reacted to the phytotoxic substances present in the wood fibre. The phytotoxicity of the wooden substrate, specifically the pine tree and its effect on the growth of lettuce and tomatoes, is confirmed by a study by Gruda et al. (2009). A response to phytotoxicity can be expected in lettuce as it is a sensitive crop that is often used for phytotoxicity testing (Zhou et al., 2021). Naasz et al. (2009) studied the phytotoxicity of waste wood from seven tree species. They attributed growth limitations to poor wood biostability and the resulting competition between plants and microbes for access to oxygen. Another possible reason for the reduction of the leaf biomass of lettuce in the wood fibre substrate is given in the study by Gruda et al. (2000), in which it was found that wood fibre can immobilise 100–350 mg (200 mg on average) of nitrogen per litre of substrate. They attribute the immobilisation to an increase in microbial activity, which was recorded in the substrate. In conclusion, the authors propose a partial reduction of the given problem by using impregnated wood fibres and increasing the doses of nitrogen during fertilisation. The lowest values of biological parameters were recorded for lettuce grown in sheep wool. Looking at the physical and chemical parameters of the substrate (Table 1), it can be observed that several parameters do not correspond to the ideal physical and chemical parameters described at the beginning of this article. The water-holding capacity for sheep wool was only 198 L · m^{-2}, which could indicate that lettuce did not have an optimal supply of water and nutrients and thus could not thrive. However, this will not be the only reason because the perlite substrate with a water-holding capacity of 300 L · m^{-2} did not result in such a vigorous reduction in leaf biomass. This is likely due to the high EC that was measured in sheep wool. Raw sheep wool naturally contains a large amount of lanolin, plant residues and other exogenic contaminants (Aitken et al., 1994; Hawkins and Ragnarsdottir, 2009). Böhme et al. (2012) used sheep wool as a fertiliser for vegetables and flowers. In their study, they focussed on the chemical properties of sheep wool and obtained EC 6.3–8.8 mS and pH 7.5–9. Since the values were too high, they had to be reduced by washing before and during the experiment. Their EC and pH values correspond to the values of the mentioned chemical parameters for sheep wool used in the present study, namely EC 6.24 mS and pH 7.97. In the study on the effect of salinity on plant stress, Kurunc (2021) found that EC values above 2.17 mS have a negative effect on the fresh yield of lettuce. According to the results, EC 6 mS can reduce the yield by approximately 40%. According to the values of leaf biomass from the present study, it follows that the reduction was 65% and 70% compared to mineral wool and perlite, respectively. The results of the analysis of elements in the drain from substrates (Table 2) show that sheep wool contained the most soluble elements of all substrates. Potassium had the highest content of all monitored elements in the drain (3,000 mg · L^{-1}). Aitken et al. (1994) also confirmed the high pH of greasy sheep wool, which reached values of 8.15 in their experiment, and a high potassium content was detected at the level of 6,090 mg · kg^{-1}. Dannehl et al. (2015) found a very high potassium content in sheep wool compared to that in other assessed substrates, that is, 54.09 g · kg^{-1} of DW. However, a high potassium content in sheep wool is not obligatory. In the study by Patkowska-Sokoła et al. (2009), the concentration of potassium ranged from 643 mg · kg^{-1} to 755 mg · kg^{-1}. Abad et al. (1993) recommended values of available potassium in the substrate of only 150–249 mg · kg^{-1} for greenhouse crops. Conversely, when testing salinity with potassium salts, Ucar et al. (2007) concluded that the yield of hydroponically grown lettuce increased, while among the experimental variants, lettuce with the highest EC (6 mS) had the best effect. However, the concentration of salts in sheep wool used in the present study consisted of many other elements, so the influence of potassium alone cannot be taken as the only reason for the reduction of leaf biomass and other biological parameters. Therefore, a combination of salinity and lack of water is probably the main reason.

