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Quality Evaluation of Mustard Microgreens Grown on Peat and Jute Substrate

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Abstract: Consumers appreciate microgreens for their vast variety of colors and flavors. Usually, they are grown employing peat, a substrate that is used in large quantities. In order to identify a more sustainable propagation protocol and to reduce the amount of peat consumed, alternative propagation protocols were evaluated. Jute is a biodegradable substrate with lower post-harvest costs because it does not leave particles on microgreens. This work evaluates the microgreen yield, flavor, texture, and phytochemical compounds when grown on jute. Green mustard (*Brassica nigra*) is one of the most popular microgreens. When growing these microgreens on jute (three repetitions), it was necessary to increase the frequency of irrigation and reduce the amount of water for each turn. In addition, the propagation time needed to be increased from 5 to 7 days. The tasters found no difference in flavor and only a slight difference in texture was observed when microgreens were grown on jute. The phenol and chlorophyll levels were unchanged, while carotenoid levels were slightly higher. Thus, the cultivation of green mustard on jute has a minimal impact on microgreens and leads to increased sustainability and reduced post-harvest costs.

Keywords: *Brassica*; texture; phenols; photosynthetic pigments

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1. Introduction

Microgreens are increasingly popular for their sensory and visual contribution to gastronomy and for their bioactive compounds that are potentially healthy in the human diet [1–3]. They are a particularly attractive crop in the modern horticultural supply chain because they are well suited to protected soilless cultivation, accelerated production, limited space for growth, and high crop turnover [1]. The most used substrate for cultivation of microgreens is peat-based, a slow renewable and relatively expensive substrate; furthermore, its future availability is being questioned, being a limiting source [4].

In the context of sustainable agriculture, and for environmental concerns, eco-sustainable substrates or alternatives to peat are inserted for the cultivation of microgreens, such as vermiculite, coconut fiber, textile fiber, and jute [5–7].

Jute, like peat, is a biodegradable substrate already sold as growth mats for microgreens [8], which, despite having a higher cost than peat, compensates for the cost in terms of working time (for shelling and, above all, for inserting and compressing the peat in the trays), and the light weight of jute makes it much more manageable than peat for processing, moving trays, and the CO_2 needed for the transportation from North Europe. The jute-based substrate also avoids leaving residues on microgreens, unlike peat, so the microgreens grown on peat need to be washed more accurately before use.

Green mustard (*Brassica nigra*), being a member of *Brassicaceae*, is rich in flavor-active compounds, exhibiting a bitter taste and a sulfurous smell, which principally determines

its acceptance by consumers [9]. Mustard microgreens, like other species of the *Brassicaceae*, are the most common choices for producing microgreens as they have easy germination, rapid growth, and diversity of colors and flavors [3,10]. Furthermore, the members of *Brassicaceae* are known and extensively studied for their health benefits, being rich in bioactive compounds such as glucosinolates, polyphenols, anthocyanins, ascorbic acid, carotenoids, and tocopherols [3,11]. One of the advantages of eating microgreens is that they are mainly consumed raw; therefore, they do not undergo the processing methods, which lead to a loss in terms of nutritional composition, especially for those thermolabile compounds [3]. The most important properties to evaluate a product include appearance and flavor, while little attention has been given to the sensory attributes of microgreens [1], which includes texture, commonly evaluated by a penetrometer [12,13].

The objective of the current study was to assess the impact of two growing media (peat and jute fiber substrate) on the quality traits and yield of microgreens of green mustard (*Brassica nigra*) and to know whether jute substrate can be used as an alternate to peat for growing microgreens with the same benefits. Mustard microgreens were grown on jute substrate along with traditional peat substrate, intending to achieve better results, and then, microgreens grown on both substrates were compared in terms of texture, total phenols, chlorophylls, total carotenoids, and their yield.

2. Materials and Methods

2.1. Plant Culture Conditions and Growing Substrates

Mustard (*Brassica nigra* L. Koch) microgreens were grown in a greenhouse in June 2022. Three replications were made for each sample, i.e., microgreens grown on jute and on peat (as control).

2.1.1. Growing Substrates

Two types of growing substrates were used; (i) a ready-to-use dry jute fiber mat weighing 4 g/126 cm^2 with a thickness of 6 mm; and (ii) brown peat substrate weighing (dry) 66 g (the amount to put in 1 tray/pot for microgreens). Both substrates are shown in Figure 1.

