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Soilless Tomato Production: Effects of Hemp Fiber and Rock Wool Growing Media on Yield, Secondary Metabolites, Substrate Characteristics and Greenhouse Gas Emissions

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Abstract: Replacement of rock wool by organic substrates is considered to reduce the environmental impact, e.g., through energy savings during production and waste prevention, caused by hydroponically produced crops. A suitable substrate for plant production is characterized by an optimal composition of air- and water-filled pores. In our study, we used hemp fibers as an organic alternative to rock wool in order to cultivate tomato plants in hydroponics for 36 weeks. The leaf area, plant length, and yields, as well as the quality of fruits including soluble solid contents, dry weight content, mineral composition, and contents of phenolic compounds caused by both substrates, were similar. Carotenoids were significantly increased in fruits from plants grown in hemp at some measuring dates. Nevertheless, higher emission rates of greenhouse gases such as N_2O, CO_2, and CH_4 caused by hemp fiber compared to those emitted by rock wool during use are rather disadvantageous for the environment. While hemp proved to be a suitable substrate in terms of some physical properties (total pore volume, bulk density), a lower volume of air and easily available water as well as very rapid microbial decomposition and the associated high nitrogen immobilization must be considered as disadvantages.

Keywords: greenhouse gases; greenhouse; organic substrates; carotenoids; phenolic compounds; carbon dioxide; nitrous oxide; methane; N_2O; CH_4

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1. Introduction

The world population will grow from 7.3 billion to 9.6 billion people by 2050 [1], which will reduce the amount of land available for growing food on a global scale. This will be accompanied by increasing urbanisation (66% of people will live in large cities [2]). Generally, population growth also increases total anthropogenic greenhouse gas (GHG) emissions, of which 23% currently derive from the agricultural sector [3]. In the future, however, the share of GHG emissions in the agricultural sector will continue to shift towards protected greenhouse production. Precise climate control can reduce plant diseases and improve the quality of products, which has led to an increase in greenhouse area, where greenhouses are mostly defined as permanent structures, excluding tunnels, row covers, etc. These greenhouse structures are common in industrialized countries and cover an area of 497,815 ha to produce vegetables worldwide [4]. Unfortunately, it is not possible to estimate the greenhouse area used in China due to the definition of greenhouses. It was reported that there the total greenhouse area covered with plastic films, including covered open vegetation fields, was 2.5 million hectares in 2004 [5].

From an energy point of view, greenhouse production caused very high CO_2 emissions caused by the heating energy required for plants in many places [6–8]. It is estimated that

a temperature increase by heating of only one degree Celsius raises CO_2 emissions by 100 t ha^{-1} [9]. In addition, there are non-negligible CO_2 emissions caused by energy input during the production of growing media [7]. In intensive hydroponic vegetable production, huge amounts of substrate residue are produced at the end of a production year. Rock wool, for instance, is the preferred growing medium of many horticultural producers [10–13]. Up to 150 m^3 of substrate residue is produced per hectare of tomato production per year [14]. This would amount to 113,700 m^3 of substrate residue from rock wool that must be disposed of in landfills every year if rock wool were to be considered on the entire acreage of tomatoes, cucumbers, and peppers (758 ha) [15] grown under glass or other walkable protective covers in Germany in 2021. In addition, the production of one cubic metre of rock wool requires an average primary energy demand of 275 kWh, with 167 kg of CO_2 being released into the environment [16].

Although rock wool is almost a perfect growing medium for hydroponic systems, alternative environmentally friendly organic substrates must be found to close the circular economy and reduce environmental impact. In this context, organic substrates used for hydroponic systems should have optimal physical properties in terms of total pore space (>85 vol%), air volume (10 to 30 vol%), bulk density (<0.4 g cm^{-3}), easily available water (20 to 30 vol%), and water buffer capacity (4 to 10 vol%) as described by Abad and Noguera [17], Abad, et al. [18], De Boodt and Verdonck [19], Boertje [20], and Jenkins and Jarrell [21]. Maintaining these optimal physical properties when using organic substrates over a very long period up to 330 days, especially for intensive tomato cultivation in hydroponics, is challenging because organic substrates can degrade due to high microbial activity [22]. On one hand, this means that the additional carbon input, in combination with abundant mineral nitrogen applications through the nutrient solution, can promote the growth of denitrifying bacteria [23]. This, in turn, means that high nitrous oxide (N_2O) emissions from denitrification are generated, e.g., when using organic growing bags, because nitrogen is typically supplied in the form of nitrate in hydroponic systems [24]. This effect can vary between different organic substrates because denitrification processes strongly depend on the quality of the C source [25] and can be additionally fueled by reduced oxygen (O_2) concentrations [26]. Under strictly anaerobic conditions, methane (CH_4) emissions from organic substrates are also possible [27], but are typically not relevant in hydroponics [24]. On the other hand, high decomposition processes can also negatively affect water and nutrient supply, as well as plant stability in the root zone, which in turn can have a negative impact on yields, dry matter, and fruit quality characteristics, e.g., soluble solid contents (SSC) [28]. Based on these facts, many organic substrates are tested for their suitability as growing media for hydroponic vegetable production. The use of coconut fiber, bark, or rice husk, for example, did not induce changes in growth, yield and SSC in tomato plants compared to those grown in rock wool [13]. In contrast, SSC in tomatoes could be increased by using almond shells as growing media, while the tomato yields did not differ when this substrate was compared to rock wool [29]. Other research groups combined organic with inorganic or different organic substrates and obtained equal tomato yields when compared to tomato plants exposed to rock wool. Among others, the following mixtures were used: peat and composted bark (66.6%:33.4%, *v*/*v*), sepiolite and leonardite (97%:3%, *v*/*v*), sieved pumice and peat-lite (85%:15%, v/v), sepiolite and perlite (80%:20%, *v*/*v*), as well as perlite and peat (85%:15%, *v*/*v*)] [22,30,31]. Some wood-based substrates, although not all, also seem to be promising alternatives to rockwool. White spruce and fir bark (40%:60%, *v*/*v*) showed high potential for greenhouse tomato production, whereas tomato yields produced with the aid of substrates consisting of fresh white spruce and fir sawdust (40%:60%, *v*/*v*) or white spruce and fir shavings (40%:60%, *v*/*v*) were lower than those achieved with rock wool [22]. This might be caused by phenols located in bark, which can have a phytotoxic effect [32].