Stress evaluation

The stress response of plants is generally due to increased AA (Oh et al., 2010; Malejane et al., 2017; Paim et al., 2020). The response of plants to drought stress is to reduce the rate of photosynthesis because it is not possible to process all the captured light (Chatterjee and Solankey, 2014). This is caused by the increased production of reactive oxygen species (ROS), which destroys chloroplasts, thus reducing carboxylation. The main sites of action of ROS are chloroplasts, mitochondria and peroxisomes. Carboxylation is also limited by a smaller leaf area (Bahadur et al., 2011). The defence reaction to the increased amount of ROS is the production of antioxidants, such as peroxidases, catalases, APX, GSH reductases and others (Mittler, 2002). The effect of excessive salinity on plant growth has two phases. In the first phase, when vacuoles are filled with Na^+ and Cl^-, the growth of leaves and roots is only partially affected, while in the second phase, when vacuoles are completely filled, the concentration of ions in cytoplasm increases, and the action of enzymes is limited (Munns, 2005; Parihar et al., 2015). Salinity stress on lettuce can manifest itself in a higher total content of phenols, anthocyanin and proline concentrations (Neocleous et al., 2014; Flores et al., 2022). GSH is concentrated in chloroplasts, cytosol, mitochondria, peroxisome and apoplasts. It occurs under the action of toxic substances or oxidative stress mainly caused by H_2O_2 (Meister and Anderson, 1983; Noctor and Foyer, 1998). GSH in the plant body can be divided into the following two categories: sulphur metabolism and a defence response against stress (Noctor and Foyer, 1998). If we consider the AA (% scavenging of DPPH) and compare it with the content of GSH (both in Table 3), it can be concluded that GSH did not have to play a major role in the stress response of lettuce to drought or salinity. Multiple regression analysis between the content of GSH and AA (% scavenging of DPPH) cannot be verified since the correlation was not statistically significant (p = 0.243). APXs are a group of enzymes that catalyse the reduction of H_2O_2 with the help of a donor of electrons (AsA). APX-driven H_2O_2 limitation, as a stress-signalling molecule, can control and influence the strength of the stress response (Maruta and Ishikawa, 2017). Like GSH, it is found in chloroplasts, cytosol, mitochondria, peroxisome and apoplasts (Noctor and Foyer, 1998). In lettuce grown in the present study, APX activity was measured between 105 μg AsA · g^{-1} FM · min^{-1} (wood fibre) and 135 μg AsA · g^{-1} FM · min^{-1} (sheep wool), and no statistically significant difference was found. From this, it can be concluded that APX activity was not a significant indicator of the response to the stress situation in lettuce caused by insufficient physical or chemical parameters of the substrates because the correlation between APX and AA was not statistically significant (p = 0.741). However, it follows from previous considerations that the reduction in leaf biomass could have been caused by the low water-holding capacity in sheep wool. This would be confirmed by the results of the study by Broñola-Hipol and Dionisio-Sese (2020), when drought stress induced by low water conditions led to an increase in APX activity by 78%–94%. However, this phenomenon did not occur in the present study. The last monitored indicator of the stress response is phenolic compounds. They are secondary plant metabolites that ensure defence reactions against bacteria, fungi and viruses, but they mainly defend against stress reactions caused by drought and salinity. The group of phenolic substances includes many classes (simple phenols, phenolic acids, aldehydes, acetophenones, etc.) and individual compounds (hydroxycinnamic, chicoric, chlorogenic, caffeic, caftaric acids, etc.) (Malejane et al., 2017; Kumar et al., 2020). Significant differences were recorded in the TPC for lettuce grown in each substrate. The highest TPC was in lettuce grown in sheep wool (74 mg GAE · g^{-1} FM), and at the same time, the highest AA (55%) and lowest values of all biological parameters were found in these lettuce. After considering the physical parameters of the sheep wool substrate, including the too-high air capacity (77%) and low water-holding capacity (198 L · m^{-3}), it could be deduced that the increased TPC was caused by drought stress. Oh et al. (2010) studied water-deficit stress in lettuce, which resulted in an increase in the TPC and an increase in antioxidant capacity. The increase in the content of phenolic substances in sheep wool could indicate drought stress, as shown by the lower water-holding capacity in sheep wool. Malejane et al. (2017) studied the response of leafy lettuce to stress induced by water deficit. Their findings show that some varieties of lettuce are more resistant to water stress, provide

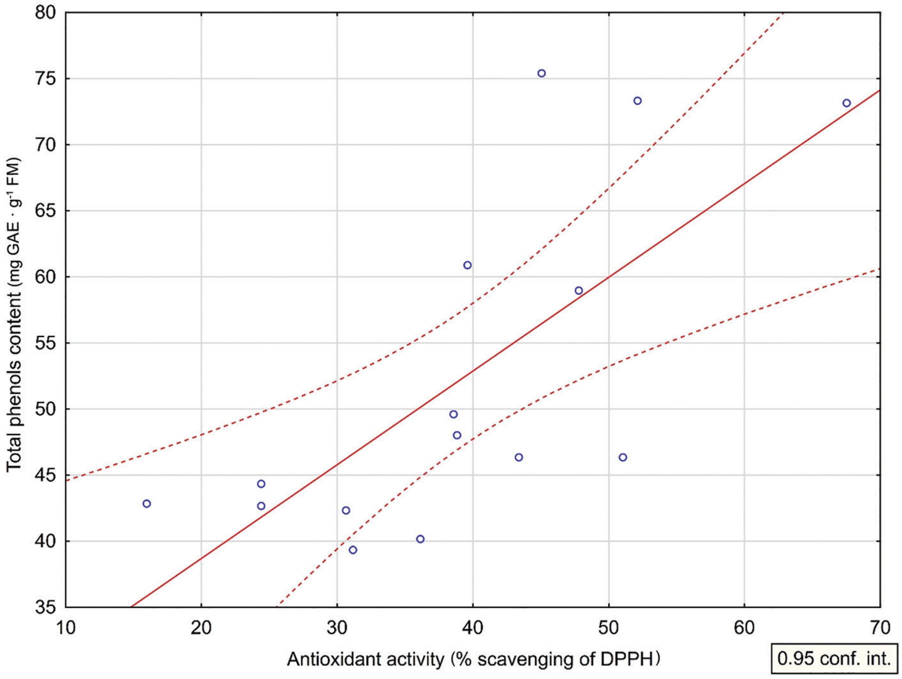

Figure 5. Graph of multiple regression analysis between AA (% scavenging of DPPH) and content of TPC ($p = 0.002449$). AA, antioxidant activity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FM, fresh mass; TPC, total phenol content.