(**a**) (**b**)

Figure 1. (**a**) Jute fiber substrate; (**b**) Peat substrate.

2.1.2. Growing Conditions

The sowings (with seeds that came from the same stock) were carried out in a fully randomized way on a total of 18 trays, 9 with peat and 9 with jute (3 repetitions with 3 trays each). Initially, microgreens on both peat and on jute (labelled as jute protocol 1) were grown with the same irrigation protocol (protocol 1), but the microgreens on jute could not grow well; therefore, the protocol was modified for jute only (jute protocol 2) aiming to achieve similar product as that obtained on peat substrate (control). For this, 9 trays were grown on jute substrate protocol 2. The experimental analyses were then carried out on 3 trays chosen at random from those reared in peat and 3 for those in jute. Protocol 1 was used for both substrates, and protocol 2 was used only for jute. The details of both protocols are as follows:

Protocol 1: Both peat and jute substrates were placed in recycled polyethylene terephthalate (R-PET) plastic trays (Area = 126 cm^2, Weight = 6 g) with 4 holes at the bottom,

pressed manually in the case of peat, and slightly watered (moistened,) and 0.5 g of mustard seeds were sown in each tray. The trays were watered again and placed in the germination chamber (at 23 ± 2 °C and 98% relative humidity). When the height of growing microgreens almost reached the edge of the tray (approximately 2.5–3 cm), they were moved to an unheated greenhouse, on benches where fertigation took place by sub-irrigation (ebb and flow) in the presence of the half-strength Hoagland nutrient solution. During irrigation, the water reached the height of approximately $\frac{1}{4}$ of the tray. The sub-irrigation took place by a commonly used irrigation pattern for microgreens at the greenhouse, which was once a day at 9:00 a.m. for 10 min, and the temperature of the greenhouse ranged between 32–35 °C with an air relative humidity below 85% (through fans and greenhouse openings).

Protocol 2: the protocol for jute was modified, decreasing the amount of water in the sowing step to not drown the seeds, and increasing the irrigation turns; the sub-irrigation was realized/performed twice a day, at 9:00 a.m. and at 2:00 p.m., for 7.5 min each. The conditions for growing microgreens on peat control and other factors, e.g., germination chamber conditions and greenhouse conditions were the same as protocol 1.

2.2. Determination of Yield

For harvesting, first, all the microgreens, along with the substrate, were removed carefully from the plastic tray and harvesting was performed with sterilized scissors by cutting just above the substrate line (a length of approximately 3.5 cm from the tip of the microgreens). To measure the height of the microgreens from both substrates, the same stage of the microgreens was considered; in other words, the same leaf size and same seedling height ≈ 3.5 cm. After that, the height (of 15 micro-seedlings taken randomly within a tray with 3 repetitions from different trays) and weight (for a whole tray with 3 repetitions) of the harvested microgreens were taken. The uniformity of the microgreens' surface/canopy was kept in consideration before the harvesting.

2.3. Sensory Test

The microgreens samples chosen to be analyzed further were the microgreens grown on peat (control) and the microgreens grown on the jute with modified protocol (jute protocol 2). The microgreens grown on jute with protocol 1 were not even considered in subsequent determinations as they were not comparable with the microgreens grown on peat in terms of yield, quality traits, and acceptability on the part of buyers/consumers due to lack of uniformity. A sensory test, recently described by Rosa et al. [14] with some modifications, was conducted by 5 Ortogourmet employees, people who have been working on microgreens for a long time and who, therefore, know the products very well. The test was conducted at three different time slots, with microgreens taken from 3 different tray sources each time; following, 2 g of mustard microgreens harvested from peat and jute (protocol 2) were placed in two unlabeled dishes so that the panelists could not recognize the source. They were asked to taste and answer with a "yes" or "no" for any difference between the two products in aroma, texture, and taste. If yes, they were required to describe the intensity of aroma, texture, and taste on a scale from 1 to 10.

2.4. Determination of Leaf Texture/Toughness

The texture of the microgreens' leaves was measured in Newtons (N) with the PCE-PTR 200, Model: FS-1001, penetrometer with a 6 mm metallic tip on 10 seedlings taken at random from each of the 3 different trays. The force required by the metallic tip to penetrate a microgreen leaflet was recorded. The penetrometer is usually used to determine the texture of fruits, for example, apples and strawberries [12,13]. However, some studies have also shown the use of a penetrometer to determine the toughness and texture of the leaves [15–19].