Based on this brief overview of possible advantages and disadvantages of using organic substrates in hydroponic systems, it becomes clear that not all organic substrates can be used for intensive vegetable production and therefore further alternatives to rock

wool must be sought. Studies on the effects of different growth media on greenhouse gas emissions hardly exist. Furthermore, there is a deficit in studies on the synthesis of secondary metabolites depending on different substrates used as growth media in hydroponic systems. Therefore, the present study is focused on the evaluation of hemp fiber bags to be used as a substitute substrate for rock wool in intensive tomato production. The main objectives of this research were to analyze the physical properties of the used renewable hemp fiber bags compared to rock wool to discern the differences in water-retention curves and possible disadvantages of using hemp fibers. Since the mineralization of hemp fibers can lead to fixation of nitrogen in microbial biomass that may not be available to the plants, nitrogen immobilization was investigated. Due to our hypothesis that organic substrates produce greenhouse gas emissions during vegetable production through their decomposition, we assessed how the degradation of hemp fibers by bacteria in hydroponic tomato cultivation affects the direct N_2O-, CO_2-, and CH_4-emissions using gas flux measurements. In addition to these study parameters, leaf area and yield development as well as mineral composition in leaves and fruits were investigated. The latter characteristics should provide information on whether nitrogen immobilization in organic substrates leads to nutrient supply bottlenecks in the plants. Based on the knowledge gap mentioned above, SSC, dry matter, carotenoids, flavonoids, and phenolic acids in tomato fruits were analyzed under consideration of the growing media used. We hypothesize that greenhouse gas emissions could cause a change in secondary metabolites in tomatoes.

2. Materials and Methods

2.1. Cultivation of Tomato Plants and Assessment of Crop Growth and Yield

Experiments were conducted in a Venlo-type greenhouse at Humboldt-Universität zu Berlin, Germany (Latitude 52° 46′ 74″, Longitude 13° 31′ 16″) from calendar week (CW) 21–47 in 2020. Hemp fiber was tested for its suitability as growing medium in bags, which were provided by Klasmann-Deilmann GmbH, (Geeste, Germany). Rock wool bags (Cutilene®; Tilburg, The Netherlands) were used as a control since rock wool is an established substrate. Tomato seedlings (*Solanum lycopersicum* L. cv. Avalantino F1) with two shoots were grown in small rock wool cubes (100 mm × 100 mm × 65 mm) and supplied by Jungpflanzen Gernert GbR (Albertshofen, Germany). Tomato transplants in small rock wool cubes were transferred to the growing bags on 13th March 2020, when four leaves were formed. Tomato plants were cultivated on high gullies, each gully equipped with 20 growing bags, and each bag planted with two plants, resulting in a distance between plants of 0.5 m. The plant experiment was conducted with three replicates, randomly selecting three gullies with rock wool bags and three gullies with hemp fiber bags. Two additional outer gutters were planted with tomatoes to ensure equal light conditions of the substrate variants.

A hydroponic system with a recirculating nutrient solution was used for a local drip irrigation that delivered a nutrient solution for 150 s, which started mainly after a light summation of 560 W m^{-2}. To obtain a water overflow of 20% after each irrigation cycle, the light summation for controlling the irrigation was regularly adjusted. Stock solution according to the recipe of Göhler and Molitor [33] was mixed with fresh water up to desired EC values and adjusted to pH 6 using phosphoric acid to obtain the nutrient solution for irrigation. The nutrient solution tank in the closed irrigation system was automatically refilled with the desired nutrient solution several times per day. Energy screens were closed at a global radiation of less than 3 W m^{-2}, in order to save energy. The floor level heating was set at 17 °C for day and night and the ventilation was opened above 23 °C to reduce the temperature inside the greenhouse. These processes were controlled using the application of proportional integral differences. CO_2 enrichment was applied and kept at a level of 800 ppm during daylight hours. When the ventilation opening of the greenhouse exceeded 10%, the CO_2 supply stopped to avoid too much loss of CO_2 into the atmosphere. Set points for cooling, heating, ventilation, and the CO_2 enrichment mentioned before were controlled

by data obtained from different sensors evenly distributed in the canopy. Measurements were forwarded to a central computer and recorded every 30 s.

Leaf number and the leaf area (LA) per plant was documented during the first six weeks after planting from three randomly selected plants per substrate. The number of leaves was noted and the leaf length (L) and width (W) of each leaf was measured with a folding ruler. The measurements were inserted in the commonly used equation A = a + b × (L × W) with a = −61.70 and b = 0.35 to estimate the LA of each individual leaf non-destructively [34]. The calculated values were added up to obtain the LA per plant, which was expressed as m^2 $plant^{-1}$.

Yields were determined by weekly harvesting ripe tomatoes corresponding to ripening stage 10 (according to the Organisation for Economic Co-operation and Development, OECD colour gauge). Yields from every week were summed to calculate the total yield per plant in kg $plant^{-1}$.

At the end of the experiments on 19 November 2020, the plant height of five plants per growing medium was measured after the stem of the plant was cut directly above the small rock wool cubes.

2.2. Analysis of Substrate Characteristics Using Water Retention Curves (pF-Curves)

Different physical parameters such as total pore space (TPS), air volume (AV), bulk density (BD), and easily available water (EAW) were examined for hemp fiber and rock wool. The respective substrate was filled into metal rings with a volume of 100 cm^3 and completely saturated with water. These prepared cylinders were placed on a ceramic pressure plate connected to a manometer. By increasing negative pressure values (pF values), different pore sizes of the previously water-saturated soil sample (pF 0) were drained. Released water volumes extracted at each pressure level (in our case pF 1.0 and pF 1.8) correspond to the pore water volume of a given pore size range. In this way, the water volume fractions (volumetric water content; θ_V (Equation (1))) of different substrate pore sizes, and thus their percentages in the soil could be determined. The density of the water was assumed to be 1 mg cm^{-3}.

$$\theta_V\ [\text{vol\%}] = \theta_g \left[\text{g g}^{-1}\right] \times \text{BD}\ \left[\text{g cm}^{-3}\right] \times 100 \quad (1)$$

The gravimetric water content (θ_g) is given in g g^{-1} and is the amount of water in gram at each suction point per g substrate (Equation (2)).

$$\theta_g\ \left[\text{g g}^{-1}\right] = \frac{m_{H_2O}\ [\text{g}]}{m_{\text{substrate}}\ [\text{g}]} \quad (2)$$

Bulk density indicates the dry mass of the substrate per 100 cm^3 (Equation (3)).

$$\text{BD}\ \left[\text{g cm}^{-3}\right] = \frac{m_{\text{substrate}}\ [\text{g}]}{100\ \text{cm}^{-3}} \quad (3)$$