a stabilised yield and improve internal quality in the form of phytochemicals with health benefits. Their results also show that increasing the water deficit leads to an increase in TPC. In their study, Broñola-Hipol and Dionisio-Sese (2020) confirmed the same trend in the TPC increase induced by drought stress in two of the three varieties that were tested. Liu et al. (2007) investigated the relationship between the TPC and DPPH radical scavenging activity of 25 varieties of lettuce. When grown under conditions causing heat stress, a higher antioxidant capacity was recorded than during the second harvest in the cold season. However, the TPC was not significantly affected. This shows that there were other AA-enhancing antioxidants in lettuce. The authors confirmed this finding using a linear correlation model, where R^2 was only 0.27. After performing the same statistical analysis in the present study, it was found that the TPC had the greatest effect on AA among the measured antioxidants. The coefficient of determination in this case was $R^2 = 0.52$ (Figure 5). The different finding in terms of the effect of antioxidants on the total antioxidant capacity may be related to a different cause of the stress response. The second highest TPC was measured for wood fibre, which is matched by the AA level. In terms of the physical and chemical parameters, wood fibre meets the requirements for an ideal substrate (see previous text). The origin of the stress reaction could therefore be found in the phytotoxicity of the wooden substrate.

Nitrate content

The nitrate content in lettuce ranged between 426 and 686 mg · kg^{-1} of FM and therefore did not exceed the limits set by commission regulation (EU) No. 1258/2011 for any substrate, where the nitrate limit is set at 4,000 mg · kg^{-1} of fresh weight. Lettuce grown in wood fibre showed the lowest nitrate values, but the significant difference was only comparable to lettuce from sheep wool. Regarding the nitrate content, none of the assessed substrates can be considered problematic at the fertilisation level of 1.5–2 mS in the present study. Kaniszewski and Sabat (2015) observed the effect of the fertilisation level on lettuce yield using different substrates. Their findings show that the higher the EC of the nutrient solution, the higher the nitrate content. The nitrate content found in the present study (see Table 3) was statistically the highest in lettuce grown in sheep wool. This substrate also had the highest EC (6.24 mS). Unfortunately, the nitrogen content in the substrate was not investigated in the present study, but other sources state that raw sheep wool is its rich source (Böhme et al., 2012; Dannehl et al., 2015; Pina et al., 2021). This could be the reason the highest nitrate content in sheep wool. In the aforementioned study by Kaniszewski and Sabat (2015), the nitrate content in the rockwool and coir substrates was compared. The authors concluded that lettuce grown in coir had a higher nitrate content in both growing seasons. The results of the present study do not confirm this trend because there was no difference in the nitrate content for these two substrates.

CONCLUSION

The results of this study showed that only coco peat can fully compete with the common substrates mineral wool and perlite in terms of biological parameters. Regarding the lettuce grown in coco peat, leaf biomass was 40% and

70% higher, respectively, than that grown in perlite and mineral wool. The leaf biomass of lettuce grown in wood fibre showed a reduction of 48% and 34% compared to that grown in perlite and mineral wool. For lettuce grown in sheep wool, the reduction was even higher at 73% than lettuce grown inperlite and 66% than lettuce grown in mineral wool. The deteriorated results of the biological parameters can be explained by the stress response of lettuce, which was confirmed by the increased AA (% scavenging of DPPH). Three stress–response indicators were monitored: GSH, APX and TPC. After the statistical analysis, it became clear that the main response to a stressful situation (increased AA) caused either by a lack of water due to the physical parameters of the substrates or by salinity stress due to high salinity was an increase in the production of phenolic substances ($R^2 = 0.52$). When investigating the role of GSH and APX on the stress reaction, no significant effect was found ($p = 0.243$ and $p = 0.741$, respectively). However, these results show that other stress enzymes, which were not analysed, must have been part of the reaction. The analysis of elements in the drain of substrates showed that sheep wool can be a good source of nutrients. However, it is necessary to consider the level of EC since high EC could partially cause the stress reaction of lettuce grown in sheep wool. Another question is the form of nutrients in sheep wool and whether they are available to plants or consist of complex compounds that are not pervious to plant roots. Sheep wool generally contains various amounts of additives (lanolin, plant residues and other exogenic contaminants), and its quality depends on many factors (Holman and Malau-Aduli, 2012). For these reasons, to achieve uniformity, it would be necessary to adjust it by cleaning and washing to achieve similar parameters from different wool sources. In general, it is necessary to focus on the physical and chemical properties of the substrates, as well as their stability and the uniformity of all parameters.

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AUTHOR CONTRIBUTIONS

V.F. contributed to methodology, validation, formal analysis, investigation, data curation and original draft preparation. T.K. assisted with conceptualisation, methodology, validation, data curation, review and editing and supervision. M.K. helped with methodology, formal analysis and data curation. M.F. contributed to methodology, formal analysis and data curation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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