2.5. Determination of Total Phenolic Compounds

For phenol determination, the analyses were performed on 3 different trays selected randomly by harvesting microgreens from each tray. First, 1 g FW of microgreens was finely crushed with 10 mL Methanol containing 1% Formic acid. The mixture was centrifuged for 5 min at 5000 rpm and then filtered; the supernatant was used as a sample solution. The total phenolic content (TPC) was determined using the spectrophotometric Folin–Ciocalteau method [20] measuring the absorbance after a 1:10 dilution with water with a Jasco V-550 UV/VIS spectrophotometer at 765 nm; the data were expressed as mg of gallic acid equivalent (GAE) per g of fresh weight (FW) and dry weight (DW).

2.6. Determination of Chlorophylls and Total Carotenoids

The chlorophyll and carotenoid levels were determined by harvesting microgreens from 3 different trays each time, and they were extracted by a methodology described by Negro et al. [21] from 1 g microgreen samples crushed with liquid nitrogen, then homogenized with 80% acetone at a ratio of 1:10 (*w*/*v*), and thereafter, stirred for 30 min. The amounts of chlorophylls and carotenoids were calculated by applying the formula [22] after the absorbance reading with a Jasco V-550 UV/VIS spectrophotometer. The values at different wavelengths (663; 646; 470 nm) were expressed in μg/g dry weight (DW). The dry weight was determined by placing the microgreens in an oven at a temperature of 105 °C to constant weight.

2.7. Statistics

All data were reported as the mean ± standard deviation (SD) with three replications for each sample taken (peat control, jute protocol 1, and jute protocol 2). An analysis of variance (ANOVA) was conducted to compare the means of all variables between the microgreens grown on two substrates, and afterwards, Duncan's multiple range test was applied with a significance level of <0.05 to determine the significant differences between variables of the microgreens grown on two substrates. Tables for all the analyzed variables with level of significance and their *p*-values are shown in S1. All statistical analyses were performed using the software Statistica (StatSoft, Tulsa, OK, USA).

3. Results

3.1. Determination of Yield

Primarily, we grew microgreens on both peat and jute substrate with the same irrigation protocol (protocol 1), but the microgreens on jute could not grow well, but later, with a modified protocol for jute only (jute protocol 2), we were able to obtain product similar to that obtained on peat substrate. With protocol no. 1 (sub-irrigation once a day for 10 min), 5 days were necessary to reach the harvesting stage (2 days for germination and 3 for growth in the greenhouse) when growing microgreens on peat, while 8 days were necessary to reach the harvesting stage on jute (4 days for germination and 4 for growth in the greenhouse). Furthermore, at harvest, the yield on jute was lower than that on peat; a whole tray of jute microgreens at harvest was approximately 5 ± 0.12 g, while for the peat microgreens, it was approximately 6.5 ± 0.42 g (Figure 2). The height of the microgreens also showed a difference, being 3.7 ± 0.13 cm and 3.1 ± 0.07 cm for the peat and jute grown microgreens, respectively (Figure 3). The main problem was that a not uniform germination of seeds was seen on the jute substrate as two or more growth layers of microgreens were evident (Figure 4b).

With protocol no. 2 applied for jute (sub-irrigation twice a day for 7.5 min), there was still a difference in growth in terms of days to reach the harvesting stage between microgreens grown on peat and jute, but the difference was 1 day less than before (3 days for germination and 4 for growth in the greenhouse). However, at harvest, the microgreens grown on jute protocol 2 showed almost a similar yield compared to those grown on peat; the weight and height reached by microgreens grown on jute were 6.2 ± 0.32 g (for a whole tray) and 3.7 ± 0.3 cm, respectively (Figures 2 and 3), with values close to microgreens

growing in peat. There was no canopy uniformity for the microgreens grown on jute, but it was visually much better than that obtained with protocol 1 (Figure 4c).