According to De Boodt and Verdonck [19] moisture content at zero suction (pf 0) is defined as TPS stated in vol% and is the product of gravimetric water content (θ_g) and the BD (Equation (4)).

$$\text{TPS}\ [\text{vol\%}] = \theta_{g\ (\text{pf0})}\ \left[\text{g g}^{-1}\right] \times \text{BD}\ \left[\text{g cm}^{-3}\right] \times 100 \quad (4)$$

The air volume is the difference of the gravimetric water content at pF 0 and pF 1 (Equation (5)). The easily available water is the difference of the gravimetric water content at pF 1 and pF 1.8 (Equation (6)).

$$\text{AV}\ [\text{vol\%}] = \theta_{g(\text{pf0})}\ \left[\text{g g}^{-1}\right] - \theta_{g(\text{pf1})}\ \left[\text{g g}^{-1}\right] \quad (5)$$

$$\text{EAW } [\text{vol\%}] = \theta_{g(pf1)} \left[\text{g g}^{-1}\right] - \theta_{g(pf1.8)} \left[\text{g g}^{-1}\right] \quad (6)$$

All physical parameters were determined in five replicates per substrate, one time before usage in hydroponic cultivation of tomatoes and one time after the cultivation period (CW 21–47 2020, 26 weeks).

2.3. Determination of Substrate Decomposition during Cultivation Period

Decomposition of the organic hemp material could result in substantial mass loss in the growing bags, thus decreasing stand stability for the plants cultivated. To determine the amount of decomposed material, growing bags were weighed in their unused condition and after usage for 16 weeks in tomato cultivation, including roots grown into the materials. The used growing bags were weighed after drying in a ventilated oven for 10 days. Differences in the weights of used and unused growing bags correspond to the decomposed amount of hemp including root biomass, or in the case of rock wool, to the root biomass grown in the rock wool.

2.4. N-Immobilization

Since it was expected that organic substrates would be mineralized during cultivation and that this could lead to the fixation of nutrients in microbial biomass, nitrogen immobilization was determined. This was necessary so that the nutrient application in the hydroponic system can be adapted to the plants' needs. The determination of the nitrogen immobilization of the substrates was carried out according to VDLUFA [35]. Sample material was mixed with a defined amount of ammonium nitrate and incubated over a period of 20 days at constant temperature and humidity. At the end of the incubation period, the contents of ammonium and nitrate nitrogen were determined separately, thus establishing the quantities in which these N compounds are released or fixed. The results are expressed as mg dm^{-3}.

2.5. Analysis of Greenhouse Gases Released by Growing Media

To evaluate the potential for substrate-related GHG emissions, three growing bags each of rock wool and hemp fiber were incubated on a greenhouse gutter with nutrient solution supplied via drippers starting in September 2020. After six weeks, the first gas flux measurement took place on 15 October 2020 and was followed by two more measurements on 9 November and 1 December 2020. For measuring the gas fluxes, the closed chamber method as described by Karlowsky, et al. [24] was used and modified to determine GHG emissions from unplanted growing bags. Briefly, acrylic glass chambers were fitted around the substrate bags and sealed with foam rubber to obtain a closed headspace on top of the growing bags with a volume of approximately 16 L. Over a period of one hour after closing, four gas samples were taken in 20 min intervals with a syringe through a sampling port on top of the chamber. The gas samples were analyzed on the same day by a gas chromatograph (GC 2010 Plus, Shimadzu Corporation, Kyoto, Japan) equipped with an electron capture detector (ECD) for N_2O, a thermal conductivity detector (TCD) for CO_2, and a flame ionization detector (FID) for CH_4.

2.6. Sample Preparation for Chemical Analyses and Determination of Dry Matter and Soluble Solid Content

Over a period of 24 weeks (11 June to 25 November 2020), fruits from 15 different plants per growing medium were harvested at intervals of three weeks and divided into three pooled samples of five tomatoes each. Only the top two ripe fruits of a panicle and panicles of the same age were considered. Each tomato was quartered. One quarter of five fruits were combined into one sample and immediately frozen in liquid nitrogen and then freeze-dried (Christ Alpha 1–4, Christ; Osterode, Germany) for seven days for analysis of secondary metabolites.

The second quarters of the same five fruits were used to determine dry mass of tomato fruits using a ventilated oven (Heraeus, Hanau, Germany) at 60 °C for seven days. The

fruit's dry matter content was calculated by the ratio of the dry mass to the fresh mass and is expressed as a percentage.

The two remaining quarters per fruit were used fresh to determine the soluble solid content (SSC). Firstly, the quarters of fresh tomatoes were mixed (KenwoodHB856, De'Longhi Deutschland GmbH; Neu-Isenburg, Germany) to obtain a homogenous starting material. Aliquots of the resulting liquid were transferred into centrifuge tubes and centrifuged for five minutes at 5000 rpm to remove coarse components and receive a clear solution for analysis. SSC was analyzed using a digital refractometer (PR101, ATAGO; Karlsruhe, Germany) according to manufacturer's protocol, which detects reducing sugars and other soluble solids. The results obtained for SSC are expressed as grams SSC per 100 g FW.

2.7. Analysis of Phenolic Compounds

To analyze phenolic acids and flavonoids, freeze-dried tomato fruits were ground to a fine powder (MM 30, Retsch GmbH, Haan, Germany) and stored at −80 °C until analysis. Extraction and determination of phenolic acids and flavonoids was performed as described by Förster, et al. [36].For analysis, an HPLC (Ultimate 3000, Thermo Scientific, Dionex Softtron GmbH, Germering, Germany) equipped with a 150 × 2.1 mm C16 column (AcclaimPA, 3 µm, Thermo Scientific, Dionex Softtron GmbH, Germering, Germany) was used. Commercially available standards from Sigma-Aldrich of single compounds were utilized as references.

Peak areas of detected phenolic acids and flavonoids were used for calculating contents of each phenolic acid/flavonoid and further summed to total phenolic acid/flavonoid content in tomato fruits in mg g^{-1} DM.