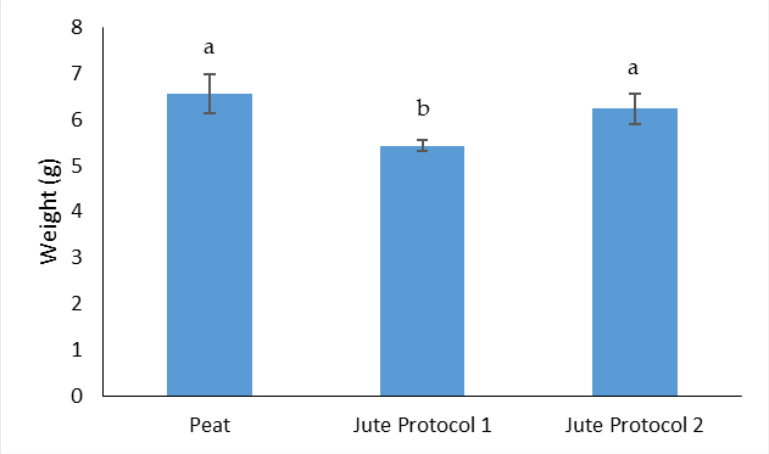


Figure 2. Weight of mustard microgreens at harvest (Different letters above the histogram correspond to statistically different means (Duncan's test, $n = 3$, $p < 0.05$)).

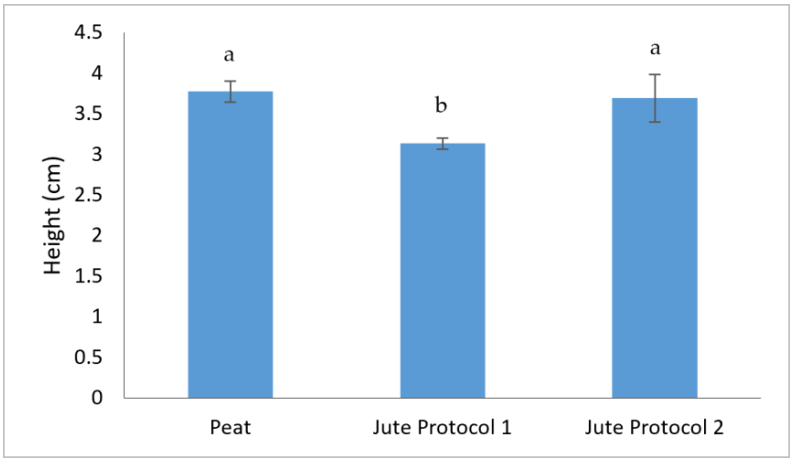
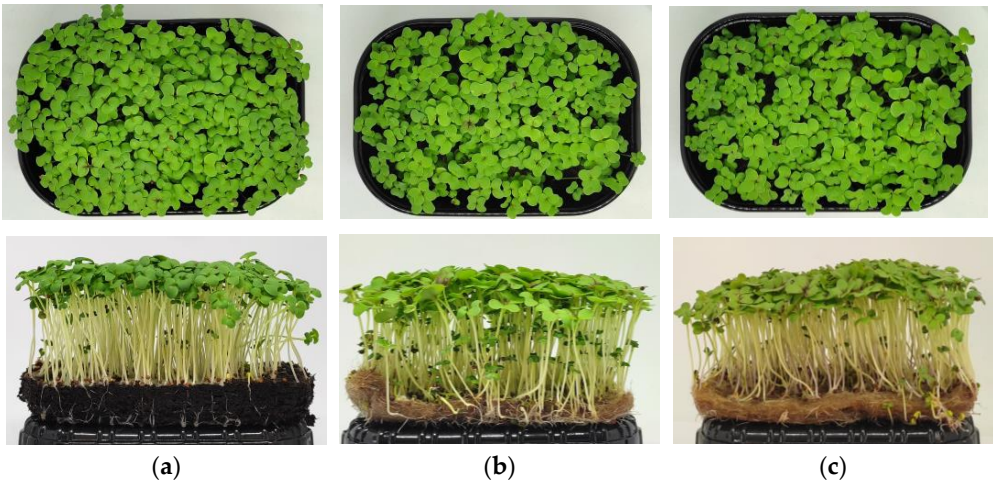


Figure 3. Height of mustard microgreens at harvest (Different letters above the histogram correspond to statistically different means (Duncan's test, $n = 3$, $p < 0.05$)).



(**a**) (**b**) (**c**)

Figure 4. Mustard microgreens at harvest on (**a**) peat, (**b**) jute protocol 1, and (**c**) jute protocol 2. (Observed from top frames and bottom frames).

3.2. Sensory Test

All the participants found no differences in taste, texture, and aroma between the two samples of green mustard microgreens from peat and jute protocol 2.

3.3. Determination of Leaf Texture/Toughness

Significant statistical differences were present between the microgreens obtained from jute protocol 2 and peat. The texture of microgreens grown on peat showed a 16.6% greater toughness than those of microgreens grown on jute. Table 1 shows the average values of microgreens' leaves.

Table 1. The texture of the microgreens' leaves obtained with the penetrometer and expressed in Newtons.

Substrate	N
Peat	0.78 ± 0.08 b
Jute protocol 2	0.65 ± 0.06 a

In the column, different lowercase letters indicate significant differences between the microgreens' texture (Duncan's test, $n = 3$, $p < 0.05$).

3.4. Determination of Total Phenolic Compounds

The total phenolic content (TPC) (both for fresh weight and dry weight) obtained from the mustard microgreens grown on peat and jute protocol 2 are shown in Table 2. The results indicate that there is no significant difference between the two products, even if the values are slightly higher in microgreens grown on jute.

Table 2. Total phenolic compounds in mustard microgreens grown on peat and jute protocol 2 expressed as mg GAE/g of fresh weight (FW) and of dry weight (DW).

Substrate	mg/g FW	mg/g DW
Peat	1.27 ± 0.42 a	24.39 ± 8.17 a
Jute protocol 2	1.54 ± 0.38 a	29.68 ± 7.23 a

In the same column, similar letters indicate no significant difference between total phenols of two types of microgreens (Duncan's test, $n = 3$, $p < 0.05$).

3.5. Determination of Chlorophylls and Total Carotenoids

The results of the photosynthetic pigments: chlorophyll *a*, chlorophyll *b,* and total carotenoids are shown in Table 3. There was no significant difference in both chlorophyll *a* and *b* in microgreens grown on peat compared to microgreens grown on jute protocol 2 substrate. Instead, a significant difference was detected for the carotenoid content in the two different microgreens; they are slightly higher in mustard microgreens grown on jute protocol 2.

Table 3. Chlorophylls and total carotenoids in mustard microgreens grown on peat and jute protocol 2 expressed in µg/g of fresh weight (FW) and dry weight (DW).

Substrate	Chlorophyll *a*		Chlorophyll *b*		Total Carotenoids	
	(µg/g FW)	(µg/g DW)	(µg/g FW)	(µg/g DW)	(µg/g FW)	(µg/g DW)
Peat	332 ± 20 a	6400 ± 370 a	166 ± 22 a	3192 ± 382 a	56 ± 3 b	1086 ± 60 b
Jute protocol 2	360 ± 23 a	6938 ± 410 a	177 ± 25 a	3413 ± 445 a	71 ± 5 a	1369 ± 83 a

In the same column different lowercase letters indicate significant differences between the total carotenoids of two types of microgreens (Duncan's test, $n = 3$, $p < 0.05$).

4. Discussion

Peat, being a slow replenished substrate for horticultural uses, is creating environmental concerns, especially in terms of future availability [4]; it is inevitable to find alternatives to peat. Jute is a soilless substrate that can be used as an alternative to peat-based substrates for growing microgreens. Di Gioia et al. [7] underwent the physicochemical, agronomical, and microbiological evaluation of Textile-fiber mat (TF) and Jute Kenaf Fiber mat (JKF), comparing those with Peat mix and STG (Sure to Grow) mats as reference media for the production of rapini microgreens. They concluded that TF and JKF may be valid alternatives to peat and STG because both ensured a competitive yield, low nitrate content, and a similar or higher microbiological quality. Bulgari et al. [5] evaluated the yield and nutritional quality traits of green basil, red basil, and rocket microgreens grown on three different substrates, i.e., coconut fiber, vermiculite, and jute. On all the three substrates tested, very fine results were obtained for the rocket, with the best data observed on jute.

In our work, the microgreens obtained on the jute substrate by using the normal irrigation protocol, i.e., protocol 1, were not worth experimenting with. With the modified protocol 2, we obtained microgreens grown on jute that were very similar to that of peat in terms of yield and canopy uniformity; therefore, we considered only those for further analyses. The germination period was reduced by 1 day in comparison to protocol 1 for jute. The yield of microgreens grown on peat was 20% higher than that obtained on jute protocol 1 and only 5% higher than the jute protocol 2 microgreens. Probably the microgreens grown on jute with protocol 1 could not grow well because of a reduced water availability.