2.8. Analysis of Carotenoids

Extraction of carotenoids was performed as described by Mageney, et al. [37] with slight adjustments. 10 mg of freeze-dried powdered plant material was weighed and shaken with 500 µL of MeOH-THF solution (1:1, *v*/*v*; extraction solution) for 5 min at 24 °C and 500 rpm. After centrifugation at 20 °C and 4500 rpm for five min, the supernatant was transferred to a glass vial and the pellet was re-extracted two more times with 500 µL of extraction solution. The collected extracts were evaporated under nitrogen flow. The obtained pellet was dissolved in 100 µL dichloromethane and 300 µL isopropyl alcohol and filtered through Corning® Costar® Spin-X® centrifuge tube filters (Merck KGaA, Darmstadt, Germany) by centrifugation at 3000 rpm for five min. At the end, the filtered extracts were transferred into dark HPLC vials with inlay. For analysis, 10 µL were injected and separated at a flow rate of 0.2 mL min^{-1} using an Ultimate 3000 HPLC system (Thermo Scientific, Waltham, MA, USA) equipped with a carotenoid column (YMC-Carotinoid column). Detection was performed at 456 nm. The oven temperature was set to 25 °C. The eluents consisted of a mixture of methanol, methyl tert-butyl ether, and Milli-Q (eluent A: 81/15/4, eluent B: 6/90/4). Separation was performed by the following gradient: 0–10 min: 0% B; 10–40 min: 0–100% B; 40–42 min: 100% B; 42–45 min: 100–0% B; 45–55 min: 0% B.

Commercially available standards from Sigma-Aldrich of single compounds were utilized as references. For each run, 5 µL of lycopene standard solution (1 nmol μL^{-1}) was injected separately, corresponding to 5 nmol. Peak areas of this lycopene standard with known concentration and determined response factors (RF) for β-carotene (RF = 0.65) and Lutein (RF = 0.79) in relation to lycopene were used to calculate the contents of each detected carotenoid. All carotenoids were summed to receive total carotenoid content in tomato fruit in µg g^{-1} DM.

2.9. Analysis of the Mineral Composition of Tomato Fruits and Leaves

Oven dried samples of tomato fruits were ground (MM 30, Retsch GmbH; Haan, Germany) and used for nutrient analysis. Elemental analysis (K, Ca, Mg, P, S) was done after microwave digestion (microwave manufacturer CEM, MARS Xpress, CEM; North

Carolina, USA) according to LUFA protocol Vol. III, 10.8.1.2. In brief, 0.2 g of dried and ground sample was weighed into deionized containers and digested with 5 mL HNO_3 (65%) and 3 mL H_2O_2 (30%) with the following program: Step 1: 20 min to reach 200 °C; step 2: 5 min at 200 °C; step 3: 1 min to reach 210 °C; step 4: 5 min at 210 °C; step 5: 1 min to reach 220 °C; step 6: 5 min at 220 °C; and step 7: 30 min to cool down. The resulting solution was transferred to 50 mL volumetric flasks using distilled water and finally filtrated into plastic flasks. Thereafter, the analysis of the elements in the digestion solution was conducted via ICP-OES with an ICP Emission Spectrometer (iCAP 6300 Duo MFC, Thermo; Waltham, MA, USA). The operating conditions employed for ICP-OES were 1150 W RF power, 0.55 L min^{-1} nebulizer gas flow with argon employed as plasmogen as well as carrier gas. Analysis was performed with a crossflow nebulizer (MIRA Mist, Thermo Scientific; Cambridge, England). For quantification of each element, a single-element calibration curve was used. The elements were analyzed in duplicate at the following wavelengths: K = 766.5 nm; Ca at 317.9 nm; Mg at 279.0 nm; P = 213.6 nm; S = 182.2 nm. Nitrogen and carbon were determined using an elemental analyzer (Vario MAX, Elementar Analysensysteme GmbH; Hanau, Germany) according to DIN-ISO-13878 (1998). An aliquot of 0.3 g of sample material was weighed into crucibles and catalytically combusted at 900 °C with pure oxygen. The combustion products and helium (as the carrier gas) passed through specific adsorption columns at a temperature of 830 °C to separate nitrogen and carbon with a thermal conductivity detector. All results are expressed as g kg^{-1} dry matter (DM) for macroelements and mg kg^{-1} DM for micronutrients.

2.10. Statistical Analyses

Data were statistically analyzed using agricolae package [38] in RStudio Version 1.2.5033 [39]. The data were first tested for normal distribution and variance homogeneity before comparisons were calculated using *t*-tests for all parameters except for greenhouse gases. Significant differences between both substrates with respect to their physical properties and influences on performances of tomato plants in terms of growth, yield, mineral content, SSC, dry matter, and secondary metabolite concentrations were calculated. Significance of statistical analyses in this research was concluded for $p < 0.05$ for a given test.

For the measured gas concentrations, gas fluxes were calculated using the R package "gasfluxes" [version 0.4–4; [40], including automatic selection of the most suitable regression method (linear, robust linear, or non-linear HMR model). Input variables used were gas concentration (µmol m^{-3}, converted from ppm values according to the ideal gas law assuming SATP conditions), chamber volume (m^3), and time after closing the chamber (h). The area was set to 1 in order to obtain gas fluxes (µmol h^{-1}) for each growing bag. Gas fluxes in g ha^{-1} d^{-1} were calculated based on molar masses and assuming a potential plant density of 2 m^{-2} (substrate slab density of 1 m^{-2}). An initial screening of the gas fluxes indicated strong deviations from normal distribution. Therefore, statistical analyses were done using exact two-sample Fisher–Pitman permutation tests from the R package "coin" [version 1.3–1; [41] with the alternative hypothesis that GHG emissions from hemp fiber growing bags are greater than GHG emissions from rock wool growing bags.

3. Results and Discussion

3.1. Substrate Characteristics

3.1.1. Water Retention Curves

A suitable substrate for the cultivation of plants is characterized by an optimal composition of air-filled and water-filled pores (physical properties). This composition can be analyzed by using water retention curves. If an organic substrate is used instead of inorganic rock wool, the degree of mineralization of the organic material may vary depending on the cultivation period and conditions, and the proportions of water- and air-filled pores may change as a result. Thus, in this study, physical properties of rock wool and hemp substrates used during hydroponic cultivation of tomatoes were studied once before their use and once afterwards (Table 1).

The total pore volume (TPV) was 90 vol% for unused rock wool and was thus above the reference value [19] given in Table 1, while unused hemp substrate was in the reference range at 76 vol%. After use in hydroponic tomato cultivation, the TPV increased for both substrates, hemp (83.1 vol%) and rock wool (95.6 vol%). Furthermore, hemp remained within, rock wool outside the reference range. An increase of the TPV can be explained by plant root growth into the substrate [42], which was observed in both substrates used.

Table 1. Physical properties of substrates before and after their use in hydroponic tomato cultivation.

	Rock Wool	**Hemp**	**Rock Wool**	**Hemp**	**Optimum ***
	unused		used		
TPV [vol%]	90.4 ± 2.6 aB	75.9 ± 2.9 bB	95.6 ± 1.6 aA	83.1 ± 2.8 bA	>85
AV [vol%]	18.9 ± 5.1 aA	13.9 ± 3.3 aA	17.7 ± 6.3 aA	10.0 ± 2.9 aA	20 to 30
EAW [vol%]	70.3 ± 5.2 aA	41.4 ± 2.0 bA	63.2 ± 4.1 aA	12.5 ± 1.6 bB	20 to 30
BD [g cm^{-3}]	0.1 ± 0.01 bB	0.1 ± 0.0 aB	0.1 ± 0.02 bA	0.2 ± 0.03 aA	<0.4

Differences between substrates are indicated by different lower-case letters, and differences between unused and used substrates are indicated by different upper-case letters (*t*-test, $p < 0.05$, n = 5, mean ± standard deviation: TPV: total pore volume; AV: air volume; BD: bulk density; EAW: readily available water. * Reference values for evaluation of our results were taken from the publication by Dannehl, et al. [28] and references therein.

The proportion of pores filled with air (air volume, AV) was in the range of values between 10 vol% and 19 vol% and showed no significant difference between both substrates and stayed similar before and after their use. All AV values obtained were not within the optimal range when compared to the reference values (20–30 vol% [43]). The AV for hemp was 10 vol% at the end of the tests and therefore much lower than rock wool. We suspect that this is related to the particle size of the substrate, which is probably smaller for the hemp substrate. The finer the material, the lower the air volume [43].

The proportion of pores with easily available water (EAW) in rock wool was highest with 70 vol% and did not change significantly during use. EAW in hemp was significantly lower (41 vol%) and during cultivation the proportion of EAW dropped drastically to 13 vol% falling below the recommended values of 20–30 vol% [19] at the end of the tests. In a study of Islam, et al. [44], where rock wool, carbonated rice husks, and coconut coir were investigated before and after usage, the air-filled pore space of rock wool didn't change over time and showed similar values as observed in our experiment. The organic materials in that study showed increased TPV and water-filled pore spaces after utilization as substrate compared to the unused material. In our study, we found an increase in TPV as well. Contrary to Islam, et al. [44] who documented increased water filled pores, the EAW declined in rock wool and hemp in our experiment after use. Since we did not analyze complete water-filled pore space but only the proportion of EAW, there is the possibility that the proportion of ultra-micropores increased and water within these pores is usually unavailable to plants. With this in mind, it might be that more water-filled pore space is present, but not taken into consideration due to the focus on EAW.

The bulk density (BD) in unused hemp substrates was 0.10 g cm^{-3} and twice that of rock wool 0.05 g cm^{-3} (Table 1). However, BD had doubled by the end of culture in both rock wool and hemp. The higher BD from hemp could result from decomposition during the culture period and the associated reduction in pore size due to degradation processes. Nevertheless, the BD of both growing substrates corresponds to values < 0.4 g cm^{-3} as recommended by Abad, et al. [18].

3.1.2. Stability of Hemp towards Decomposition

Generally, organic substrates are subjected to chemical mineralization accomplished mainly by bacteria and fungi [45]. Therefore, hemp can decompose during use as a substrate and thus lead to unfavorable properties with regard to the standing stability of the plants, as well as to the immobilization of nutrients through their fixation in microbial biomass. However, this is not the case with rock wool, which is an inorganic material that is stable with regards to degradation. Thus, rock wool bags can help to estimate the root mass

formed in the substrate. Figure 1 shows how the weight of the growing bags changed as a result of their utilization. Rock wool bags increased in mass by about 8% (from 529 g to 574 g DM), which can be attributed to the root mass. The weight of the hemp fiber bags decreased by 54% (from 1628 g to 747 g including roots, in 16 weeks). It should be noted that the total cultivation period was from week 11 to 47, i.e., 36 weeks, and not just the 16 weeks shown in Figure 1. It was very clear at the end of the trial that there was hardly any substrate left in the hemp fiber bags. This means that less nutrient solution can be stored in hemp fiber, which quickly reduces the water and nutrient supply for the plants in the event of pump failures. Therefore, when assessing growing media for suitability in hydroponic systems, the weight loss of these is at least as important as the volume loss described by Gruda and Schnitzler [46].

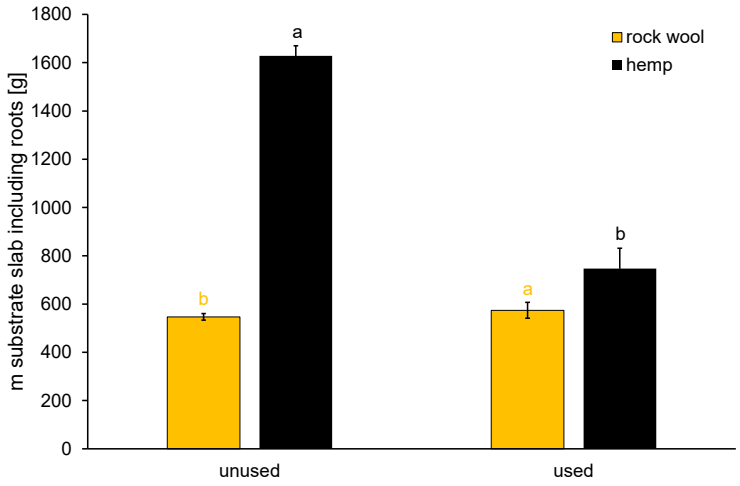

Figure 1. The mass of the substrates used as an indicator of the mineralized content of hemp and the increase in root biomass in rock wool in grams after 16 weeks of cultivation. Differences between unused and used substrates are indicated by different lowercase letters (*t*-test, $p < 0.05$, n = 3 for used substrates, n = 4 for unused hemp, n = 4 for unused rock wool, mean ± standard deviation).

3.1.3. N-Immobilization in Hemp Fiber Bags

The observed rapid degradation of hemp fiber bags (Figure 1) poses the risk that nutrients, especially nitrogen from the nutrient solution, could also be incorporated into the growing microbial biomass involved in the degradation process and thus not be available for the production of plant biomass. Therefore, nitrogen immobilization was determined, which can be triggered by the substrates. This is important in order to adapt the nutrient application in the hydroponic system to the plant. When values of nitrogen immobilization reach 250 mg dm^{-3} and more the material is not recommended to be used as substrate component [35]. It is not stable according to the evaluation of the N-balance (Table 2). According to this investigation, hemp fibers caused a N-immobilization of 601 mg L^{-1} and must therefore be classified as an unsuitable substrate. In comparison, the N-immobilization in wood is only 175 mg L^{-1} and is to be seen as an advantage over hemp fibers [47]. However, hemp fibers are cheaper to procure than wood fibers. In this context, it must be considered that the nitrogen source in hydroponic tomato production is not the substrate itself, but the nutrient solution. A constant supply of necessary nutrients could compensate for the nitrogen immobilization. Therefore, the accumulation of nutrients in the leaves and fruits must also be considered. This will be discussed later.