The microgreens of Rapini (*Brassica rapa*) grown on different substrates showed 11.5% less FW than the shoots grown on jute when compared to the shoots grown on peat [7], while in our work, the yield reduction was limited to 5%. Microgreens of coriander, kohlrabi, and pak choi grown on peat showed a 55% increase in terms of fresh yield when compared to those grown on agave fiber, coconut fiber, capillary mat, and cellulose sponge. In peat, there was higher growth and fresh yields, which favored a high production turnover; however, this was achieved at the expense of the reduced phytochemical content. They tend to favor the accumulation of nitrates in microgreens, especially in brassica vegetables, which are known as nitrate hyperaccumulators [23].

Texture, being the physical trait of plants, is an important sensory characteristic that affects consumer acceptability and can impact the marketability of fruits and other agricultural products [12,19]. One of the texture attributes is crunchiness/crispness, assessed through sensory evaluation. There are studies showing penetrometer measurement correlation with texture attributes [12]. Penetrometers are usually used for determining the texture and firmness of fruits, [12,13] but some studies have also shown the use of penetrometers, which should provide objective data compared to tasters, to determine the toughness and texture of the leaves. Takeuchi and Zalucki [16] used a penetrometer to determine *Eucalyptus* leaf toughness; Wang et al. [17] and Salgado-Luarte et al. [18] measured the leaf toughness of different spp. of oak trees and 11 spp. of trees in the temperate rainforest, respectively. A recent study [19] addressed the seagrass (*Thalassia hemprichii*) leaf toughness and found 3.259 N exerted force (n = 32). It was also used in a study [15] on forest tree leaves, and the raw data were reported (0.8 N to 1.6 N). For the texture/toughness of the microgreens' leaves, our data gave a rough indication about leaf texture. However, there were significant texture differences between mustard microgreens grown on peat and on jute protocol 2 (0.78 $\pm$ 0.08 and 0.65 $\pm$ 0.06, respectively, Table 1), showing that microgreens grown on peat are crunchier/tougher.

Total phenolic compounds, chlorophylls, and carotenoids are slightly higher in microgreens grown on jute protocol 2, probably since, in jute, the growth is slower; it takes 2 days longer than in peat (specifically, 1 day longer in the germination chamber and 1 additional day in the greenhouse), thus, more time is available to draw reserves from the seed and more time for photosynthesis. As for mustard microgreens, different amounts of total phenolic content were found in the literature, e.g., the data about total phenols from Xiao et al. [24] was in agreement with our data showing 1.8 mg GAE/g FW. The

amount of total soluble polyphenols found in hydroponic microgreens of red mustard was 18.89 mg GAE/g DW [25], nearly in agreement with our results. However, Xiao et al. [26] reported the TPC in mustard microgreens as 1.5 mg GAE/g DW, which is around 20-fold less than our findings (Table 2), and also suggested that TPC is strongly correlated with flavor attributes.

The previous findings have stated somehow comparable levels of chlorophyll *a* and *b* content in mustard microgreens; for example, Chl *a* and Chl *b* contents in mustard microgreens were reported as 490 and 190 μg/g FW, respectively, whereas total carotenoid levels were 110 μg/g FW [27]. While evaluating the phytochemical composition of different microgreen species belonging to *Apiaceae, Brassicaceae*, *Lamiaceae*, *Malvaceae*, and *Chenopodiaceae*, Kyriacou et al. [28] reported the concentration of Chl *a* and Chl *b* in mustard as 510.0 and 137.3 μg/g FW, respectively. In a study on mustard microgreens [24], the amount of total carotenoids was reported as 108 μg/g FW, while in hydroponic microgreens of red mustard, the carotenoid content was very high, i.e., 2242.7 μg/g DW [25], as compared to our results (Table 3). Kyriacou et al. [23] (when working on microgreens of coriander, kohlrabi, and pak choi using different substrates) suggested that the concentrations of chlorophylls and carotenoids are affected rather by species.

5. Conclusions

Jute fiber mats have the advantage of being light in weight, degradable, and clean growth substrates for growing microgreens as they do not leave any soil residues on the seedlings. The results obtained with the jute substrate while growing green mustard microgreens using a modified protocol (i.e., a reduced water amount during sowing and increased irrigation turns during the growth period) are sufficiently comparable with the results obtained with the microgreens grown on peat substrate. Therefore, jute substrates could represent a sustainable alternative to peat, the most used substrate to date for the cultivation of microgreens, while ensuring quality and yield and also reducing post-harvest costs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9050598/s1, Table of statistical analysis of all the analyzed variables with level of significance and their *p*-values.

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Conflicts of Interest: The authors declare no conflict of interest.

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