Table 2. Investigation of nitrogen immobilization of hemp substrates according to the VDLUFA method.

Sample	ΔNO_3-N_{20d}	ΔNH_4-N_{20d}	ΔN_{20d}	Evaluation of N Budget *
		[mg L^{-1}]		
hemp	183	418	601	not stable

* according to VDLUFA [35].

3.1.4. Greenhouse Gas Emissions Released by Hemp and Rock Wool

In general, no traceable N_2O, CO_2, or CH_4 emissions were found from rock wool growing bags incubated with nutrient solution (Table 3). The calculated gas fluxes fluctuated around zero due to background effects from gas chromatographic analyses yielding apparent positive or negative fluxes. The missing GHG emissions from rock wool were probably due to the absence of a C source, which strongly limited microbial activity in the growing bags. In contrast, clear GHG emissions were found from hemp fiber bags incubated with nutrient solution. N_2O emissions were insignificant on 15 October, after 6 weeks of incubation, but increased to a maximum on 11 November before decreasing again on 1 December (Table 3). The highest average value of 4.6 µmol h^{-1} per growing bag on 11 November would correspond to 31 g N_2O-N ha^{-1} d^{-1} (i.e., 49 g N_2O ha^{-1} d^{-1} or 14.7 kg CO_2-eqivalents ha^{-1} d^{-1}) if a plant density of 2 m^{-2} is assumed. This value lies in the upper range of N_2O emission rates reported for rock wool substrates planted with tomato or cucumber [24,48–50], and thus underpins the critical role of organic C sources for N_2O production by denitrifying bacteria. The CO_2 emissions from hemp fiber bags were highest on 15 October and decreased to a similar value at the following two measurements on 11 November and 2 December (Table 3). The average CO_2 emission rate of 3.1 mmol h^{-1} per growing bag on 15 October would correspond to 32 kg CO_2 ha^{-1} d^{-1} if a plant density of 2 m^{-2} is assumed. This value is in the lower range of CO_2 emissions found from tomato and cucumber cultivation on rock wool [24,48,49], possibly due to the missing root respiration and root exudates, which can affect the microbial community through the provision of easily degradable C [51]. In contrast to N_2O, significant CH_4 emissions from hemp fiber bags were found on 15 October (Table 3). Similar emission rates were found on 11 November before they increased to the highest values on 1 December, with on average 6.4 µmol h^{-1} per growing bag. This would correspond to 24.5 g CH_4 ha^{-1} d^{-1} (i.e., 0.7 kg CO_2-eqivalents ha^{-1} d^{-1}) if a plant density of 2 m^{-2} is assumed, which is approximately one tenth of the highest values reported for cucumber cultivation on rock wool by Hashida, et al. [49].

Table 3. Emissions of greenhouse gases from hemp and rock wool.

	Rock Wool	Hemp
	[g-N (N_2O) ha^{-1} d^{-1}]	
15 October 2020	0.17 ± 0.07 a	5.03 ± 4.79 a
9 November 2020	n.d. b	31.02 ± 21.93 a
1 December 2020	0.04 ± 0.05 b	21.97 ± 10.76 a
	[kg-CO_2 ha^{-1} d^{-1}]	
15 October 2020	0.75 ± 0.12 b	32.38 ± 1.36 a
9 November 2020	0.10 ± 0.44 b	16.23 ± 3.85 a
1 December 2020	0.29 ± 0.45 b	17.60 ± 4.01 a
	[g-CH_4 ha^{-1} d^{-1}]	
15 October 2020	n.d. b	8.11 ± 2.91 a
9 November 2020	n.d. b	6.41 ± 4.83 a
1 December 2020	n.d. b	24.49 ± 17.68 a

Measured N_2O, CO_2 and CH_4 emission rates per growing bag (mean ± SEM, n = 3) filled with hemp fiber or rock wool substrates and incubated with tomato nutrient solution. Different small letters indicate significant differences (p = 0.05) for individual measurement days (note that lower p-values are not possible in the used permutation test due to the low number of replicates). N.d.: below detection limit.

On one hand, replacing inert substrates such as rock wool with organic substrates such as hemp fiber offers a compelling opportunity to reduce the climate impact of hydroponic cultivation by lowering the energy demand for substrate production. In detail, if the CO_2 greenhouse gas emissions from the production of rock wool [16] and hemp fibers [52] are compared, they can be reduced by 84% when hemp fibers are used.

On the other hand, it must be considered that the degradation of organic C from organic substrates increases GHG emissions during cultivation. However, if residues are used that would otherwise also be degraded (e.g., in composts), the actual impact might be small, especially for CO_2 and CH_4 [53]. The higher CO_2 emissions from hemp substrates at the beginning of the measurements in October indicate a strong degradation of the hemp fibers, which could have continuously decreased oxygen availability in the substrate slabs. The presence of anoxic conditions in the substrate slabs was furthermore indicated by the perceptible odor of hydrogen sulfide. Denitrification works best under suboxic conditions and decreases again under very anaerobic conditions. However, the latter are necessary for methane formation. This would explain why the nitrous oxide emission was highest in the second measurement, while the methane emission increased again significantly in the third measurement. Thus, it would be desirable to introduce oxygen into the substrate bags to prevent or reduce these anaerobic conditions to prevent emissions of methane and nitrous oxide as suggested by Karlowsky, et al. [24].

3.2. Determination of Plant Growth Parameters

The different physical and chemical properties of both substrates could have an impact on the performance of the plants. In order to be able to make statements on this, the leaf areas during the first six weeks after planting, the plant length achieved at the end of cultivation, and the total yields of the tomato plants were documented as a function of the substrate used (Table 4). Leaf area increased to almost 3 m^2 and the length of the plants reached 9 m. Both parameters did not differ significantly between plants grown on different substrate.

Table 4. Effects of different growing media on tomato plant growth and yield during and at the end of cultivation.

	Rock Wool	**Hemp**
Leaf area per plant [m^2] 6 weeks after planting	2.97 ± 0.19 a	2.82 ± 0.22 a
Plant length [m]	9.51 ± 0.43 a	8.96 ± 0.38 a
Total yield per plant [kg]	9.98 ± 0.72 a	9.27 ± 0.16 a
SSC fruit [g 100 g^{-1} FM] CW 25	6.60 ± 0.08 a	7.07 ± 0.18 a
SSC fruit [g 100 g^{-1} FM] CW 32	5.36 ± 0.04 a	5.40 ± 0.24 a
DM fruit [%] CW 25	7.73 ± 0.34 a	9.12 ± 0.56 a
DM fruit [%] CW 32	5.64 ± 0.26 a	5.54 ± 0.16 a

Leaf area (n = 3) was measured during first 6 weeks after planting and plant length at the end of the cultivation period (n = 8). Total yield per plant was calculated by adding weekly yields (n = 60). SSC: soluble solid content (n = 3). DM: dry matter content (n = 3). CW: calendar week. Different small letters indicate significant differences between the substrates (*t*-test, $p < 0.05$, mean ± standard deviation).

Tomato fruits were harvested weekly, and yields were summed to calculate the total yield per plant at the end of cultivation. No significant differences in the cumulated yields of plants grown on rock wool and hemp were found during our cultivation period (Table 4). These results are in line with other studies that also used organic (composted white spruce and fir bark in 40%:60%, v/v ratio) rather than inorganic substrates [22]. However, a trend seems to be developing that yields from rock wool bags would be higher than yields caused by hemp fiber bags if the cultivation period were extended. Similar results were found with the use of a mixture of white spruce and fir shavings mixed in a 2:3 (v/v) ratio [22]. The reason for this observed increasing trend is probably that hemp fibers were already well advanced in mineralization and thus the conditions in the root zone, e.g., total pore and air volume, were suboptimal at the end of the cultivation period. This hypothesis is supported by Chérif, et al. [54], who showed that tomato plants are sensitive to hypoxia. In

the present study, an odor of hydrogen sulfide coming from hemp fiber bags throughout the cultivation period might be an indication that hypoxic conditions within hemp substrates existed. The rapid mineralization in which mineralizing microorganisms use most of the available oxygen could explain hypoxic conditions, which, in turn, might affect secondary metabolites or yields.

3.3. Mineral Composition of Leaves and Fruits of Tomato Plants

During the 2020 growing season, leaf samples were taken every three weeks for a period of 12 weeks, and fruit samples were taken every three weeks for a period of 24 weeks to determine the mineral composition in tomato leaves and fruits. In this context, a nutrient deficiency was neither observed visually nor detected in leaves and fruits (Table 5). If the individual nutrients are considered, there are no significant differences, neither for the macro- nor for the micronutrients in leaves and fruits. This means that the observed high nitrogen immobilization caused by the hemp substrate could be compensated by the regularly applied nutrient solution and had no negative influence on the nutrient composition in different plant organs. This also refutes the assumption made by Allaire, et al. [22] that lower yields in hydroponic tomato production using organic growing media is favored by nitrogen immobilization.

Table 5. Nutrient contents in tomato leaves and fruits in relation to different substrates.

	Nutrients in Tomato Leaves		**Nutrients in Tomato Fruits**	
	Rock Wool	**Hemp**	**Rock Wool**	**Hemp**
N [g kg^{-1}]	50.2 ± 6.0 a	50.2 ± 6.1 a	17.2 ± 2.6 a	17.0 ± 2.8 a
P [g kg^{-1}]	4.4 ± 0.6 a	4.0 ± 0.3 a	4.0 ± 0.3 a	3.9 ± 0.4 a
K [g kg^{-1}]	49.2 ± 10.8 a	50.2 ± 9.7 a	44.6 ± 3.6 a	43.4 ± 3.8 a
Ca [g kg^{-1}]	19.2 ± 5.9 a	17.2 ± 4.4 a	0.8 ± 0.1 a	0.7 ± 0.2 a
Mg [g kg^{-1}]	8.2 ± 1.2 a	9.4 ± 1.6 a	1.6 ± 0.3 a	1.6 ± 0.3 a
Cu [mg kg^{-1}]	16.7 ± 1.5 a	14.6 ± 1.8 a	9.3 ± 3.8 a	9.8 ± 4.0 a
Zn [mg kg^{-1}]	37.9 ± 7.2 a	36.9 ± 9.0 a	25.1 ± 4.6 a	22.8 ± 4.8 a
Fe [mg kg^{-1}]	174.7 ± 10.5 a	171.3 ± 35.8 a	41.4 ± 15.3 a	39.6 ± 16.3 a

Significant differences are indicated with small letters (*t*-test, $p < 0.05$, n = 3 per sampling date, 9 sampling dates, mean ± standard deviation).

3.4. Effects of Different Growing Media on Quality Parameters of Tomato Fruits

3.4.1. SSC and Dry Matter Content of Tomato Fruits

In addition to mineral content, the soluble sugar content (SSC) and the dry matter content of tomato fruits were determined. The results are shown in Table 4. For both parameters, SSC and dry matter content, no differences were found in the fruits from the different growing media at all measurement dates. Fruit dry weight consists of up to 60% reducing sugars and organic acids [55], making fruit dry weight an important tomato quality parameter. Dry matter contents in our study were between 4.7 and 9.1% and thus in the range of values already published, e.g., in Bertin, et al. [56] or Moraru, et al. [57]. SSC values ranged from 5.1 to 7.1 g 100 g^{-1} FM, which is within documented values for tomatoes from studies from Johnstone, et al. [58] or Verheul, et al. [59]. SSC and dry matter content seem to be influenced more by the measurement date than by the substrate and tend to decrease over the cultivation period. Dry matter content and SSC are influenced by the amount of sucrose produced during photosynthesis, which is transported to the fruit [60]. Photosynthesis, in turn, is closely related to solar radiation. This explains the decreasing dry matter content and SSC in tomato fruit from mid-year (CW 24, 25) to autumn (CW 31, 32).

3.4.2. Secondary Metabolites—Contents of Carotenoids

For the determination of secondary constituents (carotenoids, phenolic acids, flavonoids), fruit from 15 different plants per growing media were harvested at 3-week intervals over

24 weeks (11 June–25 November 2020). The results for the carotenoid analyses are shown in Figure 2. In tomato fruits produced on hemp fibers, there was significantly increased carotenoid content on several measurement dates compared to the fruits from rock wool (CW 25, CW 27, CW 31 and CW 32). In addition, carotenoid content at each test date varied over the culture period, which may reflect the influence of abiotic factors such as light irradiance [61] and temperature [62] on carotenoid content. Furthermore, two hypotheses are possible for significant differences in carotenoids caused by hemp and rock wool: (i) nutrient supply and/or (ii) ethylene release in the substrate. In this context, Bénard, et al. [63] found no effects of different nitrogen levels on the accumulation of carotenoids in tomatoes, whereas a high proportion of K and Mg in the nutrient solution can increase these secondary plant compounds in tomatoes [64]. Since all macronutrients in leaves and fruits were similar regardless of which substrate was used (Table 5), the first hypothesis is not valid. In terms of the second hypothesis, ethylene in organic substrates originates from decomposition of these by microorganisms, where higher rates of ethylene production were detected under anaerobic conditions [65]. Ethylene plays a central role in the ripening of tomato fruit [66]. A dramatic increase in ethylene production is correlated with the rapid accumulation of carotenoids [67]. Based on our results regarding high CO_2-emissions and weight losses caused by the use of hemp, we assume that high ethylene production existed in these growing bags followed by a higher carotenoid accumulation, especially during the end of the cultivation period (Figure 2). This hypothesis must be investigated in more detail. In particular, ethylene concentration and microbial activity and composition must be determined at close intervals during the experimental period.

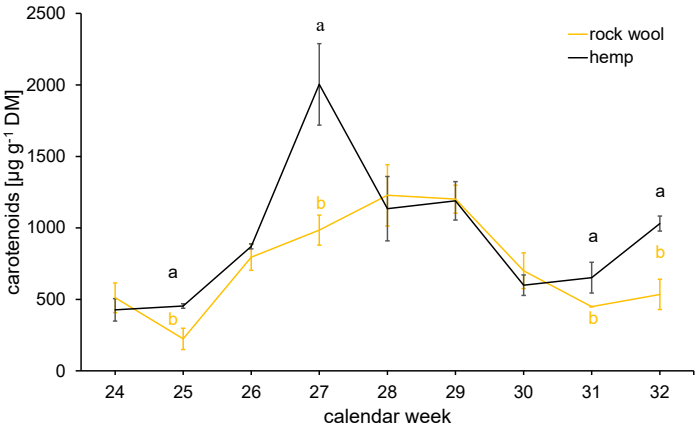

Figure 2. Influence of different substrates on the total carotenoid content in tomatoes. Different small letters indicate significance between variants (*t*-test, $p < 0.05$, n = 3).

3.4.3. Secondary Metabolites—Contents of Phenolic Acids and Flavonoids

In addition to carotenoids, phenolic substances and flavonoids were also investigated as representatives of secondary metabolites. For a first impression, the total phenolic and flavonoid content was determined. In Figure 3 it can be seen that with regard to total phenolic content in tomato fruits, no effect was triggered by the different substrates. The same applied to total flavonoid content. In this context, hypoxia in the root zone can increase phenols in plants [68,69]. In our study, it might be possible that hypoxia was not high enough to increase the phenols in tomatoes.

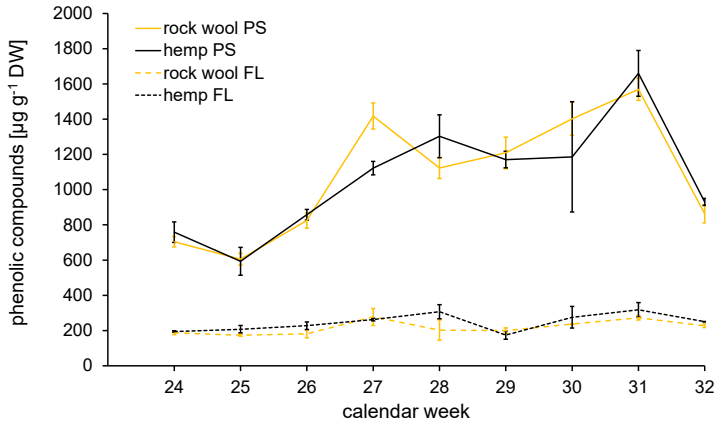

Figure 3. Mean total phenolic acid and flavonoid content in tomato fruit. No significant differences were found (*t*-test, $p < 0.05$, n = 3 over 9 harvest dates 3 weeks apart).

Furthermore, it can be stated that total phenolic content increased during the cultivation period when the last sampling date is not taken into consideration. A positive correlation was found between decreasing temperatures towards the end of the cultivation period and the accumulation of phenolic acid contents in tomato fruits (Figure 4).

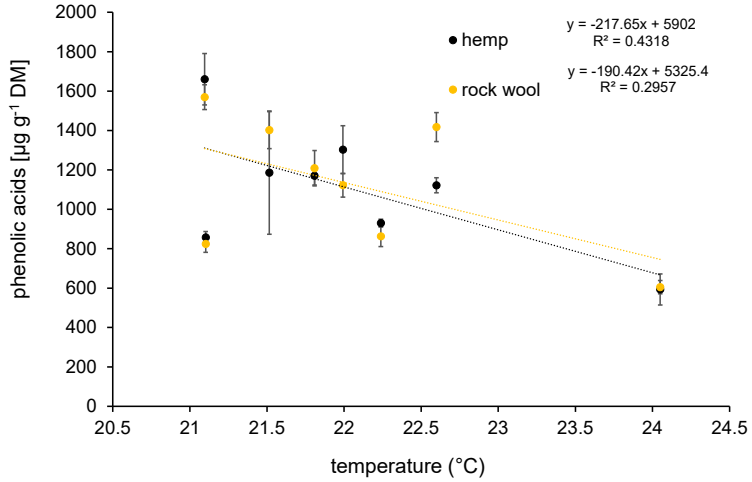

Figure 4. Correlation between phenolic compounds in tomatoes and temperature under consideration of different growing media.

4. Conclusions

The study presented here shows that hemp fibers as an organic substrate in hydroponic cultivation of tomatoes leads to similar yields to the conventionally used rock wool. Likewise, no negative effects on plant growth parameters, nutrient accumulations in leaves and fruits, or phenolic compounds were found. Carotenoids could even be increased by the use of hemp as found in some weeks. Nevertheless, hemp can only be recommended as a substrate for short-term use as the rapid mineralization can be disadvantageous for the root anchoring and thus for the stability of the plants, especially when intensive vegetable production in hydroponics is used.

A higher supply of nitrogen for plants is necessary since mineralization incorporates a significant amount of nitrogen into microbial biomass, making it unavailable to the plants. Although this N-immobilization can be compensated by regularly applied nutrient solution, this increased demand for mineral nitrogen is rather unfavorable from the point of view of sustainability. The observed release of greenhouse gases such as N_2O, CH_4, and CO_2 from hemp fibers also does not correspond to the current goal of making horticulture

more, but environmentally friendly, but could still be lower than CO_2 emissions from rock wool production.